The immense potential of xenotransplantation in surgery

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Abstract

There is a limited availability of deceased human organs and cells for the purposes of clinical transplantation. Genetically-engineered pigs may provide an alternative source. Although several immune barriers need to be overcome, considerable progress has been made in experimental models in recent years, largely through the increasing availability of pigs with new genetic modifications.

Pig heterotopic heart graft survival in nonhuman primates has extended for 8 months, with orthotopic grafts supporting life for almost 2 months. Life-supporting kidney transplants have functioned for almost 3 months. The current barriers are related to coagulation dysfunction between pig and primate that results in thrombotic microangiopathy and/or a consumptive coagulopathy, which may in part be related to molecular incompatibilities in the coagulation systems of pigs and primates. Current efforts are concentrated on genetically-modifying the organ- or islet-source pigs by the introduction of ‘anticoagulant’ or ‘anti-thrombotic’ genes to provide protection from the recipient coagulation cascade and platelet activation.

Progress with pig islet xenotransplantation has been particularly encouraging with complete control of glycemia in diabetic monkeys extending in one case for >12 months. Other areas where experimental data suggest the possibility of early clinical trials are corneal xenotransplantation and pig neuronal cell xenotransplantation, for example, in patients with Parkinson’s disease.

With the speed of advances in genetic engineering increasing steadily, it is almost certain that the remaining problems will be overcome within the foreseeable future, and clinical allotransplantation will eventually become of historical interest only.

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1. Introduction

There is a critical and increasing shortage of organs for the purposes of organ transplantation (Tx). In the USA alone approximately 110,000 patients are on the waiting list for an organ of one sort or another, and yet during the current year only approximately 30,000 organs will become available from deceased human donors. Almost 20 patients waiting for a human organ die each day, i.e., almost 7,000 per year.

If islet Tx becomes clinically more successful, as it slowly is, then the situation will be much more serious. There are an estimated 2–3 million type 1 diabetic patients taking insulin each day in the USA.

Xenotransplantation (xenoTx), i.e., the transplantation of organs, tissues, and cells across species, particularly using the pig as the source, offers the potential to resolve the critical shortage of human organs and cells (Table 1).

2. History of xenotransplantation

Clinical xenoTx has a long history going back to the 17th century when clinical blood transfusions were performed from animals. Corneal and organ xenoTx were attempted during the 19th and 20th centuries (Table 2). Since those days, however, experimental xenoTx has advanced significantly, largely due to the availability of pigs genetically-modified to protect their organs and cells from the human immune response.

3. Pathophysiology of pig organ injury in primates

Unlike alloTx, where the cellular immune response dominates, following pig organ xenoTx the graft is usually lost within minutes through an antibody-mediated response. The innate immune system, including cells, e.g., macrophages and polymorphs, plays a role in the initial damage to the graft.
a much greater role in rejection of a xenograft than of an allograft. However, the T cell response to a xenograft is also strong.

3.1. Humoral response

Natural antibodies that develop soon after birth in humans and Old World nonhuman primates recognize specific galactose (Gal), galactose-α1,3-galactosyltransferase (GalT) and α1,3-galactosyltransferase gene-knockout (GTKO) pigs allowed prolonged graft survival. This was first counteracted by the development of pigs that expressed a human complement-regulatory protein, such as CD55 (DAF), CD46 (MCP), and/or CD59, which greatly increased the protection of the pig cells to human complement. Although survival of grafts from these pigs is significantly extended, this approach is not entirely successful.

When Galα1,3Gal was recognized as being the predominant antigen to which human anti-pig antibodies are directed, the development of nuclear transfer (cloning) technology allowed steps to be taken to delete these carbohydrate antigens from the pig. In nonhuman primates, the Tx of organs from α1,3-galactosyltransferase gene-knockout (GTKO) pigs allowed prolonged graft survival.

Nevertheless, there are nonGal antigens that, although less important than Galα1,3Gal, still induce an immune response, although this is weaker than to wild-type pig organs. The nature of these nonGal antigens remains unknown. However, the combination of GTKO and transgenesis for a human complement-regulatory protein provides an advantage over either alone, and to a large extent overcomes this weaker antibody-mediated complement response.

3.2. Adaptive response

Opinions vary slightly as to the relative strengths of the primate T cell response to wild-type pig cells and to allogeneic cells. However, the response to GTKO cells is weaker than to wild-type pig cells, and is similar to that to human cells.

If, after pig organ Tx in a nonhuman primate, the immunosuppressive therapy is inadequate and a T cell-dependent elicited antibody response develops, the graft almost invariably fails from acute humoral xenograft rejection. However, this can also result from inadequate suppression of natural anti-pig antibody production.

Efforts are being made to modify pigs to express an immunosuppressive gene, such as CTLA4-Ig, and thus induce local immunosuppression around the graft, and reduce the need for exogenous immunosuppressive therapy in the recipient. Pigs expressing high levels of porcine CTLA4-Ig have been produced but, in fact, have proven too successful in that the levels of soluble CTLA4-Ig measured in their blood were many times higher than the therapeutically effective level, as a result, these pigs were to some extent immunoincompetent, and developed troublesome infections. Nevertheless, the expression of porcine CTLA4-Ig significantly reduced the primate cellular response to porcine cells. (Hara H, manuscript in preparation).

An alternative approach to reducing the primate cellular response to the pig organ is the production of dominant-negative swine leukocyte antigen (SLA) class II transactivator (CIITA) transgenic pigs. Variants of the CIITA gene were constructed to interfere with the expression of class II genes (DaI Y et al, unpublished), and CIITA dominant-negative pigs were produced by cloning. These pigs have normal lymphocyte subsets and T cell proliferative responses, but SLA class II expression after cytokine stimulation is almost completely suppressed. After cross-breeding with GTKO/CD46 pigs, these pigs will provide organs that should have a high degree of resistance to both the humoral and cellular xenoreactive responses.

3.3. Coagulation dysregulation

More recently, survival of a pig organ transplanted into a nonhuman primate has been limited, not through the immune response directly, but through the development of a thrombotic microangiopathy in the vessels of the graft (particularly seen after heart xenoTx) and/or a consumptive coagulopathy in the recipient of the graft (particularly seen after kidney xenoTx). The pathophysiology behind these complications is complex, and may be initiated by antibody binding to the vascular endothelium, thus activating the endothelium, but is certainly increased by molecular incompatibilities between the pig and primate coagulation systems. Increasingly, therefore, the efforts of the genetic engineers are being directed to insert human ‘anti-coagulant’ or ‘anti-thrombotic’ genes into GTKO pigs already transgenic for a human complement-regulatory protein.

3.4. Graft vasculopathy (chronic rejection)

In long-surviving heart transplants (>3 months), chronic graft vasculopathy has been reported, as it was in the early days of alloTx.

3.5. Sensitization

The data available to date indicate that allosensitized humans will be at no greater risk of humoral rejection of a GTKO pig organ or cell graft than other humans. Furthermore, sensitization to a failed pig organ or islet graft will not prohibit subsequent organ or islet alloTx.

4. Pig-to-primate organ transplantation - current results

GTKO/CD46 pig hearts transplanted heterotopically have been reported to function for 6–8 months.
transplanted GTKO/CD55 pig hearts have maintained the lives of baboons for periods approaching 2 months.\textsuperscript{58} and life-supporting CD55-transgenic\textsuperscript{59} and GTKO\textsuperscript{33,60} pig kidney transplants have functioned adequately for periods of 2–3 months. Survival of pig liver grafts is limited by the rapid development of a profound thrombocytopenia in the recipient (Fig. 3).\textsuperscript{61} This leads to internal hemorrhage in multiple native organs. The fragile structure of the pig lung makes it much more susceptible to primate immune-mediated injury,\textsuperscript{62–64} and graft survival is limited to hours.

\textbf{Fig. 1.} Summary of the known immunologic barriers to pig-to-primate heart transplantation. (Reproduced with permission from Zhu X, et al. J Heart Lung Transplant 2007.)

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4.1. Physiology

Even if the immune barriers are overcome, will the organ carry out all of the functions of the native organ? There is little evidence as yet,65 but the fact that pig hearts have maintained life in baboons for several weeks suggests that cardiac function will be adequate. Pig renal function appears satisfactory in most respects, but proteinuria has been documented in all nonhuman primates with pig kidneys; whether this is initiated by an immune process or not remains uncertain. The pig liver functions relatively normally in the nonhuman primate host,66 though follow-up has been limited.

Current evidence is that genetic manipulations to protect the pig organ from coagulation dysfunction, or the administration of drugs that might have the same effect, is required before consideration can be given to clinical trials.53

5. Pig-to-primate islet transplantation – current results

At least four groups have obtained survival of pig islet grafts with control of glycemia in diabetic monkeys for periods in excess of 3–6 months,67–70 and in one case for more than one year (Fig. 4).70 Encapsulation of the islets in the absence of immunosuppressive therapy has shown encouraging results in one series.69 When non-encapsulated islets are transplanted into the portal vein, there is an initial massive loss of islets – the so-called ‘instant blood-mediated inflammatory reaction’ (IBMIR).71,72 GTKO/CD46 pigs are becoming available that express tissue factor pathway inhibitor and CD39 in the islets, and it is hoped that these will induce some resistance to IBMIR. Islet xenoTx, therefore, may provide the first opportunity for a meaningful clinical trial.

An alternative approach would be to transplant the islets into a site other than the portal vein, which is the site currently used for all clinical alloTx.70

There is some evidence that neonatal islets are more resistant to injury than adult islets.73 In xenoTx this would have the advantage that the genetically-engineered islet-source pigs could be used within days of birth, and therefore would not need to be housed for several months or years until large enough to be used as sources of adult pig islets, greatly reducing the costs involved.

![Fig. 2. Correlation between platelet count and fibrinogen level (indicating the development of a consumptive coagulopathy) and tissue factor expression on platelets and peripheral blood mononuclear cells (PBMC) in a representative baboon following GTKO/CD46 pig-to-baboon kidney transplantation. The baboon was euthanized with a functioning graft (serum creatinine 2.0 mg/dL) on day 16. Microscopic examination of the kidney at necropsy showed no or minimal deposition of IgM, IgG or C3, no cellular infiltrates, but some deposition of fibrin. (Data from Lin CC, et al. Am J Transplant 2010.]

![Fig. 3. (A) Blood glucose, serum porcine-C peptide level, and exogenous insulin requirements in a streptozotocin-induced diabetic monkey recipient after transplantation of islets from an adult CD46-transgenic pig. (B) Insulin immunostaining of liver section in the same monkey >1 year after islet transplantation. (Data from van der Windt DJ, et al. Am J Transplant 2009.)

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![Graph showing correlation between platelet count and fibrinogen level](image1)

![Graph showing blood glucose, serum porcine-C peptide level, and exogenous insulin requirements](image2)
Aspects of glucose metabolism in pig-to-monkey islet xenoTx have been investigated by Casu who concluded that there are differences in glucose metabolism between cynomolgus monkeys and pigs that magnify the difficulties of obtaining normoglycemia in monkeys with porcine islet transplants. Fortunately, the data suggest that it should be easier for normoglycemia to be obtained after porcine islet Tx into humans.

6. Corneal xenotransplantation

XenoTx could provide an unlimited source of corneas for patients with corneal blindness. Although the number of deceased human donors available in most of the western world is adequate for this purpose, there is a very significant shortage of human corneas in many parts of the world (Table 3).

In vivo studies in nonhuman primates by Pan and Oh indicate that even wild-type (unmodified) pig corneas remain functional for several months when treated locally with corticosteroids. Current in vitro experimental evidence from our own group indicates that corneas from GTKO/CD46 pigs show considerable resistance to the human immune response. With new genetic modifications being introduced, it is likely that, from an immune perspective, pig corneas will soon be comparable to human corneas. They also appear to be comparable to a human cornea from a biomechanical perspective.

7. Neuronal cell xenotransplantation

The potential of pigs as sources of cells that might correct various neurodegenerative conditions is also being explored. For example, there is considerable potential for the Tx of pig dopamine-producing cells in conditions such as Parkinson’s disease. Preliminary reports from Cozzi and his colleagues indicate significant improvement in motor function in monkeys in which a Parkinson-like condition has been induced, and in which cells from the ventral mesencephalon of pig embryos have been implanted. The number of patients who would benefit from this form of therapy is clearly considerable.

Fig. 4. (A) Platelet counts in 11 baboons during the first 7 days after orthotopic GTKO/CD46 pig liver transplantation. (B) Platelet counts in two baboons during the first 5 h after orthotopic GTKO/CD46 pig liver transplantation. (Reproduced with permission from Ekser B, et al. Am J Transplant 2010.)
8. The pig as a source of red blood cells (RBCs) for clinical transfusion

There is an immense need for an alternative source of RBCs in many countries, particularly where the incidence of HIV-positivity is high in the population. RBCs from genetically-modified pigs have the potential to resolve this problem. Most characteristics of pig RBCs are similar to those of human RBCs. RBCs from GTKO pigs are superior to human ABO-incompatible red blood cells, but are not yet comparable to ABO-compatible RBCs. With the current technology that has been used to genetically-modify pigs, transgenes (e.g., those for complement-regulatory proteins) are not expressed in pig RBCs. It is likely that, if these transgenes can be directed to the pig RBCs using perhaps a hemoglobin or similar promoter, then pig RBCs may well be rendered comparable to ABO-compatible human RBCs for the purposes of transfusion.

9. Regulation of clinical xenotransplantation

With regard to the potential transfer of an infectious microorganism from the donor, there have been particular concerns about the transfer of porcine endogenous retroviruses with the organ or cells to the human recipient, as these form an integral part of each cell. However, the associated risks are now considered to be small. Furthermore, if need be, these viruses could be prevented from activation by siRNA technology that has recently been reported.

Nevertheless, for this reason and others, clinical xenoTx will require oversight by regulatory authorities, e.g., the Food and Drug Administration in the USA. Guidelines have been published in several countries and by the World Health Organization that those carrying out clinical trials will be required to follow. In particular, archiving of tissues and blood from source pigs and human recipients will be mandatory to enable retrospective investigation, if ever indicated. Ethical guidelines relating to clinical xenoTx have also been published.

If necessary, the genetically-engineered pigs that will be required to provide corneas, cells, or RBCs can be housed and bred under ideal circumstances in the more developed countries, with the corneas, cells, or RBCs being shipped to the less well-developed countries where they would be used.

10. Conclusions

Processed tissues (with no living cells), such as small intestinal submucosa, bone chips, ligaments, tendons, skin, and heart valves from wild-type pigs are already being used extensively in clinical practice. Steps are underway to replace the wild-type pigs for this purpose by GTKO pigs to which there is a reduced inflammatory response.

Nevertheless, xenoTx’s main impact will be when islets, organs, or other living tissues or cells can be used successfully in clinical practice. We are gradually drawing closer to the time when clinical trials of pig corneal, neuronal, or islet xenoTx will become fully justified. Trials of pig organ xenoTx will take a little longer until the current problems, outlined above, can be resolved. Nevertheless, with the speed of advances in genetic engineering increasing steadily, it is almost certain that the remaining problems will be overcome within the foreseeable future, and clinical alloTx will eventually become of historical interest only.

Conflict of interest statement

David Ayares, co-author, is the CEO of Revivicor, Inc.

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Ethical approval

IACUC approval of University of Pittsburgh, protocol # 0902831.

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