INTRODUCTION

The most urgent problem in clinical transplantation is the shortage of donor organs. Only 15% of patients awaiting a heart transplant in the United States can undergo the procedure in a given year; if the current criteria for cardiac transplantation were extended to all potential recipients, the donor pool would provide less than 5% of the organs needed (Evans 1991). The shortage of other organs for transplantation procedures is nearly as severe (Figure 1). The dimensions of this problem, as well as recent advances in the biomedical sciences including the ability to "genetically engineer" large animals, have provoked interest in the potential use of animals in lieu of humans as organ donors, that is, interspecies or xenotransplantation.

CLINICAL EXPERIENCE IN XENOTRANSPLANTATION

In the early 1900s, the development of surgical techniques that would allow one end of a severed blood vessel to be connected to another (vascular anastomosis) opened the door to organ transplantation. Experimental surgeons such as Alexis Carrel, Emrich Ullman, and Charles Guthrie realized that this surgical technique might be exploited to transplant healthy organs into individuals with chronic organ failure (Guthrie 1912). Because it was believed that human organs in a suitable condition for transplantation would rarely become available, initial efforts in organ replacement focused on xenotransplantation. A few procedures in which sheep or pig kidneys were connected to the circulation of patients with renal failure were performed. However, at best, the xenotransplants functioned only briefly (Ullman 1914; Neuhof 1923). Experimental allografts did function, but inevitably failed within days to weeks (Carrel 1908). Thus, further attempts at clinical transplantation were rarely undertaken until the 1950s when the immunological basis for graft failure became apparent and therapeutic approaches for dealing with immune responses began to emerge.

The availability of immunosuppressive agents in the early 1960s finally made transplantation a rational approach to the treatment of organ failure. Because human organs remained scarce, researchers turned again to the possibility of xenotransplantation. Reemtsma and others (1964) transplanted a series of chimpanzee kidneys into humans. The clinical course of the grafts was characterized by episodes of decreased renal function which, consistent with acute cellular rejection, responded to immunosuppressive therapy. In two chimpanzee-to-human renal transplants, graft loss was associated with infection; in six recipients it was associated with acute cellular rejection. However, some of the grafts lasted

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for months, the longest of which survived for 9 months. It would not be unreasonable to think that in recipients treated with modern immunosuppressive agents and with the availability of new antibiotics, these grafts might have functioned and the recipients might have survived longer.

At the same time that Reemtsma was reporting the first clinical xenografts, Starzl and others (1964) and Hitchcock and others (1964) performed baboon-to-human renal grafts. The clinical courses of the grafts were characterized by rejection episodes that responded, in part, to immunosuppression and graft irradiation. Graft survival was 10–49 days. The histologic picture of the rejected kidneys suggested that the failure of these grafts was due to acute vascular and cellular rejection. More recently, baboon livers were transplanted into two human subjects with hepatic failure (Starzl and others 1994). Although the recipients of the baboon livers ultimately died, the transplanted organs appeared to be free of rejection at the time of the patients' deaths (Nalesnik and others 1994).

While the results described above might be viewed as promising, there are some serious limitations to the use of nonhuman primates as donors for clinical transplants. First, many nonhuman primates are too small to be used as organ donors in adult patients, and large nonhuman primates are not available in sufficient numbers to address the current need for hearts or kidneys. Second, nonhuman primates may harbor viruses that would be lethal if transmitted to humans. Third, social opposition to the extensive use of nonhuman primates would pose a hurdle. Fourth, current technology does not allow genetic manipulation of primates. Even if suitable techniques were to be developed and societal concerns allayed, the long period between birth and maturity would discourage this approach.

Given the urgent need for donor organs and the problems associated with the use of nonhuman primates, many laboratories are focusing on strategies for overcoming the significant immunological barriers to using nonprimates as organ donors for clinical transplantation. The species generally viewed as being the most suitable for this purpose is the pig. Pigs are of the appropriate size and, are as far as it is known, physiologically compatible with humans. The pig harbors relatively few infectious agents that could be communicated to humans (Michaels and Simmons 1994; Ye and others 1994). Those infections that do pose a risk to humans can be detected by screening procedures. Pigs are born in litters, after a comparatively brief gestation, facilitating the breeding of animals with desired traits. Finally, current technology is available to genetically manipulate pigs, thus allowing suitable donor animals to be genetically engineered.

THE IMMUNOLOGICAL BARRIER TO XENOTRANSPLANTATION

There are some formidable immunologic barriers to transplanting organs from nonprimates into humans. In addition to the immunologic processes that cause injury to organs transplanted between individuals of the same or closely related species, there are some immunologic problems that are especially characteristic of organ transplants between species that are widely disparate. The clinical and pathologic outcomes of such xenografts, reflecting immune-mediated changes, are summarized in Figure 2.

Hyperacute xenograft rejection

Organ xenografts carried out between species that are phylogenetically distant, such as pig-to-dog or pig-to-primate, exhibit a course that is dramatically different from that of allografts, free tissue grafts, and concordant xenografts. These xenografts are subject to a rapid and violent rejection reaction, called hyperacute xenograft rejection, which abolishes organ function in minutes or a few hours (Auchincloss 1988; Perper and Najarian 1966; Platt 1995; Platt and others 1990a). Species combinations that are subject to the hyperacute xenograft rejection of vascularized xenografts are called “discordant” whereas species combinations not subject to this type of rejection are called “concordant” (Calne 1970). For a comprehensive review of hyperacute xenograft rejection, the reader is referred to a recent monograph (Platt 1995).

Hyperacute rejection of xenografts is clinically and pathologically similar to hyperacute rejection of allografts seen in recipients exposed to donor antigens (Platt 1994b). After being connected to the recipient's circulation, the xenograft
may regain color and begin to function; however, the graft rapidly takes on a mottled appearance and the function declines. The histologic features of hyperacute rejection are characterized by interstitial hemorrhage, edema, and thrombosis (Platt 1994a). The immunopathology invariably reveals the presence of complement proteins and usually antibodies of recipient origin in the blood vessel walls (Platt and others 1991b). The events which lead so quickly to hyperacute xenograft rejection involve complement-mediated injury to graft blood vessels (Platt and others 1990b). Complement activation on endothelial cells causes loss of heparan sulfate from the cells and the formation of intercellular gaps (Saadi and Platt 1994). These, and perhaps other changes lead to a loss of the normal barrier and antithrombotic functions of endothelium (Platt and others 1995).

Hyperacute rejection of allografts and of some xenografts, particularly those of porcine organs transplanted into primates, is initiated when recipient antibodies are bound to blood vessels in the donor organ (Platt 1995). Xenoreactive natural antibodies which might initiate hyperacute rejection have been found in the circulation of all mammalian species (Boyden 1964); their presence in the circulation is not linked to prior sensitization with xenogeneic cells. The binding of xenoreactive antibodies activates the recipient’s complement system which in turn mediates severe injury to the endothelial lining of blood vessels in the graft (Figure 3).

The importance of natural antibodies in triggering hyperacute xenograft rejection is widely accepted (Hardy and others 1984; Auchincloss 1988), and there is much evidence to support the concept that when a porcine organ is transplanted into a nonhuman primate or into a human, complement activation and thus organ injury is initiated predominantly by this mechanism (Platt and others 1991b; Dalmasso and others 1992; Platt 1995). For example, depletion of xenoreactive antibodies from a primate allows the prolonged survival of a vascularized xenograft (Cooper and others 1988) even if the complement system of the recipient remains intact (Dalmasso and others 1992). Moreover, porcine cardiac xenografts in newborn baboons, which have very low levels of natural antibodies but intact complement activity, are not subject to hyperacute rejection (Kaplon and others 1994).

In some experimental models, however, the complement system of the recipient is activated directly on the surface of donor cells, without the involvement of antibodies (Miyagawa and others 1988). For example, guinea pig hearts transplanted into rats that have been depleted of natural antibodies and rabbit hearts transplanted into newborn pigs which possess no natural antibodies are rejected immediately (Leventhal and others 1993b; Johnston and others 1992). Activation of complement in these models leading to hyperacute rejection of the organ grafts is thought to involve the alternative pathway of complement. The mechanism underlying activation of complement through the alternative pathway probably involves the failure of factor H in recipient plasma to control the spontaneous generation of the C3 cleaving enzyme (C3bBb) on a xenogeneic cell surface. This restricted functioning of factor H has obvious advantages in promoting host defense as it allows activation of complement on the surface of invasive organisms while protecting autologous cells from inadvertent injury. Fortunately, human factor H appears to effectively control alternative pathway activation on porcine and bovine cell surfaces (Edwards 1981).

Another mechanism that renders pig-to-primate xenografts especially susceptible to complement-mediated injury involves the impaired functioning of cell-associated complement regulatory proteins such as decay accelerating factor and CD59. These proteins, which are present in the cell membranes of all mammalian species, protect cells against inadvertent injury during the activation of complement. Thus, when complement is activated on the surface of a microorganism, soluble reaction products, which attach to adjacent endothelium, are prevented from catalyzing further reactions on endothelium. The points in the complement cascade at which complement regulatory proteins exert control are shown in Figure 4. Decay accelerating factor and membrane cofactor protein inhibit the formation and integrity of C3 convertase, the pivotal enzyme complex for the activation of complement. CD59 and homologous restriction factor inhibit the lytic properties of C8 and C9. Some complement regulatory proteins control the activation of homologous complement more effectively than heterologous complement. Thus, the limited ability of complement regulatory proteins in a xenograft to control activation of the complement system of the recipient may contribute to the susceptibility of a xenograft-to-complement-mediated injury.

Regardless of whether natural antibodies or the alternative complement pathway initiate hyperacute rejection, activation of the complement system is an essential event in the
pathogenesis of hyperacute rejection. How does complement cause within minutes the devastating vascular injury characteristic of hyperacute rejection? Figure 4 summarizes the effector mechanisms that may contribute to graft injury. One mechanism of tissue injury could involve the killing of endothelial cells caused by the formation of C5b6789n complexes, called the membrane attack complex, on the cells. Lysis of endothelial cells, mediated by the membrane attack complex, may be important in some cases of hyperacute rejection. However, in the pig-to-primate models that the author has studied, endothelial cell death is not a major lesion (Platt and others 1991b). Rather, complement-mediated changes appear to involve non-cytotoxic processes. In addition to causing cell death, the membrane attack complex of complement may trigger changes in cellular behavior. For example, the membrane attack complex activates endothelial cells leading potentially to procoagulant changes (Hamilton and others 1990).

Complement components other than the membrane attack complex may contribute to graft injury. C5a together with binding of natural antibodies heparan sulfate, an acidic polysaccharide that participates in many of the normal functions of endothelium, to be released from endothelial cells (Platt and others 1991a). The author has postulated that the loss of heparan sulfate from graft endothelium could be responsible in part for the rapid loss of endothelial integrity seen in hyperacute rejection. C5b67 complexes mediate changes in endothelial morphology leading to the formation of intercellular gaps (Saadi and others 1995). The formation of gaps in endothelium may explain the rapid onset and progression of hyperacute rejection. Formation of iC3b a proteolytic product of C3 on endothelial surfaces provides a mechanism for adhesion of neutrophils (Vercellotti and others 1991). To the extent that neutrophils are involved in xenograft rejection, iC3b generation may thus be a critical pathogenic event.

Acute Vascular Xenograft Rejection

If a xenograft recipient is depleted of natural antibodies or complement, hyperacute rejection does not occur. Instead, the vascularized xenograft is subject to a delayed type of rejection which we have called acute vascular xenograft rejection (Leventhal and others 1993b). Acute vascular rejection, which may also be seen in allografts and concordant xenografts, is characterized by the swelling of endothelial cells, prominent fibrin deposition, focal edema, and hemorrhage, changes similar to those seen in hyperacute rejection. Although the histologic picture of acute vascular rejection bears some resemblance to that of hyperacute rejection, its pathogenesis is probably quite different. We have postulated that acute vascular rejection arises as a consequence of the activation of endothelial cells in the graft. This activation leads to the endothelial cells acquiring new functions, including the elaboration of inflammatory cytokines, expression of cell adhesion molecules, and conversion of the endothelial cell surface from anticoagulant to procoagulant (Platt and others 1995). These changes cause the formation of blood clots and the infiltration of leukocytes, which are so characteristic of this type of rejection. The mechanisms contributing to the development of acute vascular xenograft rejection are an issue of current interest because this type of rejection is now viewed as the major barrier to clinical application of xenotransplantation.

Accommodation

Fortunately, acute vascular xenograft rejection is not the only outcome seen in xenografts if hyperacute rejection is prevented. In some cases, the depletion of recipient xenoreactive antibodies and the manipulation of complement allows the long-term survival of the graft even after the antibodies return to the circulation and the complement system is restored. The author has called this condition "accommodation" (Platt and others 1990a) to denote what appears to be an acquired resistance of the graft to humoral reactions, which under other circumstances would cause hyperacute or acute vascular rejection. The "accommodated" xenograft appears histologically normal. It may contain recipient immunoglobulin, but evidence of complement activation and thrombosis is not seen (Platt and others 1991b).

Accommodation was first observed in the clinical transplantation of blood group A or B kidneys into recipients who had antibodies against these blood groups (Chopek and others 1987; Alexandre and others 1987). The temporary depletion of anti-A or anti-B antibodies from the recipient allowed the engraftment of kidneys bearing A or B blood group antigens. In many instances, vascular rejection did not occur after those antibodies returned to the circulation.

Accommodation has also been observed in a few experimental xenografts. Alexandre and others (1989) used plasmapheresis and immunosuppressive therapy to prolong the survival of swine-to-baboon renal xenografts. In three cases, graft function in excess of 20 days was achieved. Fischel and others (1992) reported one case of extended survival of a pig cardiac xenograft in a rhesus monkey that had been treated with plasma exchange and organ perfusion in combination with immunosuppression.

The mechanism or mechanisms that allow accommodation to develop have not been elucidated. Potential causes of accommodation include a change in the levels or repertoire of xenoreactive antibodies or a change in the expression of the antigen or antigens they recognize. We have shown that in the accommodation of ABO-incompatible renal allografts, recipients have antibodies specific for donor blood groups, and the organ transplant continues to express that blood group antigen based on immunopathologic analysis (Chopek and others 1987; Bannett and others 1989). Since in most cases there is no evidence of recipient antibody deposited in the ABO-incompatible graft, it is likely that the mechanism of accommodation involves a qualitative alteration in the antigen leading to decreased interaction of host antibody with
the graft. Less information is available about the potential mechanisms that could bring about accommodation in a xenograft. In addition to the possible importance of a change in antigen expression, we have speculated that the slow return of natural antibodies to the circulation of the recipient and interaction of those antibodies with the graft, perhaps with the activation of small amounts of complement, may cause the endothelium of the graft to develop resistance to humoral injury. This idea is supported by in vitro studies demonstrating that endothelial cells exposed for prolonged periods to noncytotoxic stimuli such as endotoxin or cytokines develop resistance to those stimuli (Busso and others 1991). Yet another mechanism that may underlie accommodation involves recovery of the graft from injury incurred in the peri-transplant period. Thus, temporary depletion of natural antibodies from a recipient may allow the graft to recover from ischemia-reperfusion injury prior to exposure to active complement proteins (Magee and Platt 1994). Since ischemia-reperfusion injury depends in part on the activation of complement (Maroko and others 1978; Weisman and others 1990), such recovery may have the net effect of reducing the amount of complement-mediated injury at the time of transplantation. Regardless of the mechanism, the development of accommodation has an obvious impact on the ability to apply xenotransplantation clinically, without the need for continuing manipulation of the recipient.

**Cellular mechanisms of xenograft injury**

Although hyperacutely rejected organ grafts are often found to contain host leukocytes (Colman and others 1976), there is scarce evidence suggesting that leukocytes contribute to the pathogenesis of that process. Indeed, Forbes and others (1976) showed that depletion of neutrophils has no impact on the course of hyperacute rejection. Zehr and others (1994) showed that administration of an agent that inhibits neutrophil-endothelial cell interaction fails to prolong xenograft survival in otherwise untreated recipients. Furthermore, a number of cases of hyperacute xenograft rejection have been studied in which the infiltration of neutrophils is very focal and sometimes absent (Platt and others 1991b). Some studies have suggested that natural killer cells are able to accumulate in xenogeneic organs and mediate endothelial cell injury (Inverardi and others 1992); however, histologic and immunopathologic analysis fails to reveal significant numbers of these cells in hyperacutely rejecting grafts.

Given these findings it would seem reasonable to conclude that neutrophils and other inflammatory cells are not essential for the development of hyperacute rejection. On the other hand, it would seem not unlikely that the activation of neutrophils, their adherence to blood-vessel walls, and their influx into tissue adds to the injury caused by antibodies and complement.

Cellular infiltrates are commonly seen in acute vascular xenograft rejection. The infiltrating cells include neutrophils, macrophages, and lymphocytes, any combination of which may contribute to the pathogenesis of graft injury. Studies by Zehr and others (1994) demonstrate that administration of an agent that inhibits neutrophil expression of CD11b/CD18 lessens the microvascular injury in acute vascular rejection supports the importance of cellular mechanisms. Although immunopathologic studies have failed to reveal evidence that such cells are present in hyperacute rejection (Platt and others 1991b; Leventhal and others 1993b); natural killer cells may constitute an important component of the cellular infiltrate seen in acute vascular rejection (Blakely and others 1994). In fact, the extent to which these cells actually mediate tissue injury in xenografts is a subject of current inquiry.

Alexandre and others (1989) support the importance of lymphocytes in mediating xenograft injury by studying the transplantation of porcine kidneys into baboons. These studies showed that decreases in graft function can be reversed by administering immunosuppressive agents. The mechanisms by which T cells might cause the rejection of xenografts has been considered in recent years (Auchincloss 1988; Geller and others 1993).

Cellular immune responses to a xenograft might differ in certain respects from the cellular immune responses to allografts. One important difference concerns the mechanisms by which T cells become activated in response to xenogeneic cells. Owing to the events leading to development of the repertoire of mature T cells, the number of different T cells able to recognize xenogeneic cells may be fewer than the number able to recognize allogeneic cells. Furthermore, the cytokines and cell adhesion molecules synthesized by donor cells may function ineffectively on the recipient’s T cells. Thus, some have postulated that the cellular response to a xenograft might actually be less intense than the cellular response to an allograft (Auchincloss Jr., 1988; Alter and Bach, 1990). The relative intensities of cellular responses to xenogeneic and allogeneic grafts have not been compared critically; however, to the extent that cellular immune responses to xenogeneic tissues have been evaluated, they appear to be nearly as strong and sometimes stronger than the response to allogeneic tissues (Murray and others 1994). Thus, there is every reason to believe that an intense cellular response would contribute to the immunologic barrier to xenotransplantation. The most important question from a practical perspective is whether there are unique aspects of this cellular response, which might require therapeutic agents or strategies distinct from those used for allotransplantation.

**THERAPEUTIC STRATEGIES FOR XENOTRANSPLANTATION**

The last 5 years have brought much progress in understanding the immunological barriers to xenotransplantation and in developing strategies to overcome those barriers.
This progress has been summarized in several recent reviews (Platt 1994a, 1994c, 1995). The sections that follow will summarize work in the author’s laboratory which may help advance xenotransplantation toward the clinical arena.

Natural antibodies-antigens

The repertoire of natural antibodies in a serum might be expected to recognize a vast array of xenogeneic antigens, which might differ from species to species and even from individual to individual. In fact, recent studies demonstrate that human xenoreactive antibodies predominately recognize one carbohydrate antigen, Galα(1-3)Gal, which was extensively studied in the 1980s by Galili (Galili and others 1984; Galili 1993). Galα(1-3)Gal is expressed on the cells of New World monkeys and lower mammals but is not expressed by humans, apes, or baboons (Collins and others 1994b). At the same time, humans, apes, and baboons have natural antibodies specific for that structure while lower mammals do not (Galili and others 1987). Recent studies by Good and others (1992) and Neethling and others (1994) demonstrated that purified Galα(1-3)Gal and similar sugars block the binding of human xenoreactive antibodies to porcine cells and prevent complement mediated cytotoxicity. These authors also demonstrated that antibodies directed against Galα(1-3)Gal can be eluted from porcine organs perfused by human plasma. Sandrin and others (1993) demonstrated that transfection of COS cells with the murine α1,3-galactosyl transferase gene, which is responsible for adding terminal αGal residues to oligosaccharides, induces binding of human natural antibodies to the transfected cells. Collins and others (1994a) demonstrated that expression of Galα(1-3)Gal in the heart of a New World monkey provides a sufficient basis for the development of hyperacute xenograft rejection when that heart is transplanted into a baboon which has antibodies specific for αGal. Collins and others (1994b) also demonstrated that enzymatic removal of α-galactose decreases the binding of xenoreactive antibodies to porcine endothelial cells (Collins and others 1994b).

Although the importance of Galα(1-3)Gal as a target antigen is now widely accepted, the conditions that allow xenoreactive antibodies to bind to that structure are complex. Parker and others (1994), Platt and Holzknecht (1994), and Cotterell and others (1995) have shown that the affinity of natural antibodies for Galα(1-3)Gal is very low and therefore expression of that structure on a cell surface may not by itself be sufficient to result in significant binding of complement fixing in xenoreactive antibodies. Rather, it appears that the sugar must be expressed as a posttranslational modification of certain glycoproteins (Holzknecht and Platt, 1995). The apparent avidity of natural IgM antibodies for the Galα(1-3)Gal on the glycoproteins is seven orders of magnitude higher than the avidity for the simple sugar. Further evidence that the manner in which αGal is expressed dictates the extent of antibody binding to a xenogeneic cell derives from the work of Cotterell and others (1995) and Alvarado and others (1995) demonstrating that although binding of IgM to porcine cells depends on the presence of αGal. IgM binding varies over a nearly tenfold range and is independent of total expression of αGal.

Based on the finding that xenoreactive antibodies bind predominantly to Galα(1-3)Gal, it is possible to devise specific strategies for immunodepletion of those antibodies from the circulation of a xenograft recipient. A number of groups are actively pursuing approaches to antibody depletion. Affinity columns have been used previously for depleting iso-hemagglutinins from human patients allowing transplantation of organs across ABO barriers (Bannett and others 1987) and it is reasonable to think this approach could also be used for depletion of xenoreactive antibodies. One hope is that temporary depletion of xenoreactive antibodies would allow the development of accommodation for xenografts as it does for allografts.

Another way to prevent humoral injury by xenoreactive antibodies is to inhibit their binding using soluble ligands. This approach has also been used to prevent the hyperacute rejection of ABO-incompatible grafts (Chopek and others 1987; Cooper others 1993). Unfortunately, because the binding of xenoreactive antibodies to cell surfaces is very avid (Parker and others 1994), high concentrations of a monomeric inhibitor would be needed.

Yet another approach to preventing the interaction of xenoreactive antibodies with a xenograft is to seek out or develop pigs that have low levels of xenoantigen expression. With the identification and cloning of the gene for the glycosyltransferase responsible for the synthesis of Galα(1-3)Gal (Sandrin and others 1994; Strahan and others 1995), the possibility of genetically engineering donor animals with decreased expression has been advanced. Unfortunately, the most direct strategy which involves “knocking out” the gene can be achieved only in mice because it requires embryonic stem cell technology, which is not yet proven in larger animals. An alternative strategy could involve introducing another glycosyl transferase that would compete with α1,3-galactosyl transferase for the growing oligosaccharide chain. This approach has been pursued by Sandrin and others (1994).

The development of animals with low levels of expression of antigens recognized by xenoreactive natural antibodies does not necessarily require genetic engineering. Geller and others (1994) and Cotterell and others (1995) with the author found that the level of antigen expression varies over a tenfold range among pigs. Variation in antigen expression appears to have a genetic basis. Indeed, perfusion of baboon blood through organs from pigs that express low levels of antigen leads to the deposition of very little IgM and C4 in contrast with similar experiments in which organs from normal animals are perfused. These results suggest that preferred donor animals might be selected or bred.
Complement activation

Over the past 30 years a number of studies have demonstrated that if complement is depleted or inhibited, hyperacute rejection does not occur (Gewurz and others 1967; Leventhal and others 1993a; Pruitt and others 1994; Platt 1995). Most of these studies used cobra venom factor, which depletes complement components by activating the alternative pathway complement in the blood. A more recently developed agent, soluble CR1, functions by a different mechanism to inhibit complement. Soluble CR1 causes decay of active complement convertases and serves as a cofactor for proteolytic cleavage of those convertases (Weisman and others 1990). Administration of cobra venom factor or soluble CR1 at optimal doses prevents hyperacute rejection and yields graft survival of 3-4 days. The grafts ultimately succumb to acute vascular rejection (Leventhal and others 1993b). Combining the use of cobra venom factor with antibody depletion results in more prolonged graft survival (Leventhal and others 1994). However, the recipient may be subject to a heightened risk of infection as complement and natural antibodies play important roles in host defense. One way to overcome this problem might be to use agents that would selectively inhibit the classical complement pathway (Dalmasso and Platt 1993), which is used in the activation of complement in pig-to-primate xenografts (Platt and others 1991b; Dalmasso and others 1992), sparing the alternative complement pathway for host defense. Another approach that may function by a similar mechanism was recently tried by Magee and others (1995) based on the work of Frank and others (1992). This approach involved the administration of purified human IgG, which may function as an alternative acceptor for activated complement proteins by directing enzymatically-active complexes away from xenograft endothelium.

Complement regulatory proteins

The concept that a xenograft might be uniquely susceptible to complement-mediated injury because of the restricted ability of xenogeneic cells to control activation of heterologous complement was first proposed by Dalmasso (Dalmasso and others 1991; Platt and others 1990a). This concept has spurred efforts in a number of laboratories to introduce human complement regulatory proteins into potential donor animals in order to ameliorate the effects of complement activation. Although human complement regulatory proteins might be introduced extrinsically into porcine endothelium by various techniques (Dalmasso and others 1991; McClellan and others 1994), most attention has focused on the introduction of genes encoding human complement regulatory genes into potential donor animals. Cary and others (1993) developed transgenic mice and more recently transgenic pigs expressing human decay accelerating factor under control of the decay accelerating factor promoter. Subsequent studies demonstrated that cells from the transgenic mice have increased resistance to complement-mediated lysis. Transgenic mice and transgenic pigs have also been developed that express combinations of CD59, decay accelerating factor, and membrane cofactor protein under the control of various promoters (Kooyman and others 1994; Diamond and others 1994; Kagan and others 1994). Hearts from transgenic mice were shown to resist the activation of complement during perfusion with human plasma or baboon blood (McCurry and others 1995). Recently McCurry, with the author, carried out a series of transplants from transgenic pigs expressing human CD59 and decay accelerating factor into baboons. Although expression of the human proteins was lower than optimum and the experiments were preliminary, the grafts did not undergo hyperacute rejection and functioned for prolonged periods of time. Histologic analysis of the grafts revealed remarkably little tissue injury.

FUTURE PROSPECTS FOR XENOTRANSPLANTATION

What are the prospects for clinical application of xenotransplantation? The path that will lead to safe, reliable, and effective xenotransplantation is not yet clear. However, certain major steps along that path clearly have been taken and from the vantage point of 1995 it is possible to see some of the elements that will likely contribute to a successful strategy. The identification of the major antibody-antigen system involved in hyperacute rejection, and the development of effective strategies for the inhibition of complement are significant steps. The ability to genetically engineer, breed, and select donor animals will lower the immunological barriers to xenotransplantation and thus play an important role in launching it into the clinical arena. It would thus seem if one can identify a “molecular” hurdle to xenotransplantation, a strategy for overcoming that hurdle can be devised.

While hyperacute rejection was once viewed as the major hurdle to successful xenotransplantation, that view is no longer a correct one. Hyperacute rejection can be prevented reliably, reproducibly, and effectively. The next and perhaps most daunting barrier to xenotransplantation is acute vascular rejection. Although some progress has been made in elucidating the pathogenesis of acute vascular rejection, there is as yet no certain way to prevent or overcome it. Besides acute vascular rejection, humoral and cellular responses to the myriad of donor antigens remains a significant concern. Such responses as hurdles to allogeneic transplantation and in some humorally-mediated diseases have been overcome. Whether special approaches will be needed to prevent elicited responses to the xenograft is yet unknown.

If the immediate prospects for clinical xenotransplantation remain uncertain there can be little doubt that biomedical science and patient care will benefit from those efforts that are made. Studies in xenotransplantation have provided a context for testing anti-inflammatory agents (Pruitt and others 1992; Miyagawa and others 1993; Zehr...
and others 1994; Magee and others 1994) and at least one patient’s life has been saved by temporary perfusion of xenogeneic livers to correct metabolic abnormalities associated with fulminant hepatic failure (Chari and others 1994).

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For want of a nail the shoewas lost... Herbert, G. 1651.

INTRODUCTION

Organ transplantation is now an accepted, and in many instances necessary, mechanism for the preservation of human life when disease has resulted in the loss of organ function. The exact number of organs required for transplants is probably unknown, but there is unquestionably an insufficient supply of human organs, and another source or method is required. Hilts (1993) estimated that approximately 60,000-80,000 lives could be saved if organs were available. According to the United Network for Organ Sharing, in 1994 there were over 37,000 registrants for organ transplants; approximately half will die for lack of an available organ. It has been suggested that improving the logistics of delivery and matching of supply and need would be helpful in supplying human organs. Mechanical replacement is currently an inadequate option, and it is not a permanent solution. With limited human organ sources, what are the alternatives?

Leaving social and ethical aspects aside, animals could create a potential supply of temporary or perhaps permanent organs for transplantation. Assuming that xenotransplantation is viable, numerous queries enter into such a decision. For example, are infectious diseases acquired from transplanted animal organs and are body fluids the major conceivable danger? Humans are susceptible to animal-associated diseases (zoonoses). As it would appear that there is little choice between human and animal organs for transplants, and that there is an insufficient supply of human organs, problems with animal organs must be resolved.

Of the animals under consideration as organ donors, nonhuman primates and swine are most frequently suggested. Currently, nonhuman primates, because of their phylogenetic relationship to humans, are preeminent as suitable organ donors. When contemplating the nonhuman primate, anxieties that continue to counter their usage are fears engendered by the possible presence of infectious agents. It would appear, however, that “the jury is still out” with regard to this concept. Brack (1987) provides a background of infectious agents present in nonhuman primates. A potential for humans to become infected as a result of heterotransplantation exists and has been discussed in numerous reports (see references). Conceivable transfer of infectious agents as a result of xenotransplantation using nonhuman primates or pig sources is reviewed in Cooper and others (1991), Kalter (1991), and more recently by Michaels and Simmons (1994). Hardy (1989) does not consider infectious diseases.

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