There have been several advances in cardiac transplantation, but some problems remain. A major hurdle to long-term graft survival is the problem of chronic rejection or graft atherosclerosis which occurs in 30% to 60% of transplant patients within five years. In the Western world a significant imbalance between demand and supply for heart transplants exists; as a consequence, over 20% of patients awaiting a heart transplant die before receiving an organ. In xenotransplantation, over the last five years, there has been significant progress in the understanding of the mechanism of hyperacute rejection, the role of endothelium, and genetic engineering. The long-standing barrier of hyperacute rejection in xenotransplantation appears to have been conquered. Current focus is on the control of delayed xenograft rejection, and several approaches including the use of anti-platelet, immunosuppressive and/or anti-inflammatory agents are being studied. It appears that genetic engineering may have been a significant role in this control.

CONGESTIVE HEART FAILURE claimed 36,000 lives in the United States during 1992 and was implicated in 250,000 deaths. These figures can be expected to grow as the population ages. The advent of cardiac transplantation has altered the fate of many patients suffering from end-stage heart disease. In the 1990s, greater than 2000 heart transplants are performed annually in the United States. This figure can only be put into perspective when compared to the demand for organs: it is estimated that more than 16,000 patients under the age of 55 y would benefit from receiving a transplant. In the United States, one-year survival following heart transplantation is 82.4%1 and the results in our center are very comparable.

Unfortunately, the further proliferation of heart transplantation is hampered by the critical shortage of donor organs, with a wait-list mortality of 20%.
allografted heart remains the third most common cause of death, following infection and acute rejection. Presently, the only definitive therapy for allograft coronary disease is retransplantation. Patients with allografted heart remains the third most common cause of death, following infection and acute rejection. Presently, the only definitive therapy for allograft coronary disease is retransplantation. Patients with TVP may present with various signs and symptoms, including arrhythmias, congestive heart failure from myocardial ischemia, silent infarction, or sudden death. Angina is not a characteristic complaint, and initially this was attributed to graft denervation; however, it appears that reinnervation may occur over time.

Pathologically, myofibroblast proliferation and fibrosis result in circumferential intimal thickening. The intima becomes filled with lipid-laden cells. Atheromatous plaques are observed within one to two years of transplantation. These plaques are identical to those in spontaneous atherosclerosis. TVP is characterized by concentric and diffuse intimal hyperplasia, with a normal internal elastic lamina. Calcification is rare, and distal vessel occlusion occurs first.

The pathogenesis of these lesions involves an immunological process, as histological examination reveals lymphocyte accumulation within the intima and adventitia of the coronary arteries. Vascular rejection has also been correlated with allograft coronary artery disease. Patients with anti-HLA antibodies following cardiac transplantation have poorer survival and increased coronary artery disease (CAD). Anti-HLA antibodies have been correlated with allograft coronary disease. Such antibodies are not only anti-HLA in nature but may also recognize endothelial cell antigens.

Nonimmune factors have also been implicated in the development of allograft CAD. Herpesviruses, including cytomegalovirus, may initiate immunological mechanisms that culminate in endothelial damage. Donor and recipient age, donor and recipient gender, obesity, pretransplantation diagnosis, and ischemic time are also considerations. Hyperlipidemia, however, remains the most consistent link. The etiology of post-transplant hyperlipidemia has not been completely elucidated, but obesity, prednisone, and cyclosporine are related.

Investigators have reported interactions between vasoactive substances, hemodynamics, and growth factors that control vascular remodeling in response to vessel injury; thus, vasodilators may be an important treatment modality.

The association between graft coronary disease and histocompatibility is a complicated one; most studies are unable to definitively define the relationship due to their retrospective nature, small patient numbers, and limited follow-up. Furthermore, the immunosuppressive regimens are somewhat variable. A complete mismatch at the HLA-B and HLA-DR loci results in greater rejection rates and increased mortality. There is also a suggestion that TVP is increased with a complete mismatch at the HLA-DR locus. From a logistical perspective, the short donor organ ischemic time does not permit prospective donor and recipient tissue typing.

A strong case can be made for treating transplant recipients with calcium-channel blockers. Calcium-channel antagonists may be immunosuppressive, in addition to possessing cardiovascular properties. These drugs have been shown to be effective in attenuating coronary atherosclerosis. The International Nifedipine Trial on Antiatherosclerotic Therapy found that nifedipine did not influence progression or regression of new coronary lesions but did reduce the rate of appearance of new lesions.

Further, TVP is typically diagnosed with angiography, but angiograms underestimate both the extent and severity of the coronary disease. The role of intracoronary ultrasound in TVP is also relevant as this method can measure intimal thickening before it is detected by angiography.

Although hyperlipidemia control and pharmacotherapy (calcium-channel blockers, immunosuppressive agents, etc.) may serve as focal points for prevention of TVP, retransplantation remains the only effective treatment for chronic rejection. Unfortunately, retransplantation is often complicated by recurrence of CAD. With waiting lists continually increasing, alternatives to repeat transplantation are being explored. Coronary artery bypass grafting (CABG) has not been useful to date, as the disease involves the entire coronary vessel, from its origin. Coronary flow reserve measured by Doppler flow wire may define the vasodilating capacity of the vessel. This may allow identification of adequate target vessels for CABG. Transmyocardial laser revascularization is also under investigation.

Future results of investigations examining the endothelial cell response may yield clues for other therapies for TVP. The role of adhesion molecules and integrins in the interactions between cells is another area of interest. As molecular knowledge progresses, attenuation of chronic rejection may be possible.
Current Advances

Surgical Technique

The Shumway technique has been found to have certain limitations. Asynchrony in donor and atrial contractions, inconsistencies in atrial size, and incorrect orientation may contribute to atrioventricular valvular incompetence. Nevertheless, this is still preferred by most surgeons.

The most important recent innovation in surgical technique is the direct bicaval anastomosis. The method of individual caval connection permits a more anatomical atrial reconstruction. The left atrial anastomosis is modified to include a bridge of tissue retained posteriorly between the right and left pulmonary veins. Tricuspid and mitral valve insufficiency is reduced and requirements for permanent pacing are decreased with this technique. The limitation to this method is left atrial tissue bridge, which is difficult to achieve when the donor lungs are simultaneously harvested.

Donor Heart Preservation

With attempts to expand the donor pool by employing "marginal" hearts and older donors, myocardial preservation may be even more critical. While the use of marginal donor organs has permitted transplantation in patients who may not have survived to receive an ideal heart, marginal organ use has been shown to reduce graft survival. Currently, acceptable ischemic times for cardiac donor organ preservation are limited to 4 to 6 h. Investigators have developed a portable organ perfusion apparatus for organ preservation, permitting longer ischemic times. However, experimental prolongation of storage cannot be equated to clinical preservation. The heart may sustain damage during the period of donor preparation, at explantation, storage, and implantation.

Donor organ management has become further refined in recent years. Donor resuscitation to optimize hemodynamics, by manipulating preload/afterload and pharmacological support, has resulted in an expansion of the donor pool. At present, most centers employ a single-flush cardioplegia technique with hypothermic storage. This is satisfactory for short ischemic times. Prolongation of storage may permit HLA matching and if achievable, this could impact long-term survival.

Future studies therefore will likely focus on continuous perfusion storage systems and cardioplegia composition. Microvascular perfusion and endothelial cell function are important to consider during the period of myocardial ischemia, and adenosine may serve as a protective agent. Since oxygen-free radicals and white blood cells have been implicated in reperfusion injury, investigation into antioxidant therapy and leukocyte depletion may be particularly relevant.

Nitric oxide-induced vasodilatation and toxicity have implications for transplantation. Firstly, nitric oxide therapy results in reduction of pulmonary hypertension. This may potentially increase the number of candidates deemed suitable for cardiac transplantation. Secondly, nitric oxide may be useful for graft failure post-transplantation. Studies are underway in our center to assess the role of nitric oxide in donor heart preservation.

Reversing nitric oxide effects with ACE inhibitors and other agents may be protective from a vascular standpoint. Relevant to cardiac transplantation is the hypothesized role of nitric oxide in cardiomyopathy.

Pediatric Cardiac Transplantation

Infant cardiac transplantation was first attempted in 1967 but it was not until 1985 that the procedure was performed successfully in a newborn. Pediatric heart transplantation has become an important option for children suffering from cardiomyopathy or congenital heart disease. Results of cardiac transplantation in this population are comparable to those for adult recipients. The survival of neonatal and infant recipients is poorer than for older children. Technically, cardiac transplantation in children with congenital malformations remains challenging and various techniques have been well described. As in adults, the future of pediatric cardiac transplantation is limited by organ supply. Ultimately, xenografts may represent the best hope for children with end-stage heart disease.

Tolerance

Induction of donor-specific tolerance would eliminate the need for nonspecific immunosuppression following transplantation; thus, the immune response to antigens other than the graft would remain unaltered.

The strategy of creating mixed chimeras in an effort to achieve a state-of-tolerance has been successful in animal models. Mixed chimeras produced by treatment with anti-T cell monoclonal antibodies in combination with irradiation permitted engraftment of allogeneic bone marrow.
The role of gene therapy is also under investigation. This permits specific delivery of a gene to a target, using an expression vector. Local immunosuppression and induction of tolerance may be possible.55 Intimal hyperplasia can be altered with this technology36 which may have implications for chronic rejection. Gene therapy within the allograft may modulate the immune response sufficiently46 to induce tolerance. This has been proposed with respect to the CD 95 ligand.47

Immunosuppressive Drugs

Pharmacological immunosuppression continues to evolve as the immunobiology of graft rejection becomes better understood. Newer agents that may have particular relevance to cardiac transplantation include tacrolimus (FK506), mycophenolate mofetil, and rapamycin. These three agents inhibit smooth-muscle proliferation, and may, therefore, play some role in attenuating allograft CAD. FK506 is a lymphokine synthesis inhibitor, purported to be up to 100 times more potent than cyclosporine A.56 By interacting with the immunophilin FK binding proteins (FKBP), FK506 impedes calcium-dependent signal transduction, via the calcineruin-calmodulin system. The expression of IL2 and IL-7 receptors is downregulated and cytokine transcription is inhibited—lymphocyte activation is subsequently impaired. FK506 also functions to inhibit T-cell-dependent activation of B cells. Some centers are altering immunosuppressive regimens to incorporate FK506—for example, tacrolimus used in cardiac transplantation instead of cyclosporine results in comparable survival but with fewer adverse effects.57

Mycophenolate mofetil is a pro-drug and the active metabolite is mycophenolic acid. Mycophenolic acid reversibly inhibits inosine monophosphate dehydrogenase (IMPDH), an enzyme required for purine synthesis during the process of lymphocyte activation. Selective inhibition of T- and B-cell proliferation is possible, with few side effects on other organs. Mycophenolic acid is advantageous over azathioprine by increasing hematocrit, total WBC count, and absolute neutrophil count,38 while still being effective in refractory cases of allograft rejection.

Rapamycin impedes lymphokine signal transduction by suppression of IL-2 responsiveness. Rapamycin shares homology with FK506 and binds FKBPBs. Due to its lipophilic nature, rapamycin traverses the cell membrane without difficulty and then binds to cytoplasmic FKBPBs. T-cell protein synthesis is obstructed due to the inhibition of a kinase by rapamycin is less efficient in suppressing the elaboration of cytokines. The pathways affected are calcium-independent in both T and B cells. Immunoglobulin synthesis is inhibited by rapamycin, and at high concentrations, NK cells and antibody-dependent cellular cytotoxicity are suppressed. Allograft trials for renal and cardiac patients are underway. Perhaps the most encouraging finding in studies involving this agent has been the observation that rapamycin prevents TVP in a rat model.59

While innovations such as peptide sequences related to HLA are being explored,60 such studies have yet to be translated into clinically-relevant advances in cardiac transplantation.

Xenotransplantation

In the future, the demand for organs will continue to increase as the population ages. It is also likely that the organ supply will diminish as innovative safety devices prevent deaths from motor vehicle accidents, whose victims have traditionally supplied many organs. While portable ventricular-assist devices and artificial organs are drawing attention as options to allografting, these units may be most useful as bridges to transplantation. Xenografts remain the ideal solution since the patient is the recipient of a biological organ.

Historical

Man has been fascinated with the concept of crossspecies transplantation throughout history. This is reflected by allusions to xenografts in various mythologies61 and religious scriptures.62.63 The first reported clinical usage of a xenograft was in 1906 when Jaboulay performed a renal transplant.64 Since then, there have been at least thirty-five attempted xenotransplants of kidneys and livers, and seven different investigators have undertaken cardiac xenografts on eight separate occasions (Table 1).65.66 Unfortunately, the immune response to a xenograft inevitably results in a vigorous response by the recipient to the new organ. All xenotransplants performed in humans have failed shortly following surgery, although one renal xenograft has survived nine months.67

Bailey performed a cardiac xenograft in 1984 on an infant with hypoplastic left heart syndrome, utilizing a baboon donor.68 This was popularized in the media as "The Baby Fae Case," and the world watched with keen interest. Ultimately, the graft failed and the child
Table 1. Reported clinical attempts at cardiac xenotransplantation.

<table>
<thead>
<tr>
<th>Date</th>
<th>Surgeon</th>
<th>Donor</th>
<th>rt</th>
<th>Graft survival</th>
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<td>Sheep</td>
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<td>Ross</td>
<td>Porcine</td>
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<td>1991</td>
<td>Czaplicki</td>
<td>Porcine</td>
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</table>

*Modified from Markmann and Barker.70

Xenogeneic Donors

Xenograft donors may be characterized as either concordant or discordant. This classification applies to the taxonomic relationship between donor and recipient. A concordant xenograft refers to a transplant carried out between phylogenetically close species, for example, nonhuman primates and humans. Discordant xenotransplantation denotes grafting between widely disparate species, such as pigs and humans. Discordant donor-recipient xenografts are characterized by the presence of preformed circulating xenoreactive natural antibodies (XNA), while concordant combinations lack preformed antibodies. There is an inverse correlation between the phylogenetic distance and the likelihood of preformed XNA, with resulting HAR of grafted organs.70

To some extent, rejection in taxonomically closely related recipient-donor combination xenotransplantation may be controllable with currently available immunosuppressive. The challenge remains to achieve long-term xenograft survival. In the case of distantly related donors and recipients, this would appear to be a formidable task. There are no clinical reports in the literature of xenograft survival beyond 9 d in dissimilar donor-recipient combinations.67

Cost and ethical factors have led many to believe that the future of xenotransplantation will ultimately lie in discordant grafts. The practical implementation of widespread concordant xenotransplantation that relies on primate organs is severely limited by a long gestational period, resulting in only a single offspring. After considering domestication, zoonoses,71 and organ size-matching, porcine donors emerge as the most feasible for large-scale human usage.72 The large number of animals currently bred and consumed would indicate that utilization of this species is ethically acceptable. Porcine organs are large enough to be useful for grafting into human recipients while lacking the potential morbidity of zoonotic organisms that may be harbored by concordant primate donors. Another advantage of the pig is multiple gestation, producing many conceivable donors. However, like other discordant species, porcine organs are also susceptible to HAR.

Xenograft Rejection

To date, the major obstacle to the implementation of clinical xenotransplantation remains graft rejection. The stages of xenograft failure have been classified as HAR, acute vascular xenograft rejection or delayed xenograft rejection (DXR), and the T-cell mediated progression of xenograft rejection.

Figure 1. Stages of xenograft rejection. HAR = hyperacute rejection; DXR = delayed xenograft rejection or accelerated vascular xenograft rejection.
phase (Figure I). Temporally, HAR occurs within minutes to hours of graft revascularization and represents a violent response to the foreign graft.73 If the organ is able to elude the HAR response, DXR occurs after a few days. DXR represents a form of acute vascular rejection occurring days to weeks following engraftment. T-cell mediated graft rejection occurs even later in the life of the xenograft, although some researchers are now beginning to assert that the T-cell response may be more important than previously thought.74,75 Interestingly, T-cell responses across the xenogeneic barrier may not be as potent as the T-cell response in human allograft rejection.76

Briefly, XNA binds the donor endothelium, initiating the complement cascade, which inflicts damage on the organ. The endothelial cells of the graft become activated, with resulting procoagulation and thrombosis.

**XNA and HAR**

The data supporting HAR initiation from XNA activity is compelling.77 Of the primates, humans, apes, and Old World monkeys possess natural anti-porcine antibodies.78,79 These are performing circulating XNA that recognize oligosaccharide determinants on swine endothelial cell surfaces. It is known that the XNA in humans possess a carbohydrate specificity to the Galα1-3Galβ1-4GlcNAc-R structure (R=O-(CH2k CO-NH-bovine serum albumin, which is expressed on porcine endothelial cells. This epitope is variously denoted in the literature as αGal(1-3)βGal(1-4), Galα(1,3)Gal, or simply α-Gal. Although this determinant is especially prominent on vascular endothelium, it is widely distributed on pig tissues.80

Some evolutionary force, likely an infectious agent containing the Galα1-3Galα1-4GlcNAc-R epitope, caused inactivation of the α 1,3-galactosyltransferase gene.81 Neutralization of this gene obviated production of the α-galactosyl epitopes, and the human host was then free to produce antibodies against pathogens containing this epitope (anti-α-galactosyl antibodies). Coincidentally, these XNA also recognize carbohydrate moieties on porcine cell surfaces, triggering HAR.82 Some reports also imply that anti-porcine XNA may demonstrate cytotoxicity to cells even in the absence of the typical vascular events that culminate in HAR.83

XNA have been shown in various studies to be members of different immunoglobulin classes, including IgG,81 IgM,84,85 IgM and IgG2,86 and IgM, IgG, as well as IgA.87 Most cytotoxic anti-porcine XNA are probably of the IgM class,88,89 as IgG alone is not sufficient to initiate complement-mediated cytotoxicity. The predominant influence of IgM, compared to IgG, in eliciting HAR has been established by other groups as well.90 More recently, the role of IgG anti-porcine endothelial antibodies in chronic graft rejection91 has been postulated, via a mechanism of antibody-dependent cell cytotoxicity.92,93

In human beings, up to 1% circulating antibody exhibited anti-α-Gal binding activity.94 Thus, a relatively minute fraction of antibody results in a particularly fulminant rejection reaction.

**The Role of Complement in Xenograft Rejection**

Xenograft destruction is mediated by stimulation of the complement system via three possible mechanisms: alternative pathway, classical pathway activation,83,95 and by the failure of natural complement inhibitors.96 The pathway stimulated depends on the specific species combination. From a clinical perspective, the fact that porcine organs only appear to suffer from the effects of natural antibody induction of complement97,99 may be important in developing techniques to attenuate HAR. It is noteworthy that in vivo studies have found that complement in the absence of XNA does not cause HAR.99 Most recently, it has been recognized that IgA stimulation activates the alternative pathway in discordant xenograft rejection.100,101

Normally, natural complement inhibitors on the endothelial cell surface serve to protect cells from lysis. These proteins, often referred to as regulators of complement activation, include decay-accelerating factor (CD55), membrane cofactor protein (CD46), and homologous restriction factor (CD59).102 These elements are known to function in a species specific manner103 and thus may be critical in xenotransplantation.104

**Endothelial Cell Activation**

HAR has been described as a sequela of the loss of endothelial cell (EC) function. Even ifXNA depletion may prevent HAR from occurring, DXR (also referred to as acute vascular xenograft rejection) may ensue 2 to 5 d following xenotransplantation.105 While elimination of XNA and complement may result in xenograft survival beyond the HAR phase, innate immune mechanisms may still activate the EC of the grafted organ.106,107

ECs are the target of the immune reaction in xenotransplantation; activation of these cells will result
in rejection due to a loss of barrier and anticoagulant properties. Complement-induced endothelial injury results in stimulation of EC, and XNA can specifically bind to porcine endothelium, independently activating EC. It has been shown that it is the concentration of XNA - specifically IgM class - and not complement activity that determines EC activation (ECA). It is the action of human serum natural killer cells that lyses xenograft EC - this effect is augmented by the addition of IgG. Xenograft rejection is attributed to failure of organ function to cellular-level events.

The EC response is divided into two continuous phases, type I ECA which results in HAR and type II ECA which constitutes the processes that occur during DXR. Under basal conditions, ECs provide a barrier function, in addition to preventing platelet aggregation from occurring. Once an inflammatory response is evoked, the anticoagulant endothelium converts to a pro coagulant surface. These prothrombotic events are the molecular mechanisms effecting HAR and DXR.

EC integrity is regulated by certain components of the complement system. Cytoskeletal and EC-shape changes disrupt integrity, allowing intercellular gaps to occur. These changes take place within 10 to 20 min of EC exposure to XNA and complement. The concept of ECA does have a direct link to clinical transplantation. It is known that rejection of cardiac xenografts is linked to ECA. By 48 h following graft revascularization, fibrin deposition, platelet microthrombi, the presence of pro thrombotic substances, and the absence of anti-thrombotic factors are obvious. Thus, increased knowledge of ECA may aid in the development of techniques to overcome the endothelial reaction to xenografts.

**Accommodation**

When XNA or complement are depleted, either DXR or accommodation occurs. Accommodation refers to the ability to withstand EC injury, whereby HAR or DXR does not occur. To induce a state of accommodation would ensure that the graft is resistant to antibodies that normally bind to the endothelium. It has also been postulated that continuous depletion may not be requisite for graft survival: antibody-mediated rejection is less likely to occur after an initial antibody removal, even with subsequently elevated antibody titers. This state of immunocompetence is referred to as accommodation, and it has been described in animal models of discordant xenotransplantation.

Various mechanisms have been proposed to explain the development of accommodation. Firstly, antibody-antigen interactions may be changed. This may be the result of some alteration in the antibodies that are directed against EC. For example, the antibodies may no longer fix complement or participate in the antibody-dependent cell-mediated cytotoxicity response. This is not likely the etiology of accommodation, as the addition of XNA known to initiate HAR does not revert the state of accommodation. Another possibility is a decrease in the absolute XNA levels. It has also been postulated that antigen expression may be transformed. This explanation does not take into account those cases of accommodation where immunoglobulins were found deposited on the graft endothelium.

The second theory involves EC modification - EC sensitivity to injury decreases in spite of continued stimulation by antibodies or complement. This may be attributed to the action of endotoxin, IL-2, and decay-accelerating factor. The endotoxin and IL-2 continue to activate the EC, resulting in increased decay-accelerating factor secretion. Complement is, therefore, inhibited. All cases of accommodation reported in vivo have involved antibody removal, and this is currently believed to be a key for the abrogation of HAR.

The final explanation is that an organ sustains damage during harvesting and reperfusion - antibody and complement depletion during this stage allows EC recovery. Subsequent antibody binding to the graft does not induce EC damage. This theory of accommodation takes into account the clinical events preceding transplantation that may contribute to organ injury.

**Approaches to Overcome HAR**

It has been argued that the most successful stratagem to overcome xenograft rejection will involve a multi-pronged approach. Briefly, three separate areas have been identified: depletion of xenoantibodies and xenoantigen modification, complement inhibition, and EC-based strategies. The use of pharmacological immunosuppressive agents is also being examined since these may be of benefit in XNA depletion.

**Xenoantibody Depletion and Xenoantigen Modification**

In addition to the role of XNA in stimulating complement, the long-term consequences of antibody
binding are unclear. Since the target glycoproteins are related to integrins, which aid in the regulation of endothelial function, XNA binding may directly alter endothelial activity. XNA may be depleted, blocked, or modified. XNA depletion is accomplished via plasmapheresis, immunosuppressive drugs, or ex vivo human blood perfusion through a xenogeneic organ. XNA blockade is possible via carbohydrate-based immunoadsorptive techniques. Synsorbs (Alberta Research Council) are used for specific carbohydrate-immunoadsorption therapy. We have used this technology successfully in our laboratory in both in vitro and ex vivo studies. Further, xenotransfer modification has been attempted with enzymatic cleavage of the a-Gal epitope or with molecular techniques to alter endothelial expression of a-Gal.

While plasmapheresis has been shown to be effective, antibody removal is nonspecific. This may place the transplant recipient, who is already immunosuppressed with pharmacological agents, at an increased risk of acquiring infection. With the identification of the aGal(l-3)pGal(l-4) epitope, investigators have attempted to target this site directly in order to ameliorate HAR in discordant xenografts.

Immunoadsorption of XNA, which specifically removes the xenogeneic antibodies, may be an important part of the treatment strategy. Gal-type oligosaccharides have been widely used in an effort to block XNA. Our experience with Synsorbs has shown this product to be highly efficient in a-Gal XNA depletion.

The greatest advantage of specific immunoadsorption is that accommodation may result. The combination of immunosuppressive drugs and immunoadsorption has achieved some degree of accommodation in primate studies. An alternative approach to preventing XNA from initiating HAR would be to eliminate the a-Gal epitope itself, either by enzymatic removal or genetic engineering. The use of a-galactosidase to cleave aGal residues from EC results in 70% to 80% less XNA binding. Studies have failed to demonstrate complete abolition of EC reactivity. Furthermore, application of this technique to a complete organ xenograft would necessitate development of methods to prevent hypoxia and damage to the organ during the digestive process.

It is untenable to attempt to "knock out" all of the genes for all epitopes. A more attainable goal might be to inactivate a single gene that codes for the galactosyltransferase enzyme, and thus eliminate all a-Gal epitopes. The porcine a1,3-galactosyltransferase has been cloned with a cDNA library and antisense nucleotides targeted against mRNA decrease galactosyltransferase expression.

Galactosyltransferase may be eliminated by preparing a construct that inactivates a galactosyltransferase allele by homologous recombination. Another strategy that may be employed is transferase dominance. H-transferase, which influences fucose, is isolated and transfected. The H-transferase is dominant over a 1,3-galactosyltransferase. The result is that the a-Galactosyl epitope is not constructed. The a-Gal epitope is replaced by fucose, to which natural antibodies do not occur.

Transgenic mice with H-transferase, and therefore very little a-Gal expression, are currently being tested. a-Gal expression is downregulated by approximately 80% to 90%. Japanese researchers have constructed transgenic pigs that produce a(1,2)-fucosyltransferase. It is difficult to draw any conclusions regarding the true validity of such technologies until in vivo xenotransplant studies are performed, preferably in a primate model.

Complement Inhibition

Researchers have attacked the complement system from various angles. C3 depletion induced by cobra venom factor has been shown to result in a prolongation of porcine hearts undergoing ex vivo perfusion with human blood, and a comparable result was noted for cobra venom factor used in vivo. Similarly, naturally complement-deficient guinea pigs have been observed to reject rat hearts less rapidly.

Other investigators have treated animals with soluble complement receptor type 1 which causes dissociation of classical and alternative pathway C3 convertases, demonstrating a delay in graft rejection. The major limitation to cobra venom factor and soluble complement receptor type 1 is that these agents also interfere with the alternative pathway, which is necessary for defense against pathogens. Even more significant is that cobra venom factor bears a terminal a-Gal residue; repeated cobra venom factor treatment evokes significant synthesis of anti-a-Gal antibodies. Thus, the use of a more specific agent, such as the classical pathway regulatory protein C1 inhibitor may be more useful. In vitro, C1 inhibitor has been found.
to inhibit cytotoxicity and deposition of complement components and prevent ECA.153

Another method of achieving complement depletion employs genetic engineering approaches to express membrane-bound human regulators of complement activation on porcine endothelial cells.102,104 This technique has the advantage of only exerting a local effect, thus avoiding possible systemic toxicity. In the case of unaltered porcine endothelium, human IgM binds EC with subsequent fixation of C1. C1 in turn activates C2 and C4. C4b and C2a then bind to the endothelium and form the C3 convertase. This is followed by the addition of C3b to the C4b-C2a conglomerate, forming the C5 convertase. The end result is the subsequent formation of the MAC. Porcine endothelium constructed to be transgenic for human decay-accelerating factor responds in a different fashion. Once human anti-aGal IgM binds EC, C1 is still fixed, activating C2 and C4. However, the C3 convertase formed is split by the decay-accelerating factor expressed on EC. A third regulator of complement activation is CD59, and its influence is exerted even further along the complement cascade, blocking MAC formation.102

Transgenic pigs expressing human CD59 were used as heart donors in xenograft experiments involving baboons recipients.154 Unfortunately, xenograft survival was not substantially improved by using the transgenic organs. Double human regulators of complement activation are more protective than single regulator of complement activation molecules: the combination of homologous restriction factor and decay-accelerating factor is more effective than decay-accelerating factor alone for resisting EC lysis in a bovine aortic EC model.155 This has not yet been tested in vivo. Yeast artificial chromosomes have been used to create mice transgenic for multiple regulators of complement activation, including human membrane cofactor protein, CD59, and decay-accelerating factor.156 This group has also engineered transgenic pigs with this technology, with the entire human membrane cofactor protein gene contained within the yeast artificial chromosomes.157

Porcine donors transgenic for human decay-accelerating factor have been studied.158-160 Greater than 3-week survival of a human decay-accelerating factor transgenic porcine cardiac xenograft in a Cynomolgus monkey recipient was achieved by the Cambridge group, with the aid of heavy immunosuppression.161 The primates were sacrificed because of side effects from the immunosuppressive drug therapy, but the xenografts were still functional. Perhaps the most significant finding was the pristine histopathology of the heart, which failed to reveal significant immunoglobulin or complement deposition.161 This success has stimulated White and colleagues to prepare for the possibility of a relatively early clinical trial with these genetically altered organs.

The approach of inhibiting the effects of complement with transgenic expression of human regulators of complement activation on porcine endothelium has not obviated the need for a powerful immunosuppressive regime: cyclosporine A at 60 mg/kg/d was administered. The primate transplant recipients were sacrificed due to diarrhea - a side effect of the potent immunosuppression required. Interestingly, these primates were grafted with organs from porcine donors that fortuitously expressed low levels of endothelial a-Gal. Thus, this study did not examine the significance of XNA binding to the graft endothelium. Furthermore, it is noteworthy that transgenic donor heart median survival was merely 5.1 d without pharmacological immunosuppression. Nevertheless, studies in the near future will strive to improve upon this complement inhibitory technology. Presently, many researchers believe that porcine organs transgenic for human regulators of complement activation may play a pivotal role in clinical xenotransplantation.

Endothelial Cell-based Strategies

The complexity of the EC response to a xenograft has become the object of numerous experiments. In an effort to blunt the rejection response, researchers are investigating numerous potential targets in the ECA cascade.

The importance of neutrophil adhesion to the EC of the xenogeneic donor organ is well-established. NPC 15669, a new drug in the leumedin class of anti-inflammatory agents, prevents neutrophil involvement by blocking upregulation of an adhesion molecule (CD11b/CD18). When combined with complement inhibition, prevention of neutrophil adhesion with NPC 15669 prolonged xenograft function.162

P-selectin is important for platelet and neutrophil binding. The use of an antibody directed against P-selectin has been shown to prolong xenograft survival in a rat model.163 The addition of a PAF antagonist to
the P-selectin antibody further prolonged xenograft function. 163 These agents were found to suppress platelet aggregation, neutrophil, and macrophage infiltration, and to decrease deposition of C3 and C5.

Platelet gpIIbIIIa is a fibrinogen receptor that is integral to platelet aggregation and adhesion to subendothelial surfaces. GPI 562, a specific gpIIbIIIa antagonist, did not improve ex vivo cardiac function or survival but did appear to confer some degree of protection to EC by immunohistochemistry.164 The reduction in EC damage led these investigators to suggest that antiplatelet therapy may be useful to prevent DXR. The merit of platelet inhibition as it might apply to clinical xenotransplantation requires further investigation.

The stimulation of platelets and subsequent thrombosis likely requires thrombin. SDZ MTH 958, a thrombin inhibitor, was reported to prolong survival and enhance function in an ex vivo study. 165 Thrombin inhibition also improved histological features. As with the gpIIbIIIa antagonist, additional studies to define the value of a thrombin inhibitor are necessary.

The ubiquitous transcription factor NF-KB is felt to be central to gene induction in ECA.166 The agent pyrrolidine dithiocarbamate inhibits NF-KB activation. Preliminary studies have shown that pyrrolidine dithiocarbamate inhibits E-selectin, IL-8, and tissue factor. 106.167 Thus, NF-KB blockade may represent a useful modality in the modulation of EC reaction in xenotransplantation.

The Harvard group is keen on focusing on the endothelium of the xenograft for genetic modification.167 The procoagulant environment that results following ECA may be counteracted by the use of endothelium expressing human thrombomodulin. NF-KB can also be targeted for molecular alteration. If this transcription factor is blocked, upregulation of the various genes involved in ECA may also be prevented. IKBa is a naturally-occurring inhibitor of NF-KB. The use of a recombinant IKBa adenoviral vector to transduce porcine EC suppresses gene transcription. 167 This technology may be successful for creating transgenic donors for humans.

Other novel strategies include the development of monoclonal antibodies directed against porcine vascular cell adhesion molecule,168 endothelial reseeding. This approach entails enzymatic degradation of the donor endothelium with collagenase. The recipient endothelium is then used as a replacement on the graft. Initial small animal studies have been reported,169 however, to date the utility of this method in the porcine-human situation has not been established.

Results of Specific Immunoadsorption

The multitude of strategies currently being explored would suggest that multiple events must be targeted to consistently prevent HAR. Complement inhibition may be essential to ensure xenograft survival, but studies focusing on complement have failed to assess the effect of persistent XNA upon the endothelium. While technologically attractive, genetic engineering requires chance recombinant events during cell replication as well as the appropriate breeding of progeny. Such manipulations are time consuming and labor-intensive. Since XNA are initiators of a cascade of responses, including the complement system, direct deletion or blockade of antibodies may be the most effective target. This could be easily accomplished with an appropriate compound, and elimination of XNA has the added advantage of removing a potential EC stimulant. For these reasons, we elected to attack this aspect of the xenogeneic response in our lab.

One specific approach to overcome HAR is XNA adsorption by synthetic carbohydrates, which contain moieties to which the XNA are directed. Synsors are immobilized carbohydrates bound to a solid support matrix. This product is more suitable for antibody removal compared to plasmapheresis or polyclonal antibodies against human immunoglobulins, since Synsors specifically eliminate only the antibody of interest. The Synsors, which are most efficacious in reducing the cytotoxicity of human serum to porcine endothelial cells, have been identified and bear the chemical structure aGal(l-3)j3Gal(l-4)j3Glc-R.87 Synsors have been successfully employed for in vivo studies of cardiac allografting across the ABO blood group. Clinically, Synsorb immunoadsorption has permitted ABO incompatible renal allografting.170,71 Previous in vitro studies have shown Synsors to be potentially useful in xenotransplantation.87,130,72

We have demonstrated the utility of Synsorb-based immunoadsorption in an ex vivo model. Hearts perfused with human plasma, adsorbed with Synsorb 90, demonstrated prolonged function in all cases. This group survived for exactly the same duration as did the hearts perfused with porcine plasma.

The weight change during the experiment was used as a measure of edema. The amount of edema accumulated during the ex vivo perfusion was calculated relative to the duration of heart survival, which reflected the
vigor of rejection. Synsorb 90 and porcine plasma-perfused organs gained significantly less weight than controls.

Determinations by direct ELISA revealed that Synsorb 90 immunoadsorption significantly depleted anti-porcine human IgG and IgM compared to controls. We were able to demonstrate greater than 98% reduction in XNA following immunoadsorption. Anti-porcine human IgG and IgM levels were found to be significantly decreased in the Synsorb 90 immunoadsorption group compared to controls.

C3 and C4 levels were performed to confirm the presence of complement. Laboratory analysis revealed that C3 and C4 were detectable in significant quantities, even following the immunoadsorption procedure.

The Future of Xenotransplantation

In the United Kingdom, ethical approval has been granted to proceed with clinical xenografts, pending results of safety and zoonoses studies. It is possible that xenotransplantation may be attempted in 18 months time. Given the current status of organ supply and demand, the future of xenografts may be the future of organ transplantation.

References


139. Vaughan HA, McKenzie IFC, Sandrin MS. Biochemical studies of pig xenografts detected by naturally occurring human antibody.