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XENOTRANSPLANTATION - STATE OF THE ART - UPDATE 1999

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1. ABSTRACT

Organ transplantation is limited by the number of cadaveric human donor organs that become available. Xenotransplantation - the transplantation of organs and tissues between animal species - would supply an unlimited number of organs and offer many other advantages. The pig has been identified as the most suitable donor animal. Pig organs, when transplanted into humans or nonhuman primates, are, however, rejected hyperacutely within minutes by antibody-mediated complement activation. Human anti-pig antibodies have been identified as being directed against galactose α1-3galactose (αGal) epitopes on pig vascular endothelium. Methods have been successfully developed to prevent hyperacute rejection. These include (i) depletion or inhibition of recipient antibodies or complement and (ii) development of transgenic pigs that express a human complement-regulatory protein (e.g. hDAF). The persistence or return of anti-pig antibody, however, even following the use of hDAF pig organs, eventually leads to what has been variously termed "acute vascular rejection" or "delayed xenograft rejection", which is again believed to be largely antibody-dependent. Nevertheless, experimental pig-to-primate organ xenotransplantation now results in transplant function for days and weeks rather than minutes. Little is yet known of the nature of the acute cellular rejection response that is anticipated to follow, and of any subsequent chronic rejection that may develop. Tolerance to both the αGal epitope and to swine leukocyte antigens (SLA) is being explored using gene therapy techniques and by the induction of hematopoietic cell chimerism. The development of genetically engineered pigs that do not express the αGal epitope is also being pursued. Considerable progress has been made in recent years, but experimental results do not yet warrant the initiation of a clinical trial of organ xenotransplantation. However, trials are already underway of pig cell transplants in patients with diabetes and neurodegenerative conditions, such as Parkinson's disease.

2. INTRODUCTION

Organ transplantation is one of the success stories of the second part of the 20th century. During the past 15 years results have steadily improved and patients undergoing kidney, liver, or heart transplantation can realistically anticipate approximate 80%-90% and 70% one and 5-year survival, respectively (1). The major limiting factor to organ transplantation today is the increasing shortage of suitable donor organs. In the USA, 60,000 people are listed for solid organ transplantation by UNOS, and yet only 6000 cadaveric donors (and 3000 living donors) become available each year, from which approximately 20,000 donor organs are obtained (1). The discrepancy between the number of potential recipients and donor organs is increasing by approximately

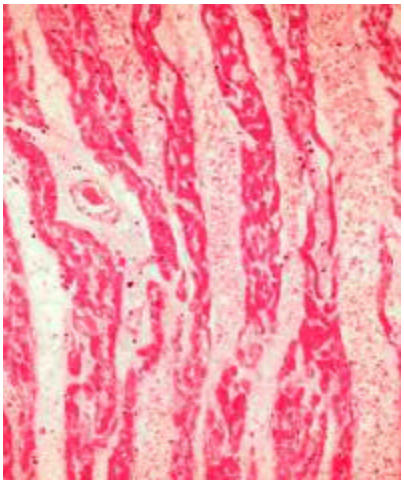

Figure 1: Photomicrograph of donor pig myocardium following xenotransplantation into a non-immunosuppressed recipient baboon. The donor heart ceased functioning after 4 hours, and histologically shows florid hyperacute rejection with severe interstitial hemorrhage, vascular thrombi, and myocyte necrosis. (Hematoxylin and eosin, x 150).

10-15% annually (2). Patients on dialysis awaiting kidney transplants are, therefore, waiting for longer and longer periods of time, and approximately 10% of patients awaiting liver or heart transplantation die before a suitable donor becomes available.

One solution to this problem would be the use of animal organs - xenotransplantation (3-5). This field of research has been undergoing intensive and increasing study during the past few years, and some encouraging progress is being made.

3. CONCORDANT AND DISCORDANT XENOTRANSPLANTATION

3.1. Definitions

Xenotransplantation refers to the transplantation of organs or tissues from an animal of one species into another species. With regard to humans, it clearly refers to the use of a donor other than a human. The terms concordant and discordant xenografting are used loosely to refer respectively to transplantation between closely-related animal species (e.g. baboon-to-human) and between distantly-related species (e.g. pig-to-human) (6).

With regard to the histopathology of the rejection that takes place, we should probably confine our terms to (i) antibody-mediated (denoting vascular or humoral) and (ii) cellular rejection, although (iii) mixed rejection can occur (7,8). Antibody-mediated rejection, however, may be hyperacute (in that it occurs within minutes or a few hours after transplantation) or delayed, occurring some days or even weeks after transplantation. The differences in histopathology are discussed below.

3.2. Pathogenesis of Xenograft Rejection

In general, pre-existing antibodies are not present in humans in high titers against closely-related species (e.g. baboon), but can develop or rapidly increase during the first few days after concordant xenotransplantation. Rejection generally occurs in an accelerated fashion (when compared to that of an allograft) within a few days, and can be of a humoral, cellular, or mixed nature (7-9).

Significant differences in the rejection process occur in different species combinations and different transplanted organs. For example, cynomolgus monkey hearts transplanted into baboons would appear to be rejected primarily by a cellular mechanism (10,11), not unlike after allografting, whereas African green monkey hearts transplanted into baboons are more likely to be rejected by a humoral (or mixed humoral and cellular) mechanism (12,13). African green monkey livers transplanted into baboons, however, have been reported to be rejected primarily by a cellular mechanism (9,14).

As most interest is now being directed towards the use of the pig as a donor of organs or cells for humans, pig-to-nonhuman primate models of xenotransplantation are those largely being investigated (15). This review will therefore concentrate largely on knowledge obtained in primates, although reference to work in other relevant models will be made.

3.2.1. Hyperacute Rejection

The presence in humans of relatively high titers of natural preformed antibodies against discordant donor species (e.g. pig) leads to immediate hyperacute rejection (HAR) (as may occur when allografting is carried out in a sensitized recipient). The HAR is initiated by the interaction of the antibodies with antigens on the vascular endothelium of the donor organ, resulting in activation of the classical pathway of complement (16,17). In some species combinations, the alternative pathway of complement activation is believed to play a role (18), and evidence has been put forward to suggest that in humans this may be due to dimeric IgA binding to the pig vascular endothelium (19).

The classical histopathologic picture of HAR consists of disruption of the vascular endothelium, with massive interstitial edema and hemorrhage (7,8) (figure 1). Intravascular fibrin thrombi are frequently present, and platelet thrombi can be observed. This picture can, however, be considerably attenuated even when early graft failure has occurred. Immunofluorescence studies demonstrate IgM, IgG, IgA and complement deposition on the vascular endothelium (20) (figure 2).

Current evidence is that all (or most) human anti-pig antibodies are directed against galactoseα1-3galactose (αGal) epitopes on the surface of pig vascular endothelium (21-28) (table 1 and figure 3). These anti-αGal antibodies, originally identified by Galili *et al*. (29), are also found in apes and Old World monkeys, but not in lower primates (e.g. New World monkeys) or non-primate mammals (including the pig), which, in contrast, express the αGal antigen (30,31). Following the transplantation of a pig organ into a human or

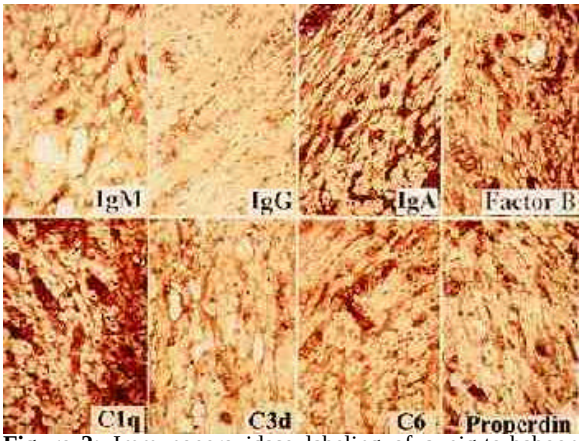

Figure 2: Immunoperoxidase labeling of a pig-to-baboon cardiac xenograft that was rejected hyperacutely. There is endothelial deposition of IgM, IgG and IgA. The graft also shows endothelial deposition of components of the classical (C1q) and alternate (Factor B, properdin) pathways of complement activation, along with C3d and terminal pathway components (e.g. C6) (Courtesy W.W. Hancock).

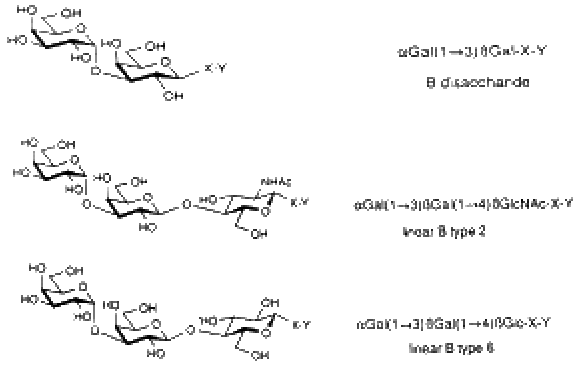

Figure 3: Three of the major carbohydrate structures that bind human antibodies eluted from pig heart, kidney and red blood cell stroma - αGal disaccharide (above), αGal trisaccharide type 2 (center), and αGal trisaccharide type 6 (below). R = $(CH_2)_8COOHCH_3$.

baboon, or the extracorporeal perfusion of human blood through a pig organ, there is a marked increase in the titer of anti-αGal antibody, increasing by <60-fold over a period of days or weeks (32-35). There is evidence for some heterogeneity within the anti-αGal antibody repertoire (36,37).

Humans are believed to develop anti-αGal antibodies during the first few weeks of life through exposure to certain microorganisms that colonize the gastrointestinal tract and which also express αGal structures on their cell membranes (38). At birth, anti-αGal IgG can frequently be detected in the plasma, presumably passively transferred from the mother, but not IgM (39). As it is predominantly IgM binding that initiates HAR, a pig organ transplanted into a neonatal baboon is not rejected hyperacutely, but does undergo acute vascular rejection over the next few days.

Other antibodies may possibly play a role in xenograft rejection once anti-αGal antibody has been removed or if the αGal epitope is successfully deleted from the donor organ, as can be achieved in αGal-knockout mice. These are listed in table 2 and have been discussed fully elsewhere (28). Their exact role, if any, remains obscure. Certainly, antibody to new pig determinants can be detected after pig organ transplantation in nonhuman primates (40,41), but the nature of the determinants has not been clearly demonstrated.

3.2.2. Acute vascular rejection

If HAR can be avoided (e.g. by depletion of complement by cobra venom factor (20,42)), current evidence is that a delayed form of rejection occurs within a few days (43), which leads to more gradual graft failure. This process has been variously termed acute vascular rejection (AVR) (44) or delayed xenograft rejection (43). The exact mechanism of AVR remains uncertain, but it appears to be antibody-mediated but complement-independent (43-45). There is increasing evidence that natural killer cells and macrophages may play significant roles (45). The histopathological features of AVR vary from those seen in HAR, with immunohistological studies revealing the presence of cytokines and various infiltrating cell types (20,40,46).

In vivo data on primate anti-porcine AVR has come from experiments in which HAR was prevented. Hearts from transgenic pigs expressing human decay-accelerating factor (hDAF) and CD59 do not undergo HAR after transplantation into nonhuman primates (47-49). However, these grafts are rejected within 5 days by a process involving formation of fibrin thrombi in the microvasculature and endothelial cell swelling, which are distinct from the platelet microthrombi and interstitial hemorrhage observed in HAR. Anti-donor antibodies would appear to play a significant role (50).

Rejection of porcine hearts by neonatal baboons, which have very low levels of preformed IgM xenoantibody, was significantly delayed compared to the rejection that has been observed in adult baboons (51). The dominant findings in hearts rejected by neonatal baboons were perivascular and interstitial mononuclear cell infiltrates, contrasting with the intravascular thrombosis typically observed in HAR in adult baboons. The cellular infiltrates were predominantly composed of host macrophages and NK cells.

HAR can be prevented in the guinea pig-to-rat discordant model by depletion of complement. The grafts are then rejected within a few days by a process which bears many similarities to the AVR seen in primates. It appears to involve a cascade of events initiated by activation of graft endothelial cells (52). This in turn leads to platelet activation, chemokine secretion, and recruitment of NK cells and monocytes/macrophages. The presence of xenoreactive immunoglobulin increases NK cell-mediated endothelial cell activation and results in increased NK cell-endothelial cell adhesion. Monocytes are the main effector cells. Very few T cells are involved. NK cells and monocytes/macrophages adhere to the activated endothelium and eventually infiltrate the graft. NK cells, which secrete cytokines including IFN γ, make up approximately 10-20% of the cellular infiltrate. Monocytes, which secrete TNFα as well as other cytokines, make up 70-80%. NK cells and monocytes participate in the

Table 1. Structures of the main carbohydrate epitopes exposed at the surface of human and porcine vascular endothelia[a]

HUMAN	PIG
Galß1-4GlcNAcß1-R[b]	Galß1-4GlcNAcß1-R[b]
ABH-Galß1-4GlcNAcß1-R[c]	Galα1-3Galß1-4GlcNAcß1-R[d]
NeuAcα2-3Galß1-4GlcNAcß1-R[e]	NeuAcα2-3Galß1-4GlcNAcß1-R[e]
	NeuGcα2-3Galß1-4GlcNAcß1-R[f]

Only the epitopes shown in bold type and underlined are different between the two species. [a] Modified from Cooper, D.K.C., *et al*. (1994). R are glycolipid or glycoprotein carrier molecules anchored in the cell membrane. [b] N acetyllactosamine [c] the A, B, H, or AB blood group antigen [d] the α-galactosyl antigen [e] N-acetylneuraminic acid [f] N-glycolylneuraminic acid (Bouhours, D., *et al*., 1997)

Table 2. Known non-α-gal carbohydrate antigens against which humans can have naturally occurring antibodies*

1. A: GalNAcα1-3(Fucα1-2)Galß1-4GlcNAcß-R
2. B: Galα1-3(Fucα1-2)Galß1-4GlcNAcß-R
3. Thomsen-Friedenreich (T or TF) Galß1-3GalNAcα1-R
4. Tn (TF precursor) GalNAcα-R
5. Sialosyl-Tn: NeuAcα2-6GalNAcα1-R
6. p^k: Galα1-4Galß1-4Glcß1-R
7. Other P antigens
8. Sulfatide I: SO_4-3Gal-R
9. Forssman: GalNAcα1-3GalNAcß1-3Galα1-4Galß1-4Glcß1-R
10. i: Galß1-4GlcNAcß1-3Galß1-4GlcNAcß-R[c]
11. I: Galß1-4GlcNAcß1-3(Galß1-4Glcß1-6)Galß1-4GlcNAcß-R[c]
12. αRhamnose-containing oligosaccharides
    L-Rhm-α-Rhm
    L-Rhm-α1-3GlcNAcß1-2L-Rhm-α-R
13. ßGlcNAc-containing oligosaccharides
    GlcNAcß-R
    GlcNAcß1-4GlcNAcß-R

[a]Modified from data collected by Tange *et al*. (1997a), Shinkel *et al*. (1997), and Cao *et al*. (1996). [b]R are glycolipid or glycoprotein carrier molecules anchored in the cell membrane. [c]The core structures of the ABH antigen system which are fucosylated by H transferase to generate H substance.

endothelial cell activation by direct cell contact and by the secretion of cytokines. This results in the induction of a wide range of endothelial cell genes including adhesion molecules and chemoattractant molecules. Molecular incompatibilities between porcine and human cytokines and adhesion molecules may be important. Platelet activation and procoagulative changes in the activated endothelium lead to diffuse microvascular thrombosis with prominent fibrin deposition. These inflammatory processes are likely exacerbated by the failure of normal downregulatory mechanisms to function due to molecular incompatibilities (53).

Although HAR and AVR appear to represent distinct clinical and pathological entities, effective strategies for overcoming the two responses may have common elements. As antibodies to donor endothelium appear to play an important role in both phenomena, complete depletion of anti-donor antibodies or the induction of donor-specific antibody tolerance may inhibit both HAR and AVR (54). Strategies involving inhibition of donor endothelial cell activation are also being investigated extensively. One promising area involves expression of anti-apoptotic genes such as hemoxygenase-1 and A20 in donor endothelial cells (55). These "protective" genes inhibit activation of endothelium and are associated with the phenomenon of "accomodation", in which a graft becomes resistant to rejection after a period of immunosuppression despite the return of anti-donor antibodies to the circulation (56). Accommodation takes place when there is an up-regulation of beneficial genes, as opposed to rejection, where there is an up-regulation of deleterious or detrimental genes.

The rejection of xenotransplanted tissue and cells, such as pig pancreatic islet cells, is rather different. Cell transplants may contain no (or little) vascular endothelium, and therefore the initial antibody-mediated complement activation does not occur (see below).

3.2.3. Acute cellular rejection

Experimental data on the cellular immune response of humans and lower primates to discordant xenografts is scarce, mainly because early graft destruction due to HAR or AVR prevents evaluation of the eventual cellular response. Therefore, most of the information which is thought to be predictive of the human cellular immune response to discordant xenografts comes from *in vitro* experiments and from rodent models.

Initial *in vitro* studies of the mouse anti-human cellular response suggested that the response to discordant xenografts might be weaker than that observed with allografts

(57). However, subsequent *in vitro* studies of the more clinically relevant human anti-porcine response have indicated that the cellular response to porcine xenografts is as strong or perhaps stronger than the corresponding allogeneic immune response (58,59). Further study has shown that human anti-porcine cell-mediated cytotoxicity is mediated *in vitro* by both NK cells and by T cells (60,61). This contrasts markedly with allogeneic cellular cytotoxicity, in which NK cells rarely play a role due to inhibition by allogeneic MHC molecules on the target cell. However, porcine MHC (SLA) is not capable of inhibiting rejection by human NK cells (62). Cytotoxicity mediated by human NK cells against porcine targets also does not appear to be specific for the SLA haplotype (62), while human T cell cytotoxicity appears to be specific for SLA class I, suggesting an interaction between human CD8 and the SLA class I molecule (60).

In vivo studies of cellular responses in rodents indicate that $CD4^+$ T cells are critical for the xenograft rejection process (63). Athymic nude rodent recipients typically accept skin or pancreatic islet xenografts indefinitely (64). $CD4^+$ T cell depletion prolongs xenograft survival in mice significantly more than it does allograft survival (65). $CD4^+$ T cell-mediated rejection of porcine skin grafts by murine recipients appears to involve the indirect pathway of antigen presentation (66). Murine $CD4^+$ T cells are also capable of inducing rejection of porcine skin or islet cell grafts after adoptive transfer into SCID recipients (63,67). This rejection process also appears to involve the indirect recognition pathway (66). Rejection of porcine cellular grafts by mice does not appear to involve humoral responses or NK cells (63,67). These findings indicate that while NK cells clearly adhere to and infiltrate donor endothelium in primarily vascularized solid organ transplants (68), they may not play an important role in rejection of cellular grafts such as pancreatic islets.

Recently, a model of the human anti-porcine cellular immune response has been developed. Mice deficient in recombinase activating gene-1 (RAG-1) due to a targeted mutation have no mature B or T lymphocytes. These R^- mice accept porcine skin xenografts indefinitely without gross or histologic evidence of rejection. However, adoptive transfer of activated human peripheral blood mononuclear cells (PBMC) results in rejection of the porcine skin grafts (69). By depleting or purifying human lymphocyte subsets from the total population of activated human PBMC before adoptive transfer, the role of individual cell types in the rejection process has been established. Purified human $CD4^+$ T cells are capable of inducing acute cellular rejection of porcine skin grafts that is histologically indistinguishable from rejection induced by total PBMC. However, human PBMC depleted of $CD4^+$ T cells are no longer capable of infiltrating porcine skin grafts or inducing rejection (Friedman, T., *et al*. - manuscript submitted for publication). These data support the hypothesis that human $CD4^+$ T cells are necessary and sufficient to induce porcine skin graft rejection. Furthermore, the fact that human antigen-presenting cells are not required for rejection to occur indicates that rejection can proceed via the direct recognition pathway.

This model has also been used to study the human immune response to porcine pancreatic islet cell grafts. Streptozocin-diabetic R^- mice can be clinically cured of diabetes within four to five weeks after transplantation of fetal porcine islet cell clusters. However, adoptive transfer of activated human PBMC one day before transplantation prevents or significantly prolongs the time to cure. Histologic examination of the islet grafts reveals infiltration by human cells and destruction of insulin-producing tissue in a pattern resembling acute cellular rejection. Preliminary data in this model also indicate that purified human $CD4^+$ T cells are sufficient to induce this rejection process (Friedman, T. *et al*. - unpublished data).

This apparent critical role for human $CD4^+$ T cells indicates that strategies to prevent cellular rejection of porcine xenografts should be specifically directed against the $CD4^+$ T cell population. Aside from pharmacological interventions, potential strategies include genetic modification of the porcine donor to confer resistance to $CD4^+$ T cell cytotoxicity, or induction of tolerance in the recipient's $CD4^+$ T cell compartment.

Moses and Auchincloss (70) have summarized the present status of the cell-mediated immune response to a xenograft. Most of their observations, however, relate to xenograft models in rodents. They have concluded that (i) helper and cytotoxic T lymphocyte responses to xenoantigen are often weaker *in vitro* than in response to an allograft; (ii) helper xenogeneic responses are CD4+ cell-mediated; (iii) vascular endothelial cells are important antigen presenting cells; (iv) cytotoxic T lymphocyte-mediated xenogeneic responses are CD8-mediated, but the CD8 cells recognize both MHC class I and class II molecules which is different from allografting - this wide recognition is thought to be due to a lack of specificity; (v) effector pathways involve mainly NK, macrophage, atypical lymphocyte, and ADCC responses; (vi) the weakness of the xenogeneic T cell response is due to defects in molecular interactions at the cell surface between T cells and xenogeneic cells; (vii) where cell surface molecular interactions are defective, T cells recognize xenoantigen indirectly as peptides in association with host MHC molecules, rather than directly; (viii) *in vivo* xenograft rejection is brisk despite weak *in vitro* responses; and (ix) the induction phase is highly dependent on CD4+ helper T cells.

In contrast to rodent models, the situation with pig-to-human or nonhuman primate xenotransplantation indicates that human T cells directly recognize porcine xenoantigen on endothelial cells and dendritic cells. The T cell recognition is through both direct and indirect pathways. Vigorous cell proliferation and cytotoxic secretions result, and the cellular rejection response is at least equivalent to that of an allograft.

3.2.4. Chronic rejection

Chronic rejection is the name given to a process of vasculopathy which occurs in the vessels of allografted organs. Intimal proliferation can eventually lead to luminal occlusion with ischemia or infarction of parts of the transplanted organ. It can also affect other structures, such as the bronchi of transplanted lungs, in which case it results in bronchiolitis obliterans. Its cause remains uncertain but it is believed to have an immune basis. Virtually nothing is known as yet of chronic rejection of discordant xenografts.

Unless tolerance can be achieved, however, it is likely to be early and aggressive.

4. CHOICE OF ANIMAL ORGAN DONOR FOR HUMAN PATIENTS

Hearts, livers and kidneys from concordant donors, transplanted from one species of nonhuman primate to another, have functioned for weeks or months or occasionally years when the recipient has been heavily immunosuppressed (10,11,71-78). However, repeated episodes of severe acute rejection have been common, and the considerable and prolonged need for immunosuppressive drug therapy has resulted in a high incidence of infectious complications.

With the current immunosuppressive agents available to us, it seems unlikely that concordant donor organs will survive for very prolonged periods of time (years) in human recipients. If standard immunosuppressive therapy is given, there will be a risk of organ failure through recurrent or severe acute rejection and/or graft vasculopathy (chronic rejection). Function for some months is likely to be achieved, but the amount of immunosuppressive therapy required is likely to be excessive and will almost certainly lead to infectious complications.

There is some prospect, however, that a combination of the immunosuppressive agents that will become available to us within the next few years may well enable concordant xenotransplantation to be carried out successfully, particularly as a bridge to allotransplantation in cases of cardiac or liver failure.

Concordant xenotransplantation in humans will be limited, however, by the relative paucity of the number of suitable donor animals that will become available and, in particular, on the size of such animals. The baboon does not grow to a size sufficient to provide organs such as hearts for adult humans, although there may be a role for it in bridging infants and children to cardiac allotransplantation. Baboons are known to carry certain infectious agents, particularly viruses, that may be hazardous if transferred to humans (79-82). In addition, there will likely be a significant public objection to the use of non-human primates in large numbers for purposes of transplantation. Increasingly, therefore, the attention of those interested in this field has been directed towards discordant xenotransplantation.

The advantages of the pig as a potential donor for organs and tissues for humans are considerable (83,84). The pig breeds in large numbers, is relatively cheap and easy to breed and maintain, rapidly grows to a size in which its organs would be suitable for the largest of human adults, miniature swine can be bred where the maximum size of the pig is between 200-300 lb, the physiology of porcine organs is similar to humans, pigs are easy to genetically engineer and, with the exception of endogenous retroviruses, are believed to be relatively free of infectious diseases that could be transferred to humans.

5. EXPERIMENTAL METHODS OF PROLONGING SURVIVAL OF DISCORDANT XENOGRAFTS

Most attention has to-date been paid towards overcoming HAR and AVR. Work in this field can loosely be divided into 4 main approaches: - (i) Depletion in the recipient of anti-pig antibodies or inhibition of their attachment to graft antigens. (ii) Depletion or inhibition of complement in the recipient. (iii) Genetic engineering of a donor pig whose organs are protected from human antibody-mediated complement activation or whose organs express no or low levels of αGal. (iv) The development of immunological tolerance in the recipient to donor tissues by the creation of mixed hematopoietic cell chimerism or by molecular chimerism.

The currently available pharmacologic immunosuppressive agents are totally ineffective in preventing HAR, but have been shown to play a role in reducing the rapidity of AVR (20,42,44,45). There is no evidence to-date, however, that they can totally prevent AVR. Clearly, until this hurdle has been overcome, their role in the prevention of the cellular responses that are likely to follow remains uncertain.

5.1. Anti-Pig Antibody Depletion or Inhibition

The potential recipient can be depleted of all antibodies by plasma exchange (85), or of some antibodies by immunoadsorption techniques using immunoaffinity columns consisting of, for example, staphyloccocal protein A (42,86,87). However, these techniques deplete the patient of antibodies that may be important in protecting against infection. Ex vivo perfusion of pig organs has also been used to deplete antibody prior to the definitive pig organ transplant (88). All of these techniques have been shown to delay rejection of the transplanted pig organ for several days, but AVR still develops.

A preferable technique is to utilize highly specific extracorporeal immunoaffinity columns where only those anti-pig antibodies that are detrimental to the transplant will be depleted (21-23). This can be achieved by utilizing an extracorporeal immunoaffinity column of an αGal oligosaccharide (40,89-95).

An alternative approach would be to carry out what has been termed "specific intravenous carbohydrate therapy," in which synthetic or natural αGal oligosaccharides are infused continuously into the recipient circulation (90,97,98). The oligosaccharides are bound by the anti-αGal antibodies in the blood, causing "neutralization" of the antibodies so that they are no longer free to attack the pig organ when it is transplanted. The limited amount of *in vivo* data available, however, suggests that binding of the anti-αGal antibody to the synthetic αGal oligosaccharide is not strong enough to prevent antibody binding to the transplanted organ. Early histopathological changes of vascular rejection develop during the oligosaccharide infusion.

An alternative to the use of αGal oligosaccharides, either in immunoaffinity columns or as an i.v. infusate, is the anti-idiotypic antibody. Koren *et al.* (36) have produced anti-

idiotypic antibodies in mice by the injection into the mouse of human anti-pig antibody (eluted from pig organs after repeated perfusion with human plasma). Several of these anti-idiotypic antibodies, when incubated with human serum, have been demonstrated to have a major inhibitory effect on serum cytotoxicity towards pig PK15 cells *in vitro*. Furthermore, when infused i.v. in combinations of two into baboons, serum cytotoxicity has again been markedly reduced (from 100% to approximately 10%).

5.2. Complement Depletion or Inhibition

Purified cobra venom factor (CVF) has been shown to be extremely effective in depleting complement (80,99). CVF can clearly protect a discordant organ from HAR (20,42). However, even when the complement level is unmeasurable by standard laboratory tests, histopathological features of AVR begin to develop within 2-3 days and lead to graft failure within a relatively short period of time (<1 week). The addition of concomitant pharmacologic immunosuppressive therapy, presumably by suppressing both B and T cell activity, delays rejection further, but AVR is seen within days with the longest survival of a pig organ in a nonhuman primate to-date being 27 days (20).

Soluble complement receptor type I (sCR1) has also had success in prolonging discordant xenograft function (100-104). Human complement receptor 1 is a single-chain cell-surface glycoprotein found on erythrocytes, some T lymphocytes, all mature B lymphocytes, neutrophils, eosinophils, basophils, monocytes/macrophages, and certain other cells (104). It is also found circulating as a soluble form in plasma at low concentrations. The interaction of complement receptor 1 with some fractions of the complement cascade regulates complement activation through its convertase decay accelerating activity and its factor 1 cofactor activity. Fearon and colleagues constructed a soluble form of complement receptor 1 which lacked the transmembrane and cytoplasmic protein domains (105). This sCR1 retains all the known activities of the native cell surface receptor, and has been demonstrated to be a potent and selective inhibitor of both the classical and alternative complement pathways. Discordant xenografts have survived for several weeks when protected by sCR1.

Other agents that inhibit complement have been explored. These include FUT and K76 (106), but these have been found to be less successful than either CVF or SCR1.

It would seem, however, that complement depletion or inhibition alone, although valuable therapeutic approaches to assist in overcoming HAR, will not be sufficient to prolong discordant xenograft survival indefinitely. The risk of infection prevents the long-term administration of any agent that depletes or inhibits complement. In any case, the presence of antibody, even in the absence of complement, appears to result in the development of AVR.

5.3. The Genetically Engineered Pig

5.3.1. Expression of human complement-regulatory proteins

Most advances in this field have come from efforts to genetically engineer a pig that expresses one or more of the human complement-regulatory proteins. Under most circumstances, complement-regulatory proteins are largely species-specific, i.e. they block autologous complement but not that of other species (107,108). For example, pig organs express complement-regulatory proteins that block pig complement, but do not adequately block human complement. The development of pigs that express a human complement-regulatory protein on their vascular endothelium has been demonstrated to successfully block human (or nonhuman primate) complement and prevent HAR when transplanted into a primate species (47-50).

The human complement-regulatory proteins include CD46 (membrane cofactor protein, MCP), CD55 (decay accelerating factor, DAF), and CD59. Pigs have been bred that express one or more of these proteins (47,109-111). The most encouraging results achieved to-date have been by the Cambridge, UK, group of White and his colleagues, who have reported transgenic (for hDAF) pig kidney or heterotopic heart survival for >2 months in a small number of nonhuman primates that were also heavily immunosuppressed (48,49). The pig-to-cynomolgus monkey model used frequently by this group, however, is unusual in that some of the control (non-transgenic) pig organs survive several days, suggesting that HAR is not uniform in this combination (47). Nevertheless, the almost consistent prevention of HAR when hDAF organs have been transplanted demonstrates the therapeutic benefit of this approach. Others are beginning to confirm this effect (reviewed in 15), particularly when transplantation of the transgenic pig organ is combined with extracorporeal immunoadsorption of anti-αGal antibody from the nonhuman primate recipient.

5.3.2. αGal 'knockout'

A second approach with regard to a genetically engineered pig would be to produce a pig that is deficient in αGal epitopes, thus leaving no target for human anti-αGal antibodies (112). In the pig, αGal is produced by the enzyme α1,3galactosyltransferase (α1,3GT) (figure 4), which is encoded by a single gene (30). If this gene could be "knocked out" by a technique such as homologous recombination, then an αGal-deficient pig would be produced. The major difference between pigs and humans with regard to the oligosaccharides expressed on the vascular endothelium is the presence of αGal in the pig where ABH oligosaccharide is expressed in the human (24) (table 1). Whether an αGal-depleted pig would be a fully viable, healthy pig remains uncertain, but the fact that there are some human subjects who are depleted of ABH antigen (the so-called "Bombay" histo-blood type) who appear to be clinically well in all respects, would suggest that αGal-depleted pigs will similarly be healthy.

The "knockout" technique, which requires the manipulation of stem cells, is not yet possible in the pig. Mice, however, have been bred which do not express αGal epitopes (113,114). In vitro and in vivo studies, however, suggest that the absence of αGal may expose the presence of underlying "cryptic" oligosaccharide epitopes against which humans also have antibodies (114).

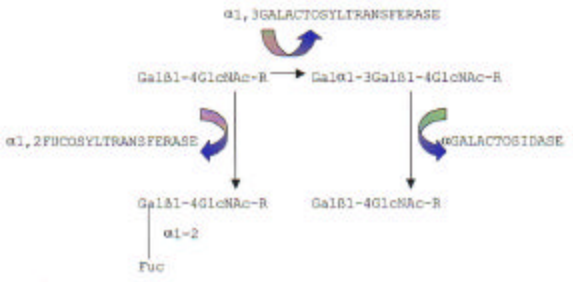

Figure 4: Biosynthetic pathway for synthesis of Galα1-3Gal. The α1,3 galactosyltransferase enzyme adds galactose to N-acetyllactosamine (Galß1-4GlcNAc) to generate Galα1-3Gal. The same substrate can be utilized by transgenically-introduced α1,2 fucosyltransferase to produce the H histo-blood group epitope. Galα1-3Gal can also be eliminated by the introduction of α-galactosidase, which enables the N-acetyllactosamine substrate to be available again for further fucosylation. (Modified from Sandrin, M.S., *et al*. (116)).

Development of anti-αGal antibodies in these mice is proving valuable in research, as they can act as a surrogate for humans. Studies involving the transplantation of wild-type mouse organs (which express αGal) into αGal knockout mice (which produce anti-αGal antibodies) have been helpful in several studies, some of which are detailed below.

5.3.3. Competitive glycosylation

An alternative approach would be to introduce the gene for an enzyme that would compete with the α1,3GT for the underlying substrate,, namely lactosamine (figure 4). Initially, suggested candidates were the genes for sialic acid (α2,3 or α2,6 neuraminic acid) or for the H histo-blood group antigen (α1,2fucosyltransferase or H transferase) (25,112). More recently, other candidate genes have been suggested (reviewed in 28). This method, involving the microinjection of a gene for the enzyme that will produce the required oligosaccharide is possible in the pig. However, it seems likely that virtually 100% of the αGal will require replacement if hyperacute rejection is to be avoided.

Good progress in this field has been made by Sandrin *et al*. (115,116) who have demonstrated *in vitro* that competition between α1,2 fucosyltransferase (H transferase) and α1,3GT takes place for the substrate N-acetyllactosamine. H transferase is significantly more successful and the H epitope predominates, reducing the presence of αGal to approximately 10-20% of its original expression.

One interesting point is that pigs do, in fact, have the gene for H transferase and express H oligosaccharide epitopes, not on vascular endothelium but in certain other tissues (24). It is therefore essential to ensure that the H transferase produced as a result of the introduction of H cDNA functions at the correct site, and this may prove to be less easy than is immediately obvious.

Unless H epitopes replace the αGal epitopes completely, the number of αGal epitopes remaining on the vascular endothelium would still make such a pig organ susceptible to HAR. The ultimate solution, therefore, may be to combine expression of H transferase with that of αgalactosidase (116,117) (figure 4). αGalactosidase is an enzyme that has the opposite effect of α1,3GT - it removes the terminal αGal molecule rather than adds it. Insertion of the gene for αgalactosidase alone results in an approximate 70% downregulation of αGal expression. Cell culture studies by Sandrin *et al*. indicate that the presence of the gene for both αgalactosidase and H transferase results in a complete absence of αGal expression. It would appear that wherever H transferase is not successful in competing for substrate with α1,3GT, any αGal epitopes that remain are removed the by αgalactosidase.

5.4. Tolerance to Donor Species by Molecular Chimerism

One approach explored at our center has been the development of what has been termed "molecular chimerism". The gene for α1,3GT has been introduced into the bone marrow of αGal knockout mice (118). This bone marrow has been returned to the mouse after a course of myeloablative radiation to deplete its own bone marrow. The autologous bone marrow graft, including cells transduced with the α1,3GT gene, has reconstituted the bone marrow. The presence of the α1,3GT gene has led to the production of αGal in these αGal knockout mice. The presence of αGal expressed by these cells has resulted in a suppression of production of anti-αGal antibodies by the mouse. B cell tolerance has therefore been achieved. This successful study in mice has been followed by preliminary studies in baboons whose bone marrow has been transduced with porcine α1,3GT. Although expression of αGal has been detected in the baboon bone marrow after autologous bone marrow transplantation, expression has been transient for a few weeks only and B cell tolerance has not yet been achieved. Nevertheless, this is a promising approach that might lead to αGal-reactive B cell tolerance.

5.5. Tolerance to Donor Species by Mixed Hematopoietic Cell Chimerism

Donor species-specific tolerance would clearly be desirable and may indeed prove essential if late rejection of a discordant xenograft proves to be significantly more severe than that of an allograft. Important studies have been carried out over a number of years in experimental animals by two groups, namely those headed by Myburgh at the University of the Witwatersrand in Johannesburg (119-121) and by Sachs, formerly at the National Institutes of Health in Bethesda and more recently at the Massachusetts General Hospital of Harvard Medical School (122-129).

The induction of donor specific tolerance would clearly eliminate the development of acute cellular or chronic rejection. The elimination of chronic rejection (e.g. graft atherosclerosis or bronchiolitis obliterans) is possibly even more important than that of acute rejection as there is no effective treatment for chronic rejection, even in allografts. If tolerance could be achieved, pharmacologic immunosuppressive therapy would not be necessary and therefore the accompanying risks of opportunistic infection, malignancy, and drug toxicity would be avoided.

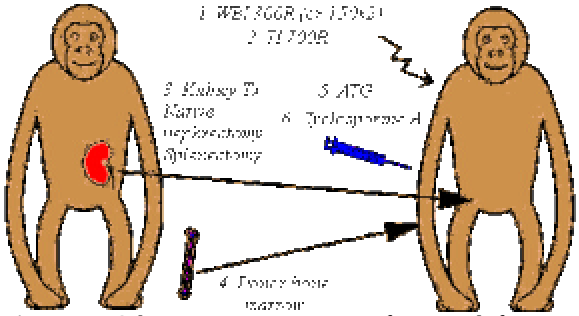

Figure 5: Schematic representation of protocol for non-myeloablative preparative regimen to induce tolerance between full MHC haplotype-mismatched cynomolgus monkeys. In attempting to induce transplantation tolerance across the discordant xenogeneic barrier (pig-to-baboon) through establishment of mixed chimerism, extracorporeal immunoadsorption of baboon blood through an immunoaffinity column of a Galα1-3Gal oligosaccharide is performed prior to the pig organ transplant in an effort to deplete anti-pig antibody. (From Sykes, M. and Sachs, D.H. (129)).

Sykes and Sachs (129) have pointed out that the tolerance approach may be well suited for xenotransplantation since animal donors are available electively (and not under emergency conditions as are cadaveric human donors) allowing for the timing of tolerance induction and transplantation to be elective. Tolerance-inducing cell populations (e.g. bone marrow) can be obtained from the donor, the recipient can undergo the procedure to induce mixed chimerism, and the organ graft from the same donor can be inserted at the optimum time. In addition, the potential for generating fully inbred xenograft donors (e.g. miniature swine) provides the possibility of using an unlimited source of genetically homogeneous tissue whenever it is required for maintenance of the tolerant state. Furthermore, xenogeneic donors could be modified using genetic engineering or cloning techniques to facilitate induction of tolerance to xenoantigens.

Two approaches are being investigated by the Harvard group (129), namely (i) the use of xenogeneic hematopoietic stem cell transplantation to induce a state of mixed chimerism, and (ii) thymectomy followed by replacement with a xenogeneic donor thymus after depletion of the preexisting peripheral T cell repertoire. In this brief review, only the mixed chimerism approach will be discussed.

After much preliminary work in rodents, the Sachs group has investigated the development of tolerance to allografts in nonhuman primates (125). The basic protocol (figure 5) consists of the nonhuman primate receiving 300 cGy of whole body irradiation (WBI), 700 cGy of thymic irradiation, and horse anti-human ATG preoperatively. Bilateral nephrectomy, splenectomy, kidney transplantation, and donor bone marrow administration are all performed on day 0. An alternative to bone marrow cells, namely mobilized peripheral blood stem cells, is also currently being explored as a source of hematopoietic cells. In order to supplement suppression of mature T cells by ATG, treatment with cyclosporine is begun on day 1 and continued for 4 weeks, but then no further immunosuppression is administered.

Clear evidence for chimerism amongst lymphoid, myeloid, and monocytic subpopulations, was generally detected first on about day 8, persisting for 1-3 weeks. Thereafter the levels of detectable chimerism decreased progressively. However, transplantation tolerance to kidney allografts was induced with organ survival for >4 years in some cases.

More recent studies have attempted to extend this non-myeloablative regimen for the production of mixed chimerism to the discordant pig-to-primate combination (40,128). The major addition to the previous protocol is the need to remove natural antibodies from the recipient's circulation in order to avoid HAR. This has been attempted by extracorporeal perfusion of the primate's blood either through an isolated liver or through specific synthetic oligosaccharide (αGal) columns, and has been carried out immediately prior to kidney or heart transplantation. Using this regimen, pig kidney and heart grafts have functioned for <15 days in cynomolgus monkeys and baboons, respectively, but have failed from the development of AVR. In contrast to the allograft model, there has been only transient evidence for pig cell chimerism, with a low level of pig cells detectable in the peripheral blood by PCR. More recent studies by Buhler *et al*. (unpublished) have used high doses of mobilized pig peripheral blood stem cells (1-3 x 10^{10} cells/kg) which have resulted in higher levels of chimerism (detectable by FACS). However, return of anti-αGal antibody has still proved a major problem.

αGal knockout mice have been used once again profitably to demonstrate that αGal-reactive B cell tolerance can be achieved by hematopoietic cell chimerism. Sykes' group has demonstrated that transplantation with bone marrow from wild-type (αGal-positive) mice into irradiated knockout (αGal-negative) mice leads to αGal-reactive B cell tolerance once engraftment takes place (130). The presence of the αGal-positive bone marrow cells leads to suppression of production of anti-αGal antibody. The mechanism by which this develops is thought to be a clonal deletion of the αGal reactive B cells.

6. EXPERIMENTAL AND CLINICAL PROGRESS IN THE XENOTRANSPLANTATION OF TISSUES AND CELLS

The transplantation of animal cells and tissues into humans might play an important role in the treatment of a great variety of disorders that result from tissue loss or dysfunction, diabetes being the most common. Diabetes is the most common endocrine disease and affects over 15 million patients in the USA. The disease is characterized by a decrease in the number of insulin-producing cells in the pancreas. Currently, insulin-requiring diabetes is treated with exogenous insulin administration, but such therapy does not necessarily prevent long-term complications, such as nephropathy, retinopathy, neuropathy, and vasculopathy. Transplantation of insulin-producing tissue is a potential

therapy and pancreas whole organ transplantation is an established procedure. However, the latter is a major surgical procedure with a significant rate of complications.

Transplantation of pancreatic islets, rather than the whole pancreas, has been proposed as a possible alternative treatment for diabetes mellitus. The advantages of using islets are (i) simplicity of transplantation (by i.v. injection), (ii) the theoretical possibility of in vitro modifications of immunogenicity of the islets before injection, (iii) potential use of various transplantation sites, and (iv) feasibility of storing the cells before use (cryopreservation, culture).

However, if islet cell allotransplantation were to be successful in humans, the availability of human pancreatic tissue would rapidly become insufficient in view of the large number of islets required for each patient. Research is, therefore, now orientated towards the use of other sources of islets, namely animal islets, with the aim of transplanting them into humans. The pig appears to be a suitable source of islets for humans. The metabolic function of pig insulin would certainly be adequate as for many years it has been used to treat diabetic patients, and its structure differs from human insulin in only one amino acid residue.

For these reasons, several teams have recently developed programs to try to isolate porcine islets on a large scale. Unfortunately, it is widely recognized that pig islets are especially difficult to isolate and to purify as the pig pancreas has a strong tendency to produce a high percentage of islet fragments and single cells after digestion. This appears to be in part due to the fact that islet capsules are absent in young pigs (<1 year-old). This renders them less resistant to injury than, for example, human islets, which are well-encapsulated. However, the xenotransplantation of islets demonstrates some possible advantages over that of the whole organ. With non-vascularized grafts, such as islets, the absence of immediate vascularization prevents contact between the recipient's circulating anti-αGal antibodies and the endothelial cells of the islets, which express the Gal antigen. Cellular immunity seems to be predominant in the rejection of tissue xenografts, but the exact mechanism remains incompletely understood. Long-term survival of pig (131,132) and human (133,134) pancreatic islets in athymic nude mice suggests a T cell-mediated rejection process. However, the use of conventional immunosuppressive agents that block the T cell response in immunocompetent recipients allows only a modest prolongation of survival of xenografted islets. To determine which T cell populations are involved in initiating rejection, anti-CD4 and anti-CD8 monoclonal antibodies (mAb) were administered to mice transplanted with pig pro-islets (135). Only treatment with anti-CD4 mAb resulted in survival of the xenografts two weeks post-transplant. Control xenografts, without any treatment, developed graft destruction at 6 to 7 days post-transplant. Thus, CD4 T cells were indirectly shown to play a fundamental role in the rejection of pig pro-islet xenografts.

Immunohistological analysis of xenografted pig islets in non-immunosuppressed rats demonstrated that the main cellular subtype infiltrating the graft is the macrophage (136). Lymphocytes were mainly located in the peripheral parts of the xenograft. It is therefore recognized that xenograft islet rejection is T cell-dependent, while the main effector cell is the macrophage. These data confirm a predominantly cell-mediated rejection of islet xenografts. There is evidence, however, that an antibody response also plays a role, but the chronology of these two reactions and their relative intensity and interaction still remain to be defined.

The first clinical experience of xenotransplantation of insulin-producing cells into human patients was by the Swedish group headed by Groth (137) using porcine fetal islets. Islet-like cell clusters were isolated by collagenase digestion and put into tissue culture. Such islet-like cell clusters were transplanted initially in 1990 into a 32 year-old woman with end-stage diabetic nephropathy who had previously been treated by combined renal and pancreatic transplantation; the pancreatic graft had failed. Thirty-nine porcine fetal pancreases were used to transplant approximately 390,000 islet-like cell clusters into the portal vein by the percutaneous trans-hepatic route. The immunosuppressive therapy she was already receiving (cyclosporine, azathioprine, prednisone) was increased and rabbit ATG was given for 7 days after transplantation. After approximately 50 days, C-peptide secretion was detectable and could be observed for 240 days, but the insulin requirement of the patient remained unchanged. This surgical team transplanted a total of 10 diabetic patients with porcine islets, but no reduction in insulin requirement was observed in any of them (138). However, the trial indicated that xenogeneic islet cells do not appear to be acutely rejected if the patient is receiving pharmacologic immunosuppressive therapy. Furthermore, xenotransplantation of porcine fetal pancreatic tissue into the human can be carried out without morbidity.

Encapsulation of islets has been proposed for preventing rejection of both allo and xeno islets. The principle is that permeability of the capsule membrane is sufficient to allow nutrients and oxygen to reach the islets and for insulin to be released into the bloodstream, but restrictive enough to exclude immune cells and antibodies. Such encapsulation can be achieved using alginate-polylysine-alginate capsules. Functional *in vitro* tests of microencapsulated islets have shown that insulin-release profiles following glucose stimulation are similar to those of free islets. Microencapsulated isl*et al*lo- and xenografts have been implanted successfully in rodents, reversing chemically-induced diabetes (139,140). Recently, porcine encapsulated islets induced normoglycemia in a spontaneously diabetic cynomolgus monkey (without immunosuppressive therapy) for more than 800 days (141).

Encapsulation of cells has a large application in medicine in the treatment, not only of diabetes, but of many other diseases. Clinical trials have been initiated using (i) encapsulated bovine adrenal chromaffin cells for the treatment of chronic pain (142) and (ii) genetically-modified hamster kidney cells in patients with amyotrophic lateral sclerosis (Lou Gehrig's disease) (143). Other disorders that might be treated by xenogeneic cell transplantation are Parkinson's,

Alzheimer's or Huntington's diseases, and have been reviewed elsewhere (144). Already, a total of 12 patients with advanced Parkinson's disease have been transplanted with porcine fetal neuronal cells (145). Several have shown significant improvement in muscular rigidity and tremor.

7. DISCUSSION

Methods that allow successful discordant xenotransplantation will clearly open up new areas of surgical therapy. Patients with native organ failure who are in need of a transplant will be able to undergo the procedure electively or immediately the need arises. They will no longer be condemned to wait anxiously in precarious health for weeks, months or even years before ultimately undergoing transplantation as an emergency procedure at a less-than-optimal time of the day or night. Patients with borderline contraindications to allotransplantation will be given the opportunity of xenotransplantation as there will no longer be a restriction on the number of donor organs. Transplantation will become a common procedure in countries such as Japan where to-date there have been cultural barriers to cadaveric allotransplantation. The ethical problem of whether retransplantation should be offered to a patient will be overcome by the abundance of donor organs. Diabetic patients may receive pig pancreatic islet cell transplants, negating the need for daily insulin injections. The existing clinical attempts to treat neurodegenerative disorders, such as Parkinson's disease, will be greatly expanded. There will, therefore, be a great expansion in organ transplantation worldwide, and it is likely that both patients and physicians will not wish to persist with inadequate medical therapy, including dialysis, if successful organ xenotransplantation is readily available.

Which of the methods and approaches briefly outlined in this review is most likely to be successful in allowing clinical organ xenotransplantation? It is unlikely that one single approach will be entirely successful. The answer will probably be a combination of techniques and/or agents, as is the case with allotransplantation today. There will be several steps of development, but the ultimate goal would be to develop tolerance in the human recipient to the transplanted pig organ.

Xenotransplantation offers us the first real opportunity for modifying the donor as opposed to the recipient. This opens up great possibilities, particularly in this era of rapidly developing techniques such as genetic engineering, gene transfer and cloning. The pig organ transplanted may be transgenic for one or more human complement-regulatory proteins and, ideally, would express no or little αGal. The breeding of a pig with a vascular endothelial structure against which humans have no preformed antibodies would be a major advance. Production of induced antibody and the cellular response would be suppressed by initiating tolerance in the recipient.

There will remain, however, several unknowns. Will the porcine organ function satisfactorily in the human environment? (146). Pig hearts have functioned successfully in the heterotopic position in nonhuman primates for several weeks (15), as have pig kidneys. It is likely that both of these organs will fulfill the functions required of them in the human host. It is much less likely that a transplanted pig liver will fulfill all of the roles expected of it. Will pig proteins, enzymes, and hormones carry out their tasks in the human? It is inconceivable that the products of a pig liver will be completely interchangeable with those of a human liver, but here again, in time, genetic engineering of the donor pig may allow some of these functions to occur satisfactorily.

We already have clinical evidence, however, that ex vivo perfusion of pig livers by blood from human patients in fulminant hepatic failure can lead to some degree of "detoxification" of the blood and improvement in cerebral activity (147). Temporary support by a pig liver, possibly as a "bridge" to allotransplantation, is therefore likely to be beneficial. After orthotopic transplantation using a pig liver, the liver will produce pig complement. This should help to protect the transplanted organ from HAR, but what effect it will have on the remaining human organs in the body and on the body's defense mechanisms to infection remains unknown.

Concerns have been raised regarding the risk of transfer of an infectious microorganism with the donor organ from the pig to the patient (148). Of greater concern is the possibility of such an organism - most probably a virus - being passed on to members of the community, and thus being a hazard to the public health. It will probably be possible to ensure that the donor herd is free of all known infectious microorganisms, but there will remain the probability of hitherto unknown microbes being transferred to a foreign host which has no immunity to the organism. Porcine endogenous retroviruses, which are believed to be present in the genome of all pig cells, have received considerable attention recently as these are spread vertically from parent to child. Whether these will prove pathogenic in human recipients of a transplanted pig organ remains unknown at present.

The ethical (149) and legal (150) questions raised by clinical xenotransplantation are numerous and relate to topics as variable as whether a pig transgenic for human tissues should have any moral or legal rights to the medico-legal consequences of an infection spread from a pig organ to a patient and then to a third party member of the community.

There are those with a cynical outlook who for many years have predicted that "the future of transplantation is xenotransplantation, and always will be!" Unfortunately, to-date they have been proved correct! In the final decade of this century, however, we at last appear to be making some real progress, and there are glimpses of light at the end of the tunnel of ignorance and failure. Nevertheless, there will undoubtedly be many pitfalls and disappointments ahead. The future has probably best been summed up by Professor Sir Roy Calne, the pioneering British transplant surgeon, who predicted that "clinical xenotransplantation is just around the corner, but unfortunately it may be a very long corner".

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