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KIDNEY XENOTRANSPLANTATION

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Abstract

Xenotransplantation using pigs as donors offers the possibility of eliminating the chronic shortage of donor kidneys, but there are several obstacles to be overcome before this goal can be achieved. Preclinical studies have shown that while porcine renal xenografts are broadly compatible physiologically, they provoke a complex rejection process involving preformed and elicited antibodies, heightened innate immune cell reactivity, dysregulated coagulation, and a strong T cell-mediated adaptive response. Furthermore, the susceptibility of the xenograft to pro-inflammatory and pro-coagulant stimuli is probably increased by cross-species molecular defects in regulatory pathways. To balance these disadvantages, xenotransplantation has at its disposal a unique tool to address particular rejection mechanisms and incompatibilities: genetic modification of the donor. This review focuses on the pathophysiology of porcine renal xenograft rejection, and on the significant genetic, pharmacological and technical progress that has been made to prolong xenograft survival.

Keywords

immunosuppression; kidney; pig, genetically modified; pig-to-primate model; transgenesis; tolerance; xenotransplantation

Introduction

Kidney transplantation, the best treatment for end-stage renal disease, is limited by the shortage of human donors. Although the donor pool has been expanded by strategies such as paired donation and the use of blood group-incompatible and non-heart-beating donors, it remains unlikely to meet the increasing demand in the foreseeable future. This has driven a search for alternative sources of donor kidneys. Much recent activity has focused on the generation of transplantable tissue from autologous stem cells, but the complexity of the

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kidney makes this a long-term prospect at best (1). In contrast, xenotransplantation using pigs as donors has been studied for several decades (2, 3), and porcine cellular xenografts have already reached the stage of clinical trials (4). The pig is the animal donor of choice for a number of reasons including relatively similar organ size and physiology, high reproductive capacity, and the potential for genetic modification to prevent rejection and correct molecular incompatibilities. Preclinical studies indicate that pig kidney xenotransplantation is feasible, with renal xenografts supporting life for several weeks or months in non-human primate recipients (5-8). However, despite considerable progress in recent years, the immunological and pathophysiological barriers have not been completely overcome. The major challenge is to place renal xenografts on at least an equal footing with allografts i.e. with comparable survival rates under similar levels of immunosuppression. This is likely to require a combination of 'humanized' donors and clinically applicable immunosuppressive protocols. Herein we will review the mechanisms of porcine renal xenograft rejection and describe recent progress in moving kidney xenotransplantation to the clinic.

THE PIG-TO-PRIMATE PRECLINICAL MODEL

Current understanding of the function and immunobiology of pig renal xenografts is primarily drawn from studies using non-human primates (NHP) as recipients. The technical challenges of this model are significant, with a relatively high rate of failure from causes unrelated to rejection (7, 9-11), but it has provided invaluable information and insights.

Physiological compatibility

Although final proof must await clinical testing, extensive *in vivo* and *ex vivo* data indicate that pig kidneys will function adequately in humans (reviewed in (12)). The most comprehensive dataset on physiological compatibility comes from a study of 22 monkeys transplanted with human CD55-transgenic pig kidneys (survival: range 21-78 days, mean 41 days, median 38 days) (13). During the period of stable xenograft function, most serum electrolytes (urea, sodium, chloride, potassium and calcium) remained within the normal range, while creatinine was modestly elevated but steady. Of some concern, phosphate and haemoglobin levels progressively fell and serum albumin was consistently low after transplantation. The cause of hypophosphatemia was not established, while anemia was postulated to be due to molecular incompatibility of porcine erythropoietin with the primate Epo receptor, and was treated using recombinant human erythropoietin (13). Hypoalbuminemia and mild to severe proteinuria have also been reported in baboon recipients (5, 7), although whether this phenomenon is due to rejection-associated injury or to an inherent physiological difference remains to be determined. In either case, the solution may be provided by further genetic modification of the donor pig and/or pharmacological intervention.

Immunological considerations

Like humans, Old World primates (e.g. macaques and baboons) possess preformed antibodies to galactose-α1,3-galactose (αGal), a xenoantigen that is abundantly expressed on the surface of most pig cells (14, 15) (see details in following section). This makes these

animals the preferred model recipients from an immunological perspective. However, two potential limitations should be noted. First, macaques appear to have a more 'hypercoaguable' phenotype than humans (16), suggesting that coagulation disturbances may be exaggerated in this model. Second, macaques and baboons lack at least some types of anti-pig antibodies that are naturally present in humans. For example, humans develop antibodies to the carbohydrate *N*-glycolylneuraminic acid (also known as Neu5Gc or Hanganutziu-Deicher antigen), which is expressed in both pigs and NHP (17, 18). Nevertheless, the macaque and baboon models have been critical to unravelling the complex immune response to renal xenografts and testing new genetic and immunosuppressive strategies.

THE IMMUNE RESPONSE TO PIG KIDNEY XENOGRAFTS

The evolutionary distance between pigs and primates has resulted in carbohydrate and protein differences that not only promote immune recognition of porcine xenografts but also affect the function of immunoregulatory pathways. The most striking example of the importance of differential glycosylation is the αGal xenoantigen. Most mammalian species including pigs express α1,3-galactosyltransferase (GalT), an enzyme that synthesises the terminal carbohydrate moiety galactose-α1,3-galactose (αGal) on glycoproteins and glycolipids (15). Humans and other higher primates have lost αGal expression due to mutations in the coding region of the GalT gene *GGTA1* (19), possibly as an evolutionary immune defence against microbial pathogens (20), and develop anti-αGal antibodies in response to gut bacteria (21). In humans, anti-αGal comprises about 80% of preformed ('natural') anti-pig IgM (22) and is the most abundant natural IgG (23). This has profound consequences for kidney xenotransplantation, as outlined below.

The innate immune response and hyperacute rejection (HAR)

Unmodified pig kidneys provoke a rapid and powerful innate immune response in primates, characterized by binding of natural anti-pig antibodies to the xenograft vascular endothelium and activation of the classical complement pathway and the coagulation cascade. The resulting congestion, oedema and massive interstitial haemorrhage are hallmark features of this 'hyperacute' rejection (HAR) (24), which occurs within hours of reperfusion (25) (Figure 1A). The pivotal role of αGal is evident from the fact that specific depletion of anti-αGal antibodies prevented HAR of pig-to-macaque renal xenografts (26). Perhaps even more salient, elimination of αGal expression in the donor pig prevented HAR in the pig-to-baboon model in the absence of any other treatment (27). It is conceivable that natural human 'non-Gal' anti-pig antibodies, including those recognising other carbohydrate antigens such as Neu5Gc, may be present at sufficient levels in some individuals to precipitate HAR. Such antibodies have been detected in human serum (28), and at least some of them can mediate complement-dependent lysis and antibody-dependent cellular cytotoxicity to pig cells (29). However, the natural anti-non-Gal titer varies considerably between individuals, and it should be possible to minimize the impact of these antibodies by careful pre-screening of recipients (30).

Differential glycosylation also contributes to direct recognition of pig cells by human NK cells and macrophages (31-33), leading to a response that may be further heightened by

cross-species molecular incompatibilities affecting particular cellular interactions (34). Gene sequence analysis suggests that swine leukocyte antigen (SLA) class I, the porcine equivalent of HLA class I, will not transmit an inhibitory signal to receptors on human NK cells (35). Similarly, although human SIRPα has been shown to bind porcine CD47 *in vitro* (36), this interaction does not send a negative signal and thus does not prevent human macrophages from phagocytosing pig cells (37). These defects in the regulation of innate immune cell activity are likely to be most problematic for cellular xenografts, but may also contribute to renal xenograft rejection.

Dysregulated coagulation and inflammation

Dysregulated coagulation is a major barrier to the survival of pig kidney xenografts post-HAR (38). Thrombotic microangiopathy is observed in rejected renal xenografts (39, 40), albeit less often than in cardiac xenografts (41, 42). Furthermore, recipients frequently develop a consumptive coagulopathy characterized by thrombocytopenia, declining fibrinogen levels, increased D-dimer and thrombin-antithrombin levels, and prolonged clotting time (25, 43-46). This condition can be fatal once established (46), and only resolves upon removal of the xenograft (44). While the cause is yet to be formally determined, it is likely that several factors converge to promote excessive coagulation and inflammation. First, xenograft endothelial cells are activated by a range of mechanisms including ischemia-reperfusion, complement, antibody binding, and interaction with recipient immune cells (47-50), and consequently express tissue factor, the primary physiological initiator of coagulation. Second, it has been proposed that *recipient* tissue factor, expressed by platelets and monocytes activated by inflammation and contact with xenograft endothelium (51), also plays a role (46), although whether human platelets express tissue factor is controversial (52). Third, pig endothelial cells express an enzyme that converts human prothrombin to thrombin in a tissue factor-independent manner (53). Expression and activity of this direct prothrombinase (fgl2) is induced by pro-inflammatory cytokines (53, 54). Rejected pig-to-baboon renal xenografts showed fgl2 expression in close association with fibrin deposition in small vessels and glomerular capillaries (53).

Finally, at least one key regulatory mechanism is compromised by molecular incompatibility. The thrombomodulin (TM) / protein C pathway is a critical regulator of coagulation and inflammation within the microvasculature (55). TM is an integral endothelial membrane protein that alters thrombin's specificity from pro-coagulant and pro-inflammatory substrates to protein C, which in its activated form inhibits coagulation and inflammation (55, 56). Pig TM binds human thrombin but is a poor cofactor for activation of human protein C, with only 1-10% of the activity of human TM (57-59). Molecular incompatibility may also promote clotting; human platelets spontaneously aggregate *in vitro* upon contact with pig von Willebrand factor (vWF) due to an aberrant interaction between human platelet glycoprotein Ib and the *O*-glycosylated A1 domain of pig vWF (60-62).

The adaptive immune response and acute humoral xenograft rejection (AHXR)

T cells play a major role in xenograft rejection (reviewed in (63)). Both the direct and indirect presentation pathways are involved (64), and there is an extensive range of potential xenoantigens (65). If HAR is prevented and conventional immunosuppression is used to

inhibit the T cell-mediated adaptive response, the survival of renal xenografts is prolonged for days to weeks before they are rejected by a second antibody-mediated process termed acute humoral xenograft rejection (AHXR), also known as acute vascular rejection or delayed xenograft rejection (66-68) (Figure 1B). AHXR is characterized by varying degrees of antibody and complement deposition, microvascular thrombosis, focal ischemic necrosis and interstitial haemorrhage, endothelial cell changes, and leukocytic infiltration (40, 69). In situations where anti-αGal has little or no role, AHXR is mediated by anti-non-Gal antibodies, either preformed (if present in sufficient titer) (11) or elicited (70, 71). Candidate non-Gal xenoantigens include carbohydrates (72-74) and proteins (75, 76) including important endothelial cell-protective regulators (77). It is reasonable to suggest that the susceptibility of renal xenografts to AHXR is increased by several of the factors described earlier, such as innate immune cell hyper-reactivity and defects in regulatory mechanisms; even low levels of anti-graft antibodies may trigger a vicious cycle of coagulation and inflammation.

Acute cellular and chronic xenograft rejection

Classical acute cellular rejection of organ xenografts is rarely observed, probably because it is usually preceded by AHXR. A histopathological analysis of pig-to-baboon renal biopsies showed increasing infiltration by T cells and macrophages, revealing cellular rejection when humoral rejection is avoided (40). However, most evidence suggests that the T cell response can be controlled by currently available immunosuppressive agents and may become less problematic as the innate immune response is better managed (see below). The frequency and characteristics of chronic xenograft rejection are unclear because few renal xenografts have survived long enough for this type of injury to develop, although one case of chronic glomerulopathy similar to that observed in allografts has been described (40).

PREVENTING KIDNEY XENOGRAFT REJECTION

It is evident from the preceding section that the rejection of pig renal xenografts is a complex and powerful process that will likely require a combination of approaches to prevent.

Genetic modification of the donor

Perhaps the key advantage of xenotransplantation over allotransplantation is the ability to genetically modify the donor to protect grafts from the human immune system. The initial method of transgenesis by pronuclear microinjection (25, 78-80) has been superseded by somatic cell nuclear transfer (cloning), which can be used to delete porcine genes and / or add transgenes (reviewed in (81)). Recent technological advances allow precise engineering of the pig genome using zinc finger nucleases (ZFNs) (82) or transcription activator-like effector nucleases (TALENs) (83), and efficient co-expression of multiple transgenes using the 2A ‘ribosome skip’ signal (84). The main types of genetic modification that have been applied to pigs for the purpose of xenotransplantation are described below and summarized in Figure 2.

Carbohydrate remodelling—The initial genetic approach to the αGal / α1,3-galactosyltransferase problem was to transgenically express alternative glycosyltransferases. The level of αGal in pigs was reduced by expressing either human α1,2-fucosyltransferase (H-transferase or HT), which adds the non-antigenic blood group O to the substrate of α1,3-galactosyltransferase (85), or *N*-acetylglucosaminyltransferase III (GnT-III), which downregulates both αGal and non-Gal xenoantigens by an unknown mechanism (86). However, the advent of cloning made it possible to eliminate αGal altogether (87-92). Renal xenografts from the resulting GalT knockout (Gal KO or GTKO) pigs were resistant to HAR (5, 10, 27), although one case of HAR of a GTKO heart in the pig-to-baboon model has been reported, presumably caused by non-Gal IgM (93). Deletion of αGal may also attenuate the innate cellular response, although this is yet to be examined *in vivo*.

With the αGal problem solved, attention has shifted to other potential carbohydrate targets, particularly Neu5Gc. Recently, ZFN technology was used in conjunction with cloning to simultaneously delete the porcine genes responsible for expression of αGal (*GGTA1*) and Neu5Gc (*CMAH*), in a process that took only 7 months (94). Peripheral blood mononuclear cells from the resulting 'double-KO' pigs showed significantly reduced binding of preformed antibodies in human sera compared to GTKO alone cells. This is very promising, although it will not be possible to test the impact of deleting Neu5Gc in the primate model because NHP express this carbohydrate and thus do not develop antibodies to it.

Regulation of complement activation—Complement activation within transplanted organs is controlled by the combined action of membrane-bound complement regulatory proteins (CRPs) on the donor organ and soluble factors of recipient origin. The former comprise CD46 (also known as membrane cofactor protein or MCP) and CD55 (decay accelerating factor or DAF), which regulate mid-pathway, and CD59, which inhibits formation of the membrane attack complex in the terminal pathway (95). Although it was once thought that pig CRPs may not efficiently inhibit primate complement, potentially contributing to the rejection of pig organ xenografts, this does not appear to be the case (96, 97). Nonetheless, transgenic expression of one or more human (h)CRPs is generally effective in preventing HAR of renal xenografts (13, 25, 98-100), presumably by providing supraphysiological regulatory capacity. Evidence from the cardiac model suggests that the combination of transgenic hCRP expression and deletion of αGal provides greater protection to organ xenografts than either alone (93).

Regulation of coagulation, inflammation and thrombosis—Inflammation and coagulation are tightly interlinked. The vascular lining of organ xenografts is the focus of antibody binding, complement activation and immune cell activity, leading to endothelial cell activation and downregulation or shedding of key protective molecules (101-103). Most genetic approaches have therefore targeted transgenic expression of anticoagulant / anti-inflammatory / anti-platelet molecules to the vascular endothelium. Mouse studies have demonstrated proof of concept for the benefits of expressing human TM and tissue factor pathway inhibitor (104, 105). Both molecules have been expressed in pigs (106-109) but efficacy in an *in vivo* transplant setting has not yet been reported. The ectonucleotidase CD39 has also been utilized because of its broad vasculoprotective functions (degradation of

extracellular ATP and ADP, and promotion of adenosine generation) (110). Transgenic expression of human CD39 protected mouse hearts and kidneys from transplant-related and other vascular injury (111-114), and protected pig hearts from myocardial ischemia-reperfusion injury (115). However, renal expression of the CD39 transgene in this line of pigs appeared to be insufficient to prolong kidney xenograft survival in the pig-to-baboon model (116).

Hemoxygenase-1 (HO-1) has been widely studied in the transplant context because of its anti-inflammatory, antioxidant and cytoprotective properties (117). The potential benefits of human HO-1 expression in transgenic pigs have been demonstrated *in vitro* and *ex vivo* (118, 119), although *in vivo* efficacy has not been reported. Other promising candidate anti-inflammatory molecules that have reached a similar stage include human A20 (120) and soluble human TNF receptor 1 (shTNFR1) (121).

Regulation of innate immune cell activity—Genetic modification has been used to tackle the molecular incompatibilities affecting the heightened reactivity of human NK cells and macrophages to porcine targets. Endothelial cells from transgenic pigs expressing the non-classical human MHC class I molecule HLA-E were partially protected from human NK cell-mediated cytotoxicity *in vitro* (122). Expression of human CD47 on a pig lymphoblastoid cell line inhibited phagocytosis of the cells by human macrophages *in vitro* (37) and protected them from rejection in a mouse tumour model (123). Intuitively, these approaches would seem to be most applicable to cellular xenografts, and it remains to be seen whether they have any relevance to renal xenotransplantation.

Regulation of adaptive immunity—Several molecules have been expressed in transgenic pigs in attempts to block the T cell-mediated adaptive response. These include human TNF-related apoptosis-inducing ligand (TRAIL), an approach designed to kill xenograft-infiltrating T cells (124); pig dominant-negative class II transactivator (CIITA-DN), to inhibit upregulation of pig SLA class II (125); human CTLA4-Ig or its high-affinity variant LEA29Y (belatacept), to block costimulation of T cells associated with indirect antigen presentation (126, 127); and pig CTLA4-Ig, to block costimulation of T cells by direct antigen presentation (128). While *in vitro* analyses and limited cellular transplant studies in mice have produced promising results, no data are yet available from the pig-to-NHP model, and adverse health effects have been associated with one of the transgenes (128).

Immunosuppression and other pharmacological treatments

Therapies directed at T and B cells—Most groups studying preclinical pig-to-NHP organ xenotransplantation have incorporated a T cell-depleting agent, most commonly anti-thymocyte globulin (ATG), in their induction regimens. An anti-human CD2 monoclonal antibody (mAb) and an anti-monkey CD3 recombinant immunotoxin (anti-CD3 rIT) have also been shown to effectively deplete T cells in the pig-to-baboon renal model (129). However, the anti-CD2 mAb did not prolong xenograft survival, and the anti-CD3 rIT-treated recipients died from complications before efficacy could be determined (129). Treatment with the specific B cell-depleting agent rituximab (anti-CD20 mAb) was

beneficial in cardiac xenotransplantation (130, 131), but rituximab has not yet been tested in the renal model. Pan-lymphocyte depletion using the anti-human CD52 mAb alemtuzumab also remains untested in NHP models because CD52 is expressed on red blood cells in all NHP except cynomolgus monkeys of Indonesian origin (132). Interestingly, induction with cyclophosphamide has been more successful than T/B cell-specific therapies in prolonging renal xenograft survival (6), although this agent is rarely used today in solid organ transplantation.

While the combination of conventional maintenance immunosuppression (e.g. tacrolimus and rapamycin) with T and B cell depletion has produced encouraging results, there has been increasing interest in costimulation blockade using agents such as anti-CD154 mAb. Following induction with ATG ± rituximab, anti-CD154 therapy extended GTKO cardiac xenograft survival to up to 6-8 months in the pig-to-baboon model (131, 133). Anti-CD154 has been less successful in the renal xenograft model, but this was related to the relatively rapid onset of consumptive coagulopathy rather than failure to block T-cell mediated rejection or the elicited antibody response (46). With the co-transplantation of donor thymic tissue, anti-CD154 has contributed to significant prolongation of GTKO renal xenograft survival in baboons (5, 7) (see below). However, the anti-CD154 mAb employed in these studies is unlikely to be used clinically because of its thrombogenic properties (134-136). This has prompted an exploration of alternative costimulation blockade agents such as belatacept and anti-CD40 mAb, which have shown efficacy in the pig-to-macaque islet xenograft model (137).

Overall, the current experimental data suggest that costimulation blockade may be the preferred immunosuppressive therapy, but this would need to include blockade of both the CD40/CD154 and CD28/B7 pathways. It is conceivable, however, that conventional pharmacologic immunosuppressive therapy with agents such as tacrolimus and rapamycin may suffice, particularly if the pig has been genetically engineered to prevent or reduce activation of xenograft endothelial cells.

Anti-inflammatory / anti-coagulant treatments—The best prospect for control of inflammation and coagulation is likely to be genetic manipulation of the donor pig, as discussed earlier. However, there may still be a need for treatment with anti-inflammatory agents. In addition to corticosteroids, there is evidence that high-dose statin therapy not only reduces the inflammatory response and platelet activation (138), but also downregulates the primate cellular response to pig antigens (139).

Similarly, judicious treatment with anti-coagulant and/or anti-platelet agents may be beneficial even when genetically modified donor pigs are used. Some groups routinely administer continuous heparin to baboon recipients of pig renal (42) and cardiac (140, 141) xenografts, although it is difficult to assess the efficacy of this treatment. Administration of recombinant human antithrombin was clearly protective in the first week after pig-to-baboon renal xenotransplantation (98), but had no apparent long-term benefit in the pig-to-macaque renal model (142), even when combined with human activated protein C (143). Other reagents have also produced mixed results, and it would be reasonable to conclude that both

genetic modification and pharmacotherapy will be necessary to fully control inflammation and coagulation in renal xenotransplantation (144).

Induction of tolerance

As in allotransplantation, the induction of tolerance is the ultimate goal of those involved in xenotransplantation research, although it should be noted that tolerance is a long-term prospect at best and is not a prerequisite for clinical renal xenotransplantation. Prolonged kidney xenograft survival under clinically acceptable chronic immunosuppression remains the initial goal. The hurdles for tolerance in xenotransplantation are in some respects greater than those in allotransplantation, but there are also some advantages. The prior identification of the donor pig will allow pre-transplant preparation of the recipient, which is not possible with transplantation using organs from deceased human donors. Furthermore, the donor pig may be able to be genetically modified to facilitate the induction of tolerance. Efforts to induce tolerance to a xenograft have largely been confined to three major approaches: mixed chimerism, co-transplantation of donor thymic tissue, and cellular therapy with regulatory T cells (Tregs) or mesenchymal stem cells (MSCs).

Mixed chimerism—Based on a successful approach in human renal allotransplantation (145), great efforts have been made to induce mixed chimerism in NHP by the infusion of pig hematopoietic stem cells (146). However, phagocytosis of pig cells by human macrophages, probably as a result of the failure of pig CD47 to transmit a negative signal to human SIRPα, may be a major barrier to the development of mixed chimerism (147). *In vitro* (37) and small animal models (148) suggest that transgenic expression of human CD47 in pigs may be the solution to this problem.

Co-transplantation of donor thymic tissue—This concept is based on the depletion of mature T cells before the combined transplantation of the organ xenograft and donor thymic tissue, thus allowing new T cells to recognize pig antigens as self, and T cells directed to pig antigenic specificities to be deleted. After recipient pre-treatment, transplantation of donor-specific pig thymus tissue with a kidney graft, either in the form of a previously-prepared ‘thymokidney’ or as a thymic lobe transplant, extended graft survival to almost three months (5, 7). Antibody-mediated rejection remained problematic, though most baboons died from the effects of the intensive immunosuppressive therapy rather than from graft failure. Whether T cell tolerance will be precluded by the additional barriers to xenotransplantation remains uncertain. It may be necessary to overcome the innate immune response and coagulation dysregulation through other means before T cell tolerance can be achieved.

Cellular therapy—Although no studies of expanded Tregs or MSCs have been carried out in pig-to-NHP organ transplantation models, there have been several *in vitro* studies (149-152), and some experience of MSC therapy in pig-to-baboon islet transplantation (153). Tregs have effects on both the direct and indirect T cell response, and inhibition of the CD40/CD154 pathway seems to be particularly beneficial in enhancing Treg activity (154, 155). MSCs have received considerable attention in recent years for their immunomodulatory, anti-inflammatory and regenerative effects. MSCs function across

species barriers (156), and pig MSCs suppress the human T cell response to pig tissues (157, 158). Pig MSCs have the advantage that they can be obtained in very large numbers from adipose tissue or bone marrow, and therefore require less expansion in vitro before infusion into the xenograft recipient. In addition, the same donor can be used for the MSCs and the xenograft, avoiding allogeneic differences.

MANAGING INFECTIOUS RISK

Transplantation carries the possibility of transmission of infectious agents from the donor organ to the immunocompromised recipient. In xenotransplantation, this potential risk is compounded by fears of a wider risk to the community posed by human-to-human transmission of novel pig-derived pathogens, such as porcine endogenous retrovirus (PERV). While the degree of risk associated with renal xenotransplantation remains unknown in the absence of clinical trials, preclinical data and the limited data from human trials indicate that infectious transmission will occur rarely, if at all (159). Nevertheless, clinical trial guidelines have been established to manage infectious risk (160), with basic principles including national and global oversight, routine screening and maintenance of source pigs in specific or defined pathogen-free facilities, pre-and post-transplant screening of recipients, and long-term archiving of animal and patient samples. Those readers interested in learning more about this topic are directed to a recent review (161). Another cross-species aspect that has received less attention is the possibility of ‘reverse’ infection: viral transmission from the recipient to the xenograft. Several human viruses are capable of productively infecting pig cells (162). However, it is likely that this potential problem will be manageable by careful monitoring and management.

One of the difficulties in extrapolating from the NHP model to predict infectious risk in the clinical setting is the markedly different environment of NHP and human recipients, both pre-and post-transplant. The recent availability of specific pathogen-free primates may help in this regard, although this option would add significantly to the cost of this already expensive model.

CONCLUSIONS AND PERSPECTIVE

Advancing kidney xenotransplantation to the clinic is a daunting challenge. The glycosylation and protein differences between pigs and humans provoke a powerful humoral and cellular immune response to porcine xenografts that cannot be controlled by conventional immunosuppression. Progress in the difficult pig-to-NHP preclinical model has been painstaking and slow, with maximum graft survival currently limited to about 3 months. However, few would disagree that the reward for success – elimination of the donor kidney shortage – warrants continued effort. The key goal is to overcome the humoral, coagulation and inflammatory responses by genetic modification of the donor pig. While clinical application is probably still several years away, the increasingly sophisticated tools for precise manipulation of the pig genome, along with the development of novel immunosuppressive agents and tolerance-inducing protocols, bode well for the future.

Several questions remain. First, what level of preclinical success would justify moving to the next stage? We suggest that the *consistent* demonstration of life-supporting renal function for at least 5-6 months in the pig-to-NHP model, with clinically applicable immunosuppression and no evidence of infection, would be sufficient. Second, which potential recipient group would be most appropriate for the first clinical trials? Highly allosensitized patients with broadly reactive preformed alloantibodies are the obvious choice; they are less unlikely than other patients on the waiting list to receive a human kidney graft, and *in vitro* analyses indicate that they are at no greater risk of xenoreactivity to porcine tissue (163). Third, will renal xenotransplantation result in sensitization to alloantigens, and thus potentially jeopardize subsequent allotransplantation? The answer to this question is less clear, although the demonstration that baboons sensitized to pig antigens did not develop increased humoral or cellular alloreactivity (164) is encouraging. Finally, what is the best way to move the field forward and efficiently address the many issues still facing the pig-to-NHP model? Ideas and technologies are not lacking, but stable funding support is difficult to obtain and the intellectual property landscape is far from clear. Real progress is likely to require expanded collaboration between groups working on different organs and tissues, including the sharing of new genetically modified pigs.

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Abbreviations

APC	activated protein C
AHXR	acute humoral xenograft rejection
CIITA-DN	dominant-negative class II transactivator
CRP	complement regulatory protein
EPCR	endothelial protein C receptor
GnT-III	*N*-acetylglucosaminyltransferase III
GTKO	GalT knockout
HAR	hyperacute rejection
HO-1	hemoxygenase-1
HT	α1,2-fucosyltransferase or H-transferase
mAb	monoclonal antibody
MSCs	mesenchymal stem cells
Neu5Gc	*N*-glycolylneuraminic acid
NHP	non-human primate

PERV	porcine endogenous retrovirus
SLA	swine leukocyte antigen
shTNFR1	soluble human TNF receptor 1
TFPI	tissue factor pathway inhibitor
TM	thrombomodulin
TRAIL	TNF-related human apoptosis-inducing ligand
Tregs	regulatory T cells
vWF	von Willebrand factor

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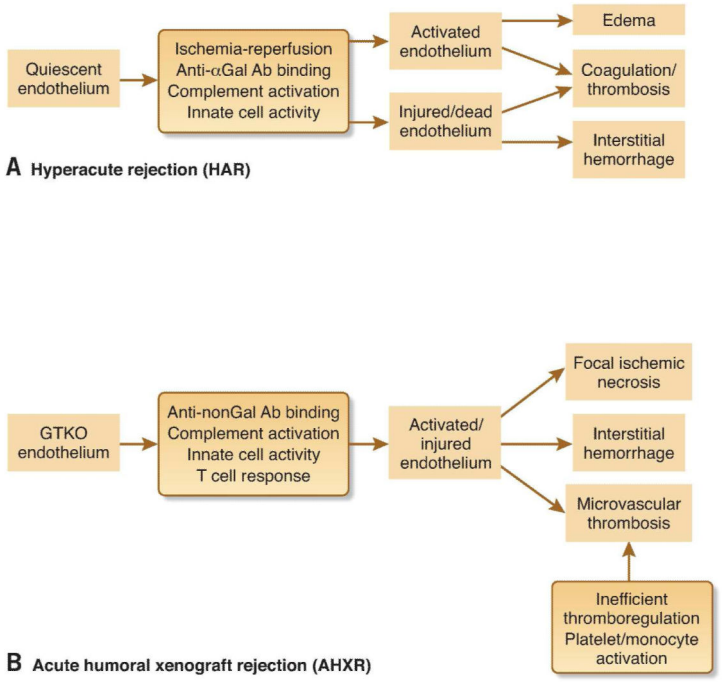

Figure 1. Phases of kidney xenograft rejection

A, Several factors contribute to hyperacute rejection of wild-type xenografts, but the key events are the binding of preformed anti-αGal antibodies (Ab) to xenograft vascular endothelial cells and subsequent activation of complement. HAR occurs within hours and can be prevented by deletion of αGal (GTKO) or transgenic expression of human complement-regulatory proteins (hCRPs). *B*, Acute humoral rejection of GTKO xenografts is also mediated by antibodies, in this case anti-non-Gal, but is a more prolonged process (days to weeks) which appears to involve the gradual development of a chronic pro-coagulant and pro-inflammatory vascular environment.

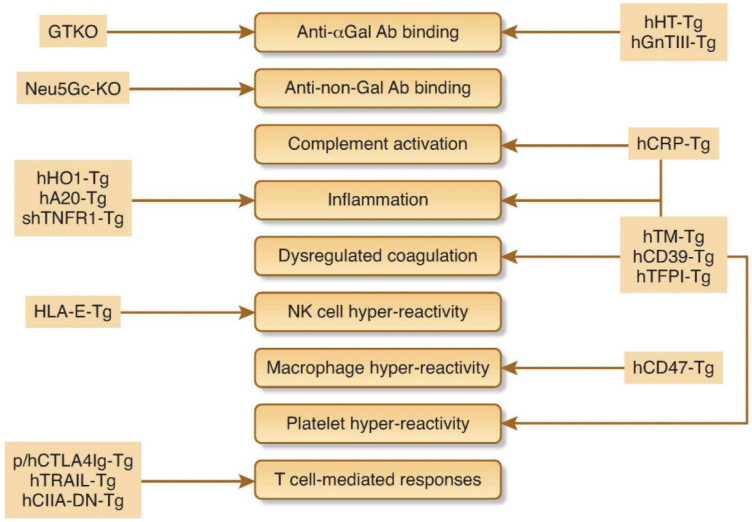

Figure 2. Donor genetic modification to prevent kidney xenograft rejection

The targets of genetic modification are shown as filled boxes. Abbreviations: CIITA-DN, dominant-negative class II transactivator; CRP, complement regulatory protein; GnT-III, *N*-acetylglucosaminyltransferase III; GTKO, GalT knockout; h: human; HO-1, hemoxygenase-1; HT, H-transferase; Neu5Gc-KO, *N*-glycolylneuraminic acid knockout; HLA, human leukocyte antigen; p: pig; shTNFR1, soluble human TNF receptor 1; Tg: transgenic; TFPI, tissue factor pathway inhibitor; TM, thrombomodulin; TRAIL, TNF-related human apoptosis-inducing ligand.