Xenotransplantation

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Introductory article

Article Contents

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The transplantation of organs and tissues across species boundaries is called xenotransplantation. The most likely source of animal organs and cells for transplantation into humans is the pig. Pigs, therefore, have been genetically engineered to provide their tissues with some protection against the human immune response. However, further immunological barriers need to be overcome. Many questions remain regarding the adequate function of pig organs in humans. The safety of xenotransplantation also remains of some concern. Is there a risk of the transfer of a pig infectious microorganism into the community? Present evidence is that this potential risk is low. This potential, however, has legal and regulatory implications.

History of Xenotransplantation

The concept of blending parts derived from different species goes back centuries. Centaurs, mermaids and other creatures from classical mythology come vividly to mind. Homer, for instance, described the chimaera as consisting of a lion in front, a serpent behind and a goat in between (**Figure 1a**). It was a fearful creature, swift of foot and strong, that spat flames at all who came within its range. In contrast, the lamassu was a benevolent beast that guarded the gates of cities and palaces (**Figure 1b**). It had a human head and the body of a lion or a bull with wings that were believed to represent spiritual elevation.

Literature aside, xenotransplantation (the transplantation of organs, tissues or cells between different species, e.g. pig-to-human) dates back more than three centuries, when in 1682 a Russian physician reportedly repaired the skull of a wounded nobleman using a piece of bone from a dog. Blood transfusions from various animals, particularly sheep, were used in humans as early as the seventeenth century, although they must undoubtedly have been associated with major complications and even death. It was not until early in the twentieth century, however, that scientists made attempts to transplant body parts across the species barrier. In 1905, a French surgeon inserted slices of rabbit kidney into a child who had renal failure and, in the years that followed, doctors attempted to transplant organs from the pig, goat, lamb and nonhuman primate into patients. Not surprisingly, all of the grafts failed rapidly, as this was at a time when the immunological basis of the rejection process was not understood. Scientific interest in the transplantation of animal and human tissues waned.

The first scientific efforts were made in the 1960s by Keith Reemtsma and Tom Starzl who respectively transplanted chimpanzee or baboon kidneys into patients in terminal renal failure when human organs were not available. Starzl went on to transplant occasional chimpanzee or baboon livers in critically ill patients. James Hardy and Leonard Bailey carried out single chimpanzee and baboon heart transplants in patients in 1964 and 1984, respectively. All of these attempts were ultimately unsuccessful, although one patient with a chimpanzee kidney and one with a baboon liver survived for months rather than days or weeks, as in the majority of cases.

Need for Xenotransplantation

Organ transplantation, using human donor organs, as a form of surgical therapy began in the middle of the twentieth century, when Joseph Murray and his colleagues performed the first truly successful renal transplant between identical twins in Boston. This success was subsequently extended to transplantation of kidneys from more distantly related and unrelated donors through the use of immunosuppressive drugs such as azathioprine and glucocorticoids and, more recently, ciclosporin and tacrolimus.

To date, it is estimated that close to 500 000 patients worldwide have received life-sustaining renal transplants. The medical applications of transplantation technology have grown significantly during the past few decades to include heart, lung, liver, pancreas and intestine transplantation. Although most attention has been directed towards transplantation of whole organs and bone marrow stem cells, isolation and transplantation of cells and tissues with specific differentiated functions (e.g. pancreatic islets, which secrete insulin) represents an important conceptual and technological advance. **See also**: Transplantation

Organ transplantation is one of the success stories of the second half of the twentieth century. Indeed, it is its very success, resulting in the referral of ever-increasing numbers of patients that has generated a crisis in donor organ

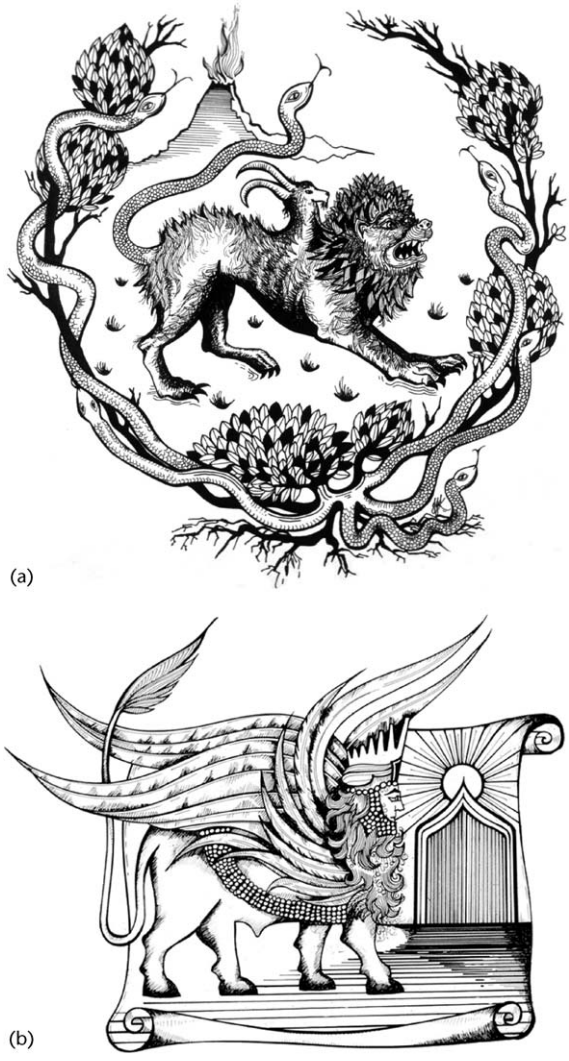

Figure 1 Mythological animals that can be considered examples of xenotransplantation. (a) The chimaera. (b) The lamassu.

supply. Even before the emergence of the new field of cell transplantation, there was a serious shortage of human donor organs and tissues. Considering the fact that, in the USA alone, more than $US400 billion are spent each year to care for patients who suffer tissue loss or end-stage organ failure, it is clear that the pressure to transplant animal tissues into humans will grow and intensify. In addition to patients with heart, liver, kidney and lung disease, over 8 million patients in the USA suffer from neurodegenerative disorders, such as Alzheimer and Parkinson diseases, over 17 million patients suffer from diabetes and millions more from immunodeficiency disorders, haemophilia and other diseases caused by the loss of specific vital differentiated functional cells.

Our improved understanding of the immune system and the immune rejection process has resulted in developing therapies that hopefully will overcome the vigorous immune responses associated with the transplantation of xenogeneic tissues. It appears likely that, during the next few years, human clinical trials utilizing animal cells and organs to treat some of these diseases will become a reality.

Indeed, the US Food and Drug Administration (FDA) has previously approved clinical trials in patients using baboon cells for the acquired immune deficiency syndrome (AIDS), pig cells for diabetes, Parkinson disease and epilepsy, cow cells for intractable pain and pig livers as a temporary support until a human organ becomes available. **See also**: Epilepsy: Management; Liver Failure; Stem Cells and Treatment of Neurodegenerative Disorders

At present, tens of thousands of patients are waiting for donor organs to become available (**Figure 2**). All too frequently, patients with life-threatening illnesses succumb while awaiting organ transplantation. In the USA, for instance, almost 90 000 patients await an organ of one type or another and yet in 2004 well under 30 000 donor organs became available. The issue facing the medical profession and society as a whole is how to resolve this dilemma.

Significant changes in attitude and law towards organ donation would make increased numbers of human organs available, but the supply would remain inadequate. At present, the deficiency in the supply of donor tissues is increasing dramatically each year, and will become even more critical if pancreatic islet transplantation develops as an effective therapy for diabetes. Diabetes alone afflicts an estimated 140 million people worldwide, whereas only a few thousand pancreatic glands become available annually. As multiple glands may be required to isolate a sufficient number of islets to treat a single diabetic patient, it is clearly imperative that techniques be developed to transplant islets from animal sources to diabetic patients on a routine basis. **See also**: Bioethics of Organ Transplantation

For this and a number of other reasons, the pig has been identified as the most suitable potential donor of organs and tissues for humans. Pigs are easy to breed and raise, they mature quickly, and have organs that are comparable in size and physiology to humans. Pigs free from certain designated pathogens have been raised for many years under carefully controlled conditions. Furthermore, fewer ethical concerns should arise than if organs are taken from nonhuman primates.

Problems Associated with Rejection of Pig Organs

The urgent and pressing need for more donor organs and tissues accounts for the current intense research activity and progress that has been achieved in the area of xenotransplantation. In the 1960s, it was clearly demonstrated that the rapid rejection of an organ across a wide species barrier (e.g. a pig kidney transplanted into a human) was the result of the

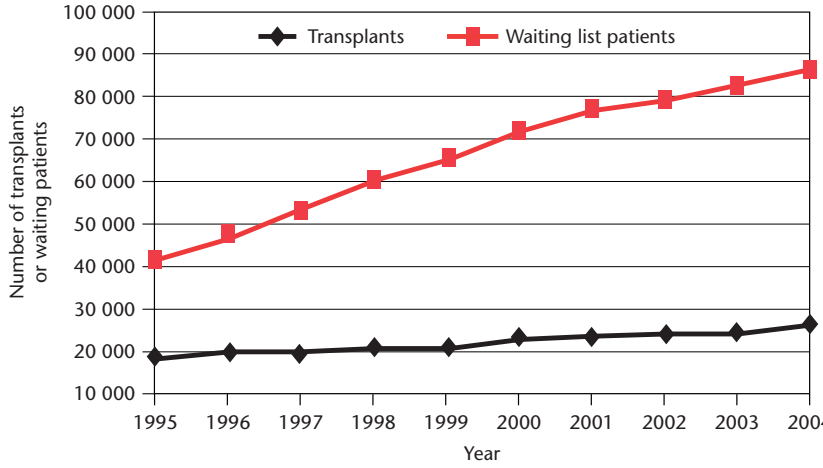

Figure 2 The increasing disparity between the number of patients on the waiting list for organ transplantation (squares) and the number of donor organs that became available (diamonds) in the USA between 1995 and 2004. *Source*: Data based on the United Network for Organ Sharing (UNOS) waiting list and transplantation files.

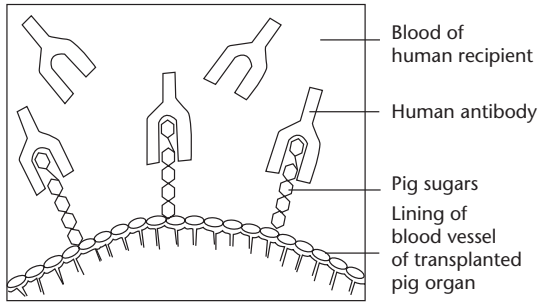

Figure 3 Drawing of human antipig antibodies binding to Gal (and non-Gal) carbohydrate antigens expressed on the pig vascular endothelial cells. This antibody-antigen interaction leads to activation of complement that causes injury to the endothelial cells, and can result in hyperacute rejection.

presence of antibodies in the recipient directed against the donor tissues. These antibodies bind to antigens, and this leads to activation of what is known as the complement cascade, a sequence of events that leads to rapid destruction of the donor tissues (**Figure 3**). This hyperacute rejection usually occurs within minutes or hours and consists of massive capillary destruction, allowing haemorrhage and oedema into the tissues. The major target for these destructive antibodies has been identified as a specific carbohydrate, Galα1-3Gal (Gal), on pig vascular endothelium. **See also**: Antibodies; Graft Rejection: Mechanisms; Natural Antibodies

It is possible to temporarily deplete antibody and/or complement or inhibit complement activation. If an organ is grafted during this critical period of antibody depletion and/or complement inactivation, the graft is not rejected hyperacutely, but survives for several days, at which time the return of antibody and/or complement causes rejection by a slightly different mechanism.

For some years it has been possible to introduce a new gene into a pig cell, but it was not possible to knockout a gene until the development of nuclear transfer/embryo transfer technology, or 'cloning' as it is known, was developed, initially in the case of Dolly the sheep.

Recently, various genetically-engineered pigs have become available for study. The first of these were pigs that were protected from human complement-mediated injury by the expression of a human complement-regulatory protein, such as human decay-accelerating factor. Pigs naturally express complement-regulatory proteins that protect their own tissues from the effects of pig complement, but these do not provide good protection against human complement. These transgenic pigs proved to be more resistant to injury by human complement than wild-type (unmodified) pigs, although not completely resistant.

The second major genetic modification was to knockout the gene for the enzyme that attaches the Gal oligosaccharide to the underlying structures on the endothelium of pig blood vessels, so-called GT-KO pigs. The absence of this Gal antigen rendered the anti-Gal antibodies in the human or nonhuman primate recipient to be void, as they no longer had a target to which they could bind. They therefore did not activate complement, reducing the early antibody-mediated injury to the pig organ.

These methods allow us to overcome the hyperacute rejection that occurs immediately after the transplantation of an animal organ. However, we already know that when hyperacute rejection is avoided, the xenograft is subjected to a second immune attack – known variously as delayed xenograft rejection, acute vascular rejection or acute humoral xenograft rejection – that develops and leads to more gradual graft failure.

A third barrier – the subsequent acute cellular response – is likely to be at least as strong as that mounted against

allografts and, using the currently available immunosuppressive drugs, may require such massive therapy that the risks of drug toxicity, infection and other related complications may prove problematic. However, some optimism can be gained from the fact that several potent new pharmacological immunosuppressive agents are in various stages of development. Some of them have proven active not only against the cellular response, but also against antibody-mediated rejection. There is a prospect, therefore, that the addition of some of our new immunosuppressive drugs will overcome these two post-hyperacute barriers. **See also**: Immunosuppression: Use in Transplantation; Immunosuppressive Drugs

However, these approaches were found to be inadequate to protect the organ in the longer term. Although early antibody-mediated injury no longer took place, there is believed to be an activation of the vascular endothelial cells of the graft that leads to coagulation in the small blood vessels of the pig organ. These thromboses cause obstruction to blood flow, with subsequent tissue injury from the lack of supply of oxygen and other nutrients.

It is believed that the abnormal coagulation/thrombosis that takes place is in part related to incompatibilities between the primate and pig anticoagulation mechanisms. Normally, our own blood vessels maintain a local anticoagulant state that protects them from the development of thrombosis. The pig has a similar mechanism, but some of the pig anticoagulant factors are ineffective in the presence of primate blood. This leads to a change from an anticoagulant to a procoagulant state, resulting in thrombosis in the small blood vessels of the graft.

Efforts are currently being made to breed pigs that express a human 'anticoagulant' gene. Organs from these pigs should be protected, at least in part, from the effects of the human coagulation system. These pigs, of necessity, will need to be crossbred with the GT-KO pigs, and are not yet available for testing in nonhuman primates.

Clearly, it would be desirable if patients could be induced to accept animal organs and tissues without the need for immunosuppressive drug therapy. Such organ acceptance, or immunological tolerance, is known to have occurred spontaneously in a few patients who have received human organs, and has allowed the dosage of immunosuppressive drugs to be reduced and ultimately withdrawn without loss of allograft function. The induction of immunological tolerance is an approach, albeit a difficult one, particularly well suited to xenotransplantation. Unlike deceased human donor organs that need to be procured urgently under emergency conditions, animal donors would be available electively at any chosen time, thus allowing physicians time (before the transplant) to reprogramme the immune system of the patient into accepting the transplant. **See also**: Immunological Tolerance: Therapeutic Induction

A number of strategies for creating tolerance are currently being investigated. One approach involves augmenting the immune system of the patient with haematopoietic (bone marrow) cells from the donor. Once introduced, the donor pig cells spread throughout the body of the recipient, creating a chimaeric immune system that is part donor, part recipient. The aim is for the patient to then recognize pig cells as 'self' and become tolerant to subsequent transplanted pig tissues. **See also**: Bone Marrow

Xenotransplantation of Cells

In patients with type I diabetes, there is a marked decrease in the number of insulin-producing β cells in the islets of the pancreas. There is hope that the transplantation of pig islets will not only eliminate the need for daily insulin injections, but will prove effective in preventing or retarding the development of complications associated with the disease. Currently, however, many pig islets are rapidly lost after transplantation into nonhuman primates, though enough have survived to maintain a normal blood sugar for several weeks or, occasionally, months.

One approach that has been investigated is to try to protect the islets from the host's immune response by encapsulating the islets. During the past several years, immunoisolation systems have been developed in which transplanted cells can be separated from the hostile immunological environment of the host by a selectively permeable membrane. Low-molecular-weight substances, such as nutrients, oxygen and biotherapeutic agents, are exchanged across the membrane, while immunocytes, antibodies, and other transplant rejection effector mechanisms are excluded.

Although not yet successful, this approach has broad application to treating common diseases such as diabetes by the introduction of pancreatic islet cells, as well as a wide range of other disorders. These include the use of hepatocytes for the treatment of liver failure, chromaffin cells for chronic pain, cells that produce clotting factors for haemophilia and cells that produce nerve growth factors for neurodegenerative disorders, such as Parkinson and Alzheimer diseases. Moreover, by using recombinant deoxyribonucleic acid (DNA) and cell engineering technologies, it may also prove possible to treat patients suffering from such disorders as immunodeficiences and cancer.

Problems Associated with Potential Infection

Our growing ability to cross the species barrier through the use of these various techniques has, however, raised a multitude of new issues. For example, there has been much media attention towards animal pathogens that can infect and kill humans, such as 'mad cow disease' (bovine spongiform encephalopathy) and Ebola infection (a haemorrhagic fever). Some experts fear that animal donor organs might harbour comparable pathogens that could not only infect the patient but also subsequently spread into the general population, resulting in an epidemic.

Baboons and monkeys are of most concern in this respect. For example, scientists now believe that the human immunodeficiency virus originated as a monkey virus that somehow infected humans. The risk of such an infection following xenotransplantation, termed a xenozoonosis, however, is considered to be markedly less if pigs are the donors of organs. Humans and pigs have been living in close association for hundreds, if not thousands, of years – the pig being raised, slaughtered, and eaten by humans throughout this time. (Annually, in the USA alone, over 90 million pigs are slaughtered for food purposes.) And yet, no serious infection primarily of swine origin appears to have arisen in humans. (Some influenza strains, that generally originate in birds, e.g. chickens or ducks, may pass through pigs before infecting humans.) **See also**: Marburg and Ebola Haemorrhagic Fevers; Prion Diseases

Nevertheless, there is still a potential risk associated with introducing any animal tissues into humans on a wide scale. The transplantation of human organs carries just such a risk, and it could well be argued that there will be much greater quality control if the pig is the donor. Indeed, it should be possible to exclude all of the significant bacteria, parasites, helminths and prions that might infect donor pigs, although it will be more difficult to exclude all of the viruses. With animal donors, however, the perceived risk is largely not from well-recognized microorganisms, but from previously unidentified pathogens.

Nearly all viruses that are known or suspected of causing health problems in pigs or humans can probably be excluded, with one major exception – the so-called endogenous retroviruses. These are essentially viral particles that have become incorporated in the genome of the cell nucleus. They therefore incorporate their genetic blueprint directly into the host cell's DNA. Estimates suggest that as much as 1% of the DNA that humans carry is made up of such viral particles.

All mammalian species probably have their own specific endogenous retroviruses. At least 30 are known in primates, although to date only a few have been positively identified in pigs. These viral sequences owe their presence in modern animals to past episodes of retroviral infection in their ancestors many thousands of years ago. As the viruses have inserted their genetic code into sperm or egg cells, the offspring of the infected animals retain these viral genes, which are then passed from generation to generation. Over time, most of these vestigial viruses have evolved into forms harmless to their hosts, yet some may remain capable of activity that potentially could cause disease in other species.

Virologists are worried about what these viruses might potentially do when the pig cells that contain them are transplanted into human patients. Not only does the transplant offer the pig virus direct access to human cells, but also it presents the virus with a uniquely susceptible victim; namely, a patient with an immune system that, in order to prevent rejection of the transplanted organ, may have been severely compromised. Under these circumstances, pig viral particles (or proviruses) might be able to give rise to active retroviruses, perhaps causing illness. It is also conceivable that these pig retroviruses could mutate in human hosts, or possibly combine with human proviruses to produce a completely new pathogen that is resistant to the human immune system. This possibility, however remote, is causing concern because, unlike the viruses that bring on a short-lived illness, such as swine influenza, some of these retroviruses may remain quiescent for many years before causing cancer or an AIDS-like immunodeficiency condition in the patient.

What is known to date is that the pig endogenous retroviruses that have been identified can, under certain ideal laboratory conditions, be transferred to human cells, which they then infect. Whether this can only take place in the laboratory or whether it will also occur if a pig organ is transplanted into a human remains unknown. It is also unknown whether pig endogenous retroviruses will cause any problems even if they do infect the cells of the human recipient. They may remain just as benign as in the pig. To date, there has been no evidence of porcine endogenous retroviral infection in any nonhuman primate or human exposed to pig organs or tissues, though graft survival, and therefore exposure to the virus, has not been maintained for longer than a few months. However, the potential for disease is sufficient to warrant caution before xenotransplantation moves ahead.

The Function of Pig Organs in Humans

There are also many other scientific issues that will require resolution if the problems of rejection and infection can be overcome. For instance, will the transplanted animal organ function normally in the environment of the human body? This involves anatomical considerations – whether the organ is structurally similar to its counterpart in the human – as well as physiological and biochemical considerations – whether the multitude of enzyme systems, hormones, etc. that function adequately in the pig will perform equally successfully when the organ is transferred to the human.

From a functional perspective, there are essentially two questions that need to be answered. First, will a transplanted pig organ continue working normally in the human milieu, or will differences in such conditions as body temperature and the acidity of the blood (pH) adversely affect the organ? Second, even if the organ works normally, will it fulfil all of the roles of a healthy human organ? The state of our knowledge at present is insufficient to answer either of these questions with confidence. Pig hearts and kidneys have functioned adequately in nonhuman primates for several weeks or months, but it seems unlikely that a pig liver will perform all of the myriad essential functions identical to that of the human liver. But here again, genetic engineering techniques may help resolve this problem. For example, a human gene could be inserted into the pig, and lead to the production of a human enzyme or hormone.

Other Issues

As with many advances in biotechnology, xenotransplantation will challenge society with difficult ethical and legal questions. For instance, is it ethical to rear animals for human replacement parts? Do 'humanized' animals have any human rights? Who would be ethically and/or legally responsible if a pig organ recipient developed an infection with a porcine microorganism and transferred it to members of the health care team or to other members of the community? The answers to these and other complex social questions could take up an entire volume, and will depend to a great degree on evolving norms and public sentiments. **See also**: Bioethics of Organ Transplantation

Undoubtedly it will be many years before all of the inherent problems in 'outwitting evolution', as German comparative biologist, Claus Hammer, has called the xenotransplant goal, are solved. Indeed, there are those such as British transplant pioneer, Sir Roy Calne, who remain cautious, and point out that, 'Clinical xenotransplantation is just around the corner, but it may be a very long corner', a prophecy, made in 1995, that is proving true.

All involved in this effort, which if successful will surely prove one of the major advances of the modern medical era, can gain strength and encouragement, however, from the elegant words of an unknown writer:

A vision without a task is a dream.
A task without a vision is drudgery.
A vision with a task is the hope of the world.

Further Reading

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