Rationale for Xenotransplantation

The compelling rationale to pursue xenotransplantation is illustrated by the treatment of cardiac disease. In the United States, an estimated 550,000 new cases of heart failure occur annually, and 38% of these patients die within 2 years. At present, the only cure for end-stage heart failure is transplantation of donated human hearts. Although there are a number of experimental therapies under development for heart failure, such as transplantation of myoblasts or stem cells, the total artificial heart, and the left ventricular assist device, none of these has achieved the same level of functional rehabilitation as whole organ transplantation. The number of patients able to benefit from transplantation, however, is severely limited by the number of human organs available for transplantation. Some current estimates suggest that fewer than 5% of patients who might benefit actually receive a cardiac transplant in the United States each year. Most of these patients die without the definitive treatment that transplantation offers. A similar statement can be made for lung transplantation.

The use of animals as a source of organs and tissues for transplantation into humans has been a goal of investigators in the field of transplantation for decades. This goal mainly reflects the belief that xenotransplantation can help alleviate the shortage of human organs for transplantation, significantly increasing the impact of transplantation on the treatment of cardiac failure. In lung disease, for which there is no effective long-term mechanical therapy, xenotransplantation offers the only hope for definitive organ replacement therapy. Xenotransplantation has also been proposed as a primary means for addressing certain diseases that selectively afflict human organs and tissues. For example, in the past, xenotransplantation of bone marrow and liver were proposed for the treatment of HIV and hepatitis respectively, as animal tissues are not susceptible to infection by the offending human viruses. Furthermore, xenotransplantation offers a powerful model system capable of elucidating fundamental aspects of the immune system, vascular biology, inflamma-
tions, etc. Experimental efforts in xenotransplantation have already led to important insights into the action of complement on blood vessels, the characteristics of "natural" antibodies, the phenomenon of accommodation, and the potential application of nuclear transfer.

Barriers to the Application of Xenotransplantation

In the sections that follow, the barriers to xenotransplantation will be discussed. For the most part, the discussion will focus on the use of pigs as a source of organs. This focus does not mean that other species of animals should be excluded as sources of xenografts. The species to be used for xenotransplantation will have to be available in large numbers, easily bred, raised to exclude infectious pathogens, and subject to genetic engineering. The pig fulfills these requirements, and its organs also have the appropriate size and physiological capacities.

Immune Response of the Recipient to the Graft

Most experts in the field see the main hurdle regarding the application of xenotransplantation to humans as the immune response of the recipient to the graft. Recent reviews have focused on the nature of the immune response and approaches to modulating it.5-8 Organ xenografts are subject to severe forms of vascular disease arising from the interaction of elements of the immune system of the recipient with blood vessels in the donor organ. The most severe and vexing type of disease is variously called acute vascular rejection or delayed xenograft rejection. This type of rejection often limits survival of porcine organs transplanted into nonhuman primates to a few months, although survival of porcine cardiac xenografts in excess of 100 days has been observed. Accordingly, a central goal of any program aimed at advancing organ xenotransplantation must be the discovery and optimization of approaches to overcoming acute vascular rejection. However, there are indications from animal studies that, when acute vascular rejection is delayed or controlled, there are other components of the host immune response, such as T cell, natural killer cell, and macrophage responses to the xenograft, that will play a role. Further studies will need to be undertaken to learn how to manipulate these.

Physiological Hurdles to Xenotransplantation

In addition to the immunologic barriers mentioned above, there may well be daunting non-immune biological barriers to successful application of xenotransplantation. Physiological barriers stem mainly from incompatibilities of the biochemical systems of disparate animals. These are exemplified by the severe barrier to engrafting bone marrow between species owing to incompatibility of growth factors, and the susceptibility of organ xenografts to diffuse intravascular coagulation owing to the incompatibility of coagulation control between species. In addition to hindering xenotransplantation directly, these problems may add to the severity of xenograft rejection reactions. Although these "molecular incongruities" are generally acknowledged to be detectable, investigators differ in their views on the significance of these. There may be tens to hundreds of proteins and processes that may be affected by imperfect compatibilities between species, and there is uncertainty about which of these might limit the function or survival of a xenograft.

Limitation of Model Systems

Advances in understanding the immunologic barriers to xenotransplantation of the heart and lungs have depended significantly on the use of nonhuman primates as model recipients. This dependence derives from the need to study immunologic reactions with Galα1-3Gal, a saccharide expressed on vascular endothelium by pigs and other lower mammals and known to be the primary target of the human antibodies directed against lower mammals, and from the variation of complement control between species. There is general agreement that primate model systems have been used advantageously in research aimed at elucidating the immunologic barriers to xenotransplantation. Some also believe these systems can be used as predictors of clinical performance and accordingly insist that investigators should demonstrate that porcine xenografts function for a certain period of time in nonhuman primates before proceeding to clinical trials. Others believe that nonhuman primate model systems may not faithfully represent what would happen if a porcine heart or lung were transplanted into a human subject.

Although the nonhuman primate may be the best model currently available, it has problems that need to be considered when making any decision about whether or not to move forward to clinical studies using solid organs. First, the genetic manipulations of pigs carried out to prolong xenograft survival may not be optimally functional in nonhuman species. For example, human decay accelerating factor, an inhibitor of complement activation, expressed in a porcine organ would be expected to function better in a human than in a nonhuman primate recipient. Second, some have found that nonhuman primates may develop immunity against human proteins expressed in pigs, which would lead to production of blocking antibodies (humans, being tolerant to these proteins, would not be expected to make such antibodies). Third, pharmaceutical and diagnostic technologies optimized for human subjects might not be optimal in nonhuman primates. For example, antibodies such as anti-CD3 used to inhibit or modulate T cell responses in humans may not function in nonhuman primates. Fourth, the routine postoperative care and management of nonhuman primates require the use of general anesthesia for procedures such as drawing of blood and invasive biopsy procedures. Human patients would not be subject to this additional stress and risk. In addition, the human patient would be expected to recognize symptoms of graft failure or complications and notify a care provider, whereas detection of these problems in the nonhuman primate must await the development of physical signs. Fifth, it has been reported that the immune response against xenogeneic cells varies between species; hence, the nonhuman primate response to porcine cells might be significantly more vigorous than the human or rat anti-pig response. Whether nonhuman primate models predict clinical performance adequately and accurately is a question of the greatest import because the use of nonhuman primates imposes a significant expense on investigators and companies and
because the application of xenotransplantation may be delayed unnecessarily by large preclinical experiments.

Risk of Infection Imposed by Xenotransplantation

Another barrier to xenotransplantation is the potential for infection. Infection is a routine complication of clinical allotransplantation owing, in part, to the requirement that the recipient be treated with immunosuppressive agents and, in part, to the frequent transfer of infectious agents from the human donor to the recipient. The latter complication should, in principle, be a less severe risk in xenotransplantation because the animal source can be fully characterized, selected, and made free of known pathogens. One exception to this conclusion may be the porcine endogenous retrovirus (PERV), which is in the genome of all pigs. Concerns about PERV have spurred much investigation in recent years, as PERV has been shown to be sometimes capable of transmission to human cells in culture. Were PERV to be transmitted from a xenotransplant to a human recipient and then spread more widely in the population, the subject of infection in xenotransplantation would become a public health concern. To date, however, patient screening has not revealed any evidence of PERV infection in xenotransplantation clinical studies. Such encouraging results have mitigated many of the concerns. Nevertheless, the matter of infectious disease has had a discouraging impact on commercial interests in developing xenotransplantation products. This impact reflects, in part, the very high costs now anticipated for microbiological screening and ongoing monitoring of xenotransplant recipients. In addition, transmission of infection beyond consenting recipients, although quite unlikely on the basis of current understanding, could create potentially devastating financial liability for commercial interests. The question of the relevance of PERV to public health cannot be entirely dismissed, but may now be viewed as one that could be resolved by careful attention to the selection of donor animals and to the monitoring of recipients of xenografts, rather than as a reason for abandoning xenotransplantation.

Role of Industry in Clinical Application of Xenotransplantation

The involvement of industry in xenotransplantation reflects the commercial opportunities afforded by the potential "sale" of animal organs and the realization that a substantial increase in the number of transplants brought about by widespread clinical application of xenotransplantation would correspondingly increase commercial opportunities for immunosuppressive agents, medical and surgical supplies, etc. In fact, the involvement of industry has been critical to recent advances in the field. Industry has applied technologies in genetic engineering to express human proteins in pigs to control activation of complement or expression of antigenic saccharides, instituted methods of animal care that exclude known human pathogens from various lines of pigs, designed methods to screen for known and potential pathogens and conducted human studies to explore infectivity of these pathogens, developed techniques for nuclear transfer and cloning of pigs that might be applied to genetic engineering, and provided financial support for both basic research and clinical studies.

Despite significant progress in the field of xenotransplantation over the past decade and a substantial investment already made, interest and support by industry for further efforts have waned in recent years. The withdrawal of the technical and financial resources of industry from this field has in turn threatened to arrest further progress toward clinical application. The waning of interest on the part of industry appears to result from the potential risk of liability for infectious disease or graft failure, the high costs associated with genetic engineering and breeding of swine, microbiological screening, and long-term follow up of patients, and/or the prolonged period of developmental work preceding commercial viability, which makes it difficult to attract venture capital or public investment dollars.

Within industry, each company has invested in its own transgenic pig modified with 1 or more human complement regulatory gene or other genes such as those regulating Galα1-3Gal expression. The ultimate animal donor, however, may require a combination of genetic modifications, and thus might be advanced by greater sharing of data and resources. Yet for companies to cooperate in developing the optimal donor animal, some means must be found to protect the proprietary interests of all parties. This legal hurdle, although not impossible to overcome, may be very complex and may require an independent mediating party.

Alternative and Competing Approaches to Organ Replacement

The potential availability of alternative approaches to the treatment of organ failure may also dampen enthusiasm for xenotransplantation. Among these other approaches are implantable artificial organs and cellular or tissue transplants, including stem cells.

The recent qualified success of the newest version of the total artificial heart and the very promising results achieved with left ventricular assist devices raise questions about whether and to what extent organ transplantation will be needed for the treatment of cardiac and pulmonary disease. Enthusiasm regarding the success of new technology, however, needs to be weighed against clinical judgment as to whether new technology is proven as effective or as safe as transplantation. It is hoped that ongoing improvements in technology will make the total artificial heart or cardiac assist device viable alternatives to cardiac transplantation. In such a setting, cardiac xenotransplantation may be a competing technology. In addition, application of implantable devices could conceivably increase the need for xenotransplantation as a means of rescue for individuals who do not thrive on devices. The main limitations to the application of devices are infection, malfunction of the device, and thrombotic complications. The total artificial heart has the disadvantages of the patient being completely reliant on the device and the function possibly not approaching the function of a normal heart. In addition, implantable devices may not be the best alternative for some diseases of the heart. The ventricular assist device may not address the problem of diffuse myocardial dysfunction, and these devices are currently not
suitable for application in infants and children. Despite some efforts in biomedical engineering, there are no implantable devices that can replace the function of the lung for the foreseeable future.

Recent years have brought exciting progress in the fields of tissue engineering and stem cell biology. Cellular transplants (eg, skeletal myoblasts) have been used to repair focal defects induced in the myocardium of experimental animals. This approach was recently applied in human subjects. Although these advances are exciting, it is unclear at this point how cellular transplantation and stem cell biology could offer an optimal treatment of diffuse myocardial failure because the anatomic configuration of transplanted myoblasts or stem cells is not likely to approximate the configuration of cardiac myocytes in the intact heart. Whereas cellular transplants or engineered tissues could be envisioned to be applicable to the repair or regeneration of localized lung tissue, their use in replacing whole lungs is not likely because of the anatomic complexity of the lung. Thus, although there are multiple approaches under development for therapy for heart and lung failure, none has been proven to be widely successful and applicable.

**Special Considerations for Lung Xenotransplantation**

Because research on lung xenotransplantation is very limited, a separate discussion was held by a subcommittee of the Working Group to explore the hurdles and opportunities in this area. The problems surrounding survival after lung transplantation are very complex. Research in the past few years has revealed 2 barriers to pulmonary xenotransplantation that do not apply to organs such as the heart. First, after xenotransplantation, the pulmonary macrophages secrete agonists that cause severe constriction of the pulmonary vasculature, leading to increased pulmonary vascular resistance that can be a severe problem early after engraftment. Second, the anatomy of the large airways of the pig differs enough from that of nonhuman primates and humans to impose some surgical challenges when using this species as the lung xenograft donor of choice.

**Recommendations: Addressing the Challenges and Opportunities of Xenotransplantation**

All members of the Working Group shared considerable enthusiasm for both the potential benefits of xenotransplantation and the likelihood of success should adequate time and resources be brought to bear. With the critical shortage of human organ donors, encouragement of xenotransplantation research is needed. Xenotransplantation could potentially offer therapy for many other diseases in addition to those affecting the heart and lungs. Xenogeneic islets of Langerhans could potentially be used for diabetes, neuronal cells for Parkinson’s disease, whole livers and/or artificial livers for liver failure, and kidneys for renal failure, to name just a few.

A crosscutting approach could accelerate progress and reduce the financial investment required. There are significant financial hurdles to producing transgenic animals, performing nonhuman primate studies, monitoring herds and recipients for infectious diseases, and archiving samples. Approaches to these should include the support of core resources for a genetic engineering and animal breeding facility, a nonhuman primate facility for testing new transgenic animals, and a central laboratory for screening for infectious agents. There is a sobering realization, however, that the time and funds to reach the goals may be more than originally estimated. These goals could be attained more quickly if the private sector, the government, and academia pooled their resources.

The Working Group thought that any potential plan to move xenotransplantation closer to clinical reality must first identify the mechanisms of the immunologic and physiological barriers and find strategies to overcome these. Second, a preclinical model should be developed to validate these strategies in humans. Although the nonhuman primate model is in many ways the model most closely related to the clinical situation, serious problems exist when it is used in the setting of solid organ xenotransplantation. A better model could potentially shorten the time to clinical application of xenotransplantation of whole organs.

Progress in understanding and treating lung ischemia, reperfusion injury, and acute lung injury could be leveraged to accelerate research in lung xenotransplantation. There is a substantial research experience in acute lung injury in primates on which to build new research collaborations between pulmonary researchers and investigators interested in pursuing lung xenotransplant research. Collaborations and cross-fertilization among these usually separate communities could facilitate accelerated progress in the lung xenotransplantation field. It may require special efforts to encourage research on lung xenotransplantation because it is such a daunting problem and the issues are not well understood. Increasing opportunities in lung xenotransplantation research for investigators with access to primate facilities should also be considered.

Development of clinically effective xenotransplantation, although ripe with challenges, promises a very high payoff. Cooperation among academic investigators, industry, and government agencies is necessary to build on the foundation of current advances and to try to bring xenotransplantation to clinical fruition. In the opinion of the Working Group, it is now a propitious time to encourage the development of xenotransplantation as a potentially effective therapy for patients with heart or lung disease.

**Appendix**

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