Xenotransplantation and the Future of Renal Replacement

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Transplantation of the kidney first became feasible in the early years of the 20th century (1). Experimental surgeons had recently devised the vascular anastomosis as a way of repairing the cut end of blood vessels, and that advance created the field of vascular surgery (2,3). Those who sought to apply the vascular anastomosis were not satisfied with the concept that it might only solve the problem of traumatic vascular wounds for which it was ostensibly developed, but rather they thought the vascular anastomosis might be the critical technical advance needed to replace a sick organ with a healthy one (4). Hence, from the first development of the vascular anastomosis, those at the forefront sought to apply it to the transplanting of organs. Of course, it was not clear then how one could obtain an organ from a human for transplantation, as it was reasoned then that because some cellular components of the kidney and other organs often remain alive long after a person is dead, harvesting a kidney from a cadaver would be unethical. Because of this concern, the first application of the vascular anastomosis to replacement of organ function was conducted using animals—swine and sheep—as a source of organs instead of humans (5). Today there may be equally compelling reasons to consider the use of animals for augmentation or replacement of renal function. This communication will review those reasons, as well as how xenotransplantation might be applied and what barriers stand in the way of application.

The most important reason for interest in xenotransplantation is that relatively few human organs are available for transplantation. By some estimates, as little as 5% of the organs needed become available (6). In the case of the kidney, this shortage may be blunted by the use of living donors. However, the demand for transplantation for the treatment of kidney disease may soon change dramatically.

The demand for kidney transplantation could increase with the broader application of molecular diagnostics, genomics, and proteomics. These tools may soon make it possible to detect tumors and other lethal diseases before the diseases are clinically manifest and before the disease can be localized by imaging. Such early diagnoses will bring to the fore the potential for preemptive transplantation. To spare the patient from the risk of waiting until the tumor can be localized and the kidney carrying it removed, one will be tempted to remove both kidneys and treat the patient for renal failure. This approach is already used for infants with genetic diseases that presage development of Wilms’ tumor.

Another advance that could increase the demand for kidney transplantation or augmentation of renal function derives from the recent observation that small decreases in glomerular filtration or microalbuminuria correlate with risk of atherosclerosis and ischemic heart disease (7,8). This correlation could simply result from some effect of vascular disease on the kidney, rather than the converse. However, these observations and recent studies in mice (9) raise the possibility that the kidney performs some occult function such as clearance of insulin, metabolism of vitamin D, or secretion of a toxin that affects cardiovascular health. The latter would explain the increase in atherosclerosis and ischemic heart disease in those who receive a kidney transplant (and thus are uninephric) and in those on dialysis. If the kidney should be found to contribute to cardiovascular health and minor decrements in function to have a biologic effect, one would be tempted to offer kidney transplantation much earlier in the course of renal disease, transplants of a larger mass (two kidneys instead of one), and transplants to a much broader group of patients. And, in this instance, the standard for success would be very much higher than it is today.

Considering the prevalence of cardiovascular disease, this scenario could increase the demand for kidney transplants, and this in turn may generate new applications for xenotransplantation. Below I shall discuss three potential ways that xenotransplantation could address the need for kidney transplants and the hurdles to clinical application.

Xenotransplantation of the Kidneys

Xenotransplantation of the kidneys has been attempted at various times during the past century for replacement of renal function (1,5). Trials conducted in the 1960s that used nonhuman primates as a source of kidneys yielded function of xenografts for weeks to months (10), and there is every reason to think that vastly better, perhaps fully acceptable, results could be achieved today. However, nonhuman primates are not generally considered as a source of organs because, among other reasons, the animals are too small and too few to address the need. Instead, most who pursue xenotransplantation as a treatment for organ failure focus on other mammals, especially...
pigs, as a source of organs. Pigs are the preferred species for several reasons. These animals are suitable in size and available in large numbers. Because they are born in litters, pigs are readily bred and can be genetically engineered. Pigs can be raised free of known pathogens. However, daunting barriers obstruct the transplantation of porcine organs into humans.

**Barriers to Xenotransplantation**

The hurdles to xenotransplantation include rejection of the xenografted kidney by a powerful immune response of the recipient, physiologic limitations inherent to the kidney functioning in a foreign recipient, and the possibility of transferring infections from the graft to the host. Of these, the immune barrier is the most difficult.

**Immune Response to Xenotransplantation**

Xenotransplantation excites nearly every immune and inflammatory pathway of the host. Some of these pathways were reviewed recently (11). All foreign grafts excite cellular and humoral immune responses. The immune response to xenografts, however, is much more severe and difficult to control than the immune response to allografts. One especially difficult facet of the immune response to xenotransplantation is innate immunity, consisting of xenoreactive natural antibodies, complement, and natural killer cells reactive with xenografts. Not only can innate immunity destroy a xenograft, but it also amplifies adaptive immune responses (11). Another reason why immune responses to xenografts are severe is that xenografts carry a diverse set of foreign antigens, against which cellular and humoral immune responses can be elicited (in allotransplants, the main foreign antigens are MHC antigens) (12). Finally, immune responses to xenografts may be severe because immune regulation, which might partially control responses to allografts, may fail to do so in responses to xenografts.

**Effect of Immunity on the Fate of Xenografts**

Although xenografts arouse potent immune responses, the effect of those immune responses depends to a considerable extent on whether the graft consists of isolated cells or free tissues on the one hand or an intact organ such as the kidney on the other. This distinction will determine how xenotransplantation of the kidney is applied and for what condition. Grafts of isolated cells are nourished and maintained by the microenvironment, growth factors, and capillaries of the recipient. These components, particularly the recipient blood vessels, can establish a barrier between the immune system of the recipient and the foreign cells (13,14).

After the transplantation of isolated cells, the first biologic barrier is known as “primary nonfunction” (Figure 1) (15). We believe that primary nonfunction of xenogeneic transplants is caused by one or more of three factors: (1) the inability of growth factors of the recipient to support newly implanted cells and/or failure of graft factors to support angiogenesis by host vessels (16); (2) the action of natural killer cells or recently activated T cells on the newly implanted graft; and (3) the action of complement on xenogeneic cells and tissues introduced into the blood (e.g., pancreatic islets injected into the portal vein) (17). Primary nonfunction can be overcome in at least some models by increasing the number of cells transplanted.

The main hurdle to xenotransplantation of cells and tissues is cellular rejection. Cell-mediated immune responses to xenotransplantation are thought to be especially severe (12,18,19) and may, in our view, be further amplified by the humoral immune reactions and by failure of immune regulation between species (11,20). Some fundamental aspects of the cellular immune response to xenotransplantation have been reviewed by us (11,21) and by others (22,23). What is pertinent here is that despite the severity of cell-mediated rejection of cell and tissue transplants between disparate species, it appears to be subject to control by immunosuppressive agents currently available (24–27).

**Barriers to Xenotransplantation of Vascularized Organs**

Xenotransplanted organs such as the intact kidney are vascularized by blood vessels of the donor. These blood vessels, and particularly the endothelial lining of these blood vessels, is directly exposed to components of the immune system of the recipient, and it is the interaction of the immune system with donor blood vessels that gives rise to distinct types of vascular disease, which have, to this point, prevented the clinical transplantation of xenogeneic organs (Figure 1). The various types
of vascular rejection of xenografts is summarized in Figure 1 and recently reviewed by us (28).

Vascularized renal xenografts are initially subject to hyperacute rejection, which destroys a xenograft within minutes to a few hours (21,24,29). Hyperacute rejection is triggered by the binding of xenoreactive natural antibodies to Galα1-3Gal, a saccharide expressed by pigs and other lower mammals (30).

One factor that makes xenografts especially susceptible to hyperacute rejection may be that complement proteins in the graft such as decay accelerating factor and CD59, which would ordinarily protect the organ from complement-mediated injury, fail to do so because the proteins function poorly against human complement (21,31). Although Hinchliffe et al. (32) could find little evidence of specific specificity of these proteins, the profound susceptibility of xenografts to hyperacute rejection is compelling evidence that such specificity exists. In fact, the problem of hyperacute rejection was solved by the genetic engineering of pigs expressing human complement regulatory proteins. Thus, kidneys and hearts from pigs expressing human decay accelerating factor or membrane cofactor protein, even at very low levels, do not undergo hyperacute rejection when transplanted into baboons (33–35). These observations establish that hyperacute rejection can be addressed by genetic engineering and without manipulation of the recipient.

Unfortunately, hyperacute rejection is not the main barrier to xenotransplantation; when it is averted, a renal xenograft undergoes acute vascular rejection (36,37). Acute vascular rejection is the main hurdle to clinical application of xenotransplantation (38–40). It is triggered by xenoreactive antibodies that bind to the xenograft, activating small amounts of complement and thus causing “activation” of endothelium in the graft (36,41,42).

Because acute vascular rejection is thought to be the main obstacle to xenotransplantation of organs, much effort has been directed at developing the means to prevent or treat this disorder. Because the antibodies triggering acute vascular rejection during the early days after transplantation, some have tried to address the problem by devising strategies to remove or suppress synthesis of those antibodies or eradicate synthesis of the antigen. Among the strategies currently used to suppress the antibodies are administration of polymerized saccharide, which absorbs the antibody and in some way appears to inhibit synthesis (43). Another approach, although not one yet applied with success in primates, is induction of tolerance (44,45).

Eradicating the synthesis of the antigen became possible with the recent cloning of pigs (46–48) and progress in gene targeting (49). These technologies have been used to disrupt the α1,3galactosyltransferase gene that encodes the enzyme that adds a terminal galactose residue and thus completes the synthesis of Galα1-3Gal (50–52). This advance has been greeted with enthusiasm; however, initial experiments that used Gal knockout pigs have not suggested that the problem of acute vascular rejection has been solved.

Another approach to preventing acute vascular rejection involves the inducing of “accommodation.” First described in organs allografted across ABO blood group barriers (53,54) and later in experimental xenografts (21,55,56), accommodation appears to be induced by temporary suppression or depletion of antidonor antibodies. Because accommodation may be vital to the success of xenotransplantation and might be exploited for treatment or prevention of vascular disease, there has been much interest in understanding how it can be reliably induced and what mechanisms underlie it. Accommodation might be explained by a change in the properties of antidonor antibodies, a change in expression of donor antigen (57), or by acquired resistance of the graft to humoral injury. Of these, acquired resistance to injury is most consistent with biologic experiments conducted to date. For example, endothelial cells exposed to xenoreactive antibodies acquire resistance to complement-mediated injury owing to increased expression of CD59 (58) and other inhibitors of injury (59). Studies in rodents have shown that accommodation is associated with expression of genes, such as Bel-2, that inhibit apoptosis and hemoxygenase-1 (HO-1) and/or carbon monoxide confer protection against toxic injury (60,61). However, efforts to prevent vascular injury by expression of these genes may not be sufficient to induce a state of accommodation because grafts with increased expression of HO-1 and/or CD59 may still undergo acute vascular rejection (62,63) (unpublished observations). This suggests that accommodation is multifactorial and still incompletely understood. Although we here discuss accommodation in the context of acute vascular rejection, it is possible accommodation will be found to mediate resistance to other forms of tissue injury (64).

When acute vascular rejection is prevented, xenografts are susceptible to cellular rejection, as discussed above, and presumably to chronic rejection (65). However, as the discussion below will indicate, cellular rejection can probably be controlled with available immunosuppressive agents, and chronic rejection, if not presently treatable, could be addressed if necessary by replacing the graft.

Another hurdle to xenotransplantation of the kidney may be the physiologic function of the organ in a foreign host. Porcine kidneys transplanted into nonhuman primates do function sufficiently to sustain life (34,66). In fact, the main functional impairment of these grafts was from rejection. Subtle physiologic defects may impair the long-term well-being of the recipient. For example, if the kidney is found to promote cardiovascular health, as discussed above, then it will be important to determine whether a xenogeneic kidney can provide this function. If, however, a physiologic defect can be defined at a molecular level, the defect might be addressed by genetic engineering (39,67).

Another barrier to xenotransplantation is infection (68,69). A regular complication of cardiac allotransplantation, infection should, in principal, be less severe a risk in xenotransplantation because the animal source can be raised free of known pathogens. However, much attention has been devoted to the possibility that an animal organ or tissue might carry the porcine endogenous retrovirus (PERV) (70), which cannot be eliminated by breeding (a recent study did suggest PERV could be present at a low level in some stocks of pigs). Were PERV to be transmitted to a xenograft recipient, the agent might be
spread more widely in the population. PERV can be transmitted to human cells in culture or to cells introduced into immunodeficient mice (71). However, studies of human subjects given temporary xenografts or exposed to porcine blood products have failed to reveal even a single instance in which PERV has been transmitted to a human subject (72). Although the question of relevance of PERV to public health cannot be entirely dismissed, the question may now be viewed as one that could be resolved by careful attention to the recipients of xenografts, rather than as a reason for abandoning xenotransplantation (69).

Renal Cellular Xenografts

It might be intuitive that cellular transplantation could be of no benefit in the treatment of renal disease given the complex structure of the kidney; however, that intuition could be incorrect. If the association of small decrements of renal function with cardiovascular disease should prove to reflect the need for a hormonal or metabolic function, then a cellular transplant might provide that function. Another potential application for cellular transplantation is tissue regeneration—presumably the diseased organ lacks the ability to regenerate itself, but regeneration might be achieved by introduction of stem cells of exogenous origin (if the disease is of immune origin, then one might wish to introduce foreign stem cells). Thus, it is not beyond imagination that cellular transplants would be applied for the treatment of renal disease, but is there any advantage to the use of xenogeneic cells over allogeneic cells?

There may be several reasons to consider the use of xenogeneic cells. First, xenogeneic cells are available and relatively inexpensive. Human cells, on the other hand, are in short supply. Although histocompatible human cell lines might be developed and engineered for a given purpose, the development and custom engineering (e.g., introduction of a gene to be expressed) is quite labor intensive and expensive, and there will always be concern about transformation and tumor formation. Finally, human cell lines might be susceptible to viral or immune-mediated diseases of the subject to be treated.

Xenogeneic cells could presumably be isolated inexpensively and in large numbers. If genetic manipulations are needed, these could be undertaken by making germline changes in founder animals, which give rise to lines of animals with the desired gene expressed at a high level or under inducible controls. Because the xenogeneic cells are not transformed, they are presumably less subject to forming tumors, and if tumors formed, they could easily be eradicated by immunotherapy. Finally, the immune barrier to transplanting xenogeneic cells is not necessarily greater than transplanting allogeneic cells. Unlike organs with highly vulnerable blood endothelium of donor origin, cellular transplants are vascularized by the recipient, and these blood vessels provide a barrier between the immune system of the recipient and the graft. Thus, cellular xenografts can be sustained with immunosuppression regimens no more rigorous than those used to sustain allografts (26,27,73,74). Hence, although organ xenografts will probably require development of new technologies to permit use in human subjects, cellular xenografts could be carried out today if a suitable application were proposed.

Role of Xenotransplantation in Future Technologies for Renal Replacement

The ideal way to replace kidney function would be to “grow” kidneys for the individual suffering from kidney disease. This idea, known as organogenesis, is being pursued in several laboratories by several technologies, and some of these technologies could require use of xenotransplantation.

Fetal kidney tissues from various sources have been found to mature after implantation into adult animals (75–79). Recently, Hammerman (80) showed that fetal porcine kidney tissue can mature in an adult rat and that the tissue exhibits some renal function. Organs grown in this way are vascularized by ingrowth of blood vessels of the “recipient.” Whether full function could be achieved by this approach is uncertain, but it does suggest at least part of a future strategy for organ replacement. One problem with the approach, however, is that it relies on the use of fetal tissue, and fetal tissue could not be obtained from a human source. Hammerman (81) proposed using the fetal pig as source of fetal kidney tissue. The fetal tissues are subject to rejection; however, for several reasons, this problem is much less than might be anticipated (79). First, because the tissue derives its vascular supply from the host, the tissue, like a cellular transplant, is subject to cellular, but not to vascular, rejection (Figure 1). Second, for reasons not as yet understood, fetal tissues may be less immunogenic (79). Thus, this strategy would require immunosuppression of the treated individual, but it could be performed with unmodified and thus relatively inexpensive tissue. If one were to find a gene such as CTLA-4, the expression of which would lessen the immunogenicity of the graft, that gene could be introduced by means of genetic engineering.

Another technology recently proposed for renal replacement involves the generation of histocompatible renal tissue by therapeutic cloning (82). Therapeutic cloning involves harvesting nuclei from the cells of the individual to be treated and implanting those nuclei into an oocyte, zygote, or other cell capable of reprogramming the DNA. Reprogramming involves removal of DNA adducts and lengthening of telomeres so that the nucleus can divide with senescence and the cells can give rise to any cell of a fetus or mature individual. Thus, reprogramming generates embryonic stem cells with the same MHC antigens as the nucleus donor (the cells do express mitochondrial antigens of the cytoplasm donor). Lanza et al. (82) recently showed that cloning could be used to generate fetal kidney tissues, which could be used to construct a kidneylike device. Now there are difficulties with this approach—the generation of human fetal cells would not be ethically acceptable, and it is not clear that the device could provide a sufficient level of function. However, these hurdles could potentially be overcome. Knowledge about nuclear reprogramming is accumulating rapidly. Thus, it may soon be possible to use a xenogeneic oocyte or to manipulate an individual’s cells directly to generate pluripotent stem cells. If these cells do

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have the capacity to become a human embryo, there should be no ethical concern.

We have suggested that cloning and organogenesis might be combined to produce a functional and histocompatible kidney (28,83). In this strategy, one would generate stem cells by nuclear transfer (perhaps by using a xenogeneic oocyte or direct manipulation of the subject’s cells). The stem cells would be implanted in a fetal porcine kidney. The stem cells might be coaxed to have the potential and commitment to form kidney and induced to form kidney tissue. The human nephrogenic cells would then be harvested and implanted in the individual from whom the nuclei were derived. The nephrogenic mesenchyme might then develop into kidney and be vascularized by the treated individual. Of course, this strategy requires some technical advances, but each of the steps has been accomplished separately.

Prospects for Application of Xenotransplantation

Recent studies in xenotransplantation have taught that if a barrier to application can be defined at a molecular level, then that barrier can probably be addressed by genetic engineering of the pig. We do not presently know all elements of the immunologic barriers to transplanting porcine organs into human; however, given the resources and time, these elements will surely be discovered and overcome. For example, if PERV should prove to be a difficult barrier, the virus might be inactivated by gene targeting. The question then is whether xenotransplantation of the kidney can be applied, but whether it will be preferable to other approaches to replacement of renal function that may be developed. We will speculate in closing about these applications.

For the treatment of renal failure, xenotransplantation would be preferred if it could be made as safe and effective as allotransplantation. Progress in understanding and overcoming the immune barriers to xenotransplantation make this goal not an impossibility. Kidney xenotransplantation might be the best and most economical option if kidney transplantation is to be offered to the very large number of subjects for whom it may soon be indicated. However, the level of function required to justify replacing functionally normal kidneys in the patient with cancer or in the case of cardiovascular disease might prove a difficult challenge.

The ideal replacement for the human kidney is a human kidney composed of the cells of the patient. Something approaching this ideal can be produced, as we have described above, by organogenesis using stem cells cloned by nuclear transfer. One can imagine applying organogenesis for treatment of renal failure or for preemptive therapy; however, the difficulty and expense may preclude application for treatment of metabolic disease, and the susceptibility of the cells to injury might preclude use for regeneration of the kidney.

For the treatment of metabolic disease and for some facets of regeneration, we might envision using cells obtained from pigs engineered to express certain proteins. We can also envision a hybrid therapy in which an implantable device might replace renal function and a cellular transplant might be used to compensate for metabolic limitations.

References


