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# Transgenic pigs as potential donors for xenografts

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The field of organ transplantation has grown dramatically over the last 35 years, and one of the key advances that has led to this growth is the introduction of Cyclosporin A as an effective immunosuppressive agent [1] So successful has allotransplantation become with improvement in terms of survival [2] quality of life [3,4] and cost benefit [5] that ever greater numbers of patients are being referred by physicians for consideration of transplantation. This has resulted in a relative shortage in the number of donor organs and in no area is this more marked than in the field of cardiopulmonary transplantation. In the United Kingdom, 454 patients received thoracic organ transplants in the year ending 31 December 1992 while the waiting list grew to 706 patients [6] and the number of patients passing through the assessment procedure is more than three times that number. 25 -30 % of patients waiting for heart or lung transplants die before suitable organs become available for them.

A similarly bleak picture is seen for kidney transplantation. The disparity in numbers between the waiting list and operations performed is shown in figure 1. It is estimated that between 2,500 and 4,000 kidneys are required annually to meet the demand [7] while a recent audit of intensive care units in England suggested an absolute maximum of 1,700 potential donors [8] Even were all of these patients consented for donation and medically suitable there would still be a shortfall in the supply compared to the demand.

The true need may be even higher than estimated since waiting lists are kept artificially low in the knowledge that there is a limited donor resource. With the unprecedented success of transplantation the operations are sought not only to save life as was initially the case, but also to improve the quality of life. Hence the indications for transplantation are widening with a dwindling donor resource.

Public education has ensured that fewer potentially transplantable organs are lost through ignorance and prejudice, but even so the shortage becomes worse each year. Artificial organs such as renal dialysis machines provide partial answers, but progress in the development of totally implantable artificial organs has perhaps been disappointing particularly in view of the huge resource invested in this area. The artificial heart is probably the most successful of the artificial organs, but even though it is a relatively simple pump problems remain with the power supply, biocompatibility, thrombosis and infection. It is likely that this will be developed to a state of clinical usefulness in the near future, but there is currently no prospect of a totally artificial alternative to the more complex metabolic organs such as the lungs, liver or kidney.

It is our belief that the field of xenotransplantation, that is the transplantation of organs between species provides the best solution to the shortage of transplantable organs. The use of animals already bred and slaughtered in large numbers for food poses fewer ethical problems than those posed using a closely related primate species for donor organs. In addition the use of an animal such as the pig which is bred easily in captivity with high parity ensures a ready supply of organs.

The phenomenon of hyperacute xenograft rejection (HXR) occurring within minutes of revascularisation of a transplanted organ has prevented the use of discordant animal organs to date. This phenomenon has several elements including the recognition of foreign tissue by preformed naturally occurring antibodies (PNAB) and the activation of the complement cascade by either alternative or classical pathways. Debate centres on the relative importance of these mechanisms in causation of the violent rejection seen in discordant species combinations. To some extent the use of non-human primate organs may over-

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come the problem since HXR is not seen in such combinations, but first set rejection still occurs. In addition it is unlikely that these animals can provide the organs in the quantity required or of an appropriate physical size and there may be deep-rooted moral objections to the use of animals which are so closely related to man.

A great deal has been written describing the discordant xenograft rejection process. Preformed naturally occurring anti-species IgM antibody binds to donor endothelium and activates complement via the classical pathway [9] although in some species combination it would appear that alternative pathway complement activation assumes greater importance. Although it is probable that antigenic determinants are glycoproteins this remains unproven at present. Tissue factor and plasminogen activator inhibitor are synthesised and heparan sulphate is lost [10] .These processes promote the accumulation of fibrin and in addition platelet activating factor is released stimulating the adhesion of platelets [11] .Neutrophil polymorphs are retained in the organ by their cell surface complement receptors and play an important role in the intravascular compartment in the generation of further inflammatory mediators and the disruption of the endothelial barriers to blood cells and proteins. The resultant platelet thrombi cause ischaemia and failure of the transplanted organ.

Discordant xenograft survival may be prolonged from minutes to hours in experimental models using techniques such as plasmapheresis or xenoabsorption to remove PNAB. Complement depletion with cobra venom factor (CVF) has a similar effect. Craft survival may also be prolonged by depletion of cellular fractions but none of these techniques have produced a xenograft which functions for more than a few hours and none can be envisaged in clinical use to provide long term maintenance of xenografted organs.

Although the role of PNAB is established another area which is becoming more clearly defined is the role of complement through both the classical and alternative pathways. It appears that the regulators of complement activation which are present on all cell surfaces are species specific and those in a xenografted organ fail to inhibit recipient complement activity. A novel approach to complement control would be to express appropriate recipient species RCA's on the xenograft cells. Indeed such an approach is already in use in nature in several micro-organisms. Schistoma Mansoni do not activate the human complement cascade after they have been in the human blood stream. The failure to activate complement is blocked by antibody against DAF (Decay Accelerating Factor – a complement control protein) suggesting that the parasites insert host DAF into their surfaces [12] .Similarly the vaccinia virus is able to encode for secretory proteins which bind host C3 and C4 products to protect against attack by complement [13]

There is experimental evidence to suggest that the manipulation of RCA's will protect an organ against xenogeneic complement. The transfection of cell li-

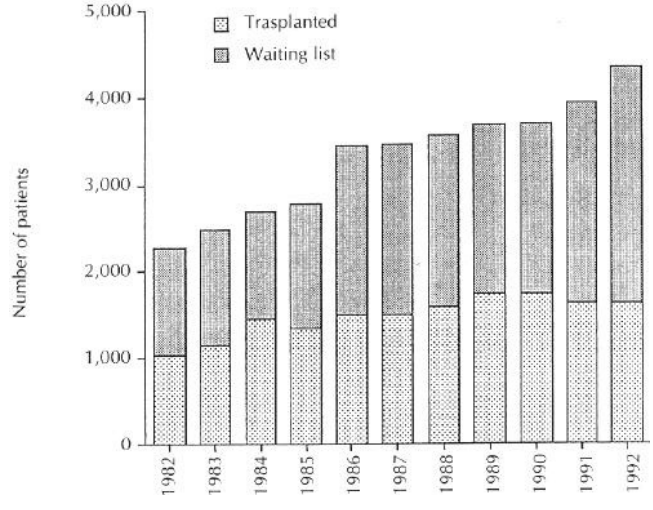


*Fig. 1.-* **Comparison of renal transplant waiting list and number of transplants performed.**

nes with gene constructs coding for human DAF and MCP (Membrane Cofactor Protein) have provided almost complete protection against human complement but not rabbit complement [14]

It is hoped that the introduction of cDNA constructs into the genome of an animal could produce transgenic animals which would express human DAF and MCP and thus protect transplanted donor organs from hyperacute complement mediated rejection. Mice transgenic for human DAF have already been produced although expression is variable[15 16] and a transgenic breeding programme in pigs incorporating the DAF cDNA construct has been undertaken. Again expression is somewhat variable. To date 27 live transgenic pigs have been born from a total of 150 piglets. This holds promise for the future.

Clearly there is a long way to go before the RCA's are reliably expressed throughout all organ systems and on the vascular endothelium and there will need to be detailed experimental assessment before the clinical application of discordant xenografting. However this approach seems likely to produce a useful strategy for the establishment of clinical xenograft programmes [15-17] and it may be as early as five years from now that the first successful transplants are performed using organs from transgenic animals.

If hyperacute rejection could be completely prevented it is not clear what would be the fate of the discordant xenograft, although studies of concordant grafts suggest that there would be many features in common with allograft rejection. Destruction by newly generated antibody and cellular rejection both occur although of course both these processes may be controlled by existing immunosuppressive techniques.

Assuming that HXR can be overcome an animal must be selected as a potential donor for man. The ideal animal must fulfil several criteria. It must be available in large numbers and able to breed easily in captivity. Clearly it must be of an adequate size to produce organs anatomically and physiologically similar to man. For preference it should already be bred as a food source since this is likely to be more readily acceptable to the public than taking an animal which is naturally wild and establishing new breeding colonies which may have highly organised social structures.

The pig has long been quoted as an animal which meets these requirements producing large litters, with rapid growth of offspring, needing relatively small areas for rearing and having low breeding and maintenance costs. In addition there is relatively easy availability of gnotobiotic animals which may be an important consideration when transplanting organs across species barriers. Clearly the transmission zoonoses with the organ is undesirable.

Other diseases too may be transmitted in the transplant organ and neoplasia must also be considered. The best information comes from a large postmortem series performed in the United States which reported an incidence of swine neoplasia of 0.004 % of which the majority were malignant lymphomas and embryonal nephromas. However this data is far from perfect since the animals were all relatively immature having come to slaughter for food.

Congenital anomalies may also prove to be an important consideration but in the same series were reported as around 0.5 % for congenital cardiac defects, and it seems unlikely that any of these considerations will ultimately prove a bar to the successful use of the pig as a cardiac xenograft donor. The question of whether or not the more complex synthetic organs such as the liver or pancreas will ever be usable remains open at this time.

Clinical experience of xenotransplantation has been limited to around 30 cases (table I) and with two notable exceptions in 1993 these attempts have been limited to concordant combinations. Although none of these grafts survived beyond nine months, and indeed most for a much shorter period a great deal of useful information has been revealed. It is clear that one species organs can function usefully in another species body although what the long term implications of for example a chronically different albumen level will prove to be remain a matter of speculation. It appears that the most likely organ to function usefully at present is the heart and comparative physiological studies of human and porcine hearts have shown similar haemodynamic function suggesting that they may work successfully in man.

**Table I.** The clinical application of xenotransplantation

| Year | Organ | Donor | Cases | Survival |
|---|---|---|---|---|
| 1964 | Kidney | Chimpanzee | 12 | < 9 months |
| 1964 | Kidney | Monkey | 1 | 10 days |
| 1964 | Kidney | Baboon | 1 | 4.5 days |
| 1964 | Kidney | Baboon | 6 | < 2 months |
| 1964 | Heart | Chimpanzee | 1 | 2 hours |
| 1968 | Heart | Sheep | 1 | 0 |
| 1968 | Heart | Pig | 1 | 4 minutes |
| 1969 | Heart | Chimpanzee | 1 | Short time |
| 1969-73 | Liver | Chimpanzee | 3 | < 14 days |
| 1977 | Heart | Baboon | 1 | 5 hours |
| 1977 | Heart | Chimpanzee | 1 | 4 days |
| 1984 | Heart | Baboon | 1 | 20days |
| 1992 | Liver | Baboon | 1 | 70 days |
| 1992 | Heart | Pig | 1 | < 24 hours |
| 1993 | Liver | Baboon | 1 | 30days |

## Conclusion

The success of allotransplantation has improved dramatically over the last 35 years. Much of this success has been dueto improvements in immunosuppression. New drugs with novel modes of action may further improve graft and patient survival, but allotransplantation remains limited by donor organ supply and xenotransplantation is probably the only way to provide an adequate number of organs for all those in need. Currently available drugs have not been shown to confer any advantage in the prevention of hyperacute xenograft rejection dictating novel approaches based on examples from the natural world. Genetic modification of donor animals may prove the solution and transgenic breeding programmes are already established. Other obstacles both physiological and moral must be overcome before widespread acceptance of xenotransplantation but there is growing impetus in this direction. It is only a matter of years before clinical xenotransplantation programmes are established.

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