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REVIEW

Clinical xenotransplantation: past, present and future

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The ability to use animal organs, such as from the pig, for the purposes of transplantation in humans, would clearly overcome the major shortage of human organs that greatly restricts transplantation programmes worldwide. Recent experimental advances have raised the possibility of renewed attempts at organ xenotransplantation in humans within the near future. Previous clinical experience, dating back to 1906, is briefly reviewed. The problems that still require resolution include the immunological barrier, the risk of transferring infectious agents with the transplanted organ, and uncertainty as to whether the transplanted animal organ will function satisfactorily in the human environment. Ironically, the answers to some of these problems may only be provided when clinical xenotransplantation is undertaken.

It is generally accepted that there is a shortage of suitable human organs for the purposes of transplantation. At present, approximately 6000 and 45 000 patients await organ transplantation in the UK and USA, respectively, and this number is increasing by about 10–15% each year. Worldwide, this number can be increased by at least a further 50% and possibly by 100% *(1)*.

An estimate of the current needs for organs each year worldwide, however, does not take into account other important factors *(1)*. It is unlikely that the number of patients on the waiting list fully reflects the number who might benefit from organ transplantation; borderline patients, who are at higher risk after transplantation, may not be accepted for transplantation in many centres. In some countries, eg Japan, allografting from cadaveric organ donors remains rare or nonexistent for religious, cultural or legal reasons, yet medical technology is advanced enough to allow transplantation to take place. The impact of successful xenotransplantation in such a society would clearly be enormous.

The advantages of xenotransplantation are obvious. The supply of donor organs would be unlimited, the organs would be available electively, pretreatment of either the donor or the recipient to enhance acceptance of the graft could be planned, donor organs would not be subjected to the potentially damaging effects of brain death, and chronic infection in the donor could be more readily excluded.

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Concordant xenografting

When xenotransplantation is carried out between closely related (concordant *(2)*) species, eg chimpanzee-to-human, there are usually no or very low detectable levels of antidonor species (xenoreactive) antibody in the host at the time of transplantation. The antibody titre generally rises rapidly during the first few days after transplantation. In a proportion of recipients, rejection will be predominantly cellular and will follow the normal sequence of events after allografting *(3)*. In others, however, rejection will be antibody-mediated (humoral, vascular) or of a mixed nature *(3)*.

Clinical experience

Kidney

Several attempts have been made to provide humans with organs from closely related species (Tables I–III). The classical early studies by Reemtsma *et al. (4)*, who used chimpanzees as donors of kidneys, and of Starzl *et al. (5)*, who used baboons as donors, demonstrated that the

Table I. World experience in clinical renal xenotransplantation*

Year	*Surgeon*	*Donor*	*n*	*Patient survival*†
1905	Princeteau	Rabbit (kidney slices)	1	16
1906	Jaboulay	Pig	1	3
		Goat	1	3
1910	Unger	Monkey	1	2
1913	Schonstadt	Monkey	1	?
1923	Neuhof	Sheep	1	9
1964	Reemtsma	Chimpanzee	12	< 9 months
		Monkey	1	10
1964	Hitchcock	Baboon	1	5
1964	Starzl	Baboon	6	< 60
1964	Hume	Chimpanzee	1	1
1964	Traeger	Chimpanzee	3	< 49
1965	Goldsmith	Chimpanzee	2	< 4 months
1966	Cortesini	Chimpanzee	1	31

* Modified from Dubernard *et al. (63)*

Table II. World experience in clinical heart xenotransplantation*

Year	*Surgeon*	*Donor*	*Type*	*Patient survival (days)*
1964	Hardy	Chimpanzee	O	< 1
1968	Cooley	Sheep	O	< 1
1968	Ross	Pig	H	< 1
1968	Ross	Pig	Perfused with human blood but not transplanted	< 1
1969	Marion	Chimpanzee	?O	< 1
1977	Barnard	Baboon	H	< 1
1977	Barnard	Chimpanzee	H	4
1984	Bailey	Baboon	O	20
1992	Religa	Pig	O	1

* Modified from Cooper and Ye *(64)*
O = Orthotopic heart transplantation
H = Heterotopic (auxiliary) heart transplantation

Table III. World experience in clinical liver xenotransplantation*

Year	*Surgeon*	*Donor*	*Type*	*Patient survival (days)*
1966	Starzl	Chimpanzee	H	< 1
1969	Starzl	Chimpanzee	O	9
		Chimpanzee	O	< 2
1969	Bertoye	Baboon	H	< 1
1970	Leger	Baboon	H	3
1970	Marion	Baboon	H	< 1
1971	Poyet	Baboon	H	< 1
1971	Motin	Baboon	H	3
1974	Starzl	Chimpanzee	O	14
1992	Starzl	Baboon	O	70
1993	Starzl	Baboon	O	26
1993	Makowka	Pig	H	< 2

* Modified and updated from Dubernard *et al. (63)*
O = Orthotopic liver transplantation
H = Heterotopic (auxiliary) liver transplantation

greater the phylogenetic disparity between donor and recipient, then the more aggressive was the rejection response. The chimpanzee donor kidneys were, in general, rejected more slowly and by a cellular mechanism, whereas the baboon donor kidneys were rejected more aggressively. Reemtsma *et al. (4)* demonstrated that acute cellular rejection of a chimpanzee kidney could be reversed by a course of increased steroid therapy. Survival of the patients ranged from 11 days to 2 months, with one patient surviving almost 9 months. The majority of deaths were related to rejection or infection. Survival of patients with baboon kidneys ranged from 19 to 60 days.

Heart

The first heart transplant performed in man by Hardy *et al.* in 1964 *(6)* utilised a chimpanzee as the donor, but the heart proved too small to support the patient's circulation. Further attempts using closely related donor species were made by Marion *(7)* and Barnard *et al. (8)* without significant success. It should be remembered that these attempts, as well as those of Reemtsma *et al. (4)* and Starzl *et al. (5)*, were carried out in the pre-cyclosporin era and therefore the immunosuppression utilised was relatively primitive. In 1984, however, Bailey *et al. (9)* performed a baboon heart transplant in a newborn infant and utilised cyclosporin therapy. The heart functioned for 20 days but failed from vascular rejection that may have been related, at least in part, to ABO incompatibility between donor and recipient.

Liver

Starzl and his colleagues *(10–14)* were again pioneers in the field of clinical liver xenotransplantation, performing four chimpanzee liver transplants in humans between 1966 and 1974 with the grafts functioning from < 1–14 days. In two of these cases, only minimal pathological lesions were seen in the xenografted liver.

In 1992 and 1993, Starzl's group utilised the baboon as donor in two orthotopic liver transplants, with patient survival for 70 days and 26 days, respectively *(15–17)*. The first of these two cases can be considered a relative success in that there was little pathological evidence of rejection in the liver at any stage, but this was achieved probably at the expense of over-immunosuppression, the patient dying of overwhelming sepsis. The second case was less successful as the patient did not regain consciousness or renal function during the postoperative period, but again there was little histopathological evidence of rejection in the transplanted liver.

These experiences, as well as the increasing amount of experimental data that are becoming available *(18,19)*, have demonstrated that transplantation of a concordant organ in humans is likely to be followed by relative success, with a reasonable prospect of organ function at least for some weeks or months. The length of survival will likely be extended further when some of the pharmacological agents that are currently under investigation become available to the clinician.

Availability and size

Concordant xenotransplantation will, however, be limited by the availability of donor animals. The great apes are endangered species and will clearly not be available as donors of organs for humans. Even with extensive breeding programmes, the logistics of these would be such as to preclude the availability of these animals in large numbers in the foreseeable future.

The baboon and several other smaller monkey species are still available in the wild in relatively large numbers and would be more easy to rear in large breeding programmes. However, the relatively small size of these animals, and the length of time required for them to reach full size, will restrict their use to children and small adults. Even the largest baboon heart will not be of a size sufficient to support the circulation in a full-grown adult human. A pair of baboon kidneys or a baboon liver, however, may be sufficient to support life in adult humans, particularly with the well-known ability of the liver to hypertrophy rapidly under such circumstances, as was clearly demonstrated by the two Pittsburgh baboon-to-human liver transplants *(15–17)*.

Organ function

Will these organs function satisfactorily in a human recipient? With regard to the heart, which has a relatively simple functional role, the answer to this question is likely to be positive. However, the early experience with kidney xenotransplants demonstrated massive diuresis on occasions and difficulty in controlling electrolyte balance *(4,5,20)*. This may not prove to be an insurmountable problem if adjunctive drug therapy, such as antidiuretic hormone, is utilised. The recent Pittsburgh experience with liver xenografting was encouraging in that the baboon livers appeared to be able to fulfil most of the metabolic roles required of the liver in humans, although there was some doubt cast on this point in view of the nature of the bile that was produced, which caused significant problems of cholestasis *(15–17)*.

Potential risk of infection

A major concern with regard to the use of non-human primate organs in humans is the potential for 'xenozoonoses'—the transfer of infectious agents, particularly viruses, with the organ. From our own experience *(21,22)*, however, and that of others *(23)*, it would appear that baboons could be selected where there is little risk of transferring *known* infectious agents to the recipient. However, the risk of transfer of a hitherto *unknown* organism, particularly a retrovirus *(24,25)*, remains and could lead to new disease in humans.

Chronic rejection

Although the pharmacological agents currently available to us might prevent both cellular and vascular rejection, it seems likely that the incidence of chronic rejection, eg

graft atherosclerosis, may prove to be an earlier and more common complication after xenografting than after allografting. Although this might lead to earlier graft failure, it could be offset by the easy availability of a second animal donor.

Discordant xenotransplantation

The early attempts at clinical xenotransplantation using a widely disparate (discordant *(2)*) animal species as a donor for humans were all doomed to early failure (Tables I–III). Rejection between widely differing species, such as pig or sheep to human, is uniformly antibody-mediated and generally hyperacute *(3)*, and results predominantly from complement activation, mainly through the classical pathway *(26)*, although there is evidence that the alternative pathway may also be involved *(27)*. Histopathologically, the features consist of massive capillary destruction with severe interstitial haemorrhage and oedema *(3)*. Intravascular thrombosis resulting from platelet and/or fibrin thrombi are relatively rarely observed by light microscopy but can be documented on electron microscopy.

If this hyperacute vascular rejection could be overcome, however, the discordant animal represents a huge and unlimited source of donor organs for man, and would be far preferable to the use of concordant species. For a number of reasons, the pig has been identified as the most likely potential organ donor for man *(28)*. These include, (1) availability in large numbers, (2) inexpensive to breed and maintain, (3) suitable size for the smallest or largest of humans, (4) availability of pathogen-free (gnotobiotic) animals if necessary, and (5) considerable similarities of anatomy and physiology with man. Furthermore, the public is more likely to accept the slaughter of large numbers of animals such as pigs than it is of non-human primates. The fact that millions of pigs are slaughtered annually for food (90 million in the USA alone) is clearly an advantage in this respect *(28)*.

Recent clinical experience

Although there has been one relatively recent attempt at pig heart transplantation in a human *(29)* (Table II) and another of auxiliary pig liver transplantation *(27,30)* (Table III), these both proved unsuccessful, and the problem of hyperacute rejection is yet to be resolved. It would seem that the most likely solution will come from one of the following approaches.

The concept of 'accommodation'

Humans have preformed antipig xenoreactive antibodies that have been demonstrated to be directed predominantly against α-galactose (αGal) oligosaccharide epitopes on pig vascular endothelium *(31–33)*. If these xenoreactive antibodies can be totally depleted temporarily, or in some other way 'neutralised', then an organ grafted during this critical period may not undergo hyperacute rejection, even when the antibody titre returns to its normal level. The period of time during which antibody depletion or neutralisation is required remains uncertain but may be as short as 1–3 weeks. The resulting state that is achieved, termed 'accommodation' *(34)*, enables survival of an organ graft in the presence of specific antibodies directed against antigens expressed on the surface of the organ and normal levels of complement.

Although this state has not been fully achieved after discordant xenografting, it has been clearly documented after the transplantation of ABO-incompatible organs, both experimentally *(35)* and clinically *(36)*, where the mechanism of antibody-mediated rejection is very similar.

Genetically engineered donor animals

The second approach that offers hope for the future success of discordant xenografting relates to the development of genetically engineered pigs that are in some way resistant to the human immune response. Two approaches are being explored, namely the expression on pig vascular endothelium of certain human complement-inhibiting proteins, and removal or masking of the αGal epitopes which are the targets for human antipig antibodies.

Expression of human complement-inhibiting proteins

Decay accelerating factor (DAF, CD55), membrane cofactor protein (MCF, CD46), and CD59 (protectin, homologous restriction factor) are membrane inhibitors of complement that are present on a wide variety of cell types, including vascular endothelium. These inhibitors block the activity of autologous complement but not of xenogeneic complement from a distantly related species. Pig complement-inhibiting proteins therefore protect against pig complement but not against human complement.

Transgenic pigs that express one or more human complement-inhibiting proteins have recently been reared. Some prolongation of pig organ survival in non-human primates has been demonstrated *(37–39)*, with the Cambridge, UK, group reporting pig heart survival for periods of < 60 days in cynomolgus monkeys *(39)*. This progress in overcoming hyperacute rejection is encouraging.

There is growing evidence, however, that even if the complement cascade is inhibited, vascular rejection might still occur within the first few weeks after transplantation *(40–42)*. This has been termed 'delayed xenograft rejection' and may be associated with other mechanisms possibly involving xenoreactive antibodies and/or cellular mechanisms, particularly involving natural killer cells and macrophages *(43)*.

Absence of α-galactose epitopes

The genetic engineering of a pig that does not express αGal on its vascular endothelium (to which antipig antibodies are directed) might prove a universal donor of organs for humans *(44–46)*. The expression of terminal

αGal depends on the proper function of a single gene encoding for the enzyme α-galactosyltransferase. If this gene were 'knocked out' by homologous recombination, then there would be no target for the human anti-αGal antibodies. Although this 'knock out' technique cannot yet be carried out in the pig, it has been established in the mouse. Unfortunately, preliminary results would suggest that the absence of the αGal epitopes exposes other oligosaccharides against which humans also have natural antibodies *(47)*.

An alternative approach would be the increased expression of another carbohydrate that would compete with αGal for expression on the vascular endothelium *(44,48)*. This has also been achieved in the mouse, where insertion of the gene for H fucosyltransferase has resulted in widespread expression of the H histo-blood group oligosaccharide, against which humans have no antibody (unless they are of the very rare Bombay phenotype) *(49)*. Such a 'universal donor' pig has not yet been bred, but this approach clearly has considerable potential.

With regard to the use of discordant animals as donors, therefore, the major problem remains the immediate immunological barrier between these species, although the report from Cambridge suggests that this barrier is crumbling *(39)*. The severity of the subsequent cellular response to a discordant organ, however, remains uncertain and there is clearly an increased risk that the early development of chronic rejection will take place.

Potential risk of infection

It would seem that the potential for the transfer of serious infectious agents is less when the pig is used as a donor than if a non-human primate is utilised. Our own studies have demonstrated that the pig is a relatively low-risk donor for humans in this respect *(50)*. Of particular importance was the fact that no nematodes were seen in any organ that could cause organ damage or that might prove difficult to treat if transferred to man. This study suggested that breeding and rearing pigs under gnotobiotic (germ-free) conditions, which are extremely expensive and time-consuming, may not be necessary. This is an important point as the production of gnotobiotic pigs would greatly add to the cost of providing donor organs. The potential risk of the transfer of pig endogenous retroviruses remains unknown *(51)*.

Organ function

Whether pig organs will function adequately in humans remains more doubtful. Pig hearts have been demonstrated to function in a heterotopic site in non-human primates for several weeks *(39,42,52)*, and it seems likely that pig heart xenotransplantation would prove successful if the immunological barrier can be overcome. The function of other organs may, however, prove less satisfactory, although once again pig kidneys have functioned adequately in non-human primates for <3 weeks *(53)*. The greatest doubt probably lies with regard to the liver as it seems inconceivable that the pig liver will produce all of the products necessary for the human subject. Here again, however, genetic engineering of the donor pig may help to resolve this problem.

Discussion

There would appear to be a growing acceptance of xenotransplantation among the public. A Partnership for Organ Donation Survey in the USA in 1993 confirmed that, whereas 85% of those questioned said they would accept an organ allograft, 50% said they would accept an organ transplant from an animal if a suitable human organ was not available *(54)*. Furthermore, in the UK, organisations that to some extent reflect public opinion, namely the Institute of Biology and the Nuffield Council on Bioethics, have recently reported positively on the ethics of xenotransplantation *(55,56)*.

Clearly, attempts at xenotransplantation can be best justified if (1) the patient will die rapidly without organ transplantation or (2) failure of the xenografted organ will not result in death of the patient. In cases of rapidly increasing cardiac failure or fulminant hepatic failure, xenotransplantation, particularly as a bridge to allotransplantation, could therefore be considered. In patients with diabetes mellitus or chronic renal failure in whom allotransplantation (of pancreatic islets or kidneys) is precluded by a high degree of allosensitisation, then once again xenografting may be justified as failure of the graft would hopefully not lead to death or significant morbidity.

This latter approach has been pursued by the Stockholm group who have performed pig islet cell transplants in patients with diabetes mellitus *(57,58)*. As the pig is a source of insulin that successfully controls hyperglycaemia in human subjects, this is a logical step forward. This clinical trial, however, has to date not been successful in providing long-term insulin production by pig islet cells, but is a sensible approach for future clinical investigation.

Are we ready for further attempts at clinical xenotransplantation at the moment? It has recently been suggested that cardiac xenotransplantation should be attempted, particularly in the infant or child for whom no form of mechanical assist device is currently available *(59,60)*. Certainly, the use of a concordant xenografted heart as a bridge to allotransplantation would seem to be a feasible approach, although it would do nothing to resolve the organ donor shortage. Current evidence is that subsequent allotransplantation is not likely to be precluded by sensitisation resulting from the previous xenotransplant *(61)*.

Heart transplant candidates who are large (>200 lb) and of blood group O wait an excessively long time and are at high risk of never receiving an allograft. A case could be made for offering this group of patients a pig xenotransplant, particularly if their cardiac status is beginning to fail despite maximal medical therapy, although the availability of mechanical left ventricular assist devices would make this a difficult ethical decision.

Fulminant hepatic failure is another condition in which

xenotransplantation may play a bridging role. A suitable allograft liver may not become available in the short period of time during which the patient rapidly deteriorates and dies, and a xenografted liver may prove the only means of supporting life until an allograft becomes available. There have been several approaches to this problem, including intermittent extracorporeal perfusion of animal livers (usually pig or baboon) to provide support for the failing native liver *(62)*.

However, a strong case can be made for the insertion of an auxiliary animal liver to support the patient until an allograft becomes available to allow for orthotopic transplantation, at which procedure the auxiliary liver would be removed. Indeed, this was the approach taken by Makowka and his colleagues in the most recent liver xenotransplant performed in a human, where the pig liver was unfortunately hyperacutely rejected before a human liver became available *(27,30)*. With the current state of immunosuppressive therapy, however, a concordant baboon liver is clearly more likely to be successful in this respect than a discordant non-primate liver. In our own laboratory, Mieles *et al. (19)* demonstrated that African green monkey auxiliary liver xenografts could support concordant recipient baboons for periods of <4 months.

The use of a xenograft kidney in a potential recipient who had a demonstrably high level of HLA sensitisation, and who was therefore unlikely ever to be transplanted successfully with a human organ, could clearly be justified. In the event of failure of the xenograft, dialysis could be resumed. Patients in whom vascular access for haemodialysis was becoming a major problem could also be considered as candidates for xenotransplantation.

The problems inherent in clinical xenotransplantation remain considerable but, if they can be overcome, the rewards will be enormous. The ready availability of a new organ to replace a diseased one would clearly be a major medical advance. In time, not only those at risk of imminent death from end-stage organ failure would prove candidates for an animal organ. If successful, xenotransplantation would probably prove too great a temptation to the average patient, or physician, to allow either to persevere with inadequate medical therapy, including dialysis, that provides only a suboptimal quality of life.

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