Physiologic and Immunologic Hurdles to Xenotransplantation

BENJAMIN SAMSTEIN*†‡ and JEFFREY L. PLATT*
Departments of *Surgery, †Immunology, and ‡Pediatrics, Mayo Clinic, Rochester, Minnesota.

Abstract. The major problem in the field of renal transplantation is currently the shortage of available kidneys. However, the use of animals as a source of kidneys, i.e., xenotransplantation, is increasingly being viewed as a potential solution to this problem. One preeminent hurdle to xenotransplantation is the immune response of the recipient against the graft; other hurdles include the physiologic limitations of the transplant, infection, and ethical considerations. This review summarizes what is currently known regarding the obstacles to xenotransplantation and some potential solutions to those problems.

Organ transplantation has been one of the preeminent successes in the field of medicine. An experimental procedure 30 yr ago, organ allotransplantation is now the preferred treatment for failure of the kidney, heart, liver, and lungs and, some would advocate, the pancreas. The 1-yr survival rate for patients receiving primary kidney transplants exceeds 95% (1), and this survival rate seems to be significantly better than the survival rate for patients treated with chronic dialysis (2).

The success of organ transplantation is such that the major problem facing the field today is neither the technical hurdles nor the medical complications but the shortage of available organs. The number of patients on the waiting lists for transplantation has increased steadily, whereas the rate of organ donation has remained constant (1). Because of this problem, the use of animals in lieu of human subjects as a source of organs, i.e., xenotransplantation, is increasingly being viewed as a potential approach to organ replacement (3).

In addition to overcoming the aggregate shortage of organs for transplantation, xenotransplantation offers several potential advantages compared with allotransplantation. First, an animal organ transplant might be, in some cases, less susceptible to the recurrence of disease or the rapid destruction caused by pre-sensitization. Second, elimination of the donor shortage would allow transplantation to be considered for patients who, because of age or underlying medical diseases, would not have been offered organ transplantation based on current standards for listing. Third, the use of an animal source of organs would provide the opportunity to plan the transplant procedure; such planning would permit approaches to immune modulation and perhaps the establishment of tolerance by pretreatment of the recipient that would not be possible with human cadaveric sources. Fourth, the use of animals instead of human subjects, particularly human cadavers, as sources of organ transplants might avert the transmission of infectious agents such as Epstein-Barr virus and cytomegalovirus, which are major causes of morbidity after allotransplantation.

Although the potential advantages of xenotransplantation generate enthusiasm, these advantages must be weighed against what may seem to be daunting hurdles to the clinical application of xenotransplantation. These hurdles include the immune response of the recipient to the transplant, the physiologic limitations of the transplant, infection, and ethical concerns. This review summarizes what is currently known regarding the hurdles to xenotransplantation and some potential solutions to those hurdles.

Early Experiences with Xenotransplantation

The technical ability to perform renal transplantation was achieved in the early years of the 20th century, when experimental surgeons such as Alexis Carrel, Charles Guthrie, Erich Ulmann, and Matheiu Joubolay developed the ability to connect the cut ends of blood vessels, i.e., vascular anastomosis (4). Although vascular anastomosis made transplantation technically feasible, it was unclear how human organs could be obtained for transplantation because there was no generally accepted definition of brain death. Thus, the initial attempts to perform transplantation in human subjects involved xenotransplants of the kidney (4). In 1906, Joubolay performed two kidney xenografts (using a pig and a goat as the sources of the transplants), in which the organ was connected to the arm or thigh of patients with chronic renal failure. These kidneys functioned only briefly (4). The lack of success in these efforts and a few subsequent attempts at allotransplantation discouraged the application of transplantation for decades.

Nearly 60 yr later, the development of immunosuppression finally allowed kidney allotransplantation to be performed effectively. Questions concerning the source of organs remained, because there were no generally applied laws regarding brain death that would allow the ready use of cadaveric sources. Therefore, for six patients for whom no human kidneys were available, Reemtsma et al. (5) transplanted kidneys from chimpanzees. An immunosuppression regimen consisting of azathioprine, actinomycin C, steroids, and graft irradiation...
was used. One recipient, a 23-yr-old female schoolteacher, was maintained for 9 mo by the xenotransplant. She died as a result of an illness of undetermined cause, and the xenograft appeared normal at the time of her death. Although this result might have been encouraging, other attempts at using nonhuman primates were less successful (6,7).

**Donor Sources**

One central question is which species would be the most suitable for use as a source of organs for xenotransplantation. Given the results achieved by Reemtsma et al. (5), particularly the survival and function of renal xenografts for periods of months, and given the revolutionary advances in immunosuppression and antimicrobial therapy in the past 37 yr, it might be intuitive that primates would be the best source of kidneys for xenotransplantation. However, for several reasons, investigators now focus on other animal sources, particularly pigs. First, pigs are sufficiently plentiful to provide any conceivable number of organs needed, whereas primates are available in only small numbers and chimpanzees are an endangered species. Although some nonhuman primates, such as baboons, might be numerous enough to meet a significant fraction of the need, these animals tend to be significantly smaller than human subjects, which limits their usefulness for adults. Second, primates may harbor infectious agents such as herpes virus B, which can be lethal for human subjects, whereas pigs can be bred in such a way that severe pathogens are excluded. Third, unlike nonhuman primates, pigs can be genetically manipulated to express extrinsic genes, which may address some of the biologic hurdles to xenotransplantation. The sections that follow focus on the hurdles to transplantation of porcine kidneys into human recipients.

**Biologic Hurdles to Xenotransplantation**

A kidney transplanted from one species, such as pigs, into a distant species, such as human patients, is subject to various types of rejection, as listed in Figure 1. The types of rejection observed for xenotransplants are the same as those observed for allotransplants; however, the incidence and severity are much greater for xenotransplants.

**Hyperacute Rejection**

Porcine organs transplanted into immunocompetent primates are subject to very rapid failure because of hyperacute rejection (8). Hyperacute rejection is thought to occur in nearly every organ transplanted between widely disparate species. Combinations of donor and recipient species in which hyperacute rejection regularly occurs are called “discordant,” and combinations of donor and recipient species in which hyperacute rejection rarely occurs are called “concordant” (9). Hyperacute rejection was originally described for human kidney allografts transplanted into recipients with circulating antibodies against the donor (10). In retrospect, hyperacute rejection was probably first observed in the early years of the 20th century when experimental kidney xenografts were attempted between disparate species.

The clinical features of hyperacute rejection are dramatic. Evidence of hyperacute rejection is usually observed upon reperfusion of the graft (11). The graft rapidly develops a beefy red or even blue appearance and swells. An early sign of hyperacute rejection is a dramatic decrease in blood flow to the transplanted organ. The kidney xenograft may emit a small volume of urine, but urine output invariably slows and then ceases in conjunction with the changes in appearance.

The pathology for hyperacute rejection is characterized by formation of platelet thrombi in small blood vessels (including glomerular capillaries), interstitial hemorrhage, and severe injury to endothelial cells (Figure 2, A and B). The immunopathology of hyperacute rejection is characterized by deposits of Ig and complement of the recipient along endothelial surfaces of graft blood vessels.

The first step in the pathogenesis of hyperacute rejection is the binding of xenoreactive natural antibodies, so named because they recognize the cells of foreign species and because they are present in the circulation without a known history of exposure to the corresponding antigen (12). The natural antibodies in human subjects that bind to pig cells are predominantly directed against galactose-α-1,3-galactose (Galα1,3Gal), a saccharide expressed on the cells of lower mammals but not on the cells of humans, apes, and Old World monkeys (13,14). These natural antibodies are not present at birth but develop soon thereafter and are present in all humans (15). The antibodies, which recognize Galα1,3Gal, represent 85 to 95% of xenoreactive natural antibodies (16). Elimination of anti-Galα1,3Gal antibodies can prevent hyperacute rejection (17).

Binding of xenoreactive natural antibodies activates the complement system, although activation of complement might, in principle, also occur via the alternative complement pathway; however, spontaneous activation of complement does not seem to occur in porcine organs transplanted into baboons and Old World monkeys (and presumably humans) (18). Activated complement causes endothelial cells to retract, leading to exposure of the underlying matrix. This disruption of the endothelial monolayer results in the loss of vascular integrity and
stimulates the adhesion and activation of platelets, leading to the typical pathology observed in hyperacute rejection.

One reason why xenografts may be especially susceptible to hyperacute rejection is because complement regulatory proteins of the graft may be incompatible with the complement system of the recipient (19). Complement activation is normally controlled by regulatory proteins on the surfaces of cells, which serve to limit the damage produced by activated complement. These complement regulatory proteins include decay-accelerating factor, membrane cofactor protein, and CD59. These proteins are inefficient in regulating the activity of complement of different species; therefore, porcine complement regulatory proteins do not effectively inhibit activated primate complement. This lack of function between pig and primate proteins contributes significantly to rejection, because it allows the activation of complement to proceed rapidly and unchecked.

Some recent work suggests that hyperacute rejection may not occur as frequently as previously thought for some combinations of donor and recipient species (20,21). However, these observations may reflect the use of very small primates without the high level of xenoreactive natural antibodies observed in humans (22).

Prevention of Hyperacute Rejection

Hyperacute rejection was once viewed as an absolute hurdle to xenotransplantation and the major barrier to the transplantation of porcine organs, such as kidneys, into human patients. Recent years have brought significant progress in overcoming this problem; therefore, hyperacute rejection is no longer considered such a daunting challenge. Indeed, three strategies have proven effective in the prevention of hyperacute rejection of kidney xenografts.

The first strategy for preventing hyperacute rejection of xenografts involves the depletion of xenoreactive antibodies from the circulation of the recipient. Depletion of xenoreactive antibodies was first accomplished by Perper and Najarian (23), who perfused the blood of experimental animals through the kidneys of potential sources of xenografts. Subsequently, Alexandre et al. (8) depleted xenoreactive antibodies and thus prevented hyperacute rejection of porcine kidney xenografts by performing plasmapheresis for baboons; however, the possibility that other elements of the blood were also depleted cannot be excluded. Recently, Lin et al. (24) and Sablinski et al. (25) demonstrated that specific depletion of xenoreactive antibodies, using affinity columns bearing Galα1,3Gal, could reliably prevent hyperacute rejection in pig-to-baboon xenograft models.

The second strategy for preventing hyperacute rejection of xenografts involves the depletion or inhibition of complement. Complement depletion has been accomplished with the administration of cobra venom factor (26), which activates and thus consumes complement. Unfortunately, cobra venom factor engenders an immune response, which can limit its duration of action. More recently, soluble CR1, a recombinant protein that inhibits the complement cascade at the level of C3 and C4 (27), has been demonstrated to effectively prevent hyperacute rejection of porcine kidneys by baboons (28). One limitation of cobra venom factor and soluble CR1 is that, by interrupting the complement cascade at the level of C3, these agents impair the ability of the treated individual to opsonize microorganisms and they thus confer a significant risk of pyogenic infection. An approach that, in principle, confers less risk is the administration of anti-C5 antibodies that inhibit the cleavage of C5, interrupting the complement cascade after the step at which opsonization occurs (29,30). In addition to these agents, there is a growing list of agents that might be used to interfere with complement; readers may refer to a recent review of this subject (31).

A third strategy for preventing hyperacute rejection, and the strategy that has emerged as the standard, is the use of organs that express complement regulatory proteins compatible with the recipient. Readers may recall from the discussion above that xenografts are especially susceptible to complement-mediated injury because complement control proteins (such as decay-accelerating factor and CD59) in the organ, which would ordinarily protect the organ from injury by complement, function poorly against complement of foreign species. To
overcome this obstacle, transgenic pigs expressing human complement regulatory proteins have been generated (32). Organs from pigs transgenic for human CD59 and human decay-accelerating factor, or human decay-accelerating factor alone, did not undergo hyperacute rejection (33), even when the level of expression was significantly lower than that in human organs (34,35). Because expression of the transgene does not influence the activity of complement in the circulation of the recipient and because expression presumably continues as long as the organ remains viable, this strategy is preferred.

A fourth strategy for preventing hyperacute rejection has been proposed but has not yet been tested. This strategy involves the development of lines of pigs that express lower levels of antigen (36,37). Efforts in this area are discussed in the next section.

Using transgenic pigs and antibody depletion, hyperacute rejection has been overcome. Once considered the most vexing problem for xenotransplantation, hyperacute rejection is now not a major factor preventing the use of porcine organs for human patients experiencing end-stage organ failure.

Acute Vascular Rejection

When hyperacute rejection is averted, xenografts become subject to acute vascular rejection (38,39), so named because of its similarity to acute vascular rejection of allografts (40). Acute vascular rejection is currently the primary obstacle to the use of porcine organ xenografts for human subjects. Acute vascular rejection has been confused with hyperacute rejection; in fact, some have referred to acute vascular rejection as “delayed” xenograft rejection, but acute vascular rejection is a distinct entity clinically and pathogenetically.

Acute vascular rejection is histologically characterized by focal ischemia, fibrinoid necrosis, and diffuse intravascular thrombosis, with the thrombi consisting mainly of fibrin (39–41) (Figure 2C). Some, but not all, cases of acute vascular rejection exhibit infiltrating cells. These cells may include natural killer cells and macrophages. Endothelial cell swelling and activation are observed in acute vascular rejection (42). Xenografted kidneys undergoing acute vascular rejection may also demonstrate acute tubular necrosis, with degenerating proximal tubules and necrotic epithelium (21). Glomeruli exhibit solidification, with loss of capillary patency, as a result of endothelial swelling and thrombi (43).

Which factors initiate acute vascular rejection has been the subject of debate. Various factors thought to play causative roles are listed in Table 1. Of these factors, xenoreactive antibodies are now widely considered to play a critical role in the pathogenesis of acute vascular rejection. A number of observations led to this conclusion. The onset of acute vascular xenograft rejection coincides with a striking increase in the synthesis of xenoreactive antibodies after exposure to porcine tissues (44). Attempts to reduce antibody and complement levels have resulted in significantly improved graft survival rates (45). In fact, using transgenic pigs and anti-Ig columns to deplete xenoreactive antibodies, acute vascular rejection has been prevented in a pig-to-baboon model (17). Additional evidence for the role of antibodies in acute vascular rejection is the finding that a similar type of rejection is observed among allograft recipients in association with the development of anti-donor antibodies.

How xenoreactive antibodies might cause acute vascular rejection is being studied in a number of laboratories. Most investigators think that xenoreactive antibodies trigger acute vascular rejection by activating endothelial cells. Xenoreactive antibodies activate endothelial cells via three potential mechanisms. First, endothelial cell activation might be induced by the binding of antibodies to cell surface antigens such as integrins, which can transduce signals (46). Second, xenoreactive antibodies could activate complement in small amounts, leading to activation of endothelial cells (47). Third, xenoreactive antibodies could serve as targets for Fc-bearing cells, which might in turn activate endothelial cells (48).

Some authors have suggested that macrophages, natural killer cells, and neutrophils may contribute to the pathogenesis of acute vascular rejection (42,49,50). The role of macrophages is supported by the observation that acute vascular rejection in rodents is associated with the influx of macrophages expressing tissue factor (42). Natural killer cells have also been identified in xenografts undergoing acute vascular rejection (42). Natural killer cells are capable of binding directly to and inducing morphologic changes in and activation of porcine endothelium (51). It is thought that host natural killer cells, whose responses are normally limited by MHC I molecules, may not recognize the MHC I molecules of pigs, which are termed swine leukocyte antigen I molecules. Neutrophils are occasionally observed during acute vascular rejection (38). Zehr et al. (50) observed that an inhibitor of CD11b/CD18 expression lessened the microvascular injury in acute vascular

<table>
<thead>
<tr>
<th>Component</th>
<th>Mechanism</th>
<th>Authors (Ref.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody</td>
<td>Activates endothelium directly or via complement</td>
<td>Palmetshofer et al., 1998 (109)</td>
</tr>
<tr>
<td>Complement</td>
<td>Activates endothelium, promotes inflammation</td>
<td>Saadi et al., 1995 (110)</td>
</tr>
<tr>
<td>Platelets</td>
<td>Release thromboxane A2, induce tissue factor and E-selectin expression</td>
<td>Bustos and Platt, 1997 (111)</td>
</tr>
<tr>
<td>Monocytes</td>
<td>Express tissue factor</td>
<td>Blakely et al., 1994 (42)</td>
</tr>
<tr>
<td>Natural killer cells</td>
<td>Activate endothelium</td>
<td>Malyguine et al., 1996 (51)</td>
</tr>
<tr>
<td>Molecular incompatibility</td>
<td>Decreases fibrinolytic activity</td>
<td>Lawson et al., 1997 (54)</td>
</tr>
</tbody>
</table>
rejection, although only minimally. However, these cellular infiltrates are often not observed in acute vascular rejection with pig-to-primate transplants (39). Moreover, recent studies in rodents demonstrated that the inflammatory cells accumulate after tissue damage has occurred (52). Therefore, inflammatory cells may be markers of tissue injury, rather than the cause of acute vascular rejection.

An additional factor in acute vascular rejection may be physiologic incompatibilities of porcine proteins, such as thrombomodulin and protein C, with human proteins (53,54). Such incompatibilities may establish a thrombotic diathesis and contribute to a procoagulant state involved in acute vascular rejection. However, because depletion of xenoreactive antibodies using absorption columns, without manipulation of the coagulation system, can prevent acute vascular rejection, as mentioned above, it is possible that these incompatibilities have limited effects on the fate of the transplant.

**Prevention and Treatment of Acute Vascular Rejection**

The prevention and treatment of acute vascular rejection now represent a central objective in xenotransplantation. For the most part, approaches are based on the current understanding of the pathogenesis of acute vascular rejection, as summarized in Table 1.

One approach to the prevention of acute vascular rejection involves depletion of xenoreactive antibodies from the recipient. Depletion of xenoreactive antibodies for this purpose has been achieved using immunoadsorption columns in conjunction with plasmapheresis (55,56). Effective application of immunoadsorption must be accompanied by immunosuppression, to prevent rebound of xenoreactive antibody levels (55,56). The approach used in our laboratory includes splenectomy and treatment with cyclophosphamide, which inhibits antibody synthesis. These methods are clearly associated with significant morbidity.

Depletion of anti-donor antibodies may allow the induction of accommodation. Accommodation is a term used to describe the apparent resistance of an organ graft to acute vascular rejection (19). Accommodation was first observed in the transplantation of ABO-incompatible kidney allografts, where it was produced by temporary depletion of anti-donor antibodies (57,58). In accommodation, the return of anti-donor antibodies to the circulation does not cause graft injury, although the corresponding antigen continues to be expressed. The mechanisms of accommodation are not certain; explanations include decreased antibody-antigen interaction, decreased sensitivity of the endothelium to stimulation by complement and antibodies, and the expression of “protective genes” in the endothelium (59). In a rodent model of discordant xenotransplantation, temporary depletion of complement and immunosuppression with cyclosporine resulted in long-term survival of heart xenografts in 75% of the animals, which was attributed to accommodation (60). Surviving hearts exhibited recipient antibodies and complement deposited on their endothelium. Bach and co-workers (60,61) proposed that accommodation is produced by the expression of protective genes, including A20, bcl-2, bcl-xL, and HO-1. Whether these genes play a causative role in accommodation has not yet been determined.

Another approach to the prevention of acute vascular rejection is induction of tolerance, leading to decreased production of antibodies against the graft. Tolerance could play a role in the prevention of both acute vascular rejection and cellular rejection. One method that has been proposed as a means to induce tolerance is to engraft bone marrow cells expressing xenogeneic antigens (62,63). The enduring engraftment of donor hematopoietic cells has proven to be difficult for widely disparate species because of incompatibilities of the bone marrow microenvironment and growth factors (64). Bracy et al. (65) used an alternative approach, which might overcome this problem; they used a retroviral vector to express α1,3galactosyltransferase in murine bone marrow cells and then transplanted those cells into isologous glucosyltransferase knockout mice, which, like humans, produce anti-Galα1,3Gal antibodies. The transplantation of α1,3galactosyltransferase transduced bone marrow led to decreased production of anti-Galα1,3Gal antibodies.

Acute vascular rejection might, in principle, be addressed by genetic engineering of source animals to reduce the expression of antigen. The prospects for the success of this approach have been advanced by evidence that the antibodies that cause acute vascular rejection are directed against Galα1,3Gal. Recent advances in nuclear transfer techniques have allowed the production of pigs cloned from adult somatic cells (66). In principle, the α1,3-galactosyltransferase gene could be targeted in the somatic cells by homologous recombination. Gene targeting in sheep and the successful development to term of ovine offspring with targeted cells have been achieved, supporting the feasibility of such an approach (67). Targeted disruption of the α1,3-galactosyltransferase gene has been achieved in primary porcine cells (66). These advances raise the possibility that pigs lacking Galα1,3Gal expression might be produced.

One major limitation to gene targeting is that it might increase the risk of infection by porcine viruses (68). Efforts to decrease antigen expression have thus far used methods other than gene targeting to decrease antigen levels. Strategies have included the expression of α1,2-fucosyltransferase, which catalyzes the formation of a nonantigenic sugar (37,69) or competes with α1,3-galactosyltransferase, and the expression of αgalactosidase, which cleaves the terminal Gal residue, yielding a less immunologically reactive saccharide. In an in vitro model, cells expressing α1,2-fucosyltransferase and αgalactosidase reduced expression of the Galα1,3Gal epitope to negligible levels and reduced reactivity with human serum to background levels (36). However, application to an in vivo model has been difficult because the addition of other transgenes decreases the expression of previous transgenes. Although further improvements will undoubtedly allow further reduction of Galα1,3Gal expression, the development of xenoreactive antibodies against antigens other than Galα1,3Gal, particularly porcine MHC antigens, will pose a continuing challenge.
Cellular Rejection

If hyperacute rejection and acute vascular rejection were prevented, then renal xenografts would be subject to cellular rejection. Cellular rejection is the most common form of rejection observed for allografts, but it has not been well characterized in xenografts, and it is not clear how similar such rejection would be to the cellular rejection of allografts. Cellular rejection of allografts is characterized by interstitial infiltrates of T cells and macrophages. Infiltrates of T cells, macrophages, and natural killer cells may characterize cellular rejection of xenografts.

One aspect of the cellular immune response to xenotransplants that has drawn attention in the past decade is the mechanism by which T cells of the recipient recognize donor antigens. The T lymphocytes, which cause acute cellular rejection of allografts, recognize predominantly MHC antigens. In doing so, alloreactive T cells recognize donor antigen by the direct pathway, with the T cell receptors of the T cells of the recipient binding directly to the MHC molecules expressed on donor cells. It was once thought that the direct pathway or recognition is deficient in xenogeneic responses, and there was thus some hope that cellular rejection of xenografts would be milder than cellular rejection of allografts. However, recent work indicates that human T cells recognize porcine cells by the direct pathway (71,72). Human T cells can also recognize xenografts and allografts by the indirect pathway, in which donor antigen is degraded to peptides and presented in association with recipient MHC molecules on recipient antigen-presenting cells. In fact, the cellular response to a xenograft may be markedly stronger than the cellular response to an allograft (73).

The cellular immune response to a xenograft may well be more potent than the cellular immune response to an allograft, for three reasons. First, a xenotransplant, in contrast to an allotransplant, may give rise to a vast array of foreign peptides, stimulating a strong T cell response. Dorling et al. (73) demonstrated that indirect presentation of porcine antigens led to a detectable primary response in cell cultures, whereas such responses were not detected with allogeneic combinations. Second, the humoral response to xenotransplants might amplify the cellular response. Kodaira et al. (74) observed that complement activation and anti-endothelial cell antibodies caused the release of heparan sulfate from the surface of endothelial cells and heparan sulfate acted on antigen-presenting cells, resulting in increased proliferation and cytolytic T cell responses. Third, immunoregulatory responses that might depend on the compatibility of donor and recipient cytokines or direct recognition by suppressor leukocytes (75) might be impaired in xenografts (76). It is possible that xenografts would not collaborate in the immunoregulatory function of an immune response, thus leading to more destructive outcomes.

Prevention of Cellular Rejection

A critical and still unanswered question is whether the prevention and treatment of cellular rejection of xenografts will require strategies different from those used, with some success, for allografts. Immunosuppression is currently quite effective in preventing cellular rejection of allografts. Whether the same immunosuppressive regimens will be adequate to prevent and/or treat cellular rejection of xenografts is not known.

One consideration in preventing or treating cellular rejection of xenografts is whether currently available immunosuppressive drugs would suffice. Rapamycin, which was recently approved for clinical use, potentiates the effects of cyclosporine A (77) and may enable tolerance to be induced (78,79). Deoxyspergualin, which affects macrophage and T and B cell function, has been demonstrated to be effective in reducing natural antibodies in a xenograft model (80). The potent ability of deoxyspergualin to suppress humoral immunity could be useful in xenotransplantation. Leflunomide inhibits proliferation of T and B cells and has been demonstrated to inhibit xenograft rejection in rodent models (81). Simultaneous blockade of the costimulatory CD28 and CD40 pathways was recently observed to prolong survival of pig-to-mouse skin and rat-to-mouse cardiac xenografts (82). Indeed, blockade of both pathways ablated IgG responses to xenotransplantation (82). However, blockade of the CD28 and CD40 pathways was not able to induce indefinite survival of xenografts.

In principle, another way to prevent cellular rejection of a xenograft would be to induce immunologic tolerance against the xenograft. Some authors have argued that, given the intensity of immune responses to xenotransplantation, tolerance induction would be necessary for the clinical application of xenotransplantation. However, it is not known how tolerance can be safely induced for this purpose. Several methods of tolerance induction have been developed for allotransplantation; however, tolerance has not been induced in pig-to-primate models.

Tolerance induction in primate allotransplantation has been approached using various methods, i.e., blockade of costimulation using monoclonal antibodies against CD40L (78), use of an immunotoxin that leads to T cell depletion (83), T cell depletion combined with donor bone marrow infusion (84,85), and establishment of mixed chimerism (43). Of those methods, the latter two, which involve infusion of donor bone marrow or donor stem cells, seem most useful for xenotransplantation, because they might suppress humoral responses to Galα1,3Gal as well as cell-mediated immune responses. Most attention has been directed to the establishment of mixed chimerism. Mixed chimerism aims to alter the immune repertoire of the recipient by transplantation of donor bone marrow, which migrates to the thymus and causes the deletion of donor-reactive T cells. Establishment of mixed chimerism through the transplantation of donor bone marrow is a considerable challenge, which requires “conditioning” of the recipient with whole-body irradiation, anti-T cell antibody treatment, thymic irradiation, and splenectomy, with or without a short course of postoperative immunosuppression (86). Although allograft survival greater than 4 yr has been achieved by establishing mixed chimerism (43), efforts to combine the induction of mixed chimerism with immunoabsorption of xenoreactive antibodies have thus far been unsuccessful in a pig-to-primate model, with survival times of only 15 d (43). Although none of the regimens mentioned above yielded tolerance in pig-to-primate xeno-
grafts, it is possible with further modifications that one of these regimens will be applicable to xenotransplantation in the future.

Physiologic Hurdles

Xenografted organs must meet the physiologic demands of the recipient and function cooperatively with the other organs. Preliminary evidence suggests that a porcine kidney transplanted into a primate would function adequately to maintain the water, electrolyte, and acid/base balances. Pig kidneys are of very similar size and structure, compared with human kidneys (87). The maximal concentrating ability of porcine kidneys (1080 mosmol/liter) and the GFR (126 to 175 ml/h) are quite similar to those of human kidneys (87).

There is limited information concerning the function of porcine kidneys in primates. Sablinski et al. (25) demonstrated survival times of up to 15 d for porcine kidneys in anephric cynomolgus monkeys, with creatine levels ranging from 0.8 to 1.3 mg/dl until the onset of rejection.

Alexandre et al. (8) transplanted porcine kidneys into baboons that had been subjected to bilateral nephrectomy. The recipients underwent preoperative plasmapheresis and splenectomy just before xenotransplantation. Three of the five baboons with porcine kidney xenografts survived more than 10 d. The longest survivor (which survived for 23 d) succumbed to vascular rejection. In these xenograft recipients, the serum creatine levels remained below 2.0 mg/dl until rejection occurred. The baboons required blood transfusions to maintain hemoglobin levels above 8 g/dl, suggesting the possibility that porcine erythropoietin might not be fully compatible with erythroid-lineage cells of baboons (8). The electrolyte levels, weight, and acid/base status were not reported (8).

The best evidence concerning the function of porcine kidneys in primates can be found in the work of Zaidi et al. (88). Using transgenic pigs that expressed human decay-accelerating factor as a source of kidney xenografts, Zaidi et al. (88) achieved graft survival times of up to 78 d in nephrectomized cynomolgus monkeys. The monkeys received immunosuppression consisting of cyclophosphamide, cyclosporine, corticosteroids, and splenectomy (88). When the monkeys were functioning, potassium, sodium, and creatine levels and the acid/base balance were within normal limits (88). Body weight and fluid balance were apparently maintained (88). In summary, the studies on xenotransplantation of porcine kidneys into primates that have been performed to date suggest that the major factor limiting kidney function is tissue injury resulting from rejection and not intrinsic differences in renal function between species.

An area in which xenotransplants may not completely replace human organs is in the interaction of hormones or complex protein cascades with the recipient. Sen et al. (89) demonstrated that heterologous renin is much less effective in eliciting the release of angiotensin than is homologous renin. We recently demonstrated that porcine thrombomodulin interacts poorly with human tissue factor and human protein C, making a porcine xenograft a potential trigger for the coagulation cascade of the recipient (90). The ability of porcine erythropoietin to function in a human milieu is uncertain; however, there is an indication, as noted above, that porcine erythropoietin is insufficient to stimulate red blood cell production (21). Thus, cynomolgus monkeys with porcine kidney xenografts experienced severe anemia despite the production of porcine erythropoietin, and human erythropoietin was required to restore the hematocrit levels of the monkeys (21).

If physiologic disturbances prove to be hurdles, it is possible that genetic engineering might be used to address such problems. For example, if erythropoietin incompatibility is a serious problem, then transgenic pigs that express human erythropoietin might be developed. As another example, genetic manipulation might be used to generate pigs that express human proteins from the coagulation cascade, to prevent coagulation.

Infection

Infectious disease contributes significantly to the morbidity and death of transplant recipients. The potential use of animals as a source of organs has increased the concerns regarding infection (91). Indeed, transfer of the influenza virus from pigs to human subjects in 1918 is thought to have sparked a pandemic that led to millions of deaths.

Issues involving the transmission of potential infectious organisms have led to the coining of new terms to describe infections potentially transferred from animal organs into human subjects. “Zoonosis” refers to an infectious disease transmitted from an animal to a human subject under “natural” conditions, such as an animal bite; the terms “xenosis” and “xenozoonosis” were developed to describe infectious illnesses introduced into human subjects through medical procedures involving xenogeneic tissue (92,93). Whether distinct terms are needed, however, is unclear.

Concerns regarding xenotransplantation fall into two categories. First, microbes endemic to the donor species might infect human subjects, especially immunosuppressed transplant recipients. Second, there is the possibility that xenotransplantation could lead to the generation of novel pathogens created by recombination of porcine retroviruses with elements of the human genome. Both concerns focus on the potential complication of infection of the recipient and the potential spread of infection to others in the population. However, it is only the second situation, i.e., the generation of novel pathogens, that might be unique to xenotransplantation. In any case, evidence gathered to date, as summarized below, suggests that the risks to patients and the public are low.

For transplant recipients, the use of xenografts might well result in fewer problems attributable to infectious organisms. The urgent nature of donor-recipient matching can result in the transplantation of organs with Epstein-Barr virus or cytomegalovirus into patients. The careful breeding and housing of animal donors could, in principle, prevent infection of the xenograft recipient by known pathogens. Therefore, xenografts might be “cleaner” than allografts, because the animals bred as a source of xenografts would be housed in pathogen-free environments and rigorously tested for known exogenous pathogens. Xenotransplants may also be less susceptible to
infection by human pathogens because animal organs frequently exhibit species-specific resistance to infection by many common human pathogens. For example, neither human hepatitis B nor HIV-1 is capable of infecting baboons or presumably pigs (94). Therefore, xenografts might be better choices in cases where infectious diseases have produced organ failure.

It is possible that some pathogenic organisms have yet to be identified. Therefore, some organisms may cause little or no morbidity in pigs but pose a risk to human subjects. For example, Hantaviruses, of which there are five known forms, produce no detectable morbidity or death in rodents, which constitute the natural reservoir; however, Hantaviruses can cause disease in human subjects, with mortality rates as high as 50% (91). Because pigs and human subjects have existed in close proximity for thousands of years, the risks of such unknown pathogens seem quite small. Two exceptions, however, may be a virus related to the human hepatitis E virus and a new torovirus identified in pigs (95,96).

Of greater concern, then, is the possibility of newly emerging pathogens developing in xenotransplant recipients and spreading to the general public. Concerns have focused particularly on endogenous retroviruses. Much significance has recently been attributed to the observation that porcine endogenous retrovirus (PERV), a type C retrovirus of pigs, is capable of infecting human cells (97). Wilson et al. (98) demonstrated that primary porcine cells (not a cell line) are capable of releasing PERV after activation, and the retrovirus is capable of infecting human cell lines. Porcine pancreatic islets transplanted into immunodeficient mice were recently demonstrated to be capable of causing transmission of PERV to the mice (99). However, the clinical relevance of this model is unclear, because the immune system of severe combined immunodeficient mice is much weaker than that of immunosuppressed human patients and mice may be more receptive to infection than human subjects (68). All pigs tested to date contain multiple copies of PERV in the genome, making breeding-out of the virus nearly impossible (100). However, a number of recent studies have failed to detect evidence that PERV can be transferred from porcine cells to human cells in vivo (101–104). For example, Paradis et al. (103) studied 160 patients with exposure to living pig tissue, including 36 patients with immunosuppression. Despite evidence of porcine cells surviving in the human subjects for up to 8.5 yr, there was no evidence of PERV infection, using PCR analysis. These studies have thus tended to diminish concerns regarding cross-species infection in xenotransplantation. However, infectious disease will always be a concern in transplantation because transplant recipients undergo immunosuppression, and differences in MHC antigens may impair the host response to infection in the transplant.

**Making Xenotransplantation a Clinical Reality**

Assuming that the biologic hurdles to renal xenotransplantation can be overcome, the next critical question is which patients would be most suitable as recipients of xenotransplants. This question is particularly challenging with respect to the kidney, because dialysis is an alternative to transplantation. However, the availability of dialysis might make xenotransplantation of the kidney appealing as an experimental procedure because the risk to the recipient would be lower than in the case of other types of xenotransplants.

We envision that xenotransplants might be considered initially for certain small groups of patients. One group that might be appropriate to consider for renal xenotransplantation consists of patients who are presensitized to multiple potential donors and thus are unlikely to receive an allograft. The use of xenotransplantation for such patients presumes that anti-HLA antibodies would not cross-react with porcine major histocompatibility antigens (swine leukocyte antigens). A second group that might be appropriate to consider for renal xenotransplantation consists of infants with kidney failure, for whom dialysis is very difficult. Such infants are also difficult to treat with transplantation because of the mismatch in size between adult kidneys and infants. A xenograft could be used in selected cases to allow the infant to grow to a size more suitable for allotransplantation. A third group that might be appropriate to consider for renal xenotransplantation consists of patients with hyperoxaluria. A fourth group might be young HIV-positive patients with HIV-associated nephropathy. A survey of 148 kidney transplant centers in the United States revealed that less than 10% would consider patients with HIV for allotransplantation and none of the 148 centers had performed a kidney transplant for a HIV-positive recipient in the past year (105).

**Ethical Considerations**

The prospective removal of an organ from one individual and its placement in a second individual evoked tremendous debate among scientists, physicians, and ethicists when allotransplantation was in its infancy. How organs could be obtained for transplantation (as discussed above), how transplantation would modify the sense of individuality of the recipient, and whether a living donor could be allowed to assume the risks involved in organ donation have stimulated debate. Today, however, the preeminent ethical question in the field of transplantation is how the very limited number of organs should be allocated. Clearly, the successful application of xenotransplantation would solve that ethical dilemma.

Xenotransplantation would raise new ethical questions. Issues related to xenotransplantation can be divided into three categories, as follows: (1) animal welfare, (2) clinical experimentation, and (3) public health implications. Although these issues are not unique to xenotransplantation, they should be considered here.

The question of animal welfare largely focuses on whether it is justifiable to use animals as a source of organs and tissues for transplantation into human subjects. Arguably, because society allows the use of animals for food, the use of animals as a source of organs or tissues is morally acceptable. It would be difficult to argue against the use of pigs for xenotransplantation, which might be the only option for the treatment of life-threatening diseases, and simultaneously countenance the use of pigs for food when nonanimal sources exist.

The ethical issues regarding clinical experimentation are partly related to the issue of informed consent. The issue of...
informed consent is particularly important for kidney transplantation because patients have other choices for therapy, such as dialysis. As with all new treatments, patients must be fully aware of all potential complications and risks. Related questions are how and to what extent patients should be allowed to assume risks. If these questions can be addressed, there is the additional question of how human organs would be allocated if xenotransplantation were an option.

The ethical issue that has received the most attention is the matter of public health. This ethical issue arises from the small risk that a xenograft might transmit to the recipient an infectious agent that has not previously been present in the human population. If, indeed, some infectious agent would warrant concern, the setting of xenotransplantation would offer an opportunity to identify and contain the agent and to develop appropriate preventative or therapeutic measures before the agent enters the human population by means other than a xenograft. Weighing an undetermined but small risk to the general population against clear and imminent risk to an individual is always difficult.

Conclusion

It seems likely that the movement of xenotransplantation into the clinical realm will be stepwise. The first step, i.e., free tissue grafts for the treatment of Parkinson’s disease and diabetes, has already begun (106,107). Porcine livers and hepatocytes are being used for ex vivo devices to treat patients with fulfillmen hepatic failure (102,108). The next critical step will be the use of xenotransplants as a bridge to allotransplantation. Whether such bridge transplants should include the use of porcine kidneys is an unresolved question. Experience with xenografts as bridges will provide information on physiologic and immunologic features and infectious disease. Xenografts would then presumably be used for patients who cannot receive allografts. These early trials would allow optimization of therapy. Finally, xenografts would replace allografts as the treatment for end-stage organ failure. Many of the questions regarding clinical xenotransplantation can be answered only by experience.

Acknowledgments

Our work is supported by grants from the National Institutes of Health. We thank Kim Barber for assistance with manuscript preparation.

References


Hurdles to Xenotransplantation


