

## Current Status of Xenotransplantation - A Review

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**ABSTRACT** : There is emerging interest in using xenotransplantation of porcine cells, tissues and organs for treatment of human illness. This article reviews the current status of xenotransplantation, with particular emphasis on the physiological and immunological barriers to xenotransplantation and genetic manipulations to overcome xenograft rejection. Preliminary success in xenotransplantation therapy for human Parkinson's disease using porcine foetal brain cells is described. Finally the zoonotic dangers of porcine xenotransplantation, most particularly porcine endogenous retroviruses (PERVs), are discussed. (*Asian-Aust. J. Anim. Sci.* 2001. Vol 14, No. 10 : 1497-1504)

**Key Words** : Xenotransplantation, Pig, Zoonoses, Porcine Endogenous Retroviruses (PERVs)

### INTRODUCTION

Xenotransplantation is the transplantation of cells, tissues or organs between different species. Interest in xenotransplantation has grown during the past decade because of the potential for using pigs as an unlimited source of donor organs and tissues to overcome the shortage of human materials for clinical transplantation. For example, in the United States alone, more than 60,000 patients awaits an organ of one type or another and a new name is added to the waiting list every 18 minutes. Of these, only one-third can be transplanted in a year and many patients do not survive the wait. For example, 6,000 US patients died waiting for a transplant in 1999 (Cooper and Lanza, 2000). The disparity between waiting lists and transplants in the United States is increasing by 10 to 15% annually (fig. 1), and similar trends are reported throughout the world.

According to White and Nicholson (1999), clinical transplantation can be divided into two eras separated at the middle of the 1960s, prior to and after availability of treatment for immunosuppression. Before the availability of appropriate immunosuppression, the results of transplants of human organs were very poor. The use of immunosuppressive chemicals like cyclosporin provided long-term survival of grafted human organs but could not eliminate progressive graft rejection (Bailey et al., 1985). There were a few attempts during the past century to transplant animal organs into humans and the results were strongly discouraging. More recently, molecular and immunological understanding of xenograft rejection and the feasibility of genetic modification of donors has made xenotransplantation more likely (Platt and Lin, 1998;

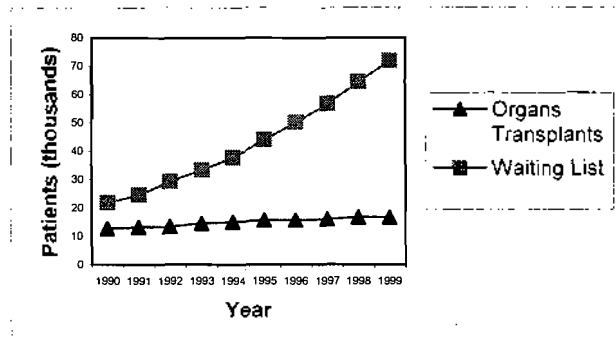
Vanhove et al., 1998). If these problems can be overcome, we are on the verge of a third era of transplantation, whose impact will be as significant as the availability of immunosuppressive drugs thirty five years ago. It is hoped that this third era of transplantation will satisfy the current shortfall in human organs, but will also open new possibilities for use of transplanted tissue for treatment of numerous other human diseases, currently not treatable by transplantation.

### CHOICE OF DONOR SPECIES

It is common sense that a xenograft donor species must have similar physiological and morphological characteristics to those of the recipient, regardless of any problems with immunological rejection. The obvious choice of donor animal was from among the non-human primates, which have close physiological similarities with humans (Cooper and Lanza, 2000), reflecting their phylogenetic closeness. However there are serious objections to use of non-human primates for clinical xenotransplantation. First, many people do not accept their use as organ donors because of the high level of their intelligence. Furthermore many of them are near extinction, have a slow rate of reproduction, and are expensive to breed and raise in comparison with domestic animals. Also their phylogenetic similarity means that they share many dangerous pathogens in common with humans. These considerations have led investigators to seek an animal donor that is less closely related.

For several reasons, pigs, rather than non-human primates, are regarded as the best sources of xenograft donors. Firstly, porcine organs are physiologically and anatomically similar to those of humans. Secondly, pigs can be bred economically and quickly in large numbers in a disease-free environment. Finally, there are few ethical qualms about breeding pigs for slaughter for xenotransplantation because pigs are already bred for human

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**Figure 1.** Discrepancy between number of patients awaiting organ transplantation and the number of transplantations performed in the United States. The number of patients awaiting organ transplantation is growing dramatically, whereas the number of cadaveric organs available for transplantation is almost static (Data from United Network for Organ Sharing: UNOS).

consumption. The detailed comparison between non-human primates and pig as potential xenograft donors for human is illustrated in table 1.

### IMMUNOLOGICAL BARRIERS TO XENOTRANSPLANTATION

#### Hyperacute rejection

Hyperacute rejection is the first major barrier to xenotransplantation and occurs immediately after exposure of the donor organs to blood flow in the recipients, similar

to that seen in ABO incompatible blood transfusion. Hyperacute rejection is characterized by microvascular thrombosis and interstitial haemorrhage. Many animals have circulating antigens that react with cells and tissues from unrelated species without any previous antigenic immunization. A single donor gene and its products are responsible for the hyperacute rejection of organs transplanted from non-primate mammals into primates. A disaccharide sugar, galactose  $\alpha$  (1,3) galactose, the so-called  $\alpha$ -gal antigen, is present as the terminal residue of glycoproteins and glycolipids on the surface of cells from pigs and other mammals, but is absent from higher apes (Galili et al., 1987). Naturally occurring antibodies in primates, formed in response to the same antigen in bacteria, recognize the  $\alpha$ -gal antigen. This antigen-antibody interaction activates the complement cascade causing hyperacute rejection. Hyperacute rejection leads to destruction of the graft within minutes to a few hours (Platt and Bach, 1991). Endothelial cells in blood vessels are the primary target of the host immunity in hyperacute rejection (Platt et al., 1990). In human, over 80% of the preformed crossspecies antibodies are directed against the  $\alpha$ -gal antigen (Parker et al., 1994).

If hyperacute rejection and complement activation can be avoided, rejection still occurs 2 to 3 days later and this process has been described as delayed xenograft rejection or acute or accelerated vascular rejection. The difference between hyperacute rejection and delayed xenograft rejection is that the latter is a slower non-complement-dependent process (Auchincloss and Sachs, 1998). It is

**Table 1.** Relative advantages and disadvantages of baboons and pigs as potential donors of organs and tissues for humans\*

	Baboon	Pig
Availability	Limited	Unlimited
Breeding potential	Poor	Good
Period to reproductive maturity	3-5 years	4-8 months
Length of pregnancy	173-193 days	114 $\pm$ 2 days
Number of offspring	1-2	5-12
Growth	Slow (9 years to reach maximum size)	Rapid (adult human size within 6 months)
Size of adult organs	Inadequate	Adequate
Cost of maintenance	High	Significantly lower
Anatomical similarity to humans	Close	Moderately close
Physiological similarity to humans	Close	Moderately close
Relationship of immune system to humans	Close	Distant
Knowledge of tissue typing	Limited	Considerable (in selected herds)
Necessity of blood type compatible with humans	Important	Probably unimportant
Experience with genetic engineering	None	Considerable
Risk of transfer of infection (xenozoonoses)	High	Low
Availability of specific pathogen-free animals	No	Yes
Public opinion	Mixed	More in favor

\* This table is based on Cooper and Lanza (2000).

known that complex events are involved in delayed xenograft rejection, like the increased expression of adhesion molecules, tissue factor, chemo-attractant cytokines and monocyte chemo-attractant (Candinas et al., 1996).

### Complement activation

Complement components provide a potent non-specific defence against foreign xenogenic tissue. Complement activation is generated via two pathways called 'classical' and 'alternative' pathways. The former pathway involves cytotoxic crossspecies antibodies against antigens like the  $\alpha$ -gal antigen, whereas the latter does not (White and Nicholson, 1999). The terminal event in both pathways is the formation of a C5 convertase complex yielding a membrane attack complex that penetrates plasma membranes causing cellular death (Kim et al., 1987). Also the by-products of the complement cascade are involved in tissue destruction by stimulating the production of potent inflammatory mediators, such as cytokines, free radicals, histamine, and prostaglandins (Baldwin et al., 1995).

Under normal circumstance, the complement cascade is inhibited by a number of proteins in the plasma and on the surface of the cells (Hourcade et al., 1989). These protective proteins include Decay accelerating factor (DAF), Membrane cofactor protein (MCP) and CD59. They are also collectively called regulators of complement activation (RCAs) (Cozzi and White, 1995).

### Cell-mediated immunity

The importance of cell-mediated rejection is becoming increasingly recognized, as it causes delayed xenograft rejection. In contrast with vascularized grafts, such as liver, heart and kidney, cellular xenografts, such as islet, bone marrow, and hepatocytes, are less susceptible to hyperacute rejection and cell-mediated xenograft rejection is more likely to be important. Major histocompatibility complex (MHC) antigens and CD4-positive T lymphocytes play a major role in eliciting this response, even though the mechanism of cell-mediated rejection of xenografts is not fully understood (White and Nicholson, 1999).

## PHYSIOLOGICAL BARRIERS TO XENOTRANSPLANTATION

Soin, Vial and Friend (2000) pointed out that some proteins and hormones are species specific and could disturb the human system in xenotransplantation. In this point of view, the organs from concordant species are more likely to control human metabolism than those from discordant species. A successful xenograft would ideally perform the homeostatic and hormonal functions as effectively as an allograft. The liver transplantation from

baboon to human is a good example where most metabolic functions are provided by the graft and synthesis of proteins are active over 2 months (Starzl et al., 1993).

The size of organs is also the matter of consideration. Fortunately, when comparing the adult pig organs with that of humans, the volumes and weight are reasonably similar (Groth, 1998). Also the measurement of cardiovascular parameters including blood pressure, cardiac output, and left ventricular stroke work for adult pigs are similar to those of adult humans (Soin et al., 2000).

## GENETIC MANIPULATION TO AVOID XENOGRFT REJECTION

### Knocking out galactose $\alpha$ (1, 3) galactosyl transferase

Since exposure of recipients to porcine tissue expressing  $\alpha$ -gal antigen causes immediate activation of the complement cascade and hyperacute rejection, an obvious question is whether it is possible to prevent pigs from expressing this antigen on the surface of their cells. Homologous recombination provides a mechanism for knocking out the responsible enzyme, galactose  $\alpha$  (1,3) galactosyl transferase in pigs. Until recently application of this technique was only possible in the mouse where the availability of embryonic stem (ES) cells allowed "reconstruction" of a mouse from cells in tissue culture. However all attempts to develop porcine embryonic stem (ES) cell culture as a prerequisite for the knockouts have failed (Vanhove et al., 1998). Thus although the molecular tools for knocking out the gene have been available since the porcine gene was cloned by Dabkowski et al. (1994), no progress could be made towards this objective. Recently, pigs were cloned by nuclear transfer (Bradbury, 2000; Onishi et al., 2000). Since it is reasonably straightforward to knockout a gene in cells in tissue culture and it is now feasible to transfer nuclei from such cells to create cloned offspring, the possibility of a knockout of galactose  $\alpha$  (1,3) galactosyl transferase is much closer. This will overcome the first major hurdle to xenotransplantation.

Knockouts of galactose  $\alpha$  (1,3) galactosyl transferase have been tested in mice. These knockout mice do not express of  $\alpha$ -gal antigen on the cell surface. However another type of antigen, which can be detected by a human natural antibody, was expressed (Tearle et al., 1996).

Due to the lack of success in creating ES cell lines in pigs, several laboratories have sought alternative ways of suppressing expression of the  $\alpha$ -gal antigen. Ogawa et al. (1999) demonstrated that transfecting porcine cells with gal transferase constructs containing deletions or mutations could suppress expression by up to 42%. However these successes are likely to be rendered irrelevant by the ability to produce complete knockouts by homologous recombination in the near future.

### Over expressing H-transferase

The most widely explored alternative mechanism for preventing expression of galactose  $\alpha$  (1,3) galactose on porcine glycoproteins is over-expression of another glycosyl transferase,  $\alpha$  (1,2) fucosyl transferase also known as H-transferase. Galactose  $\alpha$  (1,3) galactosyl transferase transfers a galactose molecule to terminal N-acetylglucosamine (N-lac) present on various glycoproteins and glycolipids. The N-lac structure also can be used as an acceptor by other glycosyltransferases, like H-transferase. The H-transferase competes with  $\alpha$  (1,3) galactosyl transferase and its over-expression suppresses the appearance of galactose  $\alpha$ (1,3) galactose in mouse and pigs (Sharma et al., 1996). However, this suppression of  $\alpha$ -gal expression is dependent on cell type in the mouse (Chen et al., 1998). Furthermore transgenics for the fucosyl transferase gene in the rat have developed carcinoma of the colon, raising fears that over-expression of this enzyme and its product might be pathogenic in pigs (Hallouin et al., 1999).

### Protective proteins

Protective proteins like DAF (Decay accelerating factor, CD55), MCP (Membrane cofactor protein, CD46) and CD59 can be over-expressed in pigs to inhibit activation of the complement cascade and thus prevent initiation of hyperacute rejection (Cozzi and White, 1995; Mollnes and Fiene, 1999). Levels of expression of human DAF in transgenic pigs are very variable, even though expression of the transgene has been detected in skin, liver, spleen, lung and heart (Langford et al., 1994). In primate recipients, porcine hearts transgenic for human DAF have survived for up to 60 days without evidence of rejection. Unfortunately, high levels of immunosuppression are also required and have led to adverse side effects in the recipients (Dabkowski et al., 1994). Human DAF transgenic pigs have also been used for renal transplantation to primates without hyperacute rejection and a maximum survival of 78 days

has been achieved (Bhatti et al., 1998; Zaidi et al., 1998).

Mollnes and Fiene (1999) indicated that there may be possible health concern arising from over-expression of membrane complement regulators, due to their affinity for different ligands. For example, DAF is a high-affinity ligand for the seven-span transmembrane molecule, CD97, which is rapidly expressed upon activation of many leukocytes and natural killer cells (Hamann et al., 1996). Thus over-expression of DAF might interfere with control of general cell mediated immunity. In addition, MCP is a receptor for measles virus and DAF is a receptor for echovirus and coxackie B picornavirus. Therefore over-expression of these proteins could affect susceptibility to virus infection and cause another danger for the recipient and donor (Weiss, 1998).

### PROGRESS IN XENOTRANSPLANTATION

Xenograft survival of transplants between more closely related species can be prolonged. For example, immunosuppressed juvenile baboon recipients of cardiac xenografts from rhesus monkey donors have survived over 500 days (Bailey and Gundry, 1997). However much more limited success has been obtained in more discordant animal models like pig to non-human primates. A maximum survival of 78 days has been recorded for pig kidneys transgenic for human DAF transplanted to monkey, and 39 days for an orthotopic cardiac xenograft from a human DAF transgenic pig transplanted to a baboon recipient (Soin et al., 2000). Until recently, organs from transgenic pigs have only been tested in non-human primate models (Byrne et al., 1997; McCurry et al., 1995; Schmoeckel et al., 1998; Zaidi et al., 1998). Moreover, various porcine cells, including foetal brain cells, pancreatic islet cells and liver cells have been tested to human recipient (Chari et al., 1994; Deacon et al., 1997; Groth et al., 1994; Heneine et al., 1998; Pitkin and Mullon, 1999). Table 2 shows the recent progress and xenotransplantation trials using porcine organs and cells to

**Table 2.** Recent progress and xenotransplantation trials using porcine organs and cells to human

Porcine cells and organs	Disease	Defect	Therapeutic effect	References
Porcine heart	Cardiac disease	Diseased or damaged heart	Replacement of non-functional heart	Czaplicki et al., 1992
Porcine liver/ Hepatocytes	Liver failure	Hepatocyte death	Liver function	Makowka et al., 1995; Chari et al., 1994; Pitkin and Mullon, 1999
Porcine neural cells	Neurological disease	Parkinson's Huntington's Epilepsy Stroke	Neuronal function	Deacon et al., 1997
Porcine islets	Diabetes	Death of insulin producing cells in pancreatic islets	Provision of insulin in response to changes in blood sugar	Groth et al., 1994; Heneine et al., 1998

human. In most cases, these are only preliminary evaluations (Phase I clinical trials), but in some cases, evidence of clinical efficacy has been obtained.

Most progress has been made in transplanting porcine foetal brain cells into humans as a therapy for Parkinson's disease. The brain is protected by the blood-brain barrier from circulating immune factors and as neural cells do not strongly express MHC class I and class II antigens, brain xenograft are relatively protected from rejection. Recently, Schumacher et al. (2000) reported results of transplantation of porcine embryonic ventral mesencephalic tissue into patients with advanced Parkinson's disease, providing statistically significant ( $p = 0.01$ ) improvements in the total Unified Parkinson's Disease Rating Scale scores of approximately 19% in a group of 10 evaluable patients.

### DANGERS OF XENOTRANSPLANTATION

Xenotransplantation provides a novel route for transmission of zoonoses via animal organs or cells into human recipients. Unlike conventional transmission of zoonoses, humans have no evolutionary history of defense against transmission via xenotransplantation and thus may be particularly vulnerable to transmission in this way. Michaels and Simmons (1994) have reviewed pathogens specific to baboons and pigs, which can infect humans. Xenotransplantation may infect human recipients with zoonotic and other infectious agents not endemic in humans. Immunosuppression agents intended to improve survival of xenotransplantation products also may inhibit immune response to infection. To reduce the potential risks of transmitting animal infections to the recipients, it is universally agreed that donor animals should be raised under specific pathogen free (SPF) conditions. In this process, any known exogenous pathogens can be excluded (Swindle, 1998). However, porcine endogenous retroviruses form part of the genome of the donor, and related viruses are present in the genome of human recipients. They are hard to detect and difficult or impossible to eliminate from the donor species, even under SPF conditions, but have the potential to cause serious disease in humans (Stoye et al., 1998).

Porcine type C viruses are classified in the genus Murine leukemia virus (MLV)-related virus (Petropoulos, 1997). Porcine endogenous retroviruses (PERVs) are proviral form of retroviruses and are inherited in a stable Mendelian fashion (Patience et al., 1997). The first description of porcine C type retroviruses was from cultured pig kidney cells (Breese, 1970; Armstrong et al., 1971) and they are associated with rare lymphosarcomas and tumors in pigs (Bostock and Owen, 1973). Suzuka et al. (1985) reported the isolation of a swine C-type retrovirus from a malignant lymphoma, which was subsequently partially

characterized by restriction digestion of the 8.8 kb viral clone (Suzuka et al., 1986). This clone has subsequently been sequenced and analyzed. The Tsukuba-1 and PERV-MSL (porcine endogenous retroviruses from miniature swine lymphocytes) sequences have more than 99% sequence similarities (Akiyoshi et al., 1998). There was little subsequent interest in porcine retroviruses until Stoye and Coffin (1995) pointed out that they represented a potential hazard for use of pig organs and tissues for xenotransplantation.

Three types of porcine endogenous retroviruses (PERVs), differentiated by their envelope (*env*) gene sequence and called PERV-A, PERV-B and PERV-C, have been recognized. They are present at approximately 50 copies in the pig genome (Le Tissier et al., 1997; Akiyoshi et al., 1998). Recently, PERVs have been mapped in the Westran (Westmead Hospital Transplantation) inbred line of pigs, specially bred for transplantation research in Australia. FISH (Fluorescence *In Situ* Hybridization) to porcine chromosome preparations, using PERV-A and PERV-B envelope probes, suggest that there are 32 significant PERV sites (19 PERV-A and 13 PERV-B). Furthermore the chromosomal locations of these in the Westran strain are quite different from European Large White pigs (Lee, 2000; Rogel-Gaillard et al., 1999). Host range and interference studies in the three classes of PERVs show that each of them recognize different receptors and all of them infect at least one human cell line (Takeuchi et al., 1998).

Co-culture and infectivity experiments have shown that PERVs released from pig kidney cell lines, from mitogenically activated porcine peripheral blood mononuclear cells (PBMCs), or from porcine endothelial cells, can infect human cells and cell lines *in vitro*, adding validity to concerns about the possibility of cross-species infection after xenotransplantation (Patience et al., 1997; Martin et al., 1998a; Wilson et al., 1998). However there has been no evidence of PERV infection *in vivo* in baboons and humans (Martin et al., 1998b; Paradis et al., 1999; Pitkin and Mullan, 1999; Switzer et al., 1999). Akiyoshi et al. (1998) suggested that the risk of viral infection would be increased in xenotransplantation by the presence of factors commonly associated with transplantation, such as immune suppression, graft-versus-host disease, graft rejection, viral co-infection, and cytotoxic therapies. Very recently, the transplantation of porcine pancreatic islets into SCID (severe combined immunodeficiency) mice led to *in vivo* expression of PERVs, further reinforcing fear about the risk of PERV infection in immunosuppressed human patients (van der Laan et al., 2000).

### CONCLUSION

We have described various aspects of xenotrans-

plantation research regarding use of porcine cells and tissues to cure human diseases. At this stage, the current knowledge of xenotransplantation falls far short of that necessary to safely carry out solid organ transplantation. However the future success of clinical xenotransplantation will depend on further research on overcoming immunological barriers, determining compatibility of physiological function of organs between species, and addressing the vexed questions of whether PERVs represent genuine hazards, and if they do, how this can be overcome.

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