Clinical Islet Xenotransplantation
How Close Are We?

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Type 1 diabetes (T1D) is a major health problem throughout the world. In the U.S., it is estimated that about 1.5 million people suffer from T1D. Even when well controlled—by frequent monitoring of blood glucose and administration of insulin, the long-term complications of the disease are significant and include cardiovascular disease, nephropathy, retinopathy, and neuropathy (1). Here we review recent progress in preclinical models of pig islet xenotransplantation and discuss the remaining challenges that need to be addressed before the application of this form of therapy can be established in patients with T1D.

During the past decade, islet allotransplantation alone (without previous kidney transplantation) using deceased human donor pancreata has been indicated mainly in patients who have had T1D for >5 years with life-threatening hypoglycemic episodes and wide fluctuations in blood glucose levels. Although the initial long-term results were rather disappointing (2), the results of islet allotransplantation have improved significantly in recent years, with 5-year insulin-independent normoglycemia achieved in >50% of patients at experienced centers (3). There is increasing evidence that successful islet allotransplantation greatly reduces the incidence of hypoglycemic episodes (2) and reduces or slows the incidence of late complications of T1D (4). This may extend the indications for islet transplantation to patients with progressive complications. For example, islet transplantation in a patient with preterminal renal failure may prevent disease progression, possibly avoiding the need for hemodialysis and kidney transplantation, provided that nonnephrotoxic immunosuppressive drug therapy is administered.

Currently, in the U.S., the median waiting time for a kidney allograft from a deceased human donor is >4 years (5). However, islets from two deceased human donor pancreata are frequently required to achieve normoglycemia in a diabetic patient. Because of the limited number of suitable deceased donor pancreata, the overall number of treated patients is small, with fewer than 1,000 procedures carried out in Western countries during the past 10 years (2). It is likely that the demand for this procedure will increase, resulting in a growing need for new sources of islets for transplantation. Although there is a prospect that this need can be filled by islets from pigs (Sus scrofa), it is unlikely that nonhuman primates (NHPs) will be used for this purpose because there are significant concerns associated with ethics, logistics, and, potentially, safety.

HISTORY OF CLINICAL ISLET XENOTRANSPLANTATION

The first scientific attempt to transplant pig islets into patients with T1D by Groth et al. (6) in 1994 resulted in detectable pig C-peptide in urine beyond 300 days and insulin-positive staining of graft biopsies in patients receiving combined human kidney and pig islet transplants. Despite these results, glucose metabolism remained unaltered. In Mexico, pediatric diabetic patients have been transplanted with pig islets that were co-transplanted with Sertoli cells placed in a stainless steel chamber that was implanted under the skin (7). In New Zealand, pig islets have been encapsulated individually in alginate and transplanted into the peritoneal cavity, an immunoisolating approach that avoids the need for immunosuppressive therapy (8). A trial of this approach is underway with appropriate regulatory oversight, and publication of the results is anticipated.

Regardless of the results of these xenotransplantation trials, lively discussion about their justification on regulatory and ethical grounds has emerged. Whereas the level of regulatory oversight of the Mexican trial was likely insufficient, the trial in New Zealand is being regulated carefully by that country’s Ministry of Health (8). Nevertheless, none of these clinical studies was preceded by peer-reviewed, preclinical data in NHPs proving the efficacy of the therapy. The World Health Organization and the International Xenotransplantation Association have both stressed that, in addition to the need for strict procedures to guarantee a safe pig product, patients should be exposed to islet xenotransplantation only if there is a relatively high expectation of benefit (9,10). Although convincing preclinical data from experiments in NHPs was not required in the past before the introduction of islet allotransplantation, these data remain the best indication of the potential therapeutic benefit of islet xenotransplantation. In recent years, significant progress has been made, with pig islets providing sustained (>1 year) normoglycemia in a small number of NHPs in which diabetes had been induced.
EXPERIENCE IN NHP MODELS

Six groups have independently reported that pig islets transplanted into NHPs can maintain normoglycemia for periods in excess of 6 months (Table 1) (refs. 55–59). When free islets have been transplanted, immunosuppressive therapy has been essential to prevent rejection. When encapsulated islets have been transplanted, however, encouraging results have been achieved in the absence of immunosuppressive therapy (11). Furthermore, 6-month graft survival has been achieved after either adult or neonatal free islet transplantation, as well as after fetal pancreas transplantation (Table 1). Collectively, these results indicate that there is reason to believe that pig islet xenotransplantation, either of free islets or encapsulated islets, will be clinically successful in due course.

REMAINING CHALLENGES FOR SUCCESSFUL CLINICAL APPLICATION OF ISLET XENOTRANSPLANTATION

Successful clinical application of islet xenotransplantation currently is inhibited by a number of barriers. These include the immediate loss of islets in an instant blood-mediated inflammatory reaction (IBMIR) and strong T cell-mediated rejection, requiring the use of excessive immunosuppression. The optimum age of donor pigs (e.g., fetal, neonatal, or adult) and the optimum anatomical site for transplantation are the subject of ongoing investigation. We discuss recent insights into these challenges and propose strategies to overcome them.

Inflammation after islet xenotransplantation. The initial hurdle faced by islets transplanted into the portal vein is IBMIR, which results in significant destruction of islets within minutes. IBMIR is believed to be a nonspecific (i.e., nonimmune) inflammatory response related to the transplantation of islets directly into the blood stream of the portal vein, which is the current site for clinical islet transplantation. Isolated islets can express tissue factor, which activates coagulation. As a result, platelets and complement are activated and the islets become infiltrated with neutrophils and macrophages (12) (Fig. 1). The extent of tissue factor expression on the islet graft negatively correlates with the clinical success of allotransplantation (13). Incompatibilities between the human and pig coagulation-anticoagulation systems render IBMIR even more problematic in xenotransplantation. Inhibition of tissue factor expression or thrombin formation prevented islet damage in vitro (14,15). However, in vivo, anticoagulation does not fully prevent IBMIR (12).

When wild-type pig organs (rather than islets or cells) are transplanted in NHPs, they are subject to hyperacute rejection. The vascular endothelium of pigs expresses the important galactose-α,1,3-galactose (Gal) oligosaccharide against which humans have natural anti-Gal antibodies (16). The binding of antibodies to Gal antigens results in almost immediate complement activation, with ensuing destruction of the graft. Although fetal and neonatal pig islets express Gal, the expression of Gal on islets is reduced as the pig matures (17). It was, therefore, originally anticipated that hyperacute rejection might not occur after transplantation of adult pig islets, although that concept is now being questioned.

Although it was first concluded that complement activation in IBMIR occurred mainly through the alternative pathway (18), recent studies suggest that human preformed IgM and IgG antibodies bind to human and, particularly, pig islets and activate complement through the classical pathway (19,20). In patients with preformed antibodies, particularly when there are high antibody titers against foreign human leukocyte antigens, success rates in achieving sustained normoglycemia after islet allotransplantation have been lower (21).

Neonatal pig islets express Gal, making them a target of anti-Gal antibodies. Neonatal islets from pigs that do not express Gal, that is, α1,3-galactosyltransferase gene-knockout (GTKO) pigs, are less susceptible to IBMIR in an NHP model (22). The expression of Gal on adult pig islets is low (13), suggesting that antibody binding to other (i.e., non-Gal) antigens may be an initiating factor in complement activation. These results suggest that IBMIR is less “nonspecific” than previously anticipated and involves a mechanism comparable to the hyperacute rejection of a pig organ.

TABLE 1

Experience with pig-to-nonhuman primate islet xenotransplantation, in which >6 months of functional graft survival has been achieved

| Reference     | Pig islet source | Islets transplanted (μ) | Site of transplantation | Immunosuppressive therapy                  |
|---------------|------------------|-------------------------|-------------------------|--------------------------------------------|
| Sun et al. (56) | WT adult, alginate encapsulated | 1–3 × 30,000–70,000 | Intraperitoneal | None                                      |
| Hering et al. (24) | WT adult | 25,000/kg | Intraportal | Anti-CD25 mAb, FTY720/tacrolimus, sirolimus |
| Cardona et al. (57) | WT neonatal | 50,000/kg | Intraportal | Anti-CD25 mAb, anti-CD154 mAb, CTLA4-Ig, sirolimus |
| Cardona et al. (58) | WT adult | 25,000/kg | Intraportal | Anti-CD25 mAb, anti-CD154 mAb, CTLA4-Ig, sirolimus |
| van der Windt et al. (30) | hCD46 adult Fetal pancreatic fragments | 6 pockets × 10 fragments/pocket | Intraportal | Omentum ATG, anti-CD154 mAb, MMF |
| Hecht et al. (59) | WT adult, alginate encapsulated | 30,000/kg | Subcutaneous | None                                      |
| Thompson et al. (60) | WT neonatal | 50,000/kg | Intraportal | Anti-CD25 mAb, anti-CD40 mAb, CTLA4-Ig, sirolimus |
| Thompson et al. (22) | GTKO neonatal | 50,000/kg | Intraportal | Anti-CD154 mAb, anti-LFA-1 mAb, CTLA4-Ig, MMF |

ATG, antithymocyte globulin; MMF, mycophenolate mofetil; WT, wild type.
To date, the identity of non-Gal antigens on pig islets has not been determined, although N-glycolylneuraminic acid is likely to be a target when clinical xenotransplantation is undertaken. However, this oligosaccharide is not important in pig-to-NHP islet transplantation because NHPs also express it and therefore do not produce natural antibodies against it (23).

Rejection of pig islets by the adaptive immune system. After theIBMIR, and likely driven, in part, by this event, the adaptive immune response to xenografted islets is largely T cell–mediated (24). Success in NHPs has been achieved only when costimulatory signals between antigen-presenting cells and T helper cells are blocked, especially with an anti-CD154 monoclonal antibody (mAb). Unfortunately, the increased risk of thromboembolic complications with the use of anti-CD154 mAb (25) prevents this biological from being applied clinically, and alternative strategies are warranted.

The autoimmunity associated with T1D is caused by self-reactive T and B cells directed against proteins expressed in pancreatic β-cells. Proinsulin, islet antigen-2, glutamic acid decarboxylase-65 and -67, and islet cell autoantigen of 69 kDa are the major targets. After allotransplantation of islets, autoimmune lymphocytes can react against the same antigens expressed on grafted islets, thereby contributing to graft failure (26). Although it is largely unknown whether this will occur after islet xenotransplantation, T cells from patients with T1D proliferate when incubated with fetal pig islet-like cell clusters (27), and they are specifically directed against pig glutamic acid decarboxylase. It can be anticipated, therefore, that xenografted pig islets will be subjected to autoimmune activity, as well as xenoinmune activity, to some extent.

The current evidence is that pig islet transplantation, even if associated with xenosensitization, would not lead to sensitization against alloantigens, and therefore would not compromise subsequent islet or kidney allotransplantation (reviewed in Cooper et al. [28]).

Donor age and preparation of pig islets. Significant debate has taken place about whether the ideal islets for clinical transplantation should be from fetal, neonatal, or adult pigs (Table 2). It generally is known that adult pig islets are more difficult to obtain consistently than adult human islets. In young adult pigs (<2 years old), islets are

TABLE 2
Advantages and disadvantages of islets derived from pigs of different ages

| Islet type            | Advantages                                      | Disadvantages                                    |
|-----------------------|-------------------------------------------------|--------------------------------------------------|
| Fetal                 | • No isolation procedure necessary              | • Not fully functional until >4 months after transplantation |
|                       | • Proliferation and maturation in vivo          | • Nonsurival C-section in sow                    |
|                       | • No need for harmful purification process      | • Need for many fetuses                          |
|                       | • Proliferation in vitro and in vivo            | • Not fully functional until >4 weeks after transplantation |
|                       | • Resistance against hypoxia                   |                                                  |
|                       | • Preferable breeding logistics                 | • Fragility of islets                            |
|                       | • More preferable breeding logistics vs. adult >2 years |                                                  |
| Neonatal              |                                                  |                                                  |
| Young adult (<2 years of age) | • Consistent islet yields                       | • Difficult to obtain consistent yields          |
|                       |                                                 | • Less preferable breeding logistics vs. neonatal |
| Adult (>2 years of age)| • Consistent islet yields                       | • Nonpreferable breeding logistics               |
|                       |                                                 | • High costs                                     |
smaller than in pigs >2 years old, making them more likely to become fragmented during the isolation procedure and reducing their in vitro and in vivo functional capacities (29). Although isolation procedures for adult islet donors have improved significantly, pigs aged >2 years, particularly retired breeder sows, may have certain benefits. The period of ex vivo culture of adult islets between isolation and transplantation has ranged from 16 to 48 h (24,30).

Neonatal islets may be preferred for several reasons (Table 2), including their higher resistance to hypoxia (31). From a logistical perspective, it is preferable to recover the pancreas from neonates during the first week of life (usually at 1–3 days of age [22,32]) than to maintain pigs under barrier conditions in a “clean” environment for >2 years, an approach that is space- and time-consuming as well as expensive. However, logistical success may depend on methods to store or cryopreserve neonatal islets. After isolation, the current approach is for the so-called neonatal islet cell clusters to be maintained in culture for 7 days, during which they proliferate, a consideration that is also important after transplantation (32).

Recent data indicate that fetal pancreata, excised at 42 days of fetal life, can result in successful implantation after transplantation into NHPs (32). However, it can take up to 5 months for the tissue to become fully functional (32), a period during which patients would be required to maintain insulin therapy as well as immunosuppressive therapy. Moreover, a large number of fetal pigs (60 fetuses as extrapolated from studies of NHP recipients [33]) would be required to provide sufficient tissue to induce normoglycemia in a single adult human.

The number of free pig islets needed to achieve normoglycemia in NHPs has been estimated at ≥25,000 islet equivalents (IEQ)/kg for adult islets and ≥50,000IEQ/kg for neonatal islets (22,24). This is significantly in excess of the number of human adult islets needed in clinical allotransplantation (10–15,000 IEQ/kg). The exact number of islets required to cure diabetes in humans is as yet uncertain, but with the numbers of pig IEQ per kilogram in NHPs, and based on a yield of 400,000 IEQ per adult pig (29), islets from several adult pigs may be required to cure one patient. However, islets from neonatal pigs maintain a proliferative capacity after transplantation (32), which may result in a functional islet mass after transplanting a smaller number of islets.

Alginated encapsulated pig islets (30,000 IEQ/kg), loaded onto a macrodevice and placed subcutaneously, reversed diabetes in NHPs (11). This is an attractive approach because encapsulation prevents the need for immunosuppressive therapy. However, several technical challenges need to be overcome, including degradation of capsules over time, reduced islet viability inside the capsules from a lack of nutrients, induction of antipig antibodies, and possibly humoral rejection.

**STRAATEGIES TO OVERCOME THE REMAINING HURDLES**

**Genetic engineering of pigs.** Our ability to genetically engineer pigs has increased significantly during the past 20 years, resulting in the production of pigs with different genetic modifications (Table 3) (refs. 61–74). These pigs can be cross-bred to produce an “ideal” pig for islet transplantation. The genetic engineering of pigs currently is aimed at providing resistance to the effects of IBMIR and to both the innate and adaptive immune responses.

Although encouraging results have been reported after transplantation of wild-type (unmodified) pig islets into NHPs, it is almost certain to be advantageous (particularly if fetal or neonatal islets are to be transplanted) to transplant islets from GTKO pigs. Development of methodology to disrupt the GT gene (34), in combination with cloning techniques (35), resulted in the first GTKO pigs in 2003 (36). A recent report indicates that there is less antibody binding and immediate injury to neonatal islets from these pigs compared with those from wild-type pigs (22). Therefore, the background for pigs to be used for clinical islet transplantation is likely to be GTKO (particularly when neonatal pig islets are used), but expression of one or more human complement-regulatory proteins (hCRPs), for example, CD46, CD55, and CD59, also will be advantageous (30). Thus, the deleterious effects of anti-Gal antibody binding will be obviated, and, although the anti–non-Gal antibody will bind to the pig islets, its effects will be mitigated by the protection offered by hCRP expression.

Theoretically, it would seem worthwhile to have GTKO/hCRP pigs in which one or more anti-inflammatory genes also are expressed, for example, CD39, *heme oxygenase-1*, and A20. To help diminish the IBMIR, expression of one or more “antithrombotic genes” (e.g., tissue factor pathway inhibitor, thrombomodulin) is likely to prove beneficial. Cells from pigs in which the major histocompatibility complex class II transactivator has been knocked down (CIITA-DN pigs) also are likely to reduce the direct T cell response to swine leukocyte antigen class II (Table 3), which is expressed on a subset of islet cells (37).

Genes can be specifically expressed in islets with the use of an insulin promoter. Expression of molecules for blockade of costimulatory pathways, such as porcine or human cytotoxic T-lymphocyte antigen 4 (CTLA4)-Ig, might provide local protection from the T cell–mediated response (Table 3). Pigs with multiple genetic modifications (e.g., GTKO/hCD46/hTFPIIns/pCTLA4-IgIns, with and without hCD39Ins) currently are available (Fig. 2), and islets from such pigs adequately correct hyperglycemia in diabetic monkeys (Fig. 3C) in an ongoing trial at our center.

**Immunosuppressive drug regimen.** To date, a clinically applicable immunosuppressive drug regimen that can prevent the xenoinmune response has not been established. In particular, an alternative to the efficacious but clinically inapplicable anti–CD154 mAb remains an obstacle. Thromboembolic complications possibly could be prevented using a fragment crystallizable region–disabled mAb (38).

Other costimulatory blockers, such as CTLA4-Ig, may be effective, especially when used in combination with endogenous “immunosuppressive” genetic manipulations (CTLA4-IgIns, CIITA). Alemtuzumab for deep lympho-depleting induction therapy currently is included in the most successful clinical regimens for islet allotransplantation. We have recently developed an NHP model for the use of alemtuzumab (39), and we plan to use alemtuzumab in our next islet xenotransplantation experiments.

**Alternative anatomical sites for islet transplantation.** Even though the pig could provide an unlimited supply of islets, the inefficiency of islet transplantation into the portal vein resulting from IBMIR remains an obstacle for applying islet xenotransplantation on a clinical scale. To avoid or minimize IBMIR, an alternative approach is to place the islets in a site where they are not immediately exposed to blood, and investigation in this area is ongoing. A number of sites have been investigated, some of which seem worthy of continued assessment.
Transplantation into the gastric submucosal space can be achieved through endoscopy (40) and offers the advantage of possible endoscopic biopsy of the graft for investigation of rejection, apoptosis, or both (41). Intra-muscular transplantation has already reached the clinical stage in islet autotransplantation (42). In diabetic monkeys, islets loaded onto a biodegradable scaffold, wrapped with omentum and placed between abdominal muscle layers, resulted in significant metabolic improvement in allo-transplantation experiments (43).

Immunomodulation. Pig islet transplantation may be enhanced by cotransplantation of mesenchymal stem cells of either recipient or donor origin (44) or of donor Sertoli cells (45). Both cell types may provide immunoprotection, revascularize the islets more quickly, and reduce inflammatory response.

**PHYSIOLOGIC COMPATIBILITY OF PIG ISLET XENOTRANSPLANTATION**

After infusion into the portal blood stream, transplanted islets depend heavily on diffusion of oxygen from the hypoxic portal blood until revascularization (mainly from the hepatic artery) is completed, a process that may take up to 14 days (reviewed in Jansson and Carlsson [46]). Both donor intra-islet endothelial cells and recipient endothelial cells contribute to this process (47,48).

Pig insulin differs from human insulin by only one amino acid and it was administered to patients with T1D for many years before recombinant human insulin became available. Nevertheless, there are differences in glucose metabolism among pigs, NHPs, and humans, and these have been discussed by Casu et al. (48). In pigs, fasting blood glucose values are higher and C-peptide levels are lower when compared with values in cynomolgus monkeys (48) (Fig. 4). As a result, when pig islets are transplanted into monkeys they have to perform at "supraphysiologic" levels. Nevertheless, maintenance of normoglycemia for up to 1 year after pig islet transplantation in a diabetic monkey has already been demonstrated (30) (Fig. 3). Because C-peptide levels in humans lie between those in the pig and monkey, it may be easier to achieve and maintain normoglycemia in humans after pig islet xenotransplantation using fewer islets per kilogram of body weight than in NHPs (49).

**TABLE 3**

Currently available pigs with genetic modifications that may be beneficial for islet transplantation

| Reference | Genetic modification | Expression on islets |
|-----------|----------------------|---------------------|
| Phelps et al. (36) | α,3-galactosyltransferase gene-knockout | Yes |
| Hara et al. (61) | Human H-transferase gene expression (expression of blood type O antigen) | Unknown |
| Miyagawa et al. (62) | N-acetylglucosaminyl-transferase III transgene | Unknown |
| Zhou et al. (63); Loveland et al. (64) | Human CD46 (membrane cofactor protein) | Yes |
| White et al. (65) | Human CD55 (decay accelerating factor) | Yes |
| Diamond et al. (66) | Human CD59 (protectin/membrane inhibitor of reactive lysis) | Unknown |
| Ayares et al. (67) | Human tissue factor pathway inhibitor | Yes |
| Petersen et al. (68) | Human thrombomodulin | Unknown |
| Le Bas-Bernardet et al. (69); Ayares et al. (67) | Human CD39 (ectonucleoside triphosphate diphosphohydrolase-1) | Yes |
| Phelps et al. (70) | Porcine CTLA4-Ig (CD152) | Yes |
| Hara et al. (71) | CTITA-DN (MHC class II transactivator knockdown, resulting in swine leukocyte antigen class II knockdown) | Unknown |
| Weiss et al. (72) | HLA-E/human β2-microglobulin (inhibits human natural killer cell cytotoxicity) | Unknown |
| Peterson et al. (73) | Human heme-oxidase 1 | Unknown |
| Oropeza et al. (74) | Human A20 (tumor necrosis factor-α–induced protein 3) | Unknown |
| Le Bas-Bernardet et al. (69) | GTKO/hCD55/hCD59/hCD39/human fucosyl transferase | Ins/TFPIIns/hCD39Ins | Yes |
| Ayares et al. (67) | GTKO/hCD46/pCTLA4-IgIns/TFPIIns/hCD39Ins | Yes |

Transplantation into the gastric submucosal space can be achieved through endoscopy (40) and offers the advantage of possible endoscopic biopsy of the graft for investigation of rejection, apoptosis, or both (41). Intra-muscular transplantation has already reached the clinical stage in islet autotransplantation (42). In diabetic monkeys, islets loaded onto a biodegradable scaffold, wrapped with omentum and placed between abdominal muscle layers, resulted in significant metabolic improvement in allo-transplantation experiments (43).
in the case of encapsulated islets. Accordingly, it is important to ascertain that islet function in these genetically engineered pigs remains within the normal range for pigs. Glucose metabolism has been investigated in GTKO pigs and has been found to be similar to that in wild-type pigs (50). More recently, pigs have become available to us (through Revivicor, Blacksburg, VA) that, through the use of an insulin promoter, have transgenes that are expressed selectively in the β-cell of the islets. Our initial studies indicate that glucose metabolism in these pigs is similar to that in wild-type pigs.

After islet allotransplantation, aggregation of islet amyloid polypeptides in the transplanted islets can promote amyloidosis and β-cell apoptosis. Differences in the amyloid polypeptide sequence between humans and pigs may explain observations showing a lack of amyloid formation after porcine islet transplantation, indicating improved survival (51) (Fig. 5).

SAFETY OF CLINICAL ISLET XENOTRANSPANTATION

Safety has been discussed for many years; much of the discourse has focused on the potential for the transfer of pig microorganisms to the islet recipient and, more importantly, to the general population. Guidelines from U.S. regulatory authorities direct that pig donor organs and cells must be free of specified bacteria, viruses, protozoa, and fungi (52). Breeding and housing of pigs in biosecure barrier facilities can eradicate many pathogens with zoonotic capabilities. For example, porcine cytomegalovirus (pCMV) can be excluded relatively easily from the islet source herd by early weaning from the sow (53). Although rare, occasional isolated pig islets have tested positive for pCMV (54), potentially constituting a risk for patients receiving immunosuppressive therapy. However, active transmission into NHPs after islet transplantation has not been documented. Efforts to continue testing for pCMV and to exclude it from the pig herd are warranted.

Even if the pigs are housed in an ideal “clean” barrier environment, they will inevitably carry the porcine endogenous retrovirus, which is integrated in the genome of pig cells and therefore will be transplanted with the islets. However, monitoring of humans exposed to pig tissues and cells and of NHP recipients of pig grafts has never identified active replication of the porcine endogenous retrovirus (10). Currently, transfer of this virus is not considered to be a serious risk, and although national regulatory authorities (e.g., the U.S. Food and Drug Administration) will insist on monitoring for the virus, these bodies are unlikely to preclude clinical xenotransplantation on the basis of the presence of the virus alone (52). Furthermore, if absolutely essential, techniques of small interfering RNA have been developed in which activation of the virus could be prevented successfully after transplantation (55).
FIG. 3. A: Functional survival of pig islets with a single genetic modification, that is, the transgenic expression of a human complement regulatory protein (hCD46), after transplantation in a cohort of five diabetic cynomolgus monkey recipients. Immunosuppression consisted of induction with antithymocyte globulin and was maintained with an anti-CD154 mAb and mycophenolate mofetil. All experiments were electively terminated; all monkeys were healthy when they were killed. Partial graft function (white bars) and full graft function (insulin independence) (black bars) are shown. Arrowheads indicate retransplantation. B: Serum acute C-peptide responses (ACR) of monkey C-peptide (white bars) and pig C-peptide (black bars) in nanograms per milliliter after a metabolic challenge with intravenous glucose (glu) during follow-up of case 5. The ACR was calculated as the mean of postchallenge C-peptide values obtained at 5 and 15 min minus the corresponding prechallenge value. Pre, the acute monkey C-peptide response before diabetes induction; post, the average of acute monkey C-peptide responses during follow-up after transplantation, monitored until day 372. C: Blood glucose values and insulin requirements in a monkey transplanted with pig islets carrying four genetic modifications—GTKO/hCD46/hTFPIns/pCTLA4-IgIns. The monkey is currently insulin independent for >150 days. Immunosuppression is identical to the regimen in A.
safe. If this proves to be the case and is acceptable to regulatory authorities, then source pigs may not need to be maintained under such rigorous conditions. Regardless, a “clean” environment and regular monitoring of the pigs for microorganisms obviously will be essential.

Regulatory requirements relating to xenotransplantation are intricately interwoven with the microbiologic safety of the procedure itself and will require additional discussion when immunologic problems have been overcome and clinical islet xenotransplantation is fully warranted.

CONCLUSIONS

With the increasingly promising results from both clinical islet allotransplantation and experimental islet xenotransplantation, we can be cautiously optimistic that genetically engineered pigs will provide islets in sufficient numbers to allow treatment of T1D within years rather than decades. Just as with allotransplantation, patients with episodes of severe hypoglycemia or patients with stable kidney grafts probably would be the first candidates. However, problems associated with IBMIR need to be overcome, either by further genetic manipulation of the pig islets, therapy with drugs that reduce its severity, or identification of a successful alternative site for islet transplantation.

Against this backdrop, it should be stressed that the need for intensive, long-term immunosuppressive therapy needs to be reduced. This might be achieved by successful encapsulation or by further genetic engineering of the pig that will provide at least some local “endogenous” protection from the T cell immune response. The more that the pig islets can be genetically manipulated in this respect, the less exogenous immunosuppressive therapy will be required.

We suggest that the transplantation of islets from GTKO/hCD46/pCTLA4-Ig/fln/FPIfl/hCD39fln pigs combined with an effective, clinically applicable immunosuppressive regimen (e.g., induction alemtuzumab and maintenance tacrolimus and mycophenolate mofetil, which is currently associated with favorable results after clinical islet allotransplantation [3]) may be sufficiently successful in an NHP model to fulfill published criteria for a clinical trial (9). Results of such studies of NHPs should be available within the next 18 months. If the results do not fulfill the criteria for a clinical trial, then testing of pigs with further genetic modification, for example, expression of heme oxygenase-1, and alternative immunosuppressive regimens will be required.

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REFERENCES

1. Nathan DM, Cleary PA, Backlund JY, et al.; Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med 2005; 353:2643–2653
2. Collaborative Islet Transplant Registry. Sixth Annual Report. 1 November 2009. Available from http://www.citregistry.com/. Accessed 29 July 2011
3. Shapiro AM. State of the art of clinical islet transplantation and novel protocols of immunosuppression. Curr Diab Rep 2011;11:345–354
4. Thompson DM, Meloche M, Ao Z, et al. Reduced progression of diabetic microvascular complications with islet cell transplantation compared with intensive medical therapy. Transplantation 2011;91:373–378

FIG. 4. Fasting blood glucose and C-peptide levels in humans, cynomolgus monkeys (cyno), and pigs. Data are presented as mean ± SE. Adapted from Casu et al. (48).

FIG. 5. A: Absence of islet amyloid polypeptide deposition in pig islets >1 year after transplantation into the liver of a cynomolgus monkey. B: Native islets from a deceased, nondiabetic adult human in which there is significant amyloid deposition (red immunofluorescence). Blue, cell nuclei; green, insulin. (A high-quality digital representation of this figure is available in the online issue.)
5. US Department of Health & Human Services. Organ Procurement and Transplantation Network. Available from http://optn.transplant.hrsa.gov/ latestData/step2.aspx? Accessed 20th July 2011.

6. Groth CG, Korsgren O, Tibell A, et al. Transplantation of porcine fetal pancreas to diabetic patients. Lancet 1994;344:1402–1404.

7. Valdés-González RA, Dorantes LM, Garibay GN, et al. Xenotransplantation of porcine neonatal islets of Langerhans and Sertoli cells: a 4-year study. Eur J Endocrinol 2005;153:419–427.

8. Elliott RB. Living Cell Technologies. Towards xenotransplantation of pig islets in the clinic. Curr Opin Organ Transplant 2011;16:195–200.

9. First WHO Global Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials: Changsha, China, 19–21 November 2008. The Changsha Communiqué. Xenotransplantation 2009;16:61–63.

10. Hering BJ, editor. The International Xenotransplantation Association Consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes. Xenotransplantation 2009;16:196–262.

11. Dufrane D, Goebel RM, Gianello P. Allogeneic macronencapsulation of pig islets allows correction of streptozotocin-induced diabetes in primates up to 6 months without immunosuppression. Transplantation 2010;90:1054–1062.

12. Moberg L, Johansson H, Lukinuis A, et al. Production of tissue factor by the endocrine cells of the islets of Langerhans is associated with a negative outcome of clinical islet transplantation. Diabetes 2005;54:1755–1762.

13. Oznin L, Ekdahl KN, Elgque G, Larsson R, Korsgren O, Nilsson B. Inhibition of thrombin abrogates the instant blood-mediated inflammatory reaction triggered by isolated human islets: possible application of the thrombin inhibitor melagatran in clinical islet transplantation. Diabetes 2002;51:1779–1784.

14. Ji M, Yi S, Smith-Hurst H, et al. The importance of tissue factor expression by porcine NICC in triggering BMMIR in the xenograft setting. Transplantation 2011;91:841–846.

15. Good AIH, Cooper DK, Malcolm AJ, et al. Identification of carbohydrate structures that bind human antiporcine antibodies: implications for discordant xenografting in humans. Transplant Proc 1992;24:559–562.

16. Rayat GR, Rajotte RV, Hering BJ, Binette TM, Korbutt GS. In vitro and in vivo expression of Galalpha-(1,3)Gal on porcine islet cells is age dependent. J Endocrinol 2005;183:127–135.

17. Bennett W, Sundberg B, Lundgren T, et al. Damage to porcine islets of Langerhans after exposure to human blood in vitro, or after intraportal transplantation to cynomolgus monkeys: protective effects of sCR1 and heparin. Transplantation 2000;69:711–719.

18. Goto M, Tjemberg J, Dufrane D, et al. Dissecting the instant blood-mediated inflammatory reaction in islet xenotransplantation. Xenotransplantation 2008;15:225–234.

19. van der Windt DJ, Marigliano M, He J, et al. Islet damage after direct exposure of pig islets to blood: has humoral immunity been underestimated? Cell Transplant 5 July 2012 [Epub ahead of print].

20. Mohanakumar T, Narayanan K, Desai N, et al. A significant role for histocompatibility in human islet transplantation. Transplantation 2006;82:180–187.

21. Thompson P, Badell IR, Lowe M, et al. Islet xenotransplantation using gel-deficient neonatal donors improves engraftment and function. Am J Transplant 2011;11:2093–2092.

22. Padler-Karavani V, Varki A. Potential impact of the non-human sialogic acid N-glycolylneuraminic acid on transplant rejection risk. Xenotransplantation 2011;18:1–5.

23. Hering BJ, Wijkstrom M, Graham ML, et al. Prolonged diabetes reversal after intraportal xenotransplantation of wild-type porcine islets in immunosuppressed nonhuman primates after transplantation with hCD46 transgenic porcine islets. Am J Transplant 2009;9:2716–2726.

24. Missale MM, Shapiro AM, Frost GL, Rajotte RV. Large scale isolation, growth, and function of porcine neonatal islet cells. J Clin Invest 1996;97:2119–2129.

25. Kawai T, Andrews D, Colvin RB, Sachs DH, Cosimi AB. Thromboembolic complications after treatment with alemtuzumab (Campath-1H) in cyclosporine-resistant renal allograft recipients. Transplantation Proc 1992;24:559–562.

26. Rayat GR, Rajotte RV, Hering BJ, Binette TM, Korbutt GS. In vitro and in vivo expression of Galalpha-(1,3)Gal on porcine islet cells is age dependent. J Endocrinol 2005;183:127–135.

27. Rayat GR, Rajotte RV, Hering BJ, Binette TM, Korbutt GS. In vitro and in vivo expression of Galalpha-(1,3)Gal on porcine islet cells is age dependent. J Endocrinol 2005;183:127–135.

28. Rayat GR, Rajotte RV, Hering BJ, Binette TM, Korbutt GS. In vitro and in vivo expression of Galalpha-(1,3)Gal on porcine islet cells is age dependent. J Endocrinol 2005;183:127–135.

29. Bottino R, Balamurugan AN, Smetanka C, et al. Isolation outcome and functional characteristics of young and adult pig pancreatic islets for transplantation studies. Xenotransplantation 2007;14:74–82.

30. van der Windt DJ, Bottino R, Casu A, et al. Long-term controlled normoglycemia in diabetic non-human primates after transplantation with hCD46 transgenic porcine islets. Am J Transplant 2009;9:2716–2726.

31. Emanuellaue JA, Shapiro AM, Rajotte RV, Korbitt G, Elliot JF. Neonatal porcine islets exhibit natural resistance to hypoxia-induced apoptosis. Transplantation 2006;82:945–952.

32. Korbutt GS, Elliott JF, Ao Z, Smith DK, Warnock GL, Rajotte RV. Large scale isolation, growth, and function of porcine neonatal islet cells. J Clin Invest 1996;97:2119–2129.

33. Hecht G, Eventov-Friedman S, Rosen C, et al. Embryonic pig pancreatic tissue for the treatment of diabetes in a nonhuman primate model. Proc Natl Acad Sci U S A 2009;106:8659–8664.

34. Koike C, Fung JJ, Geller DA, et al. Molecular basis of evolutionary loss of the alpha-1,3-galactosyltransferase gene in higher primates. J Biol Chem 2002;277:10114–10120.

35. Dai Y, Vaught TD, Boone J, et al. Targeted disruption of the alpha1,3-galactosyltransferase gene in cloned pigs. Nat Biotechnol 2002;20:251–255.

36. Phelps CJ, Koike C, Vaught TD, et al. Production of alpha-1,3-galactosyltransferase-deficient pigs. Science 2003;299:411–414.

37. Murray AG, Nelson RC, Rayat GR, Elliott JF, Korbutt GS. Neonatal porcine islet allografts transplanted into the gastric submucosal space in pigs. Cell Transplant 2003;12:225–231.

38. Dufour JM, Daas B, Halley KB, Korbitt GS, Dixon DE, Rajotte RV. Sertoli cell line lacks the immunoprotective properties associated with primary Sertoli cells. Cell Transplant 2008;17:525–534.

39. Christoffersson G, Henrikssons J, Johansson L, et al. Clinical and experimental pancreatic islet transplantation to striated muscle: establishment of a vascular system similar to that in native islets. Diabetes 2010;59:2569–2578.

40. Bottino R, Balamurugan AN, Smetanka C, et al. Isolation outcome and functional characteristics of young and adult pig pancreatic islets for transplantation studies. Xenotransplantation 2007;14:74–82.

41. Casu A, Bortolo R, Balamurugan AN, et al. Metabolic aspects of pig-to-monkey (Macaca fascicularis) islet transplantation: implications for translation into clinical practice. Diabetologia 2008;51:120–129.

42. Graham ML, Bellin MD, Papas KK, Hering BJ, Schuurman H. Species incompatibilities in the pig-to-macaque islet xenotransplant model affect transplant outcome: a comparison with allotransplantation. Xenotransplantation 2011;18:329–342.

43. Casu A, Echeverri GJ, Bottolo R, et al. Insulin secretion and glucose metabolism in alpha1,3-galactosyltransferase knock-out pigs compared to wild-type pigs. Xenotransplantation 2010;17:131–139.

44. Potter KJ, Abedini A, Marek P, et al. Islet amyloid deposition limits the viability of human islet grafts but not porcine islet grafts. Proc Natl Acad Sci USA 2010;107:4305–4310.

45. Elliott RB. Living Cell Technologies. Towards xenotransplantation of pig islets in the clinic. Curr Opin Organ Transplant 2011;16:201–206.
54. Abrahante JE, Martins K, Papas KK, Hering BJ, Schuurman HJ, Murtaugh MP. Microbiological safety of porcine islets: comparison with source pig. Xenotransplantation 2011;18:88–93
55. Dieckhoff B, Petersen B, Kues WA, Kurth R, Niemann H, Denner J. Knockdown of porcine endogenous retrovirus (PERV) expression by PERV-specific shRNA in transgenic pigs. Xenotransplantation 2008;15:36–45
56. Sun Y, Ma X, Zhou D, Vacek I, Sun AM. Normalization of diabetes in spontaneously diabetic cynomologus monkeys by xenografts of microencapsulated porcine islets without immunosuppression. J Clin Invest. 1996;98:1417–1422
57. Cardona K, Korbutt GS, Milas Z, et al. Long-term survival of neonatal porcine islets in nonhuman primates by targeting costimulation pathways. Nat Med. 2006;12:304–306
58. Cardona K, Milas Z, Strobert E, et al. Engraftment of adult porcine islet xenografts in diabetic nonhuman primates through targeting of costimulation pathways. Am J Transplant. 2007;7:2260–2268
59. Hecht G, Eventov-Friedman S, Rosen C, et al. Embryonic pig pancreatic tissue for the treatment of diabetes in a nonhuman primate model. Proc Natl Acad Sci USA. 2009;106:8659–8664
60. Thompson P, Cardona K, Russell M, et al. CD40-specific costimulation blockade enhances neonatal porcine islet survival in nonhuman primates. Am J Transplant. 2011;11:947–957
61. Hara H, Long C, Lin YJ, et al. In vitro investigation of pig cells for resistance to human antibody-mediated rejection. Transpl Int. 2008;21:1163–1174
62. Miyagawa S, Murakami H, Takahagi Y, et al. Remodeling of the major pig xenoantigen by N-acetylglucosaminyltransferase III in transgenic pig. J Biol Chem. 2001;276:39310–39319
63. Zhou CY, McInnes E, Parsons N, et al. Production and characterization of a pig line transgenic for human membrane cofactor protein. Xenotransplantation. 2002;9:183–190
64. Loveland BE, Milland J, Kyriakou P, et al. Characterization of a CD46 transgenic pig and protection of transgenic kidneys against hyperacute rejection in non-immunosuppressed baboons. Xenotransplantation. 2004;11:171–183
65. White IM, Yannoutsos N. Production of pigs transgenic for human DAF to overcome complement-mediated hyperacute xenograft rejection in man. Res Immunol. 1996;147:88–94
66. Diamond LE, McCurry KR, Martin MJ, et al. Characterization of transgenic pigs expressing functionally active human CD59 on cardiac endothelium. Transplantation. 1996;61:1241–1249
67. Ayares D, Phelps C, Vaught T, et al. Multi-transgenic pigs for vascularized pig organ xenografts. Xenotransplantation 2011;18:269
68. Petersen B, Ramackers W, Tiede A, et al. Pigs transgenic for human thrombomodulin have elevated production of activated protein C. Xenotransplantation. 2009;16:486–495
69. Le Bas-Bernardet S, Tillou X, Poirier N, et al. Xenotransplantation of galactosyltransferase knockout, CD55, CD59, CD39, and fucosyltransferase transgenic pig kidneys into baboons. Transplant Proc. 2011;43:3426–3430
70. Phelps C, Ball S, Vaught T, et al. Production and characterization of transgenic pigs expressing porcine CTLA4-Ig. Xenotransplantation. 2009;16:477–485
71. Ezzelarab M, Ezzelarab C, Hara H, Ayares D, Cooper DKC. Characterization of mesenchymal stromal cells (PMSC) from GTKO pigs transgenic for CD46 and their modulatory effect on the primate cellular response. Am J Transplant 2010;10(Suppl. 4):188.
72. Weiss EH, Lilienfeld BG, Müller S, et al. HLA-E/human beta2-microglobulin transgenic pigs: protection against xenogeneic human anti-pig natural killer cell cytotoxicity. Transplantation. 2009;87:35–43
73. Petersen B, Lucas-Harn A, Lemme E, et al. Generation and characterization of pigs transgenic for human hemeoxygenase-1 (hHO-1). Xenotransplantation. 2010;17:102–103
74. Oropeza M, Petersen B, Carnwath JW, et al. Transgenic expression of the human A20 gene in cloned pigs provides protection against apoptotic and inflammatory stimuli. Xenotransplantation. 2009;16:522–534