The Heart Surgery Forum®

EDITORIAL

Xenotransplantation Update

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The Clinical Problem

End-stage thoracic organ disease is currently a major health care issue in the United States. Heart disease itself is the leading cause of death in the U.S., and the incidence of heart disease is rising with the increasing age of the baby boomers. In a recent report on xenotransplantation, Cooper et al. cite estimates of 250,000 patients in the U.S. with "advanced heart failure" despite current recommended therapy [Cooper 2000]. In addition, they state that as many as 25,000 to 60,000 patients could benefit from a left ventricular assist device (LVAD) and 10,000 to 20,000 patients need a total artificial heart. Upon analyzing the data from the UNOS Registry [UNOS 2001], one can clearly see that the number of patients on the waiting list for heart transplants has dramatically increased over the past decade from around 1,000 in 1988 to over 4,000 in 2001. This phenomenon, combined with a fairly constant number of hearts transplanted annually, has led to an increasing organ shortage (Figure 1, ◎) [UNOS 2001].

Similar situations exist for heart-lung and lung transplants, with the demand for organs far outweighing the supply. Approximately 1,000 lung transplants per year are performed in the United States, while there is an ever-increasing waiting list of almost 4,000 (Figure 2, ◎) [UNOS 2001]. Cooper et al. cite a median waiting time of 567 days for patients on the lung transplant list, which is more than double the heart transplant waiting period [Cooper 2000]. The long waiting time, combined with the increasing supply and demand mismatch, results in many patients succumbing to end-stage organ failure before transplantation. We can expect to see the number of deaths on the waiting list rise as heart and lung disease continue to increase while the supply of allografts remains fixed. In fact, the current waiting lists actually underestimate the true need for thoracic organs because many institutions do not list patients with end-stage disease due to age or strict eligibility criteria resulting from the organ shortage. Chronic obstructive pulmonary disease (COPD), the leading indication for lung transplantation, affects 14 million persons in the United States alone. The prevalence of end-stage lung disease, like heart disease, is increasing [Cooper 2000].

Clearly, our present system of allograft transplantation is not meeting the needs of the patients with end-stage thoracic disease (Figure 3, ◎) [UNOS 2001]. Cooper et al. briefly review the current treatment options for these patients. Medical therapy presently is unable in many cases to prevent or reverse the disease process [Szabo 1997, Follath 1998]. Likewise, the Batista operation has met with limited clinical success [Cooper 2000]. Alternative surgical procedures such as coronary artery bypass grafting (CABG) or valve repair have limited applicability to the majority of patients on the transplant list. For heart failure patients, mechanical devices such as LVADs and artificial hearts hold much promise, with patients today able to survive for more than one year on an LVAD. However, LVADs and artificial hearts have serious problems yet to be overcome, such as hemostatic difficulties and a lack of long-term power supply. Many LVADs require battery changes every four hours and have power supply connections that exit the skin, so that infection remains an ever-present concern. Nevertheless, LVADs currently may be considered reasonable bridging devices prior to heart transplantation for some patients.

Unfortunately, for lung transplantation there is no good bridging device. The extracorporeal membrane oxygenator (ECMO) is at best a very temporary bridge. Artificial lung development is in its infancy and has problems of biocompatibility and hemostatic or thrombotic issues similar to the ECMO. Thus, for lung transplant candidates, an even greater reason exists to explore alternatives to allografts for organ replacement.

In the report that is the subject of this editorial, Cooper et al. perform a comprehensive review of the current state of affairs in xenotransplantation. Xenotransplantation has the potential to alleviate our organ shortage problem overnight and make thoracic organ replacement available to many who are currently denied allografts. However, many barriers still remain.

Xenotransplantation Clinical Experience

Current medical use of nonliving animal tissue is widespread. The most common example is the use of porcine valve tissue. In addition, many medicines such as insulin and

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Table 1. History of Cardiac Xenotransplantation

Physician/Date	Species	Position	Duration of function	Notes
Hardy, 1964	Chimpanzee	orthotopic	2 hours	First reported clinical heart transplant of any kind
Cooley, 1968	Sheep	orthotopic	10 minutes	
Ross, 1968	Pig	heterotopic	4 minutes	
Marion, 1969	Chimpanzee	unpublished	Failed immediately	
Barnard, 1977	Baboon	heterotopic	5 _ hours	
Barnard, 1977	Chimpanzee	heterotopic	4 days	First use of "immunosuppression"
Bailey, 1984 (Baby Fay: neonate)	Baboon	orthotopic	20 days	Cyclosporine-based immunosuppression, HLA typing
Czaplicki, 1992	Pig	orthotopic	4 hours	Used cross-circulation scheme to remove preformed antibodies

heparin have long been obtained from animals. Use of animal organs transplanted into humans is seen as a possible solution to the shortage of human organs for transplantation. Xenotransplantation remains quite a distance from clinical reality but is nevertheless an area of active research.

Eight cases of cardiac xenotransplantation have been reported in the literature (Table 1, ◉) [Adams 2000]. The first took place in 1964 when James Hardy placed a chimpanzee heart into a 64-year-old man with ischemic cardiomyopathy who was in cardiogenic shock. The orthotopically-placed graft failed after two hours. There was no ABO or HLA typing and no immunosuppression. This case represents the first reported case of clinical heart transplant of any kind. In addition, no case of clinical cardiac xenotransplantation has been reported in the literature since 1992, although a report exists of an unpublished porcine orthotopic transplant in India, which failed in less than 24 hours [Cooper 2000]. There is no clinical experience with lung xenotransplantation. Despite this limited experience, research interest in xenotransplantation has continued to grow, and the number of publications devoted to xenotransplantation clearly suggests that research funding is available.

Scientific Obstacles

The scientific obstacles to successful clinical thoracic xenotransplantation are daunting—in some ways they serve as testimony to the fortitude of the investigators in this field. The first problem, from a practical standpoint, is the choice of animal to use as donor. Intuitively, one would think that the species most closely related to man phylogenetically, non-human primates, would be the obvious choice. However, slow growth rates as well as poor breeding in captivity limit the use of primates, as producing the number of needed organs would be difficult [Adams 2000, Cooper 2000, Samstein 2001]. The smaller size of most primates also limits organ suitability for humans, and the potential for effective genetic manipulation is poor. Primates also harbor infectious agents that are known to be lethal to humans, such as herpes simplex B [Samstein 2001].

Research is now focused on pigs as the primary organ source. Pigs attain fertility at an early age, have a brief generation time, and breed in large numbers. Humans have long experience in maintaining large numbers of pigs in captivity. Furthermore, pigs have a reasonably good cardiac size match with humans and their organs grow to human size quickly. Genetic manipulation potential is good, which may help researchers deal with some of the inherent immunologic barriers to xenotransplantation. Pigs have long been used to produce medicines such as heparin and insulin and are used as a source of heart valves. Finally, there is likely to be less of a perceived ethical dilemma in using pigs as an organ source given their widespread use as food, compared to the use of the more phylogenetically advanced primates.

Major immunologic barriers exist to transplantation of organs across species. These barriers intensify as one proceeds away from humans phylogenetically. Xenotransplanted organs are subject to hyperacute rejection, acute vascular rejection, cellular rejection, and chronic rejection. Hyperacute rejection is caused by the presence of preformed antibodies to species antigens (xenoreactive antibodies). In the case of pig to human transplants, the presence of anti-pig antibodies directed against alpha-1,3-galactose (GAL) moieties expressed on pig vascular endothelium results in immediate antibody-mediated organ rejection [Cooper 2000]. Once anti-GAL antibodies bind to vascular endothelial cells, they activate the complement cascade, trigger endothelial cell dysfunction, platelet aggregation, and vascular thrombosis [Adams 2000]. Current methods have been successful in abating hyperacute rejection of the pig heart. Methods used include depletion or inhibition of xenoreactive antibodies in the recipient, depletion or inhibition of complement, and the use of pigs that are transgenic for human complement regulatory proteins [Cooper 2000, Samstein 2001]. Despite the success with inhibition of the hyperacute rejection of the pig heart, no such success has been seen in pig lung transplantation to date. However, genetic manipulation of the pig genome, creating a "knockout" pig lacking the gene that encodes for enzyme alpha-1,3-galactosyl transferase has been proposed by Polejaeva et al. With the knockout pig a reality today, the hurdle of hyperacute rejection may be surmountable for all xenotransplants [Polejaeva 2000].

Xenotransplanted organs are subject to acute vascular rejection when hyperacute rejection is averted. This now represents the major obstacle to cardiac xenotransplantation. Despite intense immunotherapy, acute vascular rejection, or "delayed xenograft rejection," occurs within days or weeks. Acute vascular rejection is thought to be a form of antibody-dependent, cell-mediated immunity, but its exact mechanism is currently not known. Acute vascular rejection is characterized by focal ischemia, mononuclear cellular infiltration, endothelial cell activation, fibrinoid necrosis, and diffuse

intravascular thrombosis [Adams 2000, Samstein 2001]. At present, no effective therapy for acute vascular rejection exists. Attempted therapies for acute vascular rejection have included depletion or inhibition of xenoreactive antibodies, anti–B-cell/plasma cell therapies, and the induction of B-cell tolerance. It is hoped that improved therapies will follow when the exact mechanism of acute vascular rejection is understood.

Knowledge of the role of the cellular immune response in the rejection of porcine to primate xenografts is sparse. In vitro data predict that this response will be at least as robust as that encountered in allotransplantation [Auchincloss 1998]. Only after further study reveals more information about the role and mechanisms of cellular xenograft rejection will strategies involving immunosuppressive agents, genetic modification, and/or tolerance induction begin to be targeted against this response [Cozzi 2000, Samstein 2001]. Chronic rejection also remains largely a mystery, but it is feared that this reaction will also be vigorous and will possibly lead to early graft failure [Cooper 2000]. This form of rejection may be partially offset by a larger donor pool and the possibility of retransplantation.

The survival rates for pig-to-nonhuman primate heart transplantation cited by the International Society for Heart and Lung Transplantation (ISHLT) are dismal. The longest survival time for a functioning heart in the heterotopic position is 99 days [Bhatti 1999, Cooper 2000]. Median survival for that group is 26 days. In the orthotopic position, the longest survival is 39 days, with a median of 12 days [Schmoeckel 1998, Vial 1999, Vial 2000]. Data for pig-to-nonhuman primate lung transplantation is even more discouraging. There is no organ survival beyond a few hours and no case of a transplanted lung being able to sustain life for any period of time [Dalmasso 1991, Yeatman 1998, Yeatman 1999]. Kidney xenograft data is slightly better, survival being comparable to heterotopic heart transplantation. However, lymphoproliferative disease has been a frequent consequence of immunosuppression in these experiments, which, in addition to involving the use of cyclosporine and corticosteroids, has required splenectomy and cyclophosphamide induction therapy. Clearly, a great deal of improvement in immunologic strategies will be needed in order to make xenotransplantation a practical transplant procedure.

In addition to the immunologic challenges of xenotransplantation, there are several questions related to the function of pig organs in the human host that arise from important physiological differences between the species. These include the function of porcine heart/lungs in the upright human as opposed to the horizontal pig, metabolic differences that include a higher basal body temperature in the pig (102.5°F), and different acid/base physiology. Additionally, the pig has a significantly lower serum cholesterol level than humans, which likely would place the heart at risk for accelerated atherosclerotic disease. These and a host of other physiological questions remain largely unanswered.

Xenozoonoses

Among the major challenges to xenotransplantation is the potential for the passage of animal infections to man. The possibility of such "xenozoonoses" will pose an ethical dilemma to be faced if clinical trials of xenotransplantation to humans become imminent. The possibility that not only the organ recipient may become infected but that the infection may be passed to the human population in general is also a matter of concern. As yet, no in vivo evidence of transmission of porcine infection to humans exists. However, studies have been limited by the lack of long-term organ survival [Cooper 2000].

It should be possible to limit the transmission of known infections from pig to man by controlling the conditions of handling and breeding pigs for xenotransplantation. The authors of the ISHLT paper point out that pigs raised in captivity will likely represent less of an infectious threat than the current human donor pool for hearts, which frequently carries cytomegalovirus and Epstein-Barr virus, as well as hepatitis and HIV. The possibility of the passage of unknown infections from animal donors to heavily immunosuppressed human recipients, and from them to the human population in general, is still poorly understood.

Porcine endogenous retroviruses (PERVs) make up approximately 1% of the pig cell genome and have been shown to infect human cells in vitro [Cooper 2000]. No passage of PERVs to humans has ever occurred in vivo and no human disease associated with PERVs has ever been observed [Adams 2000]. Furthermore, follow-up of 160 patients who underwent extracorporeal perfusion of human blood through pig organs or hepatocytes demonstrated no evidence of active PERV infection by reverse transcriptase polymerase chain reaction (RT-PCR). It is to be noted that only 36 of the 160 patients were immunosuppressed. However, 23 of the 100 patients who were treated by spleen perfusion had evidence of microchimerism. In other words, PCR revealed pig DNA in their blood [Paradis 1999, Takeuchi 2000]. A genotype of pigs unable to pass PERVs to human cells has been identified by Patience et al. [Patience 1999], raising some hope that the danger of PERV transmission will be eliminated in the future. The meaning of these findings is unclear; it remains to be seen whether PERV infection in humans occurs under conditions of immunosuppression and prolonged exposure to porcine organs. Nevertheless, recent data demonstrates that PERVs are able to infect human cells in vitro [Patience 1997, Martin 1998, van der Laan 2000].

Even less well understood is the possibility of the generation of recombinant viruses after passage of porcine viral elements into human cells. Potentially long latent periods would mean that new endogenous retroviral infections might not be seen for years after transplantation and could affect patients, the community, and commercial pig herds [Cooper 2000]. However, the fact that humans have been living close to pigs for many years makes disease transmission less likely.

In summary, no hard evidence of significant risk to patients or the general population from xenozoonoses presently exists. Known infections are likely to be eliminated by the conditions of captivity and breeding of animals for xenotransplantation. Knowledge regarding novel and/or recombinant viral infections is at an early stage. Much may remain unknown until actual human clinical trials are under-

taken and until xenografts have been in place for clinically relevant periods of time.

The Ethics of Xenotransplatation

Before discussing the ethics of xenotransplantation, one must consider the ethical issue posed by humans taking advantage of animals at all. C.S. Lewis, in his famous collection of essays entitled "God in the Dock," addressed the issue of vivisection [Lewis 1970]. He first asserts that he has never heard a rational discussion about the subject. Needless to say, there are strongly held opinions for and against the use of animals for research or for any benefit to humans at all. Lewis makes the point that this argument cannot be settled in any kind of logical way, but, at the very least, if people do choose to take advantage of animals (and it can be argued that they have the prerogative to do so from a philosophical, ethical, or religious point of view), they incur the obligation to take care of those animals as best they can. Thus, it would seem that those who wish to debate the ethics of transplantation can get no further than C.S. Lewis does in his essay. This is clearly an issue about which many people must agree to disagree.

Medical professionals generally have little ethical objection to using animals for research. Although opinion is not unanimous, most agree that research for the benefit of humankind is justifiable if done with proper observance of animal care, professional ethics, and assurances of the scientific validity of the research. There is little philosophical and ethical difference between causing an animal to have a human disease in order to study vaccines and utilizing parts of that animal for transplantation. It can be argued that if humans are willing to use animals for food, we should certainly be willing to use parts of animals to save human lives.

Interestingly, in the only textbook of surgical ethics published to date, the issue of xenotransplantation is not discussed, although other difficult issues of transplantation ethics are addressed [McCullough 1998]. A more difficult dilemma in xenotransplantation is the problem of zoonoses. Despite the fact that most human diseases (measles, smallpox, etc.) were originally animal diseases acquired by humans when the animals were domesticated, we are likely to find some new diseases caused by transplanting animal organs to people and then immunosuppressing them. Surely we have had a glimpse of this with the retrovirus infections. Furthermore, though we think of diseases like measles and smallpox as being either benign or controllable, throughout most of human history they were not. Diseases of this sort probably killed 90% of all Native Americans living in the Western Hemisphere when Europeans arrived. Though the epidemiology of these epidemics is still debated by historians, there is no doubt that these diseases, which Europeans had been able to live with for generations, were devastating to populations with no prior exposure to them.

Surely, some of these ethical and practical issues can be dealt with by scientific expedients such as genetic manipulations and raising suitable animals in environments free of animal diseases. Still, we will be left with some uncertainty about many of these issues until we actually try to use animal organs for humans in significant numbers. Obstacles of this sort have not stopped scientific inquiry in the past and should not now. However, the scientific, philosophical, and patient communities must carefully consider each step in this process to see if it is the next logical, ethical, and scientifically appropriate step to take. If this approach is followed, we can be confident that the results of the research will justify the ethical and practical risks that are undertaken, even if success is limited.

Conclusion

The problem of thoracic organ shortage will almost certainly grow worse, and strategies to increase human allografts are unlikely to meet clinical needs. Furthermore, one should realize that the problems encountered in end-stage heart and lung diseases might have different solutions. The artificial heart appears to hold much promise. The simplicity of the organ's function as a pump lends itself better to mechanical replacement than other organs, such as the liver or lungs. A mechanical heart is also less antagonistic to the immune system's barriers. Although the artificial heart is not without its own set of obstacles, technology is likely to overcome power supply issues, and the biological problem of hemostasis appears more easily surmountable than the immunological barriers of xenotransplantation. While the artificial heart does show promise, we should bear in mind the words of Dr. Starzl that "the future of xenotransplantation is brighter that at any previous time because what must be done to succeed has become remarkably clear." [Starzl 1998.]

Clinically, there has been more human experience with cardiac xenotransplantation than with most other organs. Replacing the lung seems considerably more daunting given that there is currently no viable mechanical replacement and that there have been such poor results with xenotransplantation of lungs. Ultimately, xenotransplantation may provide the solution as the problems are further defined and barriers gradually overcome. Indeed, the availability of animals as organ donors seems to hold forth the best long-term promise for solving the global problem of organ shortage.

Both mechanical and biological avenues are worthy of pursuit. Only the future will decide which one ultimately solves our organ shortage problem. Indeed, there is the real possibility that both may play a role. Xenotransplantation research itself will produce many collateral benefits to other fields of medicine as we learn more about the immune system. Benefits may be anticipated in infectious disease research as well as current allograft transplantation research. In addition, it is possible that other fields will benefit in ways that we cannot yet realize.

The ethics of animal stewardship are also likely to become more complex. One can imagine the creation of a new hybrid species as we manipulate an animal's genome to be more like a human. At what point will these genetically altered animals be more like a human than an animal? With our new abilities to clone species and alter genomes with gene knockouts and insertions, the day when these questions will be upon us may be closer than we imagine.

Although the future may hold difficult decisions if our research and manpower resources become more limited, the future of xenotransplantation is nevertheless a bright one

and worthy of pursuit. We commend the great investigators who have made advances in this field since the first clinical human xenotransplantation in 1963. Although xenotransplantation is still in its infancy, we believe that it may hold the promise of alleviating the donor shortage problem.

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