Solid organ xenotransplantation: Progress, promise and regulatory issues

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Abstract Xenotransplantation, the process of transplanting live cells, tissues and organs from one species into another, is an emerging novel area in medicine. It is proposed as a treatment for human end-stage organ failure and for other applications such as, for example, the treatment of neurodegenerative and liver disorders, meeting the large demand for donor organs. The pig is considered to be the most suitable donor species. However, the rejection of a pig transplant is much more severe and multifactorial compared with that of a human graft and involves almost all branches of the human immune system. Major rejection mechanisms include complement-mediated rejection, antibody-mediated rejection and cellular rejection. Prevention of rejection requires not only improved immunosuppressive agents, but also innovative approaches including the genetic modification of the pig donor by which organs become more easily acceptable, and the modulation of the human recipient's immune system so that it tolerates the xenograft without the need of extensive immunosuppression. Regarding safety, concerns have been raised on the potential risk of transmission of infectious microorganisms by a pig transplant to a human patient, and subsequent spread of infectious microorganisms into the population by an infected individual. This in particular concerns porcine endogenous retrovirus: to date intensive research has shown no evidence that this virus is transmitted *in vivo* from pig to humans; also a miniature swine line has been identified which does not give detectable *in vitro* transmission to human cells. Based on concerns on xenotransplantation, especially the safety of porcine-to-human transplants, many countries in Europe, the USA and Canada have instituted stringent policies for the development of xenotransplantation products, which include guidelines and regulations of clinical trials and additionally include public discussions on the issues associated with xenotransplantation. Based on these initiatives of regulatory authorities, xenotransplantation could advance towards a clinical application by a closely monitored stepwise approach. It is expected that advances in preclinical research during the coming years will give a further basis for such a stepwise development from a promising tool into a new well-accepted clinical entity.

Keywords: genetic modification, immunology, microbiological safety, regulatory aspects, xenotransplantation, xenozoonosis

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Introduction

Xenotransplantation is the process of transplanting live cells, tissues and organs from one species into another. It is proposed as a treatment for human end-stage organ failure and for other applications such as, for example, the treatment of neurodegenerative and liver disorders.[1] Interest in xenotransplantation emerged following the successful implementation of allotransplantation, ie the transplantation of human cells, tissues or organs into unrelated human patients, when it became evident that the number of available organ donors was insufficient by far to meet the growing demand. To illustrate this point, according to the United Network for Organ Sharing annual report, the number of patients awaiting transplants in USA has risen more than five times as fast as the number of transplant operations in the 1990s, documenting an increasingly acute need for livers, hearts and other organs. At the end of 1999, more than 72,310 patients were on the US national transplant waiting list, three times as many people as in 1990. As of February 2001, it had climbed even higher, to 74,073. The number of deaths on the waiting list has also more than tripled – from 1,958 in 1990 to 6,125 in 1999. The figures worldwide are about twice those in the USA.

The success of allotransplantation is largely due to the introduction of powerful immunosuppressive drugs to suppress the rejection of the grafted organ or tissue by the host. When kidney transplantation was introduced in the 1950s, a small number of immunosuppressive agents were available that had many unwanted side effects. Heart transplantation was first performed in the early 1960s, and after an enthusiastic growth it came almost to a stand-still as rejection proved to be difficult to manage. When the immunosuppressant cyclosporine (Sandimmun Neoral, Novartis Pharma) reached the market in the early 1980s, clinical transplantation again showed substantial growth, until a steady level was imposed by the limited availability of donor organs. The transplantation area has taken a forefront position in the development of new immunosuppressive agents, starting with the introduction of cyclosporine. Subsequently, the first approval for clinical use of a monoclonal antibody was for an anti-T-cell antibody in transplantation (muronomab-CD3, Orthoclone OKT3®, mid-1980s), and more recently the first approval of a humanised monoclonal antibody (daclizumab, Zenapax®) and a chimeric monoclonal antibody (basiliximab, Simulect®) directed to activated T cells were also indicated for transplantation. A number of other powerful immunosuppressive agents (Table 1) have become available, allowing optimal management of the balance between suppression of graft rejection and avoidance of adverse side effects.[2]

This development in allotransplantation is described to illustrate the long time period and inherent complications in developing a new procedure from an experimental anecdote to a well-accepted clinical entity. After a few heroic exploratory trials in patients, solid organ xenotransplantation is currently essentially at the stage between advanced preclinical research and first controlled clinical trials. Although some original clinical experiments were conducted using organs from non-human primates, it is now widely accepted that non-human primates are not suitable as a donor species: rather the major focus towards potential clinical development is on the pig as a donor species because of its close similarity to humans regarding physiology, its size and breeding characteristics.[3] Also, ethical concerns relating to the use of the pig as donor animal may be reduced in view of the fact that the pig is a highly domesticated animal and purpose-bred as source of food. A few clinical trials have been performed thus far using porcine cells or tissues, under which foetal porcine islets in diabetic patents[4] and porcine foetal neural cells in patients with Parkinson's or Huntingdon's disease.[5]

Major issues in xenotransplantation that are currently the subject of extensive research are related to the control of different types of rejection that in strength outweigh those observed in rejection of

Table 1 Main immunosuppressive agents presently on the market or in advanced clinical development

Compound	Trade name	Mechanism of action
Xenobiotics		
Cyclosporine	Neoral	Calcineurin inhibitor
FK506, tacrolimus	Prograf	Calcineurin inhibitor
Rapamycin, sirolimus	Rapamune	Inhibitor of growth factor-driven cell proliferation
RAD, everolimus	Certican	
Cyclophosphamide		Inhibitor of cell proliferation
Methotrexate		Inhibitor of cell proliferation: anti-inflammatory
Azathioprine	Imuran	Inhibitor of cell proliferation
Mizoribine	Bredinin	Inhibitor of inosine monophosphate dehydrogenase
Mycophenolate mofetil	Cellcept	Inhibitor of inosine monophosphate dehydrogenase
Mycophenolate Sodium	Myfortic	
Leflunomide	Arava	Inhibitor of dihydroorotate dehydrogenase
15-Deoxyspergualin	Spanidin	Inhibitor of cell differentiation
Biologicals		
OKT3	Orthoclone-OKT3	T-cell depletion
CD25 antibody, basiliximab	Simulect	Depletion of CD25-positive T cells
CD25 antibody, daclizumab	Zenapax	Depletion of CD25-positive T cells
CD4 antibody		Depletion of CD4-positive cells
CD20 antibody	Rituximab	Depletion of CD20-positive B cells
CD52 antibody	Enlimomab	Depletion of leukocytes
anti-TNFα antibody	Remicade	TNF blockade
CTLA4-Ig		Inhibition of costimulation

Adapted from Table 1 in the chapter by Calne and White in Shuurman *et al*.[2]

allografts, and the identification of potential pathogens that could be transmitted from an animal graft into a human. Because of the progress made and the novel issues that xenotransplantation engenders, many governmental institutions have instituted tightly regulated policies for the development of products for xenotransplantation, similar to those in other emerging technologies like gene therapy. In the following we will address progress and promises in the immunobiology and safety of xenotransplantation, as well as present an overview of governmental regulations. Only key references will be mentioned; for a recent overview the reader is referred to two recent issues of the journal *Graft*.[6]

Immunobiology of xenotransplantation

In allotransplantation donor and recipient are generally matched for ABO blood group and the absence of antibodies in the recipient to donor leucocytes: the major mechanism of rejection is that mediated by the cell-mediated immune system, ie T lymphocytes. Hence, most of presently available immunosuppressive drugs primarily target this branch of the immune system. In contrast, the human immune system can utilise almost all branches of its immune system in rejecting a porcine graft. This involves components of the innate system (ie not primed by specific antigen sensitisation) and the acquired system, ie cell-mediated and antibody-mediated processes becoming functional after sensitisation.

It is generally assumed that the rejection of a porcine graft can vary between organs and cells or tissues, and also depends on the site of injection in case of cells or tissues. For instance, hyperacute rejection (HAR, further described above) is particularly evident for solid organs that are directly connected with the vasculature of the recipient, while it is less evident for cells and tissue when injected at sites outside the blood circulation. Also rejection is expected to be less severe when injection is done in so-called 'immune-privileged' sites that are normally not accessible to all components of the immune system: the brain is given as an example, being 'immune-privileged' thanks to the blood–brain barrier.

In the following description of various branches of the immune system involved in xenograft rejection, it is worth mentioning that present knowledge is mainly based on human anti-pig reactivity demonstrated in *in vitro* models, while *in vivo* data are based on models in rodents (pig to mouse/rat transplantation) or transplantation in non-human primates (pig transplants in baboons or cynomolgus monkeys).

Complement-mediated rejection

In the first defence to, for example, pathogenic microorganisms, non-specific cytolytic cells including macrophages play a pivotal role. There is little evidence that this defence system has a pivotal role in the immediate rejection of a xenograft. At least, this is overshadowed by humoral factors, in particular those of the complement system. This system comprises a number of factors that in a well-defined sequence of activation, as in enzyme–substrate reactions, yield split products giving inflammatory reactions and tissue injury. Besides activation and release of pro-inflammatory split products, the system is tightly regulated, among others by molecules expressed on cell surfaces. Such regulators of complement activation (RCA) generally do not work across the species barrier between pig and human. Since the complement system in a healthy individual is almost always in a low state of activation (but regulated by RCA), or otherwise can become activated upon trauma (like in a surgical procedure), complement activation occurs almost immediately upon reperfusion of a xenografted organ after implantation, yielding the disruption of the vasculature and subsequent destruction of the organ. This process, called hyperacute rejection (HAR), can be substantially accelerated and magnified by the simultaneous action of naturally existing anti-pig antibodies discussed below.

A number of strategies have been developed to circumvent this potential immediate loss due to complement-mediated lysis. A number of complement inhibitors have been identified and are presently in development. Most advanced are TP10, the soluble form of the complement receptor 1,[7] and a monoclonal antibody to complement component C5.[8] These molecules act by inhibiting activation of the complement cascade. The efficacy of TP10 in prevention of the immediate destruction of a porcine xenograft in non-human primate models has been shown.[7] A

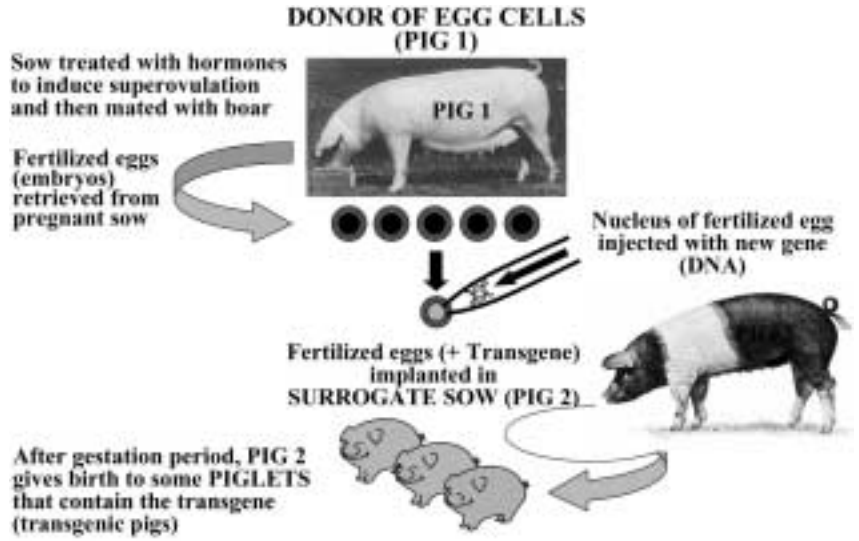

Fig. 1 Schematic presentation of the procedure for producing transgenic pigs

major disadvantage in using this pharmacological inhibition is the systemic action. Blockade of complement activation throughout the body may prevent the potential beneficial action of complement in the early phase of an infection. For this reason it is expected that the chronic administration of a complement-inhibiting drug will not be considered as a feasible option to prevent xenograft destruction. However, short-term administration in the context of a transplant may be feasible.

As an alternative, complement regulation in the graft has been exploited (Fig. 1). Pigs transgenic for human RCA have been created, with human decay-accelerating factor (hDAF, CD55), membrane cofactor protein (CD46) and protectin (CD59) (Fig. 2). The expression of these RCAs at the surface of endothelium lining blood vessels has been associated with protection from hyperacute rejection of organs in pig-to-non-human primate transplantation models.[9–11] These transgenic pigs were considered a major breakthrough for xenotransplantation and elicited high expectations for subsequent development. This has been slightly tempered as follow-up studies showed that 100 per cent protection from immediate graft destruction cannot be achieved by using organs from RCA-transgenic pigs, and also by the observations that subsequent immune-mediated rejection apart from complement-mediated lysis continues to cause graft loss.

Antibody-mediated rejection

Xenoreactive antibodies occur in almost every xenogeneic combination. The larger the distance between the species on the evolutionary tree, the more diverse the xenogeneic antibody spectrum can be. For human and Old World monkeys a peculiar situation exists, namely the presence of naturally occurring antibodies directed to terminal Galα1,3Gal carbohydrate structures (Gal) present both on glycoproteins and glycolipids of the pig (so-called anti-Gal antibody) (Fig. 3). Such terminal carbohydrate molecules are present in almost all mammalian species but not in Old World monkeys and humans as these species lack the enzyme involved in their synthesis (α1,3-galactosyl transferase). The Gal carbohydrate structures are similar to blood group substances, ie respective antibodies are elicited by cross-immunisation with intestinal bacterial flora and first become evident after birth when the intestinal tract is colonised by microorganisms. Most of the antibodies are

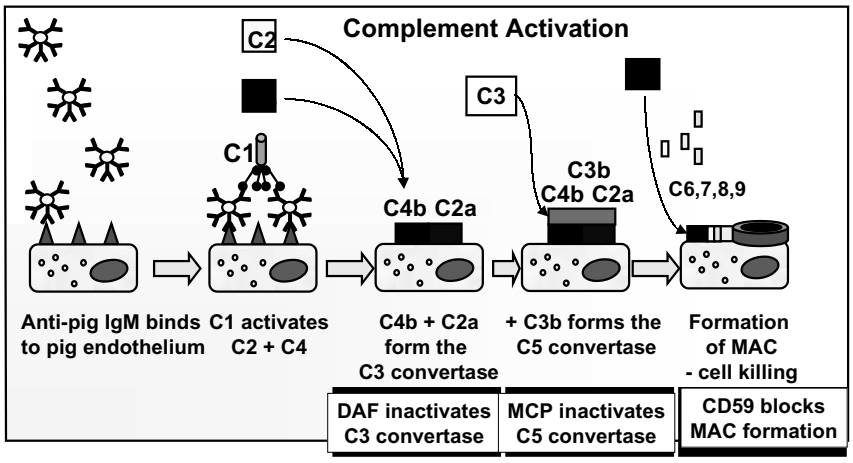

Fig. 2 Mechanism by which regulators of complement activation modulate the destruction of endothelium lining blood vessels by antibody and complement. C1–C9 = complement components; MAC = membrane attack complex. Regulators of complement activation decay accelerating factor (DAF), membrane cofactor protein (MCP) and CD59 interfere in this pathway.

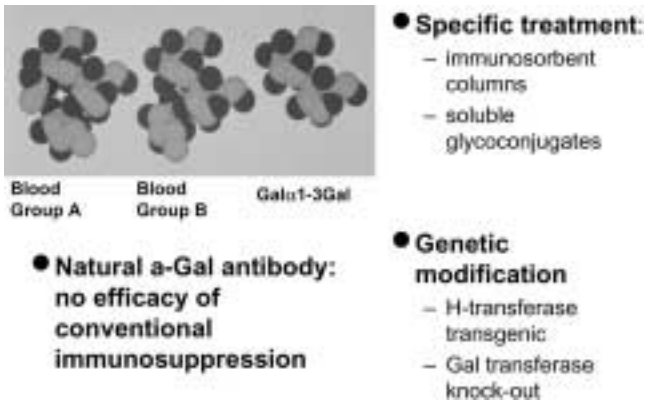

Fig. 3 Scheme presenting the close resemblance in structure between blood group substances A and B, and the Galα1,3 Gal carbohydrate structures.

of IgM immunoglobulin class, acounting for 5–10 per cent of the total body's IgM. It has been well established that these antibodies play an important role in the immediate graft destruction after implantation and reperfusion of a xenograft. HAR occurs upon antibody binding to the cell surface and subsequent complement activation causing graft destruction within minutes to hours after reperfusion. HAR mediated by naturally occurring antibodies appears to have a larger role in graft destruction than the antibody-independent complement activation mentioned above.[12]

The presence of anti-Gal specificity as the major, if not only, component in the spectrum of naturally existing anti-porcine antibodies enables a specific intervention to avoid antibody-mediated destruction of a xenograft. Extracorporeal perfusion through columns filled with Gal-containing resin,[13] and infusion with soluble Gal-containing glycoconjugates have been introduced to temporarily remove anti-Gal antibodies from the circulation. This removal has shown efficacy in prevention or management of HAR. Chronic treatment with soluble glycoconjugates seems feasible as it affects only the anti-Gal antibody population. However, it is not clear whether chronic inhibition of anti-Gal antibody has long-term consequences. In a reverse approach, the aim is to eliminate the Gal epitope from the graft. This can be done in different ways, like pharmacological inhibition of the Gal-transferase enzyme, or by generating pigs transgenic for another transferase resulting in dimished Gal expression due to competitive inhibition with other sugar residues.[14,15] The most radical approach is to eliminate/inactivate the gene encoding Gal-transferase. Procedures for gene targeting using embryonic stem cells exist in mice, but it has proved to be difficult to establish similar procedures in other species. However, recent work has demonstrated the establishment of nuclear transfer in cloning procedures in pigs, enabling a route to specifically knock-out distinct genes (Fig. 4).[16] It is expected that Gal-transferase knock-out pigs will become available in the coming years.

Although the main component of anti-porcine antibody in a normal individual comprises anti-Gal antibody, this situation can change upon introduction of a xenograft: upon sensitisation antibodies are elicited to pig antigens. Since many more antigens in a transplant can be recognised by the host as foreign, a huge spectrum of antibody specificities can arise, and in addition various types of antibody-mediated rejection reactions are initiated. Besides antibody and complement, this can include antibody-dependent cellular cytotoxicity, and attraction of polymorphonuclear granulocytes and

macrophages which can contribute to tissue destruction. This rejection reaction is difficult to control; complement inhibition and anti-Gal neutralisation will not be effective as these target only part of a large spectrum of potential rejection reactions.

Antibodies are synthesised by plasma cells, ie terminally differentiated B lymphocytes. There are no clear modalities yet available to inhibit plasma cells, either immunosuppressive drugs or anti-plasma cell biologicals; also, it turns out to be quite difficult to chronically immunosuppress B lymphocytes. There are immunosuppressant agents available that by virtue of their mechanism of action target both T and B cells (Table 1), such as mycophenolic mofetil (Cellcept, Roche), leflunomide (Arawa, Aventis) and 15-deoxyspergualin (Spanidin®, Nikkon-Kayaku): these compounds have shown efficacy in yielding prolongation of xenograft survival in pig-to-non-human primate models, but long-term survival with a functioning graft is not yet achieved. Some groups have worked with a B-cell antibody approved for the treatment of B-lymphoid malignancies (Rituximab, IDEC Pharmaceutical Corp.), with variable success. In pig-to-baboon xenografts it appears that chronic blockade of co-stimulatory signalling by an anti-CD154 antibody (formerly called anti-CD40 ligand antibody) as part of the immunosuppressive regimen reproducibly adds to the prevention of sensitisation and generation of antibodies to other structures than Gal.[17]

Cellular rejection

In the sequence of events after xenograft transplantation, cellular rejection including the generation of cytolytic T lymphocytes can emerge at a similar time after transplantation as elicited antibody towards Gal and non-Gal antigens. Most studies on cellular rejection have been conducted in *in vitro* models and studies in small laboratory animals. Interestingly, products of the major histocompatibility complex, in human called HLA (human leucocyte antigens), appear an important target in xenogeneic cellular responses as they are in allogeneic cellular responses. In other words, cellular reactions are mostly directed towards products of the pig major histocompatibility complex, called SLA (swine leucocyte antigens). This is somewhat remarkable, as the cell surface of swine cells carries many other antigens besides SLA, which could serve as target for

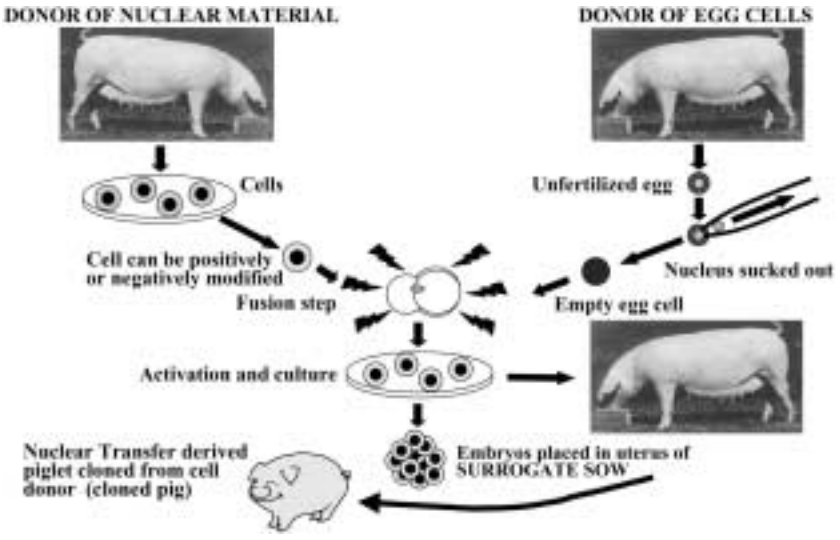

Fig. 4 Schematic presentation of gene targeting and pig cloning.

cellular reactivity. Essentially, similar therapeutic modalities are applied to suppress cellular rejection in the xenogeneic setting as in the allogeneic setting. However, in multiple *in vitro* and *in vivo* models it has been demonstrated that the suppression of a xenogeneic cellular response requires more immunosuppression than that of an allogeneic response. This not only regards cellular rejection utilising cytolytic cells, but also also antibody-mediated rejection involving T cell-dependent antibody synthesis. Indeed, in Novartis's experience in a large-sized pig-to-non-human primate solid organ transplantation programme using organs from hDAF-transgenic pigs, cellular rejection as the cause of graft dysfunction was a relatively rare phenomenon in cases with prolonged graft survival. Most grafts with prolonged survival but finally lost owing to rejection showed histological signs compatible with antibody-mediated rejection, often accompanied by the presence of elicited antibodies in the circulation. This indicates that it appears even more difficult to suppress B-cell reactivity and subsequent antibody synthesis, whether or not being dependent on T-cell regulation, than to suppress T-cell reactivity resulting in cellular rejection by cytolytic T cells. Hence, in these large animal models the therapeutic window between optimal immunosuppression and adverse side effects appears to be small, ie under similar aggressive immunosuppression animals could show adverse side effects such as drug toxicity or infection, and/or rejection (in most cases antibody-mediated). This means that immunosuppressive regimens (Fig. 5), which are nowadays so successful in allograft transplantation, might need substantial improvement for the case of xenotransplantation before being clinically acceptable: in this respect the present status of xenograft immunosuppression appears similar to that of allograft immunosuppression in the pre-cyclosporine era (Fig. 5, Table 1).

There is, however, an alternative approach to immunosuppression, namely tolerance, ie creating a state of graft

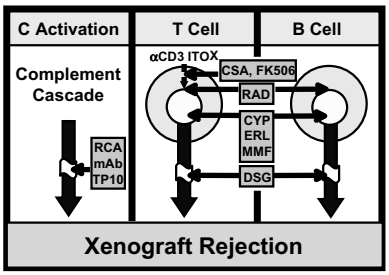

Fig. 5 Schematic presentation of different immunosuppresive approaches to prevent xenograft rejection. (C Activation = complement activation. RCA, mAb, TP-10 = complement activation inhibitors regulators of complement activation, monoclonal antibody and soluble complement receptor 1 respectively. T- and B-cell inhibitors include CD3-immunotoxin (α CD3 ITOX), cyclosporine A (CSA), tacrolimus (FK506), Sirolimus or everolimus (RAD), cyclophosphamide (CYP), mycophenolate mofeti/ (MMF), mycophenolate sodium (ERL) and 15-deoxyspergualin (DSG).

unresponsiveness in the presence of low or no immunosuppressive treatment.[18] This could be a state in which reactive cells are present but actively suppressed by reactive cells in the regulatory T-cell network or being intrinsically anergic (peripheral tolerance), or a state in which reactive cells are deleted from the spectrum of lymphoid cells (central or deletional tolerance). This latter state is preferred, as controlling the maintenance of peripheral tolerance in the clinical setting might be difficult. Deletional tolerance is also called central tolerance as it involves so-called central lymphoid organs such as bone marrow and thymus that have a function in shaping the immune repertoire. Deletional tolerance can be achieved by depleting the immune system of the host followed by transplantation of the bone marrow or the thymus of the donor. In the bone marrow approach, the recipient is not completely depleted of its own haematopoietic stem cells (ie cells with the capacity to build a competent immune system), so that a condition is created in which both host and donor stem cells repopulate the immune system. This condition is called 'mixed haematopoietic chimerism'. In the case of thymus transplantation, the recipient's immune system is re-educated by the donor (pig)

thymus, resulting in deletion of relevant anti-donor (anti-pig) reactive T cells from the repertoire. Both methods have shown promising results in rodent models including pig-to-mouse transplantation, as well as in xenogeneic transplants between different non-human primate species, like cynomolgus monkeys and baboons: after creating a tolerant state by so-called conditioning, a skin or solid organ transplant is permanently accepted without the need of any immunosuppression.[19] These approaches are nowadays actively investigated in pig-to-non-human primate transplantation models. It is expected that effective T-cell tolerance induced by thymus transplantation will not only prevent cellular rejection, but also rejection by antibodies elicited in a T cell-dependent immune reaction: bone marrow transplantation might in addition create a condition in which the B-lymphocyte population is tolerant as well.

Conclusion

The immunological hurdles in prevention of (solid organ) xenograft rejection are substantial and require intervention in almost all branches of the immune system, in particular complement-mediated damage, antibody-mediated rejection and cellular rejection. The small therapeutic window of the presently available armamentarium of immunosuppressive drugs makes it difficult to control xenograft rejection and achieve long-term survival in animal models such as pig-to-non-human primate solid organ transplantation. It is evident that modification of the donor (removal of relevant antigens by gene targeting, insertion of human complement regulatory molecules by transgenesis) or host (tolerance induction) will be crucial in reducing the hurdles associated with the species barrier. It can be anticipated that these innovative approaches in genetic modification and modulation of the immune system, together with the development of new immunosuppressive agents with a broader therapeutic window, will finally result in the optimal management of a porcine graft in a patient.

Safety of xenotransplantation

For any product intended for clinical use, safety of the product is paramount. For porcine tissues, cells and organs this includes microbiological safety, or assessment of the risk of any pathogenic disease spread by the pig tissue to human (xenozoonosis).[20,21] A substantial list of potentially infectious bacterial, fungal, protozoal and viral microorganisms has been created, which can be eliminated from the production herd without too much difficulty. This also applies for so-called prion disease (bovine spongiform encephalopathy in cattle, scrapie in sheep, Creutzfeldt–Jacob disease in humans), which appears to be not of major concern in pigs.

However, elimination appears more difficult for latent and endogenous viruses. It is well known that pigs carry viruses that cause disease in humans. Some viruses can be transmitted vertically (ie from mother to foetus) such as porcine circoviruses, cytomegalovirus and lymphotropic herpesviruses. Although these viruses do not cause disease when present in the latent state in healthy individuals, they can be upregulated under certain conditions such as with immunosuppression, and then cause disease. This phenomenon is well characterised in humans: for example, upregulation of cytomegalovirus can contribute to chronic rejection reactions, and upregulation of Epstein–Barr virus can cause so-called post-transplant lymphoproliferative disease, especially if the donor organ is virus-positive while the recipient is virus-negative. Cross-species transmission of the viruses of major concern (hepatitis virus, circovirus, cytomegalovirus, gamma-herpesvirus) has not been unequivocally proven, but essentially cannot be ruled out, particularly in view of viral adaptation once transmitted to a recipient. It is therefore expected that these viruses be eliminated from the herd in a final xenotransplantation product. This elimination is largely possible, for instance

by hysterotomy delivery of piglets by which the risk of cross-placental transfer is reduced, followed by rearing in a barrier-controlled facility. Exploratory studies in a herd of hDAF-transgenic animals and miniature swine has shown the feasibility of this approach, and this is presently under investigation for other pig herds.

Porcine endogenous retrovirus (PERV)

Some viruses are not acquired by transmission but are endogenously present in animals as part of the genome. Like other species, pigs harbour endogenous retroviruses (porcine endogenous retrovirus, PERV).[22,23] PERV is related to so-called type B and type C retroviruses, bearing similarity to mouse mammary tumour viruses and murine leukaemia viruses: it is not closely related to lentiviruses such as human immunodeficiency virus. There is no disease condition yet ascribed to PERV. Three infectious PERV subfamilies have been identified, called PERV-A, PERV-B and PERV-C. Using *in vitro* transmission studies it has been documented that PERV-A and PERV-B can infect human and pig cells, while PERV-C is only transmissible to porcine cells.[23] Cytopathology as a consequence of infection in these *in vitro* experiments has not been observed thus far.

Transmission of PERV into a human being via a xenotransplant product is presently considered as a potential risk factor. This regards not only potential infection of a transplanted patient, but also the potential of subsequent spread in the human population by infected individuals. To address this hypothesis, a number of studies on *in vivo* infection have been initiated, either evaluating PERV infection in transplanted individuals or conducting infectivity studies in naïve or immunosuppressed animals. A large retrospective study has been carried out on approximately 160 patients that had previously been directly exposed to various living porcine tissue up to 12 years earlier, eg by tissue transplantation or by extracorporeal blood perfusion through porcine organs. Using the most sensitive molecular biology assays that are presently available, none of these patients showed any evidence of PERV infection.[24] Similar data have been reported in patients exposed to porcine foetal neuronal cells, porcine islet xenografts or to a bioartificial liver support system with a membrane device containing porcine hepatocytes. Also, the analysis of immunosuppressed non-human primates transplanted with porcine endothelial cells, porcine solid organs or directly exposed to cell-free virus in an infectivity experiment did not give any indication of PERV infection.

There appear to be differences between lines of pigs with respect to their potential for PERV infection *in vitro*. Interesting data were obtained in the analysis of miniature swine lines inbred for certain SLA types, namely infection of human cells could not be demonstrated in *in vitro* infection studies using cells from a miniature swine line. This does not exclude the presence of PERV elements in the genome of these pigs, but apparently the genomic loci of PERV are defective and therefore incapable of establishing productive infections. Such a situation is quite likely as the vast majority of endogenous retroviral elements in the genome of other better-documented species (mouse, human) is also defective.

A number of approaches have been proposed to reduce the risks and consequences of potential PERV infection in xenotransplantation. As stated above, these approaches are considered not only in view of the risk to individuals receiving potentially infectious transplants, but also the risk of spreading the infection through the population once an individual is infected. As for other virus infections, vaccination has been proposed, but is generally not considered feasible in view of the difficulties in development of an effective vaccine, and the fact that virus vaccines do not give full protection in immunocompromised individuals (like transplant patients under immunosuppression). Drugs inhibiting viral replication have been evaluated, demonstrating effective inhibition of PERV

replication *in vitro* by the nucleoside analogues azidothymidine and dideoxyinosine.[25,26] These drugs are already used for anti-retroviral treatment in humans. The data offer the potential to interfere with the possible consequences of infection in infected individuals, but are not considered feasible in the prevention of possible infection. This is because of drug side effects and a lack of knowledge on the pathogenicity of the virus. Rather, a more radical approach, ie the avoidance of infection, is preferred.

Based on results of the *in vitro* infectivity studies, PERV research is currently focused on the elucidation of the genomic organisation, that is the identification of replication-competent viral sequences. Dependent on the outcome of such studies an active intervention strategy can be envisaged with the aim of inactivating or removing such loci in the genome. It is well known that endogenous retroviral sequences can form a substantial part of the whole genome, about 1 per cent in humans, making it impossible to remove all such sequences. However, the vast majority of these loci comprises defective sequences. Studies on identification of replication-competent viral sequences are underway to determine the feasibility of preparing an animal that is non-infectious regarding PERV. Particularly interesting in these studies is the genomic analysis of the miniature swine line for which PERV transmission to human cells *in vitro* could not be demonstrated.

Conclusion

The present status of research indicates the feasibility of developing a xenotransplantation product with minimal safety risks regarding infection of the recipient with porcine microorganisms. On the one hand, potential pathogens can be eliminated from the herd by selective breeding and, on the other hand, the assessment of endogenous pathogens could yield data that these bear less risk than generally perceived. In particular the risk assessment of endogenous porcine retrovirus transmission to human beings needs further study before a definite conclusion can be drawn. A relevant factor to be considered in risk assessment is the fact that the recipient of a xenotransplantation product is generally immunosuppressed and hence has increased vulnerability to infection, not only by microorganisms acquired from the environment or present in the recipient itself, but also by microorganisms from the graft.

Regulatory aspects of xenotransplantation

The last decade has witnessed not only some major breakthroughs in xenotransplantation research, but also growing concern on the potential risks. This concern principally relates to two issues: genetic modification of living organisms and risk of zoonosis and subsequent spread to the human population. The first issue is related to the concerns of the public about food safety and appropriate consumer health protection, and the second issue to the possibility of infection by PERV or other as yet unknown microorganisms, including avoidance of prions in pigs. Regulatory authorities and other institutions have therefore proposed strict policies and guidelines in proceeding with these innovative technologies towards early clinical trials, to ensure optimal safety in balance with the proposed efficacy.

Initiatives on steering clinical xenotransplantation research were not only taken by regulatory authorities, but also by the research community itself. For instance, scientists presenting results of first baboon liver grafts into patients with liver failure already proposed in 1994 a moratorium on further clinical trials using grafts from non-human primate donors. Recently, in December 2000 the International Society for Heart and Lung Transplantation published comprehensive guidelines on how and when to proceed with animal-to-human heart and lung transplants.[27] Among other things it proposed only considering a clinical trial when approximately a 60 per cent survival

rate of life-supporting pig organs in non-human primates has been achieved for a minimum of 3 months, with at least 10 animals surviving for this period of time. It was also suggested that there should be evidence that longer survival, ie exceeding 6 months (ideally in 50 per cent of transplants), can be achieved. Such results have not been reported in these models to date.

Europe

The Parliamentary Assembly of the Council of Europe voted in January 1999 to recommend a temporary moratorium on clinical trials in xenotransplantation. This was not followed by the member states but a Working Party on Xenotransplantation was created, which is expected to draft guidelines for clinical xenotransplantation research. Individual countries in Europe have developed specific regulations and legislation and installed regulatory bodies in xenotransplantation. Specific legislation regarding clinical trials in xenotransplantation exists or is drafted in France, the Netherlands, Germany and Switzerland, while other countries have developed advisory guidelines. In France, any clinical trial needs approval by the Ministry of Health, after assessment by both the Regulatory Authority and the French Transplant Establishment. In the Netherlands the Health Council has issued a report on the scientific status of xenotransplantation, considering clinical trials ethically acceptable if requirements of clinical viability are met that will be assessed by a central committee: a legally binding moratorium on clinical trials is presently under discussion. In Switzerland xenotransplantation is included in the legislation: pending the adoption of a new transplantation law in 2004 clinical trials need permission from the Federal Public Health Office. Other European countries, such as Norway, Sweden, Spain and the UK, have installed xenotransplantation advisory committees, which have a major role in drafting guidelines and assessment of applications for clinical trials. Most advanced in this respect is the UK since the initiation of the UK Xenotransplantation Interim Regulatory Authority (UKXIRA) in 1997.[28] This authority advises government departments on the regulation of xenotransplantation, in particular regarding safety, efficacy and animal welfare considerations. UKXIRA has published a number of documents, including a draft guidance on patient surveillance and biosecurity considerations. The UKXIRA has the remit of assessing any clinical trial proposals in the UK: however, the final decision to approve or reject an application remains with the relevant minister.

USA and Canada

In the USA a somewhat different approach has been followed in the development of a xenotransplantation policy. Four agencies of the US Public Health Service, the Centers for Disease Control and Prevention, Food and Drug Administration (FDA), Health Resource Services Administration and National Institutes of Health (NIH), worked together to prepare the 'Draft Guideline on Infectious Disease Issues in Xenotransplantation' in 1996. This guideline has been reviewed after various public comments and discussions, and the final version was published in January 2001.[29] The guideline addresses not only safety issues of xenotransplantation into humans, but also items on protocol designs and review, ethical consent, quality control of a xenotransplantation product, specimen archiving and medical records. The responsibility for the regulation of xenotransplantation lies within the existing regulatory authority, the FDA. As such, the FDA has initiated the Xenotransplantation Subcommittee of the Biological Modifiers Advisory Committee, which is intimately involved in the review of developments in the field, clinical trial design and approval, and provision of advice and proposals for guidance to the FDA. In addition, the NIH has established the Secretary's Advisory Committee on Xenotransplantation (SACX),[30] which will provide a public forum for discussions on xenotransplantation. Its role might be

considered similar to that of the Recombinant Advisory Committee: it is involved in considering the public health aspects of clinical trial protocols and other issues associated with xenotransplantation into humans. As SACX is only recently established and had its first meeting in February 2001, its exact role in the field will become clearer in the future. Most recently an important draft document regarding changes in the regulation of xenotransplantation has been sent out by the FDA for consultation in January 2001.[31] In contrast to the normal policy of the FDA relating to the filing of an investigational new drug (IND) application with the FDA, it is proposed that clinical trials in xenotransplantation are to be disclosed in public. This not only applies to xenotransplantation, but also to trials in the area of gene therapy. In addition to clinical protocols, this change in disclosure includes patient enrolment, safety data, annual progress reports and filings of adverse effects. This radical change from the normal FDA policy in dealing with clinical trials, especially early and exploratory clinical trials, is not expected to affect such trials in xenotransplantation, as these are anyway to be presented in public forums such as SACX. It remains unclear how this proposal will evolve, but it continues to support the generation of clear regulatory guidance, transparency and education of the public in the field of xenotransplantation.

Finally the approach followed in Canada should be mentioned. Xenotransplantation is regulated by the Therapeutic Products Program (TTP) of the federal department Health Canada under the requirements of the Food and Drugs Act. The Canadian National Forum on Xenotransplantation was held in 1997 and its recommendations have been published.[32] The TTP also brought together a group of experts to establish the the Canadian Standards for Xenotransplantation.[33] Based on these reports, as well as the 1999 report of the Standing Committee on Health 'Organ and Tissue Donation and Transplantation: a Canadian Approach', a Public Advisory Group funded by the Canadian Public Health Association was initiated in August 2000, with the task of conducting public consultations across Canada on the issue of xenotransplantation.[34] It is anticipated that these consultations that are ongoing in 2001 will yield recommendations on future government policy on xenotransplantation.

Conclusion

Regulatory authorities in many European countries, the USA and Canada have taken initiatives to regulate pig-to-human transplantation, in particular producing guidelines for clinical trial proposals. These initiatives are mainly based on concern about safety, ie the risk of transmission of potential infectious microorganisms such as PERV. In most countries special advisory committees have been created to advise on design and monitoring of clinical trials. Interestingly, in particular in the USA and Canada, the governmental initiatives include public consultations and discussions on various issues of xenotransplantation, which has the added advantage of educating the public in this novel area in transplantation medicine. Although the approaches taken in individual countries differ, under the current regulations and guidelines in several countries it is possible to obtain approval for clinical trials to transplant porcine cells, tissues or organs into patients with carefully designed trials based on proven efficacy and safety data in animal models, and which include extensive safety monitoring. This means that the progress of xenotransplantation towards a clinical application will be conducted in a carefully controlled stepwise fashion. It can be expected that further developments in regulatory activities will be highly influenced by progress in preclinical science in the field, and in this regard initiatives taken by the scientific community illustrated by the recent publication of the International Society for Heart and Lung Transplantation[27] are relevant as well.

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