Review

The immense potential of xenotransplantation in surgery

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ARTICLE INFO

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).[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.[2] Corneal[3] and organ[4,5] 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.[6,7] The innate immune system, including cells, e.g, macrophages and polymorphs, plays

Table 1
Major advantages of xenotransplantation over allotransplantation.

Unlimited supply of organs, tissues, and cells
Unlimited supply will allow transplantation procedures in 'borderline' candidates who might otherwise be declined
Organs available electively
Avoids the detrimental effects of brain death on donor organs
Provides exogenous infection-free sources of organs, tissues, and cells
Obviates the 'cultural' barriers to deceased human donation present in some countries, e.g., Japan

a much greater role in rejection of a xenograft than of an allograft.[8] However, the T cell response to a xenograft is also strong.[9,10]

3.1. Humoral response

Natural antibodies that develop soon after birth in humans and Old World nonhuman primates[11] recognize specific galactose (Galα1,3Gal) antigens on the surface of vascular endothelial cells[12–16] to which they bind, activating complement and initiating rapid graft destruction, of which vascular thrombosis is a key factor[17–19] (Fig. 1).

This was first counteracted by the development of pigs that expressed a human complement-regulatory protein,[20,21] such as CD55 (DAF),[22]CD46 (MCP),[23,24] and/or CD59,[25,26] which greatly increased the protection of the pig cells to human complement.[27] 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,[13–15]the development of nuclear transfer (cloning) technology allowed steps to be taken to delete these carbohydrate antigens from the pig.[28–30] In nonhuman primates, the Tx of organs from α1,3-galactosyl-transferase gene-knockout (GTKO) pigs allowed prolonged graft survival.[31–33]

Nevertheless, there are nonGal antigens that, although less important than Galα1,3Gal, still induce an immune response, although this is weaker than the response to wild-type pig organs.[34–36]The nature of these nonGal antigens remains unknown.[37] 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.[38]

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.[9,10] However, the response to GTKO cells is weaker than to wild-type pig cells, and is similar to that to human cells.[10]

If, after pig organ Tx in a nonhuman primate, the immunosuppressive therapy is inadequate and a T cell-dependent elicited

Table 2
Summary of clinical xenotransplantation of organs in the 20th century.*

Donor	n	Survival
Kidney - Primate	(30)	1 day–9 months
Non-Primate	(3)	3–9 days
Heart - Primate	(5)	<1–20 days
Non-Primate	(4)	<1 day
Liver-Primate	(11)	<1–70 days
Non-Primate	(1)	<2 days

*Data from Taniguchi S, Cooper, DKC. Ann R Coll Surg Engl. 1997;79:13-19.

antibody response develops, the graft almost invariably fails from acute humoral xenograft rejection.[39] However, this can also result from inadequate suppression of natural anti-pig antibody production.[8]

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 therapeutic level[40]; 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.[41] 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)[31,32,42,43] and/or a consumptive coagulopathy in the recipient of the graft (particularly seen after kidney xenoTx) (Fig. 2).[44–46] This observation supports the current opinion that pig cardiac and renal grafts respond in different ways after XTx.[47] The pathophysiology behind these complications is complex, and may be initiated by antibody binding to the vascular endothelium, thus activating the endothelium,[48,49] but is certainly increased by molecular incompatibilities between the pig and primate coagulation systems.[50–53]Increasingly, therefore, the efforts of the genetic engineers are being directed to insert human 'anticoagulant' 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,[31,32,42,54] 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.[34,55] Furthermore, sensitization to a failed pig organ or islet graft will not prohibit subsequent organ or islet alloTx.[56]

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

GTKO/CD46 pig hearts transplanted heterotopically have been reported to function for 6–8 months.[31,32,57] Orthotopically

A Hyperacute Rejection (HAR)

Complement-mediated injury associated with the immediate binding of preformed anti-pig antibodies to antigens expressed on the vascular endothelium of the pig organ, e.g., Galα1,3Gal. Defined as occurring within the first 24 hours.

Acute Humoral Xenograft Rejection (AHXR)

A delayed antibody-mediated response often, but not always, associated with the production of elicited antibodies, that leads to vascular endothelial activation and injury caused by complement and cellular components of the immune system, e.g., NK cells and macrophages. Histopathology similar to that of HAR. May take days or weeks to develop and cause graft failure.

Acute Cellular Rejection (ACR)

A cellular response, including T cells, that is similar, but not identical, to that seen after allotransplantation. Perhaps surprisingly, it has rarely been described after pig-to-primate organ xenotransplantation, and has not proved a common problem. This is possibly because T cell activation leads to a more rapid antibody response that results in AHXR before significant T cell infiltration occurs in the graft.

B Thrombotic Microangiopathy (TM)

Fibrin deposition and platelet aggregation result in thrombosis within the vessels of the graft, even in the absence of an elicited antibody response. Endothelial cell activation, e.g., by antibodies, may result in a change from the normal anticoagulant state of the vascular endothelium to a procoagulant state, resulting in fibrin and platelet deposition. This is probably associated with the numerous molecular incompatibilities between the pig and primate coagulation systems that prevent maintenance of an anticoagulant state after xenotransplantation.

C Chronic Rejection

Has been documented in pig cardiac grafts that survive for >3months in baboons. Similar histopathological appearance to that seen after allotransplantation. Pathogenesis is poorly understood.

Hyperacute Rejection

Thrombotic Microangiopathy
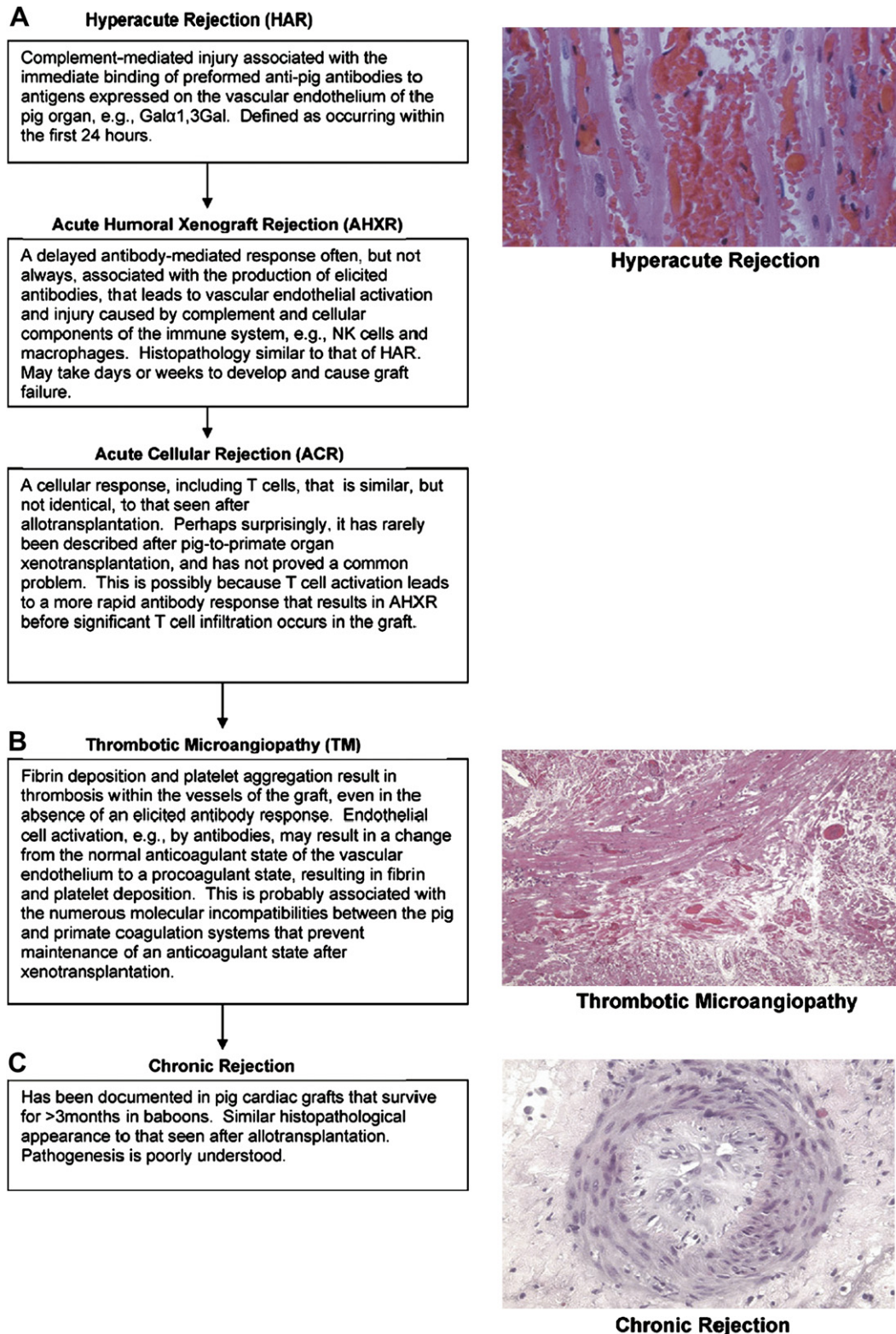
Chronic Rejection

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.)

transplanted GTKO/CD55 pig hearts have maintained the lives of baboons for periods approaching 2 months,[58] and life-supporting CD55-transgenic[59] and GTKO[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).[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,[62–64] and graft survival is limited to hours.
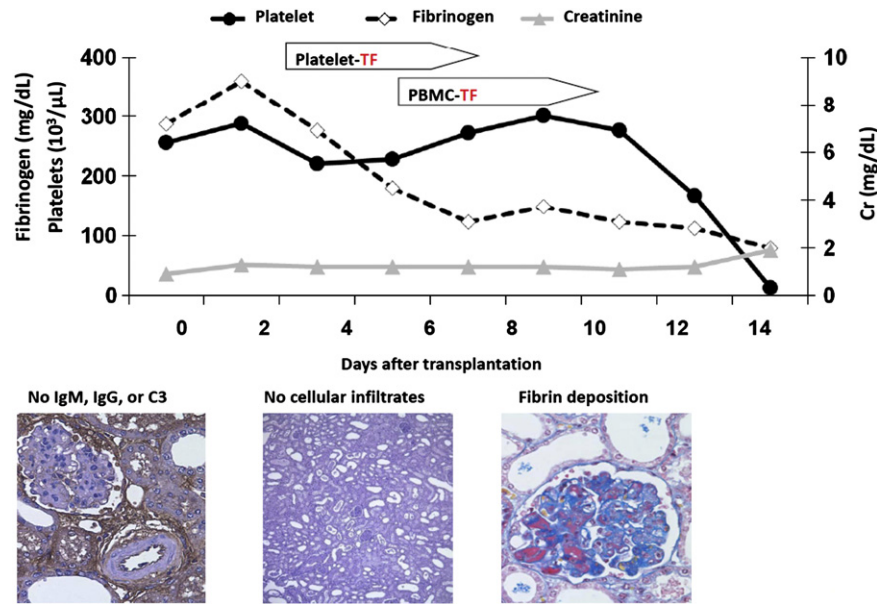
Fig. 2. Correlation between platelet count and fibrinogen level (indicating the development of a consumptive coagulopathy) and tissue factor expression on platelets and peripheral blood mononuclear cells (PBMC) in a representative baboon following GTKO/CD46 pig-to-baboon kidney transplantation. The baboon 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.)

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 immuno-suppressive 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.
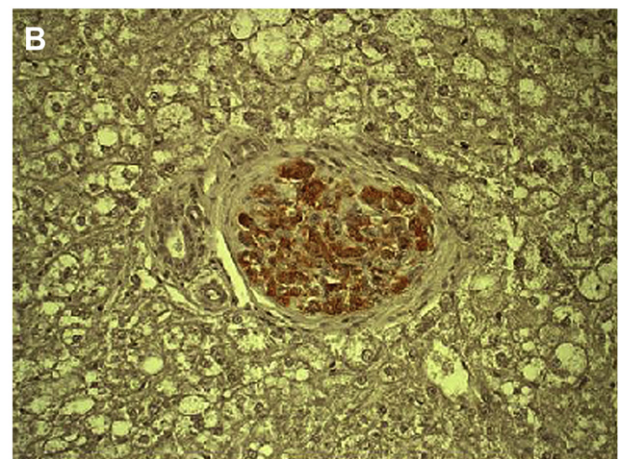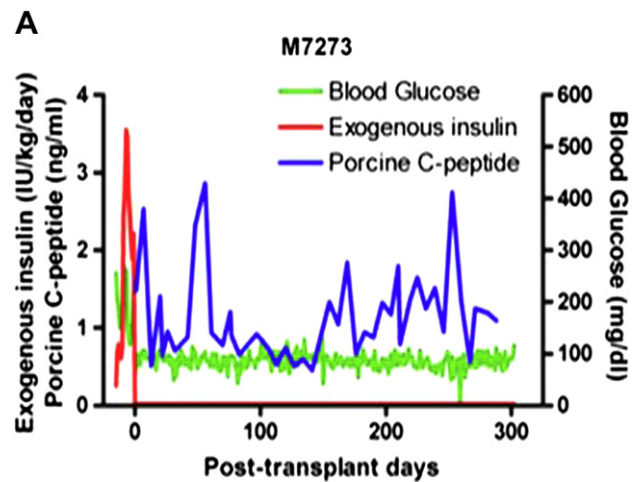

Fig. 3. (A) Blood glucose, serum porcine-C peptide level, and exogenous insulin requirements in a streptozotocin-induced diabetic monkey recipient after transplantation of islets from an adult CD46-transgenic pig. (B) Insulin immunostaining of liver section in the same monkey >1 year after islet transplantation.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

Aspects of glucose metabolism in pig-to-monkey islet xenoTx have been investigated by Casu[74] 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.[3] 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[75] and Oh[76] 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.[77] 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.[3]

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.[78] 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.
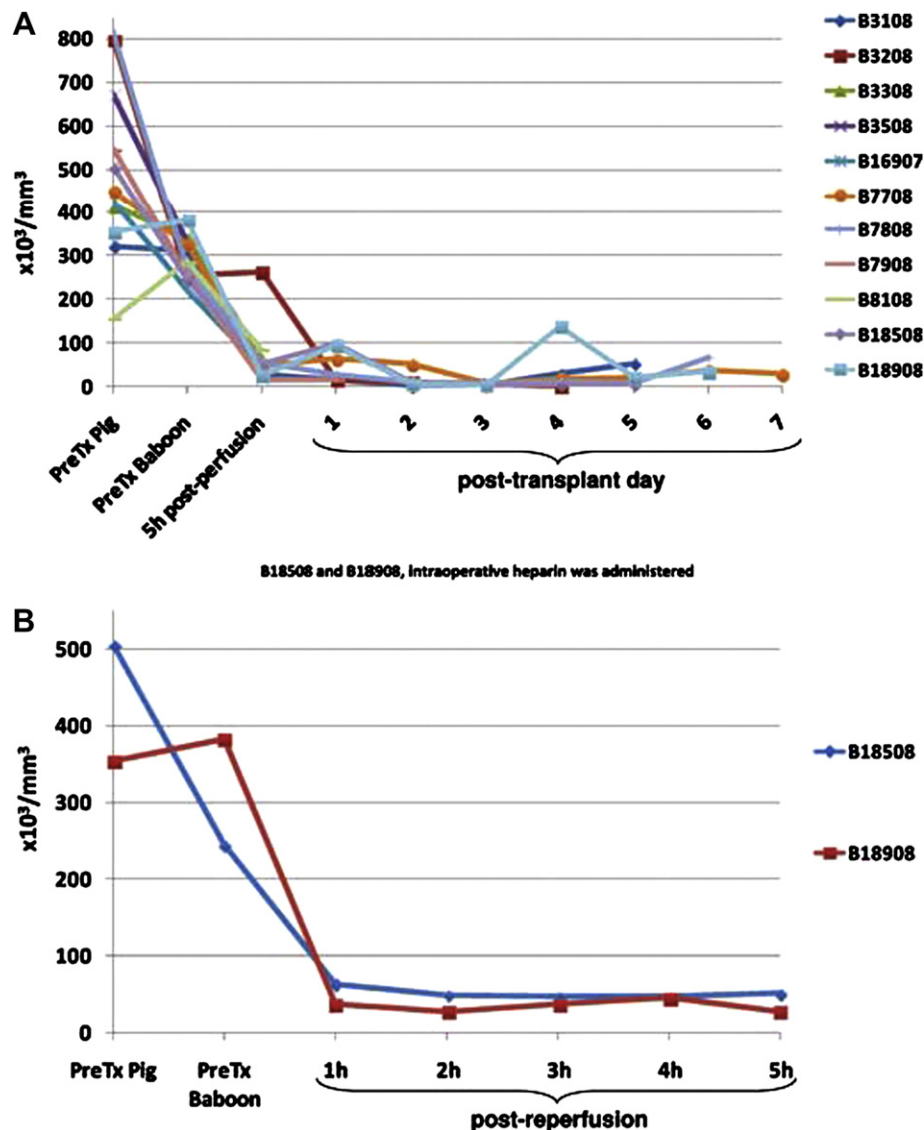

Fig. 4. (A) Platelet counts in 11 baboons during the first 7 days after orthotopic GTKO/CD46 pig liver transplantation. (B) Platelet counts in two baboons during the first 5 h after orthotopic GTKO/CD46 pig liver transplantation (Reproduced with permission from Ekser B, et al. *Am J Transplant* 2010.)

Table 3
Estimated numbers of corneal allotransplants carried out in 2008 and numbers of patients awaiting corneal transplantation in selected countries*

Country	Estimated number of cases per year	Waiting list
United States of America	41,652	Saturated
United Kingdom	2,711	500
South Africa**	330	1,884
India	15,000	300,000
China	101	4,000,000
Taiwan	263	637
Korea	480	3,630
Japan	1,634	2,769
Australia	1,096	Saturated

*Based on Eye Bank data in individual countries and personal communications.
**In sub-Saharan Africa, the number of corneal transplants carried out annually has been falling for several years because of the high incidence of human immunodeficiency virus (HIV)-positivity in the potential donor population.

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.[79–81]

RBCs from GTKO pigs are superior to human ABO-incompatible red blood cells, but are not yet comparable to ABO-compatible RBCs.[82] 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,[83] 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.[84] Furthermore, if need be, these viruses could be prevented from activation by siRNA technology that has recently been reported.[85–87]

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[88,89] and by the World Health Organization[90] 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.[91]

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.[92]

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.

References

1. Cooper DKC, Lanza RP. *Xeno-The promise of transplanting animal organs into humans.* New York: Oxford University Press; 2000. 1–274.
2. Roux FA, Sai P, Deschamps JY. Xenotransfusions, past and present. *Xenotransplantation* 2007;**14**:208–16.
3. Hara H, Cooper DKC. Xenotransplantation – the future of corneal transplantation? Cornea. in press.
4. Taniguchi S, Cooper DKC. Clinical xenotransplantation - past, present and future. *Ann R Coll Surg Engl* 1997;**79**:13–9.
5. Deschamps JY, Roux FA, Sai P, Gouin E. History of xenotransplantation. *Xenotransplantation* 2005;**12**:91–109.
6. Lexer G, Cooper DKC, Rose AG, Wicomb WN, Rees J, Keraan M, et al. Hyperacute rejection in a discordant (pig to baboon) cardiac xenograft model. *J Heart Transplant* 1986;**5**:411–8.
7. Cooper DKC, Human PA, Lexer G, Rose AG, Rees J, Keraan M, et al. Effects of cyclosporine and antibody adsorption on pig cardiac xenograft survival in the baboon. *J Heart Transplant* 1988;**7**:238–46.
8. Ezzelarab M, Garcia B, Azimzadeh A, Sun H, Lin CC, Hara H, et al. The innate immune response and activation of coagulation in α1,3-galactosyltransferase gene-knockout xenograft recipients. *Transplantation* 2009;**87**:805–12.
9. Yamada K, Sachs DH, DerSimonian H. Human anti-porcine xenogeneic T cell response. Evidence for allelic specificity of mixed leukocyte reaction and for both direct and indirect pathways of recognition. *J Immunol* 1995;**1**(155):5249–56.
10. Lin YJ, Hara H, Tai HC, Long C, Tokita D, Yeh P, et al. Suppressive efficacy and proliferative capacity of human regulatory T cells in allogeneic and xenogeneic responses. *Transplantation* 2008;**86**:1452–62.
11. Rood PPM, Tai HC, Hara H, Long C, Ezzelarab M, Lin YJ, et al. Late onset of development of natural anti-nonGal antibodies in infant humans and baboons: implications for xenotransplantation in infants. *Transplant Int* 2007;**20**:1050–8.

12. Galili U, Shohet SB, Kobrin E, Stults CL, Macher BA. Man, apes, and old world monkeys differ from other mammals in the expression of alpha-galactosyl epitopes on nucleated cells. J Biol Chem 1988;**263**:17755–62.
13. Good AH, Cooper DKC, Malcolm AJ, Ippolito RM, Koren E, Neethling FA, et al. Identification of carbohydrate structures that bind human antiporcine antibodies: implications for discordant xenografting in man. Transplant Proc 1992;**24**:559–62.
14. Cooper DKC. Depletion of natural antibodies in non-human primates - a step towards successful discordant xenografting in man. Clin Transplantation 1992;**6**:178–83.
15. Cooper DKC, Good AH, Koren E, Oriol R, Malcolm AJ, Ippolito RM, et al. Identification of α-galactosyl and other carbohydrate epitopes that are bound by human anti-pig antibodies: relevance to discordant xenografting in man. Transpl Immunol 1993;**1**:198–205.
16. Oriol R, Ye Y, Koren E, Cooper DKC. Carbohydrate antigens of pig tissues reacting with human natural antibodies as potential targets for hyperacute vascular rejection in pig-to-man organ xenotransplantation. Transplantation 1993;**56**:1433–42.
17. Rose AG, Cooper DKC, Human PA, Reichenspurner H, Reichart B. Histopathology of hyperacute rejection of the heart − experimental and clinical observation in allografts and xenografts. J Heart Transplant 1991;**10**:223–34.
18. Rose AG, Cooper DKC. A histopathologic grading system of hyperacute (humoral, antibody-mediated) cardiac xenograft and allograft rejection. J Heart Lung Transplant 1996;**15**:804–17.
19. Rose AG, Cooper DKC. Venular thrombosis is the key event in the pathogenesis of antibody-mediated cardiac rejection. Xenotransplantation 2000;**7**:31–41.
20. Dalmasso AP, Vercellotti GM, Platt JL, Bach FH. Inhibition of complement-mediated endothelial cell cytotoxicity by decay-accelerating factor. Potential for prevention of xenograft hyperacute rejection. Transplantation 1991;**52**:530–3.
21. White DJ, Oglesby T, Liszewski MK, Tedja I, Hourcade D, Wang MW, et al. Expression of human decay accelerating factor or membrane cofactor protein genes on mouse cells inhibits lysis by human complement. Transpl Int 1992;**5**:648–50.
22. Cozzi E, White DJG. The generation of transgenic pigs as potential organ donors for humans. Nat Med 1995;**1**:964–9.
23. Loveland BE, Milland J, Kyriakou P, Thorley BR, Christiansen D, Lanteri MB, et al. Characterization of a CD46 transgenic pig and protection of transgenic kidneys against hyperacute rejection in non-immunosuppressed baboons. Xenotransplantation 2004;**11**:171–83.
24. McGregor CG, Davies WR, Oi K, Teotia SS, Schirmer JM, Risdahl JM, et al. Cardiac xenotransplantation:recent preclinical progress with 3-month median survival. J Thorac Cardiovasc Surg 2005;**130**:844–51.
25. Byrne GW, McCurry KR, Martin MJ, McClellan SM, Platt JL, Logan JS. Transgenic pigs expressing human CD59 and decay-accelerating factor produce an intrinsic barrier to complement-mediated damage. Transplantation 1997;**63**:149–55.
26. Cowan PJ, Aminian A, Barlow H, Brown AA, Chen CG, Fisicaro N, et al. Renal xenografts from triple-transgenic pigs are not hyperacutely rejected but cause coagulopathy in non-immunosuppressed baboons. Transplantation 2000;**69**:2504–15.
27. Hara H, Cooper DKC, Lin YJ, Tai HC, Ezzelarab M, Ayares D, et al. In vitro investigation of pig cells for resistance to human antibody-mediated rejection. Transplant Int 2008;**21**:1163–74.
28. Cooper DKC, Koren E, Oriol R. Genetically engineered pigs. Lancet 1993;**342**:682–3.
29. Phelps CJ, Koike C, Vaught TD, Boone J, Wells KD, Chen SH, et al. Production of α1,3-galactosyltransferase-deficient pigs. Science 2003;**299**:411–4.
30. Kolber-Simonds D, Lai L, Watt SR, Denaro M, Arn S, Augenstein ML, et al. Production of α1,3-galactosyltransferase null pigs via nuclear transfer with fibroblasts bearing loss of heterozygosity mutations. Proc Natl Acad Sci USA 2004;**19**:7335–40.
31. Kuwaki K, Tseng YL, Dor FJ, Shimizu A, Houser SL, Sanderson TM, et al. Heart transplantation in baboons using α1,3-galactosyltransferase gene-knockout pigs as donors:initial experience. Nat Med 2005;**11**:29–31.
32. Tseng YL, Kuwaki K, Dor FJ, Shimizu A, Houser S, Hisashi Y, et al. α 1,3-galactosyltransferase gene-knockout pig heart transplantation in baboons with survival approaching six months. Transplantation 2005;**80**:1493–500.
33. Yamada K, Yazawa K, Shimizu A, Iwanaga T, Hisashi Y, Nuhn M, et al. Marked prolongation of porcine renal xenograft survival in baboons through the use of α1,3-galactosyltransferase gene-knockout donors and the cotransplantation of vascularized thymic tissue. Nat Med 2005;**11**:32–4.
34. Hara H, Ezzelarab M, Rood PPM, Lin YJ, Busch J, Ibrahim Z, et al. Allosensitized humans are at no greater risk of humoral rejection of GT-KO pig organs than other humans. Xenotransplantation 2006;**13**:357–65.
35. Ezzelarab M, Hara H, Busch J, Rood PP, Zhu X, Ibrahim Z, et al. Antibodies directed to pig nonGal antigens in naïve and sensitized baboons. Xenotransplantation 2006;**13**:400–7.
36. Rood PPM, Hara H, Busch JL, Ezzelarab M, Zhu X, Ball S, et al. Incidence and cytotoxicity of antibodies in cynomolgus monkeys directed to nonGal antigens, and their relevance for experimental models. Transpl Int 2006;**19**:158–65.
37. Yeh P, Ezzelarab M, Bovin N, Hara H, Long C, Tomiyama K, et al. Investigation of potential carbohydrate antigen targets for human and baboon antibodies. Xenotransplantation 2010;**17**:197–206.
38. Azimzadeh AM, Kelishadi S, Ezzelarab M, Singh AK, Stoddard T, Zhang T, et al. Early graft failure of GTKO pig organs in baboons is reduced by HCPRP expression. Am J Transplant 2010;**10**(Suppl. 4):186 (Abstract 499).
39. Chen G, Qian H, Starzl T, Sun H, Garcia B, Wang X, et al. Acute rejection is associated with antibodies to non-Gal antigens in baboons using Gal-knockout pig kidneys. Nat Med 2005;**11**:1295–8.
40. Phelps C, Ball S, Vaught T, Vance AM, Mendicino M, Monahan JA, et al. Production and characterization of transgenic pigs expressing porcine CTLA4-Ig. Xenotransplantation 2009;**16**:477–85.
41. Hara H, Crossley T, Witt W, Long C, Fang J, Ezzelarab M, et al. Dominant-negative CIITA transgenic pigs − effect on the human anti-pig T cell immune response and immune status. Am J Transplant 2010;**10**(Suppl. 4):187. Abstract 503.
42. Kuwaki K, Knosalla C, Dor FJ, Gollackner B, Tseng YL, Houser S, et al. Suppression of natural and elicited antibodies in pig-to-baboon heart transplantation using a human anti-CD154 monoclonal antibody-based regimen. Transplant 2004;**4**:363–72.
43. Ierino FL, Kozlowski T, Siegel JB, Shimizu A, Colvin RB, Banerjee PT, et al. Disseminated intravascular coagulation in association with the delayed rejection of pig-to-baboon renal xenografts. Transplantation 1998;**66**:1439–50.
44. Kozlowski T, Shimizu A, Lambrigts D, Yamada K, Fuchimoto Y, Glaser R, et al. Porcine kidney and heart transplantation in baboons undergoing a tolerance induction regimen and antibody adsorption. Transplantation 1999;**67**:18–30.
45. Bühler L, Basker M, Alwayn IP, Goepfert C, Kitamura H, Kawai T, et al. Coagulation and thrombotic disorders associated with pig organ and hematopoietic cell transplantation in nonhuman primates. Transplantation 2000;**70**:1323–31.
46. Lin CC, Ezzelarab M, Shapiro R, Ekser B, Long C, Hara H, et al. Tissue factor expression on recipient platelets is associated with consumptive coagulopathy in pig-to-primate kidney xenotransplantation. Am J Transplant 2010;**10**:1556–68.
47. Knosalla C, Yazawa K, Behdad A, Bodyak N, Shang H, Bühler L, et al. Renal and cardiac endothelial heterogeneity impact acute vascular rejection in pig-to-baboon xenotransplantation. Am J Transplant 2009;**9**:1006–16.
48. Bach FH, RobsonSC Ferran C, Winkler H, Millan MT, Stuhlmeier KM, et al. Endothelial cell activation and thromboregulation during xenograft rejection. Immunol Rev 1994;**141**:5–303.
49. Gollackner B, GohSK Qawi I, Buhler L, Knosalla C, Daniel S, et al. Acute vascular rejection of xenografts:roles of natural and elicited xenoreactive antibodies in activation of vascular endothelial cells and induction of procoagulant activity. Transplantation 2004;**77**:1735–41.
50. Robson SC, Cooper DKC, d'Apice AJF. Disordered regulation of coagulation and platelet activation in xenotransplantation. Xenotransplantation 2000;**7**:166–76.
51. Schulte am Esch II J, Rogiers X, Robson SC. Molecular incompatibilities in hemostasis between swine and men—impact on xenografting. Ann Transplant 2001;**6**:12–6.
52. Cowan PJ. Coagulation and the xenograft endothelium. Xenotransplantation 2007;**14**:7–12.
53. Pierson III RN, Dorling A, Ayares D, Rees MA, Seebach JD, Fishman JA, et al. Current status of xenotransplantation and prospects for clinical application. Xenotransplantation 2009;**16**:263–80.
54. Houser SL, Kuwaki K, Knosalla C, Dor FJ, Gollackner B, Cheng J, et al. Thrombotic microangiopathy and graft arteriopathy in pig hearts following transplantation into baboons. Xenotransplantation 2004;**11**:416–25.
55. Wong BS, Yamada K, Koumi M, Weiner J, O'Malley PE, Tseng YL, et al. Allo-sensitization does not increase the risk of xenoreactivity to α1,3-Galactosyl-transferase gene-knockout (GalT-KO) miniature swine in patients on transplantation waiting lists. Transplantation 2006;**82**:314–9.
56. Cooper DKC, Tseng YL, Saidman SL. Allo- and xeno-antibody cross-reactivity in transplantation. Transplantation 2004;**77**:1–5.
57. Mohiuddin MM, Corcoran PC, Singh AK, Hoyt RF, Thomas ML, Eckhaus MA, et al. Pig to baboon cardiac xenotransplantation:essential role of B cell depletion in prolonging cardiac xenograft survival. Am J Transplant 2010;**10**(Suppl. 4):186 (Abstract 498).
58. McGregor CGA, Byrne GW, Vlasin M, Walker RC, Tazelaar HD, Davies WR, et al. Early cardiac function and gene expression after orthotopic cardiac xenotransplantation. Xenotransplantation 2009;**16**:356 (Abstract IXA-O-2.4).
59. Cozzi E, Vial C, Ostlie D, Farah B, Chavez G, Smith KG, et al. Maintenance triple immunosuppression with cyclosporin A, mycophenolate sodium and steroids allows prolonged survival of primate recipients of hDAF porcine renal xenografts. Xenotransplantation 2003;**10**:300–10.
60. Griesemer AD, Hirakata A, Shimizu A, Moran S, Tena A, Iwaki H, et al. Results of gal-knockout porcine thymokidney xenografts. Am J Transplant 2009;**9**:2669–78.
61. Ekser B, Long C, Echeverri GJ, Hara H, Ezzelarab M, Lin CC, et al. Impact of thrombocytopenia on survival of baboons with genetically-modified pig liver transplants:clinical relevance. Am J Transplant 2010;**10**:273–85.
62. Schroeder C, Allan JS, Nguyen BN, Wu G, Zhang T, Azimzadeh AM, et al. Hyperacute rejection is attenuated in GalTKO swine lungs perfused ex vivo with human blood. Transplant Proc 2005;**37**:512–3.
63. Nguyen BH, Zwets E, Schroeder C, Pierson III RN, Azimzadeh AM. Beyond antibody-mediated rejection: hyperacute lung rejection as a paradigm for dysregulated inflammation. Curr Drug Targets:Cardiovascular Hematological Disord 2005;**5**:255–69.

64. Nguyen BN, Azimzadeh AM, Zhang T, Wu G, Schuurman HJ, Sachs DH, et al. Life-supporting function of genetically modified swine lungs in baboons. *J Thorac CV Surg* 2007;**133**:1354–63.
65. Ibrahim Z, Busch J, Awwad M, Wagner R, Wells K, Cooper DKC. Selected physiologic compatibilities and incompatibilities between human and porcine organ systems. *Xenotransplantation* 2006;**13**:488–99.
66. Ekser B, Echeverri GJ, Cortese-Hassett A, Yazer MH, Long C, Meyer M, et al. Hepatic function after genetically-engineered pig liver transplantation baboons. *Transplantation* 2010;**90**:483–93.
67. Hering BJ, Wijkstrom M, Graham ML, Hårdstedt M, Aasheim TC, Jie T, et al. Prolonged diabetes reversal after intraportal xenotransplantation of wild-type porcine islets in immunosuppressed nonhuman primates. *Nat Med* 2006;**12**:301–3.
68. Cardona K, Korbutt GS, Milas Z, Lyon J, Cano J, Jiang W, et al. Long-term survival of neonatal porcine islets in nonhuman primates by targeting costimulation pathways. *Nat Med* 2006;**12**:304–6.
69. Dufrane D, Goebbels RM, Saliez A, Gulot Y, Gianello P. Six-month survival of microencapsulated pig islets and alginate biocompatibility in primates:proof of concept. *Transplantation* 2006;**81**:1345–53.
70. van der Windt DJ, Bottino R, Casu A, Campanile N, Smetanka C, He J, et al. Long-term controlled normoglycemia in diabetic non-human primates after transplantation with hCD46 transgenic porcine islets. *Am J Transplant* 2009;**9**:2716–26.
71. Bennet W, Sundberg B, Lundgren T, Tibell A, Groth CG, Richards A, et al. Damage to porcine islets of langerhans after exposure to human blood in vitro, or after intraportal transplantation to cynomologus monkeys:protective effects of sCR1 and heparin. *Transplantation* 2000;**69**:711–9.
72. van der Windt DJ, Echeverri G, Cooper DKC. The choice of anatomical site for islet transplantation. *Cell Transplant* 2008;**17**:1005–14.
73. Rayat GR, Rajotte RV, Korbutt GS. Potential application of neonatal porcine islets as treatment for type 1 diabetes:a review. *Ann N Y Acad Sci* 1999;**18**:175–88.
74. Casu A, Bottino R, Balamurugan AN, Hara H, van der Windt DJ, Campanile N, et al. Metabolic aspects of pig-to-monkey *(Macaca fascicularis)* islet transplantation: implications for translation into clinical practice. *Diabetologia* 2008;**51**:120–9.
75. Pan Z, Sun C, Jie Y, Wang N, Wang L. WZS-pig is a potential donor alternative in corneal xenotransplantation. *Xenotransplantation* 2007;**14**:603–11.
76. Oh JY, Kim MK, Ko JH, Lee HJ, Park CG, Kim SJ, et al. Histological differences in full-thickness vs. lamellar corneal pig-to-rabbit xenotransplantation. *Vet Ophthalmol* 2009;**12**:78–82.
77. Hara H, Koike N, Long C, Piluex J, Roh DS, Sundarraj N, et al. Initial in vitro investigation of the human immune response to corneal cells from genetically-engineered pigs. 2010. [Submitted for publication].
78. Badin RA, Padoan A, Vadori M, Boldrin M, De Benedictis GM, Fante F, et al. Porcine embryonic xenografts transgenic for CTLA4-Ig enable longterm recovery in Parkinsonian macaques. *Am J Transplant* 2010;**10**(Suppl. 4):208 (Abstract LB01).
79. Zhu A. Introduction to porcine red cells;implications for xenotransplantation. *Sem Hematol* 2000;**37**:143–9.
80. Cooper DKC, Hara H, Yazer M. Genetically-engineered pigs as a source for clinical red blood cell transfusion. *Clin Lab Med* 2010;**30**:365–80.
81. Cooper DKC. Porcine red blood cells as a source of blood transfusion in humans. *Xenotransplantation* 2003;**10**:384–5.
82. Long C, Hara H, Pawlikowski Z, Koike N, d'Arville T, Yeh P, et al. Genetically-engineered pig red blood cells for clinical transfusion:initial in vitro studies. *Transfusion* 2009;**49**:2418–29.
83. Onions D, Cooper DKC, Alexander TJL, Brown C, Claassen E, Foweraker JE, et al. An assessment of the risk of xenozoonotic disease in pig-to-human xeno-transplantation. *Xenotransplantation* 2000;**7**:143–55.
84. Fishman JA, Patience C. Xenotransplantation:infectious risk revisited. *Am J Transplant* 2004;**4**:1383–90.
85. Dieckhoff B, Karlas A, Hofmann A, Kues WA, Petersen B, Pfeifer A, et al. Inhi-bition of porcine endogenous retroviruses (PERVs) in primary porcine cells by RNA interference using lentiviral vectors. *Arch Virol* 2007;**152**:629–34.
86. 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.
87. Ramsoondar J, Vaught T, Ball S, Mendicino M, Monahan J, Jobst P, et al. Production of transgenic pigs that express porcine endogenous retrovirus small interfering RNAs. *Xenotransplantation* 2009;**16**:164–80.
88. Tibell A, Lundgren T. Xenotransplantation–clinical activities and regulatory development. *Acta Vet Scand* 2004;**99**(Suppl.):19–23.
89. Schuurman HJ. Regulatory aspects of pig-to-human islet transplantation. *Xenotransplantation* 2008;**15**:116–20.
90. World Health Organization. First WHO global consultation on regulatory require-ments for xenotransplantation clinical trials, Changsha, China, 19–21 November 2008. *Xenotransplantation* 2009;**16**:58–60. The Changsha Communiqué.
91. Sykes M, d'Apice A, Sandrin M. IXA ethics committee. position paper of the ethics committee of the international xenotransplantation association. *Trans-plantation* 2004;**78**:1101–7.
92. Daly KA, Stewart-Akers AM, Hara H, Ezzelarab M, Long C, Cordero K, et al. Effect of the αGal epitope on the response to small intestinal submucosa extracellular matrix in a nonhuman primate model. *Tissue Eng Part A* 2009;**15**:3877–88.