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REPORT ON THE STATE OF THE SCIENCE IN XENOTRANSPLANTATION

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SACX REPORT ON THE STATE OF THE SCIENCE IN XENOTRANSPLANTATION

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REPORT ON THE STATE OF THE SCIENCE IN XENOTRANSPLANTATION

EXECUTIVE SUMMARY

Xenotransplantation holds promise for the treatment of many human diseases and disorders and could potentially alleviate the shortage of human organs and tissues available for transplantation. It also raises many interesting and complex scientific, medical, ethical, legal, and social issues. This report focuses on the science of xenotransplantation, an area in which major advances have been made in the last few decades. The report outlines the considerable progress that has been made in understanding and overcoming some of the major hurdles to xenotransplantation and also discusses the public health concerns associated with xenotransplantation.

Aim of This Report

This report was produced in response to two critical mandates of the U.S. Department of Health and Human Services (DHHS) Secretary's Advisory Committee on Xenotransplantation (SACX): to advise the DHHS on the current state of scientific knowledge about xenotransplantation and on the potential for transmission of infectious diseases as a consequence of xenotransplantation.

This report provides an overview of the potential impact of xenotransplantation, the types of xenotransplantation procedures currently being used in research, and the source animals for xenotransplantation products. It then discusses some of the major challenges posed by immunologic and physiologic incompatibilities, as well as strategies to address those challenges. Also discussed are the infectious disease risks associated with animal-to-human transplantation and management strategies to cope with them. Some alternative strategies to xenotransplantation are described to provide a contextual perspective on the field. The report also describes public health concerns about "xenotourism," a term coined to describe personal travel outside of a country of residence for the purpose of participating in xenotransplantation programs or attending clinics to obtain therapies not presently available or acceptable in the home country. Finally, the SACX presents its recommendations regarding xenotransplantation as an experimental therapeutic strategy.

Types of Xenotransplantation Procedures

The term *xenotransplantation* refers to the transplantation, implantation, or infusion into a human recipient of live cells, tissues, or organs derived from non-human animals. The procedure includes the use of human body fluids, cells, tissues, or organs that have had *ex vivo* contact with live, non-human animal cells, tissues, or organs. The source animals or their cells may or may not be genetically modified. The different types of xenotransplantation procedures being performed or considered include the following:

- **Solid-organ xenotransplantation** is a procedure in which a source animal organ such as a heart, lung, kidney, or liver is transplanted into a human. In such cases, the vascular supplies of the source animal and the recipient are connected.

- **Cellular and tissue xenotransplantation** is the grafting of tissues and cells from a source animal without surgical connection of any animal blood vessels to the recipient's vessels. These xenotransplantation products may be implanted directly into a recipient's organ.

- **Extracorporeal (natural and artificial organ) perfusion** occurs when human blood is circulated outside of the human body through an animal organ, such as a liver or kidney, or through a bioartificial organ produced by culturing animal cells on an artificial matrix.

- **Exposure to living animal-derived material** occurs when any of a variety of human cell types are grown ex vivo with non-human animal cells. If these human cells are subsequently transplanted or infused into a human patient, the procedure is considered a form of xenotransplantation.

Scientific Challenges in Xenotransplantation

Technical challenges to xenotransplantation are primarily immunologic and physiologic in nature. Although these challenges are common to both human-to-human transplantation (allotransplantation) and xenotransplantation procedures, the various differences and disparities between species tend to exacerbate these difficulties in xenotransplantation.

Immunologic rejection processes are faced in any transplantation procedure and are exacerbated by the differences between species. In xenotransplantation, however, a number of factors arising from disparities between species intensify the immunologic processes that mediate rejection:

- **Hyperacute rejection** is the nearly immediate and catastrophic destruction of a graft, a process that is initiated by natural antibodies directed to a specific sugar molecule called galactose-(a1-3)-galactose (Gala1-3Gal) (abbreviated aGal). Because human cells do not express this sugar molecule, the human immune system recognizes it as "non-self," or foreign, and produces antibodies against it in response to its presence on common microorganisms. These antibodies and the process of complement-mediated destruction are elements of natural human immunity against invading microorganisms.

- **Delayed vascular rejection** occurs over days to a few months and involves the vascular system of the xenotransplantation product. Pig kidneys and hearts transplanted into non-human primates stimulate progressive destruction of the pig blood vessels. Antibodies and inflammatory immune cells are found in these vascular lesions. The exact immune mechanisms responsible for delayed vascular rejection are not yet fully known.

- **Acute cellular rejection** occurs over weeks or months after transplantation. This phenomenon is mediated predominantly by T-cells, which constitute the cellular arm of the immune system. Acute cellular rejection can target blood vessels but usually involves infiltration of attacking host T-cells (and other immune cells) into the transplant tissues and

destruction of the epithelial cells that are responsible for the function of the xenotransplanted organ.

- **Chronic rejection** is the progressive destruction of a transplant over months to many years. Antibodies induced by the organ graft may play a role in the process. For some transplanted organs (e.g., hearts), a poorly understood, distinct pathological process results in such severe narrowing of blood vessels that the underlying tissue is starved of essential nutrients. This process, as well as direct attacks on the cells that form the structure and maintain the function of the transplant, may eventually produce extensive tissue destruction and replacement by fibrotic scars.

Additional species differences that may affect the function of a xenograft include complement and coagulation systems; adhesion molecules, cytokines, and growth factors; organ-specific physiologic considerations; and size considerations. Current approaches to the challenges facing xenotransplantation include genetic modification of source animals, encapsulation and other bioartificial isolation devices, methods to induce tolerance in xenotransplantation recipients, gene therapy to modify either source animals or recipient cells, targeted molecular therapies to inhibit the activation of the complement and blood clotting systems that follow antibody binding and complement activation, and a number of other host treatments, such as removal of anti-aGal antibodies before xenotransplantation.

Risks of Infectious Diseases Associated with Xenotransplantation

Although all allotransplant procedures are associated with a risk of infectious diseases caused by known and emerging human pathogens, the risks associated with xenotransplantation have unique features that could pose a threat to public health. Xenotransplantation may allow infectious agents from source animals to circumvent natural barriers, such as mucosal surfaces and skin, through the surgical placement of a xenograft product into a human. The magnitude of the risk associated with xenotransplantation would be influenced by the requirement for immunosuppressive agents, the species of the source animal and the applicable husbandry practices, the type of tissue or organ used, and the duration and type of recipient exposure. If an infectious agent from a source animal is transmitted to a human recipient, the agent could result in the undetected spread and establishment of novel infections in humans. Risks from xenotransplantation include both acute and chronic persistent viral infections. Importantly, some infectious agents may cause no disease in the source animal and may remain unrecognized. Person-to-person spread of infection without detection for years represents a significant problem due to potential long clinical latency periods that can extend to decades, thus allowing for the spread of an animal virus to the general population. As an example, HIV-1 is asymptomatic in chimpanzees, but its transmission to humans resulted in a new clinical disease (AIDS) after a long clinical latency. Therefore, the risks from xenogeneic infections pose unique concerns that need to be addressed during research and clinical trials involving xenotransplantation.

Risks from Porcine Xenotransplant Products

At present, pigs are the source animal of choice for whole-organ xenotransplants. It is recommended that nonhuman primates not be used as source animals for xenotransplants because of problems associated with infectious diseases in monkeys and their risk to humans. Porcine infectious diseases are currently being studied and assays are being developed to detect infection in pigs and humans. Many porcine agents are non-infectious for humans, and others can be eliminated through screening and husbandry practices, including closed breeding colonies. Unrecognized infections are likely to circulate in porcine populations and could be a potential risk to humans in xenotransplantation. Efforts should be directed toward preventive measures by 1) developing new assays and methods to detect novel porcine agents and 2) developing methods to reduce infections in swine colonies.

Of particular concern as a potential emerging infection from xenotransplantation has been porcine endogenous retrovirus (PERV). PERV is essentially embedded in all pig genomes and could potentially be transmitted to humans with the xenotransplant product. Previous retrospective studies to detect transmission to humans exposed to porcine products have failed to detect transmission of PERV; however, as clinical trials become successful, longer exposure to xenotransplant products may enhance the risk from PERV. Recent studies suggest that the risk from PERV might be reduced through selective breeding practices using pigs that do not transmit the virus to human cells (e.g. some MGH mini-pigs have been shown to be non-transmitters).

The overall risk from porcine xenografts can be minimized through good animal husbandry practices, construction of barrier-contained breeding facilities, and screening of source animals for known infections. Because of the potential for an infected blood donation or other recipient contact with an infected individual to spread new, unidentified pathogens, infectious risks from animal sources will continue to warrant more intensive investigation as the field of xenotransplantation matures.

"Xenotourism": An Emerging Global Public Health Concern

The SACX defines "xenotourism" as the travel of U.S. residents to foreign nations to participate in xenotransplantation programs or clinics for the purpose of obtaining therapies not presently available or acceptable in the United States. The SACX believes that xenotourism constitutes a public health risk, because the xenotransplantation procedures may be performed under circumstances that would not be allowed in the U.S. After the xenotransplantation procedure, American xenotourists return to the U.S., where they receive the majority of their health care and participate in the daily activities of life. Xenotourists unaware of the potential for transmitting infections are not likely to take appropriate precautions to reduce the risk of transmission of a potential xenogeneic infection. For example, they might donate blood or organs or engage in other behaviors that might be associated with risk of transmission of viral infection. Furthermore, unless they inform their health care providers of their participation in a xenotransplantation procedure, a potential xenogeneic infection may not be recognized and properly monitored.

The SACX believes that a systematic effort should be undertaken to identify xenotransplantation programs in other countries and to assess their use by U.S. citizens. This will inform U.S. efforts

to educate the public about the potential dangers of xenotourism and to develop strategies for further discussions with the international community on the regulation of xenotransplantation.

Knowledge Gaps and Resource Limitations

Although increased understanding of molecular immunobiology and cell and organ physiology has permitted significant progress in xenotransplantation in recent years, many challenges must be addressed before organ and cellular xenotransplantation can be clinically valuable. Following are several areas in which major gaps in knowledge or limitations in resources may hinder the progress of xenotransplantation:

- **Molecular incompatibilities between species** will require more research to generate new molecular and genetic strategies for avoiding delayed vascular rejection and other deleterious outcomes.

- **Animal models**, in particular non-human primate models, are particularly important in the setting of xenotransplantation, in which the unknown risk of infection from the source animal imposes an unusual ethical requirement to justify clinical trials. Efforts are needed to overcome the limitations of non-human primate models that diminish the utility and predictive value of these models.

- **Sharing of resources** through new partnership arrangements between industry and government, as well as public support of research leading to future modifications, might better ensure the sharing of genetically modified pigs and other reagents that are in limited supply and are costly and time-consuming to generate. The science of xenotransplantation is unlikely to proceed expeditiously without sharing of such reagents.

- **Support for xenotransplantation** from the biotechnology industry is currently low. In addition, there are significant challenges regarding sharing of data, animals, and reagents. Extensive additional basic research in xenotransplantation and a long-term investment of resources and effort will be required to determine if xenotransplantation is a viable clinical approach.

Parallel or Alternative Strategies to Xenotransplantation

In addition to xenotransplantation, a number of other approaches are under development for the treatment of conditions involving cellular, tissue, and organ destruction:

- **Prevention** of the acute and chronic conditions that lead to the need for replacement organs, cells, and tissue is the ideal approach, and prevention activities need to be promoted by all available means.

- **Gene therapy**, a relatively new and highly experimental technology for treating human disease, has recently enjoyed some limited clinical success. In organ transplantation, gene therapy approaches could one day be useful in preventing transplant rejection, inducing

tolerance, prolonging graft survival, and ameliorating some of the problems associated with systemic immunosuppression.

- **Stem cell therapy** offers the possibility of treatment for a variety of diseases and disorders involving tissue destruction or cellular injury and dysfunction. It offers hope for treating a multitude of clinical diseases and has both advantages and disadvantages when compared with xenotransplantation. Stem cells may have considerable potential for cellular replacement and repair, but their potential for whole-organ replacement is currently unknown.

- **Artificial organs** include left ventricular assist devices, which can improve cardiac function in patients with isolated left-sided heart failure, and an artificial heart (Abiocor) has been developed and evaluated in a small group of patients. Such devices, however, currently face several obstacles, and the potential for the success of these technologies in terms of improving quality of life and longevity are currently unknown.

Findings and Recommendations

The SACX makes the following recommendations for pursuing xenotransplantation as a strategy for treating a variety of medical disorders:

1. Continue to evaluate pigs as a suitable source animal for xenotransplantation. Due to heightened risks and ethical concerns apparent with nonhuman primates, these animals should not be considered as source animals for xenotransplantation. The establishment of specific pathogen-free closed colonies of pigs will ultimately be needed to raise animals for clinical trials.

2. Support existing federal guidelines on source animals for xenotransplantation.

3. Further development of diagnostic tools, including antibody and nucleic acid-based assays, to detect known and unrecognized porcine pathogens that pose a risk to humans should be supported. Continue research on the risks of zoonotic infection in xenotransplantation recipients and gauging the potential for new emerging diseases is needed.

4. Initiate research studies that will use the new tools of molecular biology and genetics to reveal physiologic and immunologic incompatibilities between source animals and humans.

5. Develop facilities where pig–to–non-human primate models could be used to gauge the efficacy of xenotransplantation of pig organs, tissues, and cells to humans.

6. Encourage scientists from diverse disciplines to apply their expertise in the discovery of solutions for successful xenotransplantation.

7. Establish repositories in which reagents, genetically modified pigs, and other valuable materials can be maintained and distributed to researchers and laboratories engaged in xenotransplantation research.

8. Build government-industrial-academic partnerships that ensure the sharing of reagents and research animals.

9. Provide counseling to industry early in their development of xenotransplantation products on issues related to compliance with federal regulatory and safety issues.

10. The problem of broad liability for the consequences of possible zoonotic infections is perceived by some as a deterrent to participation by industry in xenotransplantation research. Investigate this issue and identify solutions.

11. Periodically re-evaluate federal guidelines on xenotransplantation and institute a system of review and oversight of regulations.

12. Investigate the scope of xenotransplantation in countries lacking stringent oversight and the extent of risks posed by entry into the United States of persons receiving xenotransplants in such countries.

13. Educate U.S. residents about the risks of unregulated xenotransplantation procedures and discourage their participation in those lacking regulatory oversight as stringent as that in the United States.

14. Work closely with international health agencies to promote regulations and guidelines for xenotransplantation that are as rigorous as those developed by the PHS and assist other countries in implementing them.

REPORT ON THE STATE OF THE SCIENCE IN XENOTRANSPLANTATION

INTRODUCTION

Xenotransplantation holds promise for the treatment of many human diseases and disorders and could potentially alleviate the shortage of human organs and tissues available for transplantation. Xenotransplantation also raises many interesting and complex scientific, medical, ethical, legal, and social issues. This report focuses on the science of xenotransplantation, an area in which major advances have been made in the last few decades. The report outlines the considerable progress that has been made in understanding and overcoming some of the major hurdles to xenotransplantation, including hyperacute rejection, delayed xenograft rejection, the role of antibodies against the aGal sugar in these processes, and the potential infectious complications of xenotransplantation.

Xenotransplantation refers to procedures in which live cells, tissues, or organs derived from a non-human animal are transplanted, implanted, or infused into a human patient. It also includes procedures in which human body fluids, cells, tissues, or organs are removed from the body, come into contact with live animal cells, tissues, or organs, and are then placed back into a human patient.[a] Xenotransplantation is raised to a level of special public interest not only because of its potential to meet a critical health care need but also for the potential risk it poses: that new disease-causing agents could be transmitted from a xenotransplantation product to patients and their close contacts and, ultimately, to the public at large. Major headway has been made toward minimizing these risks through both the development of regulatory guidelines by the U.S. Public Health Service and scientific research that has improved our understanding of and ability to diminish the risk of transmitting endogenous infections from source animals to humans. Despite all of the encouraging progress, many major hurdles remain before the potential of clinical organ and tissue xenotransplantation can be fully realized. In this report, we summarize the limitations of the progress that has been made and identify areas in which considerable effort may still be needed in order to optimize progress in xenotransplantation.

Aim of This Report

The U.S. Department of Health and Human Services (DHHS), which includes the U.S. Public Health Service (PHS), has been developing tools for the oversight and coordination of xenotransplantation activities. These public health tools include a DHHS interagency working group; a regulatory framework; the PHS *Guideline on Infectious Disease Issues in Xenotransplantation* (http://www.fda.gov/cber/gdlns/xenophs1000.htm);[1] a national database of xenotransplantation clinical trials and biomedical animal facilities supplying animals and tissues for these trials; a national centralized archive of biological specimens for public health

[a] This is a paraphrase of the definition of xenotransplantation used by the DHHS and set forth in *PHS Guideline on Infectious Disease Issues in Xenotransplantation*[1]: "Any procedure that involves the transplantation, implantation, or infusion into a human recipient of either (a) live cells, tissues, or organs from a non-human animal source or (b) human body fluids, cells, tissues, or organs that have had ex vivo contact with live nonhuman animal cells, tissues, or organs" (p. 4).

investigations; and a national advisory panel on xenotransplantation: the Secretary's Advisory Committee on Xenotransplantation (SACX), which has produced this report.

In the mid- to late 1990s, there were a number of calls for a national advisory body on xenotransplantation to serve as a public forum for input, public education, and deliberation on this topic by researchers, health care providers, policy makers, patients and their families, public health officials, ethicists, animal welfare representatives, and other members of the public. The SACX was established by the DHHS in 1999 and first convened in 2001. The Committee is charged with advising the Department on the scientific, medical, social, ethical, and public health issues and concerns raised by xenotransplantation, as well as with recommending policies and procedures related to the scientific development and clinical application of xenotransplantation procedures.

This report was produced in direct response to two critical mandates of the SACX: to advise the Department on (1) the current state of scientific knowledge about xenotransplantation and (2) the potential for transmission of infectious diseases as a consequence of xenotransplantation. In keeping with these and related mandates, this report also addresses certain ongoing practices that the SACX considers to be global public health risks and provides recommendations for appropriate responses.

This report provides an overview of the potential impact of xenotransplantation, the types of xenotransplantation procedures currently being used in research, and the source animals for xenotransplantation products. It then discusses some of the major challenges posed by immunologic and physiologic incompatibilities, as well as strategies to address those challenges. Also discussed are the infectious disease risks associated with animal-to-human transplantation and management strategies to cope with them. Some alternative strategies to xenotransplantation are described to provide a contextual perspective on the field. The report also describes public health concerns about "xenotourism," a term coined to describe personal travel outside of a country of residence for the purpose of participating in xenotransplantation programs or attending clinics to obtain therapies not presently available or acceptable in the home country. Finally, SACX presents its recommendations for pursuing xenotransplantation as a therapeutic strategy.

Background

Although sporadic (and unsuccessful) attempts at xenotransplantation can be documented as far back as the 1600s,[2] the field is still very much in its infancy. In the United States, xenotransplantation products are regulated using applicable guidelines by the U.S. Food and Drug Administration (FDA). The FDA has received approximately 40 investigational new drug (IND) applications for xenotransplantation in the last decade. Approximately 500 patients in clinical studies in the United States have received experimental xenotransplantation products[b] for liver failure, Parkinson's disease, Huntington's disease, diabetes, intractable pain of cancer, melanoma, or burns. At least 1,000 burn patients have been treated with autologous skin cells that have been grown on mouse feeder layer cells.

[b] Xenotransplantation products are the live cells, tissues or organs used in xenotransplantation (as defined in *PHS Guideline on Infectious Disease Issues in Xenotransplantation*[2]).

Potential Impact of Xenotransplantation on U.S. Chronic Disease Burden

Statistics from the United Network for Organ Sharing (UNOS) provide some indication of the number of people who suffer from organ failure.[3] Nearly 83,000 patients were on the UNOS waiting lists at the end of 2002, and more than 6,000 died before an organ from a human donor became available (see Table 1). Approximately 2 million people die in the United States each year. However, a recent study found that only 10,500–13,800 deceased individuals are eligible to donate organs each year, and only about 42% of eligible donors during the period studied became actual donors.[4] Even if all eligible donors became actual donors, it would not meet the demand for transplantable human organs.

The waiting list reflects only those individuals who are listed as candidates for transplantation and so is not a reflection of the prevalence of organ failure. For example, the vast majority of people with type 1 diabetes, which is endocrine pancreas failure, are not listed as candidates for transplant because there is no widely available, reliable source of acceptable donor material.

Table 1. Patients on UNOS waiting list, transplants, and death while waiting in 2002

Organ	No. of patients on list*	No. of patients transplanted	No. of deaths
Total	111,716	24,544	6,385
Kidney	68,468	14,523	3,396
Liver	26,326	5,060	1,818
Heart	6,990	2,111	558
Pancreas†	1,884	517	29

* Includes patients on waiting list at start of 2002 and patients added to the list at any time during 2002.
† Includes pancreas transplant alone and pancreas after kidney transplant candidates.
Source: U.S. Department of Health and Human Services/United Network for Organ Sharing/Universal Renal Research and Education Association. 2003 Organ Procurement and Transportation Network/Scientific Registry of Transplant Recipients Annual Report: Transplant Data 1993–2002. Available at: http://www.ustransplant.org. Retrieved June 16, 2004.

Type 1 diabetes provides an example of a medical condition that could be significantly affected, both on an individual and a public health level, if xenotransplantation were to become a standard medical treatment. This disease results from the failure of pancreatic islet cells to produce insulin, which regulates blood glucose. It affects approximately 1 million Americans, who must take daily injections of insulin to survive, and results in lost work, high medical costs, and diminished quality of life.[5]

Type 1 diabetes could potentially be treated by transplantation of either pancreatic islet cells or the entire pancreas. Because this form of diabetes can also cause kidney damage or failure, xenotransplantation of an animal kidney could also be considered. Preliminary research[7] suggests that it may be possible to use non-human islet cells to produce levels of insulin that would be sufficient to reduce or eliminate the need for insulin injections. In solid-organ xenotransplantation, the transplanted organ might completely replace the malfunctioning pancreas. Human adult pancreatic tissue cannot meet this need because of the inadequate availability of human organs or tissues for transplantation.

Some chronic and degenerative diseases that might be treated with xenotransplantation:

- **Congestive heart failure** affects 4.8 million Americans, half of whom die within 5 years of diagnosis.[8]
- More than 1.5 million people in the United States have **Parkinson's disease**, a progressive disorder of the central nervous system characterized by the loss of dopamine-producing brain cells.[9,10]
- About 30,000 Americans have **Huntington's disease**, and at least 150,000 others have a 50% chance of developing the disease because they have an affected parent. There is no effective treatment for this devastating disorder, which results from genetically programmed degeneration of neurons in certain areas of the brain.[11]
- About 2,500 babies are born with **cystic fibrosis** each year in the United States. Approximately 30,000 Americans of all ages have this chronic, progressive, and frequently fatal genetic disease of the body's mucous glands.[12]
- An estimated 250,000–400,000 Americans have **spinal cord injuries**, about 60% of which occur before age 30. These injuries account for millions of dollars in medical costs and unemployment.[13]
- In 2002, 6,385 patients **died while on the waiting list** for human organ transplantation. At the end of 2002, more than 82,000 patients were **still on the waiting list** for human organs.[3]

In 2002, almost 100,000 people in the United States progressed to end-stage kidney failure, with the result that 300,000 patients were sustained on dialysis and 80,000 had functioning transplanted kidneys. These numbers had doubled since 1990 and are expected to double again by 2010.[6] More than 40% of cases of end-stage kidney failure are caused by diabetes.[7] Current standard treatment for kidney failure is hemodialysis or kidney transplantation of a kidney from a living or deceased human donor (kidney allotransplant). Survival is greatly increased after a kidney allotransplant: up to 83% of these patients are alive after 5 years. In contrast, only about 33% of patients receiving dialysis treatment survive for 5 years.[8] The annual cost of hemodialysis for one person is approximately $73,500 per year, and the cost after kidney transplantation is approximately $8,000–10,000 per year.[9] Islet-kidney xenografts, in which porcine islets are engrafted under the pig's kidney capsule before transplantation,[10] has the potential to cure both end-stage kidney disease and diabetes simultaneously. The potential effectiveness, safety, and cost of kidney xenotransplantation require further investigation and evaluation.

Diabetes is just one of many disorders that could be treated with xenotransplantation were this procedure to become proven and accepted. Among the others are congestive heart failure, Parkinson's disease, cystic fibrosis, Alzheimer's disease, and Huntington's chorea (see box).[5,11,12,13,14,15,16] However, significant technical challenges and public health risks remain and

must be addressed before xenotransplantation can become a clinical reality. These technical issues and public health considerations are discussed in detail in the following section.

Types of Xenotransplantation Procedures

The term *xenotransplantation* refers to a complex array of procedures involving the transplantation, implantation, or infusion into a human recipient of live cells, tissues, or organs derived from a non-human animal source. The procedure includes the use of human body fluids, cells, tissues, or organs that have had ex vivo contact with live, non-human animal cells, tissues, or organs. The source animals or their cells may or may not be genetically modified. A number of different types of xenotransplantation procedures are being performed or considered and are associated with various challenges. The categories of xenotransplantation procedures, as well as some of the challenges involved in their use, include the following:

- **Solid-organ xenotransplantation** is a procedure in which a source animal organ such as a heart, lung, kidney, or liver is transplanted into a human. In such cases, the vascular supplies of the source animal and the recipient are connected. As a result, the recipient's blood circulates through the animal organ, where it encounters endothelial cells that line the animal organ's blood vessels. This contact between components of the recipient's circulating blood and the source animal's endothelial cells may initiate certain types of rejection processes, which are described below.

- **Cellular and tissue xenotransplantation** is the grafting of tissues and cells from a source animal without surgical connection of any animal blood vessels to the recipient's vessels. These xenotransplantation products may be implanted directly into a recipient's organ. Examples of this type of procedure are the implantation of porcine neural tissue into a recipient's brain or of animal-derived cartilage into a recipient's joint. Alternatively, animal cells may be infused into the recipient's bloodstream, such as when islet cells are infused into the portal vein (and are intended ultimately to take up residence in the liver) or hematopoietic cells, such as bone marrow cells, are infused into the recipient's peripheral veins (and ultimately take up residence in the bone marrow or other sites of hematopoietic tissue). All cellular and tissue xenotransplants are subject to immune-mediated rejection, but the mechanisms of rejection differ from those that are activated specifically by endothelial cells (see "Immunologic Rejection Processes").

- **Extracorporeal (natural and artificial organ) perfusion** occurs when human blood is circulated outside of the human body through an animal organ, such as a liver or kidney, or through a bioartificial organ produced by culturing animal cells on an artificial matrix. To date, extracorporeal perfusion has been used in patients with hepatic failure to keep them alive until an allogenic liver transplant becomes available or until the patient's liver regenerates sufficient function to support life.

- **Exposure to living animal-derived material** occurs when any of a variety of human cell types are grown ex vivo with non-human animal cells. If these human cells are subsequently transplanted or infused into a human patient, the procedure is considered a form of xenotransplantation. An example of this technique is the growth of human skin cells

(keratinocytes) on a mouse cell line in order to generate a layer of autologous tissue to provide a temporary wound covering in patients with severe burns. Another example is the exposure of human immune cells to animal cells for sensitization. Finally, some existing human embryonic stem cell lines, if used to generate cells or tissues for treatment of patients, would be defined as xenografts if they have been previously grown on mouse feeder cell layers. The considerations regarding these types of transplants mainly relate to the risks of infectious disease (see "Infectious Disease Risks Associated with Xenotransplantation").

Potential Source Animals for Xenotransplantation

Recent research in xenotransplantation has focused largely on the pig as a potential source animal for a number of reasons. In particular, the widespread availability and excellent breeding characteristics of pigs make it possible to generate large numbers of animals in closed colonies and to develop transgenic and cloned animals. In addition, the similar size of pig and human organs (particularly those of certain breeds of pig known as miniature swine) and the similar physiology of the two species make the pig particularly suitable as a potential xenograft source.[17] Considerable human experience with the husbandry and veterinary care of pigs also favors their use as source animals. Sheep and cows have equally long histories of commercial breeding, genetic manipulations, and animal health experience, and they might be considered as source animals in the future.

A number of other diverse source species also appear to be suitable. Certain species of fish, such as tilapia (*Oreochromis* spp.), are a potential source of pancreatic islets.[18] Mouse and insect cells have been used for growing human cells in culture. Animal cells that produce viral gene delivery vectors have been injected into humans in a type of cancer therapy. Genetically altered mammal, fish, or insect cells that produce therapeutic biological agents might be placed within special isolation capsules to be injected or introduced into humans for the treatment of genetic or chronic inflammatory diseases.

Non-human primates may seem to be logical source animals because their genetic proximity to humans might present less formidable immunological barriers to successful xenotransplantation. Major barriers to the use of non-human primates, however, have effectively removed them from consideration. The major reasons are the risks of transmitting viruses to humans[19] and the realization that most monkeys and baboons are considerably smaller than adult humans. Moreover, it would be difficult, expensive, and time-consuming to breed large numbers of captive non-human primates in germ-free isolation facilities. In addition, the breeding of non-human primates as a source of xenografts is considered unethical and unacceptable by many members of the lay and scientific community.

The importance of the ethical and humane treatment of animals in xenotransplantation, both those used in research and those eventually serving as source animals for human recipients, is a topic that deserves consideration in itself and is beyond the scope of this report. Animals used in research, and eventually in therapeutic applications of xenotransplantation, should be treated humanely and with respect.

SCIENTIFIC CHALLENGES IN XENOTRANSPLANTATION

Technical challenges to xenotransplantation are primarily immunologic and physiologic in nature. Although these challenges are common to both human-to-human transplantation (allotransplantation) and xenotransplantation procedures, the various differences and disparities between species tend to exacerbate these issues in xenotransplantation.

Immunologic Rejection Processes

Immunologic problems such as acute cellular rejection and chronic rejection are faced in any transplantation procedure. In xenotransplantation, however, a number of factors arising from disparities between species intensify the immunologic processes that mediate rejection.

Hyperacute Rejection

Hyperacute rejection[20] is the nearly immediate and catastrophic destruction of a graft. This process is initiated by antibodies, often called natural antibodies, that are present and circulating in all humans. For xenotransplantation, the most important natural antibodies are directed to a specific sugar molecule expressed by most species, including pigs, called galactose-(a1-3)-galactose (Gala1-3Gal) (abbreviated aGal). Because human cells do not express this sugar molecule, the human immune system recognizes it as "non-self," or foreign, and produces antibodies against it in response to its presence on common microorganisms. Most other mammalian species (except Old World monkeys and Chinese hamsters) express aGal on the proteins and lipids that are present on their cell surfaces. When a vascularized xenotransplantation product that expresses these aGal sugars is placed into a human or Old-World, non-human primate recipient, the circulating natural antibodies quickly bind to the sugars and activate a destructive cascade of protein interactions along what is known as the complement pathway. These antibodies and the process of complement-mediated destruction are elements of natural human immunity against invading microorganisms.

Most antibody binding occurs first in the bloodstream, on the surface of endothelial cells that line the blood vessels. With complement activation, these endothelial cells are activated, injured, and often killed. Simultaneous activation of the blood coagulation systems leads to the generation of fibrin clots, which can obstruct the blood vessels feeding the xenotransplant and cause tissue destruction due to lack of blood flow.

Both the complement and coagulation cascades are normal responses to infection and injury. An example of these processes at work in human-to-human transplantation is the severe reaction that occurs when organs are transplanted across ABO blood group types. These events are typically down-regulated by other proteins that are secreted into the serum by endothelial cells. However, some of these down-regulatory proteins do not work uniformly well between species. For this reason, the complement and coagulation cascades may be more powerful in xenotransplantation than in allotransplantation across human blood group barriers.

Antibody-mediated rejection can also affect non-vascularized transplants (e.g., islets), but not in the same rapid, dramatic manner. Moreover, islets from adult pigs do not express the aGal

carbohydrate. As is discussed later in the report, genetically engineered pigs have recently been generated that lack the expression of the aGal sugar.

Delayed Vascular Rejection

Major advances have been made in the prevention of hyperacute rejection by using organs from pigs that are genetically engineered to express proteins that regulate human complement and transplanting these organs into non-human primates. With these advances, however, another immunological barrier to successful xenotransplantation, termed delayed vascular rejection, was revealed.[21,22] This form of rejection occurs over days to a few months and involves the vascular system of the xenotransplantation product. Pig kidneys and hearts transplanted into non-human primates stimulate progressive destruction of the pig blood vessels. Antibodies and inflammatory immune cells are found in these vascular lesions. The exact immune mechanisms responsible for delayed vascular rejection are not yet fully known. Anti-aGal antibodies (despite absorption procedures to remove them), along with other types of anti-pig antibodies responding to the xenotransplant, attack the graft by localizing to its blood vessels. Still other cellular components of the innate immune system rapidly attack and remove invading organisms. This system includes macrophages, natural killer cells, and primitive types of T-cells. Some of these elements are activated by the products of antibody binding and complement activation just described, or they may be activated independently by xenogeneic cells. Because delayed vascular rejection has limited the maximum survival time of most organ xenotransplants to 2–3 months, the future success of these procedures will require an improved understanding of the mechanisms of this reaction and the formulation of effective therapeutic strategies.

Acute Cellular Rejection

Acute cellular rejection[7, 23] occurs over weeks or months after transplantation. This phenomenon is mediated predominantly by T-cells, which constitute the cellular arm of the immune system. Acute cellular rejection can target blood vessels but usually involves infiltration of attacking host T-cells (and other immune cells) into the transplant tissues and destruction of the epithelial cells that are responsible for the function of the xenotransplanted organ.

Cellular rejection, though a major problem after allotransplantation, has not yet presented a major barrier to the survival of experimental xenotransplants. This may be due to the high doses of immunosuppressive drugs that are currently used in xenotransplantation studies to block activation of the cells that mediate this process. A number of studies have nonetheless provided evidence that the T-cell immune response to xenogeneic (including porcine) antigens is stronger than the response to non-self antigens of the same species. This may be because human anti-pig T-cell responses appear to involve a greater number of antigenic disparities than do pig anti-pig T-cell responses. The problem is compounded by the fact that many of the molecular interactions between T-cells and foreign cells that express antigens seen by the T-cells seem to function perfectly well between pig and human. This makes the human anti-pig T-cell response highly effective. Since massive doses of immunosuppressive drugs will not remain acceptable as long-term management of this vigorous human anti-pig response, its continued prominence will necessitate other strategies to make widespread xenotransplantation feasible. Thus acute cellular rejection may yet become an issue in future xenotransplantation studies.

Chronic Rejection

Chronic rejection is the progressive and relentless destruction of a transplant over months to many years. As one of the major causes of late graft loss from human donors, chronic rejection is currently regarded as a major hurdle in allotransplantation. Antibodies induced by the organ graft may play a role in the process. For some transplanted organs (e.g., hearts), a poorly understood, distinct pathological process results in such severe narrowing of blood vessels that the underlying tissue is starved of essential nutrients. This process, as well as direct attacks on the cells that create and maintain the structure and functions of the transplant, may eventually result in large areas of dead tissue, which are ultimately replaced by fibrotic scars.

Chronic rejection has not been widely observed in xenotransplantation because of the generally short survival of xenotransplants. Because the mechanisms of chronic rejection are incompletely understood and there is no effective therapy at present, this problem is likely to become more prominent when the initial barriers are surmounted and graft survival is extended.

Additional Species Differences That May Affect Xenograft Function

Other differences between species are also of concern in xenotransplantation. In addition to their relevance to organ function, physiologic differences between species also compound immunologic incompatibilities. Species differences in responses to hormones and growth and other regulatory factors are additional potential barriers to xenotransplantation, which would ordinarily require little consideration in allotransplantation. Current knowledge of these potential challenges is extremely limited.

Complement and Coagulation Systems

An important advance in elucidating the critical roles of the complement, coagulation, and clotting systems[24,25] came with the transgenic engineering of pigs to express human complement regulatory proteins on their blood vessels. Although pig complement regulatory proteins that are broadly homologous to their human counterparts are normally present on pig blood vessels, the porcine versions of these molecules are sufficiently different so as to be unable to fully regulate the activation of human complement. The transgenic addition of the human proteins to pig endothelial cells increases the total amount of complement regulatory protein that is expressed and adds molecules that may be more effective than their porcine counterparts in inhibiting human or non-human primate complement.[26,27,28,29]

Other incompatibilities in the mechanisms by which the complement and coagulation system is activated in pigs and primates are also likely to be important. For example, investigators performing xenotransplants from pigs to non-human primates have noted a systemic coagulation disorder that begins with excessive coagulation and clotting, leads to consumption of clotting factors and platelets, and ends in a bleeding disorder.[17] The endothelial cells that line blood vessels produce several types of anti-coagulation molecules that are critical to normal blood flow. Patients with genetic defects in the production of these anti-clotting molecules have a high risk of clotting and tissue injury. If pig anti-clotting proteins show similar incompatibility with

human blood coagulation factors, the increased risk of clotting might contribute to xenotransplant rejection. This process appears to occur along at least one anti-coagulant pathway, and other, undiscovered incompatibilities are also likely to exist in this complex system.

Adhesion Molecules, Cytokines, and Growth Factors

The normal function of any organ or tissue requires the regulation of its mass and architecture in addition to repair of injury. These processes involve dynamic interactions at the cellular level among factors that are carried in the circulation from a distance or are produced locally. It is therefore critical to consider potential incompatibilities between growth and regulatory factors, adhesion molecules, and their receptors in humans and animal organ sources.

These types of interactions may come into play in the transplant of non-vascularized tissue grafts (e.g., cartilage, neural tissue, thymus), which require the growth of new blood vessels from the recipient to maintain their nutrition and survival. In addition, the survival of intravenously injected cellular transplants (e.g., islets, bone marrow cells) depends on their ability to "home" to the right microenvironment, a process that depends on the interactions of specific adhesion and chemoattractant molecules and their ligands as well as the ability to respond to growth factors once they reach that microenvironment. Some researchers have already identified adhesion molecules and growth factors that affect pig bone marrow homing and function but that do not show normal interactions between pigs and primates.[30]

Organ-Specific Physiologic Considerations

Critical physiologic and/or metabolic functions are performed by the cells of every organ. The cells of the heart do not merely constitute a biological pump, however, nor do the cells of a kidney merely constitute a biological filter. The complexity of organ function presents a challenge to both xenotransplantation and the development of artificial organs.[31,32,33] For example, cells in the liver, lungs, and kidneys take up and process drugs and body toxins. Failure of a porcine graft to perform these functions normally could have profound and unexpected side effects. Kidneys and other organs maintain normal levels of circulating electrolytes, water, sugar, and other biochemical products, and these levels may be subtly different in pigs and primates. In addition, because they have an upright posture, humans' heart valve size, pulmonary circulation, and other physiologic functions are different from those of four-legged animals.

Human organs that are frequently transplanted (i.e., kidney, liver, heart) also produce a variety of growth and metabolic factors that are largely unrelated to their primary function. For example, in addition to fulfilling its primary role as a filter, the kidney produces the hormone erythropoietin, which regulates normal blood cell production in the bone marrow and prevents life-threatening anemia. Some evidence suggests that pig erythropoietin may not work or may be destroyed by an immune response in non-human primates. This has resulted in severe anemia in non-human primates maintained on porcine kidney xenografts.[34] This limitation could theoretically be overcome by using transgenic pigs that produce human erythropoietin, but the

requirements for generating many such organ-specific factors could impose significant constraints on the applicability of xenotransplantation.

Liver xenotransplantation poses the greatest challenge in regard to organ-specific differences between species.[33] The liver produces innumerable proteins that are involved in normal clotting, complement function, drug metabolism, metabolism of normal biological waste products, digestion, lipid metabolism, and more. It will be critical to determine which of the normal human homeostatic mechanisms can and cannot be maintained by the pig liver by elucidating the interactions of pig and human molecules that regulate these functions and characterizing the similarities and differences between pig and human physiology. It may be prudent to undertake such research even before long-term organ graft survival has been achieved in non-human primates in vivo, when these problems might come to light. Early initiation of molecular and comparative physiologic studies would allow investigators to anticipate potential dysfunction between species and to potentially circumvent problems by genetically engineering the source animal.

Size Considerations

Adult pigs typically weigh 1,000 pounds and obviously have organs that are too large for use in humans. The use of very young porcine source animals has resolved the problem of size in non-human primates. However, studies have not yet involved orthotopic, functioning heart transplants (i.e., organs transplanted in place of the recipient's original organ), and survival of heterotopic transplants (organs transplanted alongside the recipient's original organ) has not exceeded a few months. It is unknown whether the growth potential of these porcine organs could ultimately result in physiologic problems in recipients. A potential solution to this problem would be to use adult miniature swine, whose maximum weight of about 250 pounds more closely approximates adult human size.

Current Approaches to Xenotransplantation Challenges

The issues described in the preceding sections would have to be successfully resolved in order for xenotransplantation to be considered as a human therapy. It is likely that a combination of strategies will be needed to address these challenges. Some of the most promising strategies are genetic modification of source animals, development of devices to encapsulate or otherwise isolate the transplant, creation of tolerance to the xenotransplantation product, gene therapy, targeted molecular therapy, and several others.

Genetic Modification of Source Animals

One of the major advantages of the use of pigs as source animals for xenotransplantation products is their excellent breeding capabilities in captivity. These breeding characteristics facilitate genetic engineering and allow the rapid transmission of introduced genetic modifications into the herd, as well as their combination with other genetic modifications. Several human genes have already been introduced into pigs as transgenes, and advances in nuclear transfer techniques have permitted the recent development of pigs that do not express aGal on cell surfaces (referred to as *aGal knockout pigs*; see "Other Host Treatments" under

"Current Approaches to Xenotransplantation Challenges"). It is likely that the optimal pig for xenotransplantation will require multiple genetic manipulations, which could be facilitated by increasing experience with the techniques for porcine genetic modification, developing more efficient techniques for genetic modification, and sharing of proprietary genes or genetically modified pigs to allow their combination in a single animal.

Transgenic source animals. Investigators have produced several strains of transgenic pigs that express human proteins that down-regulate activity of the human complement cascade involved in hyperacute rejection. These human proteins were selected because some of the corresponding proteins in pigs do not fully down-regulate human complement activity. It is thought that the expression of human complement regulatory proteins at higher than physiologic levels might significantly diminish antibody-mediated destruction of the graft. Some of these proteins have been introduced into pigs, and all showed some efficacy. In some cases, the proteins reduced or prevented hyperacute rejection of porcine solid organs that had been transplanted into non-human primates.[26,27,28,29,35,36,37] Another transgenic approach involves the expression in porcine source animals of an enzyme that glycosylates porcine glycoproteins and glycolipids in a manner that masks the expression of the aGal carbohydrate on these molecules.[35,38]

Survival of up to approximately 135 days has been achieved for genetically engineered pig organs implanted into non-human primates. Organs from these transgenic pigs may function even better in humans, because the humanized pig organs may induce immune responses in non-human primates that would not occur in humans. Similarly, the human proteins may not interact optimally with the non-human primate complement components that they are expected to regulate.

Although these studies have demonstrated the efficacy of the transgenic approach in preclinical models, they have also highlighted the fact that hyperacute rejection is only one of several rejection processes that can rapidly destroy solid-organ xenografts. Transgenic pig heart and kidney grafts have been lost in days to months to the process of delayed vascular rejection, probably because of the binding of antibodies to the endothelium of the xenogeneic organ. Delayed vascular rejection may also involve cells of the innate immune system, such as macrophages and natural killer cells, and these may also play a role in graft loss.

Islets from the tilapia fish have been shown to be capable of maintaining normal blood sugar in mammals. These animals are an attractive source of islets in part because of their abundance and ease of isolation. They can also be genetically modified to improve their suitability as a xenograft source and, indeed, their insulin genes have been effectively "humanized" to make them less likely to incite immune responses in humans.[18]

Researchers have recently developed a technique of nuclear transfer in which a genetically modified donor cell nucleus can be used to replace the nucleus of a germ cell (an oocyte). This development provides an approach to making site-specific genetic modifications. The technique has been used successfully to generate transgenic animals and may be more efficient than earlier strategies.

Additional transgenic strategies are also under consideration or development. One of these is genetic modification to protect the source organ's endothelial cells from activation and death in the face of antibody and complement activation. Another is engineering pigs to express molecules on the cell surface that are inhibitory of immune responses. Still other transgenic modifications that are currently being considered would make porcine bone marrow cells more receptive to adhesion molecules and growth factors in the human marrow microenvironment, where bone marrow homing and function are regulated. Such modifications would be aimed at making porcine bone marrow cells more effective for the induction of immune tolerance to the source animal (see "Tolerance").

Knockout pigs. Until recently, the development of genetic modifications in which specific genes are targeted for mutation has been an elusive goal in the field of xenotransplantation. Researchers consider the most important target of gene knockout strategies to be aGal transferase, the gene that leads to the production of the ubiquitous aGal carbohydrate that is the target of hyperacute rejection and delayed vascular rejection of porcine xenografts in primates (see "Scientific Challenges in Xenotransplantation"). A major advance in this direction has been made with the recent success of a nuclear transfer approach to develop knockout pigs that lack the aGal transferase gene.[39,40] The viability of pigs with both copies of the gene knocked out is very encouraging.[41] Pig-to-primate transplant studies using these knockout source animals are now in progress, and promising preliminary data provide proof of principle that porcine renal xenografts can survive in non-human primates for longer than 80 days with no evidence of any type of rejection (public presentation to the SACX). Although antibodies to aGal are currently a major challenge in pig-to-primate xenotransplantation, it should be borne in mind that the absence of aGal in knockout pigs may expose other antigens that may evoke an immune response.

Encapsulation and Other Bioartificial Isolation Devices

Encapsulation of xenotransplantation products represents an approach to protecting cellular transplants, such as islets, from destruction by proteins and cells of the immune system. In this technique, the transplant is encapsulated in a material that is impermeable to destructive factors but allows the diffusion of nutrients from the recipient's body fluids into the encapsulated cells, as well as the diffusion of the desired product of the encapsulated cells (e.g., insulin) into the recipient's bloodstream.[42] This approach has been under investigation for many years in the allotransplantation field. Although no product with demonstrated clinical utility is yet available, research in both academia and the biotechnology industry may ultimately yield useful technologies.

Another approach to protecting cellular xenografts includes the in vivo production of a bioartificial isolation environment. This could be accomplished by, for example, implanting a tubular scaffold that would become coated with collagen from the recipient and neovascularized. The xenograft would then be implanted inside this device.

Tolerance

Although numerous approaches to inducing tolerance between individuals of the same species have been developed in rodents, only a few of these methods have been successfully applied in large animals and in xenotransplantation models. The approaches that have shown success in these latter, more difficult settings are hematopoietic cell transplantation and thymic transplantation. In addition, "costimulatory blockade" has shown some success with islet xenotransplantation in rodents. However, costimulatory blockade alone and other approaches, including a variety of monoclonal antibodies, donor antigen infusions, and certain drugs, have not yet been successfully applied in more stringent large-animal or human transplantation settings, even within a species. In contrast, hematopoietic cell transplantation has shown to successfully achieve tolerance in humans.[43,44]

Because of the potency of the human anti-pig antibody and T-cell responses, some researchers believe that the induction of tolerance will be necessary for the clinical success of xenotransplantation. Without tolerance, highly toxic, broadly immunosuppressive drugs would be needed to prevent rejection.

Hematopoietic cell transplantation has been shown to induce simultaneous tolerance among all classes of antibody-producing cells as well as of T-cells in xenogeneic combinations.[45,46,47] An advantage of this approach over those that aim to remove aGal from the source animal or to specifically suppress the immune response to aGal is that cell transplantation tolerizes to all donor antigens expressed by hematopoietic cells, including those that might become important targets of rejection in the absence of aGal. Xenogeneic bone marrow transplantation to aGal-negative recipients (aGal knockout mice) has been shown to lead to tolerance not only of cells that make antibodies to aGal, but also to those recognizing other antigens on the xenogeneic source animal.

The use of hematopoietic cell transplantation has previously been limited by the requirement for very toxic host treatments to achieve successful engraftment. However, investigators have recently developed animal models of allogenic and xenogeneic transplantation, and even clinical approaches to allogenic bone marrow transplantation, that achieve marrow engraftment with less toxic treatment of the recipient. An immunodeficient mouse model has recently provided proof of principle that porcine marrow engraftment tolerizes human T-cells developing in a human thymus.[48] However, success has not yet been achieved in non-human primate models of pig hematopoietic cell transplantation. The reasons for this lack of success are largely because the barriers to engraftment posed by the immune system have not been fully addressed, and because physiological incompatibilities limit porcine marrow function in primate recipients.[17] This approach would be moved forward by the development of better reagents for temporarily depleting cells of the immune system in primates, as well as more information on the nature of the physiologic incompatibilities described here (see "Physiologic Issues").

Another promising approach for the induction of T-cell tolerance involves the transplantation of porcine thymus tissue. T-cells develop in the thymus, and studies have shown that pig thymus grafts can induce tolerance among developing T-cells of other species, including humans.[49,50] This approach, however, would have to be used in combination with some other strategy for overcoming the antibody problem, because antibody-producing cells are not tolerized by thymic transplantation. In addition to hematopoietic cell transplantation, another approach that has

induced tolerance of the B cells that produce natural antibodies to a xenograft in rodents is the infusion of donor antigens along with treatment with certain drugs.[51]

Gene Therapy

Gene therapy involves the transfer of genetic material (DNA or RNA) in order to provide a copy of a gene to alter cells in a manner that is beneficial. This technique could be used to modify either source animals or recipient cells. An example of the former is the in vitro transfection of porcine neural stem cells with a gene to protect the cells from immune destruction in the xenogeneic environment. Many technical hurdles must be overcome, however, before this technique will be practical.

Recipient cells could also be modified to facilitate xenotransplantation. Recipient bone marrow can be engineered to incorporate the aGal transferase gene. After a conditioning procedure to prepare the recipient, reimplanted recipient marrow will express aGal and induce tolerance of anti-aGal antibody-producing cells.[52] Although this approach is promising as a component of a xenotransplantation strategy, it is limited to tolerization to known antigens for which a gene can be identified. It is unlikely on its own to permit the induction of tolerance to the wide variety of the many unknown foreign antigens expressed by the pig. In addition, gene therapy has its own set of safety issues, often associated with viral vectors used for gene transfer (see "Gene Therapy" under the section "Parallel or Alternative Strategies to Xenotransplantation").

Targeted Molecular Therapies

A number of new drugs are being developed to inhibit the activation of the complement and blood clotting systems that follow antibody binding and complement activation. These agents include soluble inhibitors of complement activation-some of which are quite promising-and inhibitors of coagulation.[17] Further knowledge of species incompatibilities in the regulation of coagulation and clotting could permit the development of additional, useful agents of this type.

Other Host Treatments

Removal of anti-aGal antibodies before xenotransplantation has been used to prevent hyperacute rejection.[17] This can be accomplished by perfusion of pig organs with the non-human primate recipient's blood, perfusion of extracorporeal columns bearing aGal sugars that can absorb natural antibodies from the plasma, or infusion into the recipient of conjugated aGal sugars or substitutes that absorb the anti-aGal antibodies in vivo. Success in preventing hyperacute rejection and even delayed vascular rejection in some non-human primate studies has been tempered by the inevitable occurrence of delayed vascular rejection once the antibody removal procedure is discontinued and antibodies are allowed to recover. This rebound of anti-aGal antibodies has been a recurring problem in all such studies.

INFECTIOUS DISEASE RISKS ASSOCIATED WITH XENOTRANSPLANTATION

Allotransplant procedures have associated risks caused by known human pathogens. Infectious disease risks associated with xenotransplantation, however, have unique features that could pose a threat to public health. Pathogens in source animal organs, tissues, cells, or body fluids could result in the undetected spread and establishment of novel infections in the humans.

Xenotransplantation may allow infectious agents from source animals to circumvent natural barriers, such as mucosal surfaces and skin, through the surgical placement of an animal organ in a human. If an infectious agent from a source animal is transmitted to a human recipient, the agent may then be passed on to other humans. Risks from xenotransplantation include both acute and chronic persistent viral infections. The infectious agents may not have caused disease in the source animal. Infections that are asymptomatic in the animal but pathogenic in humans have occurred. HIV-1, for example, originated from chimpanzees, where it causes no disease, but the virus induces AIDS in humans.

Parasitic, bacterial, and mycotic infections also pose a risk but are of less concern because of the availability of diagnostic screening methods, effective animal husbandry practices, and antimicrobial treatments to eliminate known potential pathogens. Nevertheless, oversight of the emerging field of xenotransplantation requires vigilance in preventing all infections.

The magnitude of the risk associated with xenotransplantation would be influenced by a number of factors:

- The requirement for immunosuppressive agents
- The species of the source animal and the applicable husbandry practices
- The type of tissue or organ used and the duration and type of recipient exposure

Many of these issues have been addressed in earlier publications,[53,54,55,56,57,58,59] including peer-reviewed manuscripts, chapters, and FDA and PHS guidelines. This report highlights the areas considered most critical and in need of further research and action.

General Properties of Infectious Agents Relevant to Xenotransplantation

The microbial agents that are of concern in the transplant setting can be broadly classified as exogenous and endogenous. Exogenous agents are transmitted from an infected individual or animal to a susceptible host. Examples include viruses (e.g., HIV, herpes simplex virus, and viruses that cause the common cold); bacteria (e.g., *Streptococcus pneumoniae* and *Mycobacterium tuberculosis*); fungi (e.g. *Aspergillus*); and parasites (e.g., *Toxoplasma gondii*). Some exogenous microbes can be transmitted across the placenta. The organisms that pose the greatest hazard to public health in xenotransplantation are those that persist asymptomatically in quiescent or latent phases in source animals. Active screening for agents such as herpesviruses, arenaviruses, and retroviruses will be essential. Although fungi and parasites show similar persistence and transmission patterns, these exogenous microbes are more visible and well recognized and can be more readily excluded or removed from the chain of transmission by screening and husbandry techniques.

In contrast to exogenous viruses, endogenous agents are essentially embedded in the host genome and therefore are considerably more difficult to eliminate from source animals. Many endogenous viruses are defective, but a few do replicate and may be capable of infecting other animal species, including humans. These endogenous viruses have been detected in all animal species and have not yet been eliminated from source animals by either classical husbandry techniques or more sophisticated knockout technologies.

There are many known infectious agents in source animals and many diagnostic tests and husbandry practices capable of reducing the risk. Although these agents represent a major risk in unregulated xenotransplantation, they can be largely controlled by measures discussed elsewhere in this report (see "Viral Persistence, Latency, and Species-Specific Virulence"). However, there is no doubt about the future emergence of unknown exogenous and endogenous infectious agents, for which no means of detection currently exist. Examples include recent outbreaks of severe acute respiratory syndrome (SARS) and Nipah and Hendra viruses, which were transmitted from animals to humans. As yet undiscovered, novel transmissible agents constitute a potential risk to the transplant recipients themselves, their intimate contacts, health care workers, and the population at large.

Non-Human Primate and Porcine Source Animals and Infectious Disease Risk

Current strategies in xenotransplantation focus on swine as source animals, although several other species have been proposed. Heightened concerns over the potential transmission of infection from apes and monkeys to humans have further focused xenotransplantation studies on porcine cells, tissues, and organs. Concern about the use of non-human primates comes partly from prior experience with xenografting of their organs and cells.[60] In experiments carried out in the 1960s and 1970s, for example, humans with transplanted chimpanzee kidneys survived for as long as 9 months. However, the compelling reason to assess the risk of infection in these recipients was not appreciated before the identification of latent retroviral infections in non-human primate source animals. In 1993, simian retroviruses and herpesviruses were detected in two human recipients of baboon livers.[61] Because both patients survived only briefly after transplantation, the ability to determine the level of risk from these infections was limited. Evidence of simian foamy virus (SFV) was detected in several tissues taken from both autopsied patients; however, whether retroviral transmission from non-human primate cells to human cells occurred could not be resolved.[62] SFV is an apparently nonpathogenic retrovirus found in most non-human primates but represents a risk of uncertain magnitude in xenotransplantation. A second simian virus, baboon cytomegalovirus, was also isolated from the blood of one of the two liver recipients.[63] Human cytomegaloviruses are routinely transmitted from human donors to recipients and can cause serious complications, and although cytomegaloviruses have generally proven to be species-specific with regard to pathogenicity, concern remains that the baboon homologue might result in human disease.

A 1996 baboon-to-human bone marrow xenotransplant highlighted uncertainty about transmission of infectious disease.[64] The recipient was a patient with AIDS whose bone marrow transplant survived for only 13 days. The source material contained two known simian viruses (baboon endogenous virus and baboon gamma herpesvirus), but there was no evidence of recipient infection. The patient may have been spared from infection because the transplanted

baboon cells were present only transiently. With knowledge of these and other simian viruses harbored by non-human primates, along with ethical considerations on rearing animals in closed environments, the FDA published *Guidance for Industry* in 1999 urging researchers not to consider non-human primates as source animals for human xenotransplantation.[65]

Another source of concern regarding transmission of simian viruses to humans is the historical record of the development of the childhood viral vaccines in the 1950–1970 time frame, in which monkey kidneys were used as a source of cell substrates for the propagation of vaccine viruses. Dozens of novel viruses were identified as exogenous contaminants of cells used for vaccine production and represented actual or potential contaminants of early viral vaccine preparations.

Swine also carry several infectious agents that are transmissible to humans. Although transfer of viruses from pigs to humans has been documented and has public health implications (e.g., the worldwide influenza epidemic of 1918 is thought to have originated in pigs), these types of infections would be excluded from an FDA-approved source animal colony. Accordingly, many experts believe that the pig generally poses less of a risk of infection than does the non-human primate.

The anxiety about transmission of pig viruses to humans through xenotransplantation was recently rekindled by studies demonstrating that a virus called porcine endogenous retrovirus (PERV) could infect human cells in culture.[66,67,68] As with any zoonotic infections, the risk of PERV transmission from carriers to contacts (e.g., sexual contacts, health care workers) presents a potential risk. Current efforts have focused on understanding the determinants of PERV infection and disease in transplant recipients, but determining the potential risk from endogenous retroviruses remains a daunting task. The overall lower risk from porcine xenografts can be minimized through good animal husbandry practices, construction of barrier-contained breeding facilities, appropriate controls for surgical procedures to harvest organs, and screening of source animals for known infections. It is also possible that an infected blood donation or other recipient contact with an infected individual could spread new, unidentified pathogens. Infectious risks from animal sources will continue to warrant more intensive investigation as the field of xenotransplantation matures.

Other Sources of Xenotransplantation Products

As discussed earlier, pigs are currently considered the most likely sources for xenotransplantation products. Other species, however, may also prove useful, including the fish tilapia as a source of islet cells, and mice as a source of primary cell feeder layers for some stem cell applications.[69] FDA guidance specifies that even autologous transplants of human tissues are considered xenotransplants when feeder layers employ cells of a different species.[67]

The principles for preventing contamination of the porcine-derived xenotransplantation products by endogenous and exogenous infectious agents are essentially the same as those for products derived from other source animals and their production processes (e.g., reagents, facilities, equipment, staff, product containers). Details of the control procedures may require adjustment for the specific properties of the endogenous and exogenous infectious agents associated with the source species, their mode of transmission, and the most efficient means for their monitoring and

control. However, appropriate methods to control potential infectious agents in the environment, in the reagents and raw materials, and harbored by staff who handle the animals and animal-derived materials remain largely the same. These controls are needed because the organ, tissue, or primary cells often cannot be characterized in detail before the product is used. The use of source animal procedural and environmental controls should help to limit or prevent contamination of the transplant product.

Some products in use in humans are actually xenotransplantation products, as defined by the FDA and the PHS, because they use animal cell feeder layers (e.g., autologous human skin cells grown on murine 3T3 feeder layers) based on characterized banked cells. Xenotransplantation products could use characterized banked cells either as a direct source of transplanted cells or as feeder layers for autologous human tissues. Xenotransplantation products based on cell lines that can propagate and maintain their differentiating characteristics (e.g., murine embryonic stem cells) may be developed in the future.

Appropriate controls rely on characterization of the banked cells as well as on procedural and environmental management of the application process (e.g., scale-up of cells from the characterized cell bank and their harvest for intended use). Decades of experience with producing viral vaccines, recombinant DNA products, and monoclonal antibodies in cells from characterized cell banks testify to the success of rigorous characterization efforts in keeping these products free from the risks of infectious agents.[70,71] The specific tests used for characterization depend on the species of origin for the non-human cells, the exogenous and endogenous viruses found therein, and the most appropriate microbiological methods for their detection. In general, products made from characterized banked cells have significantly lower risks of contamination and are safer to use than products using primary cells or other uncharacterized cells or intact organs, due to the ability to perform more extensive testing and characterization on banked cells.

Viral Persistence, Latency, and Species-Specific Virulence

Acute symptomatic viral infections can usually be identified and eliminated from source animals. In addition, if a source animal infection is transmitted and leads to a rapid clinical course in the patient (e.g., within days or weeks of exposure), the main risk posed is to the patient and health care workers in the hospital setting. Hospital containment practices are generally useful in restricting the spread of most acute infections.

Some viral infections, however, can remain in a latent state within the source animal and, potentially, in transplant recipients for prolonged periods, even decades.[72] These persistent or chronic infections are of concern because they may not be recognized during the post-transplant recovery period. Because infections by viruses in their natural host species commonly produce little or no disease, it is difficult to make predictions about the relative virulence of an animal virus in human recipients on the basis of its virulence in the natural host. Several examples exist of asymptomatic infections in non-human animals that have become important emerging diseases in humans. One is HIV, where natural infection of African nonhuman primates does not lead to disease, but transmission to humans led to the establishment and spread of HIV-1 and a global AIDS epidemic.[73] Source animals may harbor other known and unknown infectious

agents that might elude attempts at identification, possibly threatening public health.

Many new, unidentified infections could be avoided by establishing closed colonies of source animals through cesarean derivation. Considerable attention has been given to intensive breeding practices and maintenance of closed herds to minimize infections carried by pigs. Pigs may carry viral infections that pose a measurable risk in xenotransplantation, and they may also carry undefined viral infections with unknown potential consequences to public health. Although many of these risks can be reduced with good herd management, the endogenous infection PERV remains a current concern. To date, subjects who have received experimental xenotransplants have not been found to be PERV infected. Although this is reassuring, some of these studies were limited in the types of samples that could be evaluated. Recently identified, genetically distinct strains of pigs appear to be incapable of producing replication-competent PERVs.[74] A greater understanding of the biology of infectious PERV could lead to strategies for avoiding such infections in human recipients of xenotransplantation products. Because of its ability to replicate in human cells and the possibility that transplanted organs and cells harbor and express PERV, efforts have been directed at establishing a basic understanding of the infectious nature of PERV and its risk to humans in the transplant setting.

Studies to Address the Risk of PERV in Humans

Several studies have assessed the risk of infection with PERV by examining archived samples from humans who had received porcine xenografts, had been exposed to porcine cells by ex vivo perfusion of their blood through porcine liver or spleen, or had been exposed through a bioartificial device containing porcine cells. More than 160 patients with various degrees of exposure to pig cells have been studied.[75]

To date, PERV infection in humans exposed to porcine cells or tissues has not been demonstrated by either molecular or antibody-based methods. However, porcine cellular DNA and PERV have been detected in human recipients exposed to porcine spleens.[75] In some cases, porcine cells have persisted in these patients for as long as 8 years after transplantation. Since PERV, and possible other porcine infections, could be associated with porcine cells circulating in human patients, those patients may be at continual risk for PERV infection. Other studies have failed to demonstrate PERV transmission to human recipients of pancreatic islet cells or to patients exposed to extracorporeal perfusion from either transgenic porcine livers or bioartificial devices containing pig hepatocytes.[76]

These studies provided no evidence for transmission of PERV or other porcine virus to human xenograft recipients. Although these results are reassuring, the studies were performed on archived samples from recipients who had various degrees of exposure. Furthermore, no patients have survived whole-organ porcine xenotransplants, which may confer a greater risk of infection than other forms of xenotransplantation. Further work is necessary to assess the risk of PERV transmission from porcine xenografts, especially with tissue expected to survive for long periods.

Animal Models to Assess the Risk of PERV

Animal model systems for xenotransplantation represent an important strategy for determining

the risk of PERV infection in the transplant setting. In a seminal study, mice implanted with porcine pancreatic islet cells became infected with PERV.[77,78] Genetic immunodeficiencies in these mice may have facilitated their acquisition of PERV. (Mice do not generate the anti-aGal antibodies that occur naturally and that neutralize PERV in humans and non-human primates and thus would be more likely to become infected.) The study illustrates that PERV is actively expressed during xenografting and can be infectious to other animal species. Further in vivo testing and long-term xenograft survival will be needed to determine whether xenografts from pigs to non-human primates and humans would have similar consequences.

Xenotransplantation models using pig source animals and non-human primate recipients have been used to develop protocols for similar procedures in humans. These models have focused primarily on strategies to overcome immunologic rejection. The advantage of using Old World, non-human primates is that they, like humans, produce anti-aGal antibodies. As such, porcine xenografts induce hyperacute rejection in both Old World primates and humans.

Despite biochemical and physiological differences between non-human primates and humans, this model remains an important source for developing methods to overcome immunologic rejection and to study the risk of infectious disease. The average survival to date for whole-organ xenografts in a non-human primate has been short, although one recent study demonstrated a 76-day median survival with heterotopic heart transplantation from swine (MacGregor, personal communication, February 2003). Several monkeys succumbed from reactivation of simian cytomegalovirus, as could occur in humans undergoing immunosuppression in the absence of anti-viral agents, because of widespread latent human cytomegalovirus infection of the human population.

As with previous studies in humans exposed to porcine tissues or cells, there is also no evidence for PERV transmission to monkeys experimentally treated with porcine tissues.[79] It is not known whether baboons are susceptible to PERV, although one report suggests that PERV does not replicate in non-human primate cells.[80] Long-term survival of porcine xenografts in monkeys would set the stage for clinical trials in humans but may be limited in their true assessment of the infectious disease risk from PERV.

Although all pig genomes carry PERV, some studies have raised the possibility that development of inbred lines of pigs may lead to reduced risk for PERV transmission.[81] These studies demonstrate that certain inbred herds harbor PERV strains that are not infectious for human cells, suggesting that these swine breeds may represent a reduced risk in the transplant setting. Recent advances in understanding the biology of PERV infection of human cells raise the possibility that the relevant PERV could be genetically engineered out of a source animal herd. As encouraging as these findings may be, the risk from PERV in pigs has not yet been eliminated and further studies will be necessary to characterize endogenous infections that are transmissible to humans.

Another potential infectious disease concern may arise as a byproduct of efforts to develop genetically altered pigs (e.g., aGal knockout pigs) to minimize immunologic rejection.[82] Naturally occurring aGal antibodies render PERV non-infectious by attacking the aGal incorporated on the surface of the enveloped virus during budding of the aGal-positive pig cell

membrane.[83] Production of pigs that lack aGal may have the unintended effect of increasing the infectious nature of PERV in humans. [84,85] Further consideration must be given to strategies that seek to overcome rejection but that may enhance associated infectious risks.

Potential for Xenogeneic Infections Other Than PERV

Many types of infectious agents may be transmitted from animals to humans via xenotransplantation. Depending on the type of infection and the degree of risk, different strategies can be adopted for prevention or control. The greatest attention should be paid to whether an infectious agent is present in the source animal population and, more importantly, in the graft itself. Next to be considered should be whether the microbial agent is known to infect humans or human cells in vitro and, by extension, its potential risk to a transplant recipient.

All animals, including pigs, harbor bacteria in their upper respiratory and gastrointestinal tracts as part of their normal flora. Most of these bacteria are harmless to the host animal. An example is the bacterium *Pasteurella multocida*, which normally resides in the pig respiratory tract. Although harmless to the pig, this organism can cause disease in humans. In the xenotransplantation setting, the risk for transmission to humans via a xenograft would be negligible as long as bacteria are absent from the bloodstream of a swine source animal. Control measures to ensure the absence of occult bacteremia in source animal organs, tissues, or cells can include blood cultures before harvest of a xenograft.[1,51]

In contrast, porcine cytomegalovirus (PCMV), though not proven to infect humans, is a potential pathogen via xenotransplantation. This virus is found in most swine tissues, can be transmitted horizontally and *in utero* in swine, and leads to lifelong infection.[86] Persistently infected pigs generally show no signs of disease, but like immunosuppressed humans, infected pigs treated with immunosuppressive agents develop severe CMV-related disease.[87] The virus can be excluded from porcine source animals by early weaning or cesarean delivery. Screening procedures are available to exclude source animals that are positive for cytomegalovirus.[63]

Other porcine herpesviruses include the porcine gamma-herpesviruses residing in cells or organs that are likely to be harvested for xenotransplant. Among them are the recently identified porcine lymphotropic herpesvirus (PLHV) types 1 and 2.[88] Fortunately, these viruses are typically acquired postnatally. Infection should therefore be preventable by cesarean section, removal of piglets from infected mothers soon after birth, and maintaining the animals in a closed herd.

Other types of infectious agents that could be transmitted from source animals to humans include parasites, such as *Toxoplasma gondii*, and viruses, such as hepatitis E virus, rabies, or infections for which swine can act as an intermediate host (e.g., influenza viruses).[89,90] Methods of controlling these agents would vary according to the organism but would generally include primary prevention of exposure of the pig to the outdoors, where *T. gondii* cysts reside, or maintenance of a closed colony in which screening can exclude the agents.

For many infectious agents of animals, diagnostic tests remain in development or are unsuited

for testing in humans. Antibody-based assays to detect porcine infections of xenotransplant recipients are important but have limitations. In some cases, immunologic profiles suggestive of infection have not been validated, making verification of active infection difficult. Another problem with antibody-based assays is that transplant recipients are generally given immunosuppressive therapies that could obscure immune responses to possible infections. Nucleic acid-based methods have not been standardized for most porcine infections and may not be suitable for detecting viruses that replicate in tissues other than blood. In response to concerns that tests should be standardized, in 2002 the FDA convened a workshop to address problems associated with diagnostic assays to detect PERV and recommended further efforts to develop standardized reagents and assays for PERV testing.[91]

Control of Infectious Disease Risks

Primary and secondary prevention of infection in source animals will be important to the safety of xenotransplantation procedures. A number of methods are available to mitigate some of the risks. No single method is capable of removing them all, but judicious combinations of control methods are likely to provide the best possible results.

Current technology is probably adequate for the task of minimizing the known risks presented by infectious agents transmitted by xenotransplantation. Differing combinations of control methods will be needed for each product category. For example, the approach of controlling exogenous contamination of cell-based transplants prepared from well-established cryopreserved cell lines is likely to be more robust than that which is achievable with current technology for controlling exogenous contamination of cells, tissues, and organs derived directly from the source animal.

Approaches to infection control in cryopreserved cell-based transplants can be summarized as follows:

- Cell-based xenotransplantation products prepared from well-established cryopreserved cell lines can be fully characterized for a spectrum of infectious agents before they are used in production.

- Cells can be propagated by using biological safety cabinets housed in properly designed rooms to prevent microbial contamination from the environment. Cell culture reagents can be tested, prepared, and stored using standard technology for preventing microbial contamination.

- If needed, existing technology for contained fermentation devices can be used to prepare very large numbers of animal cells in culture.

- Aseptic processing technologies can be used to transfer animal cells from their growth vessels to the patient for implantation.

- Specific tests for infectious agents can be performed at various stages of production, some as soon as within half a day after harvest.

When appropriate measures are used, the risk of contamination by infectious agents can be reduced to a very low level. This is evident from ongoing research involving cell-based xenotransplantation products, produced under IND, for which no evidence of infection has been found in recipients of swine or other animal products. Similar safety records exist for these technologies when applied to the preparation of FDA-approved autologous human cell products such as Apligraf and Carticel.

As with characterized cell lines, when cells derived directly from an animal are used to create a xenotransplantation product, substantial influence can be exerted on the risk of contamination during cell processing. However, control of microbial contamination is more difficult during animal procurement and tissue removal. Full characterization of the cell banks may be possible when a cell suspension can be created and aliquots of it can be cryopreserved for use after characterization. If cryopreserved primary cell suspensions cannot be used (i.e., the product is administered immediately after procurement with minimal processing), the risk of infection for primary cells would approach that for organs and tissues.

Managing the risk of contamination of xenotransplanted organs or tissues used directly from source animals is heavily dependent on animal husbandry techniques and conformity to federal guidelines. Guidelines for selection and maintenance of source animals for xenotransplantation[92] are documented in *Xenotransplantation: Guidance for Industry: Source Animal Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans*[58] from the FDA Center for Biologics Evaluation and Research; the PHS *Guideline on Infectious Disease Issues in Xenotransplantation*[4]; the World Health Organization's *Xenotransplantation: Guidance on Infectious Disease Prevention and Management*[56]; and guidelines from various countries and professional societies. Guidelines pertain to the use of closed herds to exclude random source animals of unknown status. Cesarean section or animal cloning to establish initial breeding colonies of source animals adds another layer of safety by minimizing the possibility of perinatal transmission.

Yet another safety measure is uniform standards for maintaining source animals in a biosecure environment, requiring all source animals to have a PHS assurance for laboratory animals and U.S. Department of Agriculture (USDA) registration with periodic inspections. Accreditation of facilities by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) would ensure that facilities comply with all regulations. Conformity to current regulations is considered an essential requirement for conducting clinical xenotransplantation.

Among the methods available to control infectious agents among herds of source animals are the following:

- Use of specific pathogen-free animals, typically derived by cesarean section and raised under barrier-sustained conditions with high-efficiency particulate air (HEPA) filters

- Use of genetic methods to remove or neutralize endogenous retroviruses

- Employment of closed herds to minimize the entry of infectious agents from other animals. New animals would enter the herd only after quarantine and testing for the presence of

specific pathogens

- Use of pest control programs and sanitation methods

- Use of sentinel animals and antibody monitoring programs to screen for infectious agents that could contaminate the herd, staff, and xenotransplant product

- Employment of environmental controls over potential infectious agents that might be spread by air, drinking water, and staff

- Control of human infections, including influenza, through vaccination to prevent contamination of herds

- Accreditation of animal facilities by AAALAC, approval of registration by the USDA, and maintenance of inspection records to ensure compliance

The current PHS guidelines are generally believed to be adequate at present but should be reviewed and amended on a regular basis to reflect updated scientific knowledge and experience.

"XENOTOURISM": AN EMERGING GLOBAL PUBLIC HEALTH CONCERN

The SACX believes that xenotourism constitutes a potential emerging public health risk. The SACX defines "xenotourism" as the travel of U.S. residents to foreign nations to participate in xenotransplantation programs or clinics for the purpose of obtaining therapies not presently available or acceptable in the United States. Such xenotransplantation programs have offered various animal cells, including rabbit, goat, pig, and shark, with claims of treating a multitude of disorders. Some of these programs advertise the availability of luxury accommodations and travel packages, often listing U.S. addresses and contacts that can facilitate patient evaluation. Ready access to unregulated or inadequately regulated xenotransplantation clinics could have global infectious disease implications.

Xenotourism is likely to be associated with violation of the principles underlying U.S. recommendations. The consensus reached after many years of public discussions conducted by the PHS is that transmission of animal pathogens to humans constitutes an infectious disease risk, including a public health risk, that warrants caution and regulatory oversight. Oversight within the United States will be incomplete if individuals receiving unregulated xenotransplantation products abroad can potentially introduce a xenogeneic pathogen into the population upon reentry into the country. At present, information is insufficient to permit review of efficacy and safety data for these clinics or to determine whether the level of regulation of these practices is appropriate in many of the host countries, nor have mechanisms been established to obtain this critical information.

Concerns relate to the possibility that xenotransplantation procedures might be performed under circumstances that would not be allowed to proceed in the United States for lack of compliance with the PHS *Guideline on Infectious Disease Issues in Xenotransplantation*. The forms of non-

compliance might include the absence of measures intended to ensure the safety of using the source animals or of procedures for monitoring recipients for the potential to transmit xenotransplantation-related infectious diseases. American xenotourists would be likely to return to the United States after receiving their transplants, where they would receive the majority of their health care. These patients will participate in the daily activities of society, such as school attendance, commercial preparation of food, social interaction, and sexual activity, without recognizing or taking precautions to reduce the risk of transmission of a potential xenogeneic infection. Likewise, they might also inadvertently donate blood, tissues, and organs for transplantation. Furthermore, unless the foreign xenotransplantation product is revealed by the patient, there is no reliable way to recognize its importation or to ensure proper monitoring of the patient, who may seek care at a hospital or medical clinic for a complication or unexpected illness.

Xenotransplantation procedures offered in the context of xenotourism could be available in two different forms. First, the procedure could be offered as a clinical trial. Although the level of regulation, oversight, consent procedures, infection monitoring, sample archiving, and/or follow-up might not meet U.S. guidelines, the procedure would be presented as an experimental medical procedure. In that situation, it is possible that U.S. citizens might assume that the high level of FDA oversight of the safety and potential benefit that is expected in U.S. clinical trials is comparable to that of the FDA counterparts in other countries.

Alternatively, xenotourism may involve unconventional procedures described as therapies offered by luxury spas or clinics, where the connection to xenotransplantation and a clear sense of the potential danger to the recipient and to society are likely to be obscure to the average citizen. Recipients might well assume that any "therapy" offered in the context of a clinic or hospital would already have been approved as safe and efficacious by medical authorities and regulatory agencies. The foregoing examples of confusion or lack of awareness on the part of potential participants highlight the dangers implicit in xenotourism.

There is currently no way to determine how many U.S. citizens receive xenotransplantation products outside the United States and no systematic way to track the serious health consequences of these procedures. There is limited information on the number of foreign xenotransplantation clinics or their location. Likewise, the actual validity or quality of what patients receive in these xenotransplants, even though they are advertised as cells and tissues from animals, is unknown. The SACX believes that a systematic effort should be undertaken to identify these programs and to assess their use by U.S. citizens. The objectives of this effort would be to determine the scope of the problem, including an estimate of the number of U.S. citizens who receive xenotransplantation products abroad; to direct efforts to inform and educate U.S. citizens of the potential dangers of this practice; and to develop strategies for further discussions with the international community on the regulation of xenotransplantation.

Health policy issues involving foreign and sovereign nations are complex. However, the risks of xenotourism to U.S. citizens merit serious consideration. Appropriate agencies should review policy options and communicate their judgment to the public, particularly to the patient groups most likely to seek xenotransplantation therapies. Educational materials explaining the potential problems and risks of xenotourism can be developed. Screening questions about exposure to

xenotourism could be addressed to individuals entering the United States, and public health reporting and health monitoring procedures could be considered for those who reply positively to the queries. In addition, the considerable expertise in the oversight of clinical xenotransplantation trials in the United States should be offered constructively to appropriate governmental agencies in nations interested in developing such procedures. The SACX is encouraged that both the U.S. State Department and the World Health Organization have initiated efforts to promote international cooperation toward the harmonization of xenotransplantation regulations.

KNOWLEDGE GAPS AND RESOURCE LIMITATIONS

Although increased understanding of molecular immunobiology and cell and organ physiology has permitted significant progress in xenotransplantation in recent years, many challenges must be addressed before organ and cellular xenotransplantation can be clinically valuable. Following are several areas in which major gaps in knowledge or limitations in resources may hinder the progress of xenotransplantation.

Molecular Incompatibilities Between Species

Currently available investigative and technological tools should allow the identification and development of strategies for addressing the challenges in xenotransplantation. Expansion of research resources in the form of funding and expertise would optimize the ability to exploit the opportunities provided by these new tools and would accelerate the pace of discovery.

More research into species incompatibilities in innate immune system (e.g., recognition of porcine sugars and lipids by human macrophages and natural killer cells) could generate important new molecular and genetic strategies for avoiding delayed vascular rejection and other deleterious outcomes. A greater understanding of the factors that limit the function of porcine hematopoietic cells in primates should improve adaptation of porcine cells to the human microenvironment. Efforts should be initiated to anticipate potential molecular incompatibilities in the physiologic functions of xenotransplanted organs. Many of the specific physiologic problems associated with each type of xenograft will not be revealed until we have succeeded in achieving long-term xenograft survival in human or non-human primates. Waiting until this point to discover such incompatibilities, which may then require additional years for the development of new strategies to overcome them, could greatly delay the success of clinical xenotransplantation. For example, a failure of interaction between a porcine apoprotein ligand and its human lipoprotein receptor could impair vital steroid hormone synthesis in the liver transplant recipient. Early recognition of this incompatibility through basic research could stimulate earlier development of a porcine source animal that produces the human apoprotein. Scientists with expertise in the physiology of specific transplantable organs, tissues, and cells and their products should be encouraged to explore such potential incompatibilities.

Animal Models

Non-human primate models play a unique and critical role in advancing many therapeutic interventions, including allotransplantation. These models are particularly important in the setting of xenotransplantation, in which the unknown risk of infection from the source animal imposes an unusual ethical requirement to justify clinical trials. Unlike most clinical trials, xenotransplantation clinical trials entail risks that are borne by society in addition to the study participants because of the potential for transmission of infectious agents to family members and the community. The potential for societal risk imposes a higher standard of expected benefit.[93] Therefore, before clinical trials are undertaken, extensive preclinical data should support a high expectation of significant benefit to the participants in the trial. Efforts should be made to overcome the limitations of non-human primate models (e.g., clinical and biological monitoring methods that are inferior to those available for humans; limited disease models in non-human primates) that diminish the utility and predictive value of these models.

The limitations of non-human primates as models for studying xenotransplantation in humans include species differences in physiology, immunology, and susceptibility to infections, as well as difficulty in monitoring non-human primates in a clinically relevant manner. Knowledge about optimal drug levels and the ability to monitor drug levels and biochemical and physiologic parameters are far less sophisticated for non-human primates than for humans. Although some of these limitations are insurmountable, others could be minimized by the development of assays and facilities with advanced monitoring capabilities for non-human primates. Core facilities and regional centers could be developed for these purposes. Such developments would require a significant financial investment and access by all investigators to the requisite knowledge, facilities, and reagents. Unfortunately, even simple primate studies are extremely expensive to conduct, and the funding available for them is limited. Models that are more sophisticated will require resources at a level well beyond those currently available.

Improved knowledge about the ability of human transgenes in porcine source animals to perform their functions in non-human primates could reveal a need for the development of transgenic pigs that are more specifically engineered to function in non-human primate species. The development of pigs expressing non-human primate transgenes would require a major investment of resources and expertise.

Sharing of Resources

It is probable that pigs will ultimately be the most widely tested xenograft source animals. However, it is also likely that success will require that source pigs be genetically modified in several ways, perhaps differently for transplantation of different organs, cells, and tissues. This expectation raises a more general concern, since different biotechnology firms have already invested heavily in the development of various genetically modified pigs and are understandably reluctant to share their product animals with other commercial entities that might bring additional modifications to the same animal. In anticipation that additional modifications will become necessary, public support of research leading to future modifications might best ensure that newly developed reagents would be shared. Alternatively, creative new partnership arrangements between industry and government might better ensure the sharing of genetically modified pigs and other reagents. Without such initiatives, optimal genetically modified pigs may never be developed. By analogy, none of the scientific advances made with the many

knockout mice that were generated and crossed to other knockouts would have been possible if the animals were each privately owned. Similarly, the science of xenotransplantation is unlikely to proceed expeditiously without sharing of reagents between investigative teams.

Support for Xenotransplantation

In addition to major concerns about inadequate sharing of data, animals, and reagents if industry continues to be relied upon to fund technological advances in xenotransplantation, there are other good reasons why the private sector can no longer be expected to play the major role. At present, funding for xenotransplantation from the biotechnology industry is low. In recent years, many of the major biotechnology companies involved in xenotransplantation research have either discontinued or suspended their efforts in this area (e.g., PPL Therapeutics, GenVec [formerly Diacrin], Circe; Novartis closed its subsidiary biotechnology company Imutran). This turn of events cannot be attributed to a lack of significant advances in xenotransplantation, especially as the long-awaited aGal knockout pig has only just been successfully generated and shown to be viable. Unfortunately, a serious obstacle is the incompatibility of the typical short-term horizons for return on venture capital with the long-term investment required for large-scale, successful organ xenotransplantation to become feasible. Investor enthusiasm for xenotransplantation is further diminished by concerns about product liability from potential infectious risks and by disappointment over earlier failure following exaggerated expectations. Many investigators who have relied on industry funding for xenotransplantation research can no longer do so. If the field is to advance, imaginative new strategies for revitalizing the biotechnology sector's interest in xenotransplantation or other sources of funding are needed to fill the void.

Although non-profit organizations would be responsive to a potential breakthrough that would benefit their constituency, these organizations are not in a position to make a financial commitment of the magnitude required. The intellectual commitment needed by academia to reinvigorate the field of xenotransplantation is also constrained by the level of support from the traditional sources. The public has a very high stake in the products of the xenotransplantation enterprise and may be the logical source of the requisite additional support. In other medical advancements, the NIH has typically underwritten the basic research that has led to translational efforts by foundations or industry in collaboration with basic scientists, which has often led to clinical development of therapies by the industrial sector. The SACX's analysis of the state of the science of xenotransplantation underlines the need for extensive additional basic research in xenotransplantation. Major program initiatives in xenotransplantation, the development of a reagent and information repository, core facilities to serve common technical requirements, and incentives for scientists in alternative career paths to apply their expertise to xenotransplantation are currently needed.

PARALLEL OR ALTERNATIVE STRATEGIES TO XENOTRANSPLANTATION

In addition to xenotransplantation, a number of other approaches are under development for the treatment of conditions involving cellular, tissue, and organ destruction. These other approaches include prevention of the diseases that lead to end-organ failure, and in the absence or failure of

prevention, gene therapy, stem cell therapy, and artificial organs. In the sections that follow, these other approaches are briefly reviewed to provide a perspective on xenotransplantation within the context of other potential treatments.

Prevention of End-Organ Failure

The best alternative to the need to treat end-organ failure is the prevention of the acquisition and/or progression of the chronic diseases that lead to it in the first place. Prevention activities need to be promoted by all available means. For example, the current epidemic of obesity in the United States is a major factor in the increase of type 2 diabetes mellitus and contributes to risk factors for end-stage renal disease, hypertension, coronary artery disease, and osteoarthritis. Control of this epidemic and an increase in physical activity (e.g., Shape Up America's 10,000 Steps Program) could substantially reduce the incidence of renal and cardiac failure and, along with it, the need for organ transplantation.

Gene Therapy

Gene therapy[94] remains a relatively new and highly experimental technology for treating human disease. It involves the transfer of genetic material (DNA or RNA) into a patient in order to provide a copy of a normal gene to compensate for a defective gene or to direct the body to produce a potentially therapeutic substance. Viruses are often used as the vehicle (vector) for delivering the new genetic material to human cells. The viruses are genetically engineered so that they retain their ability to infect human cells (and thereby deliver the new genetic material), but lose their ability to cause disease. Nonviral vectors for delivering genes into cells are also being explored, including the use of plain DNA and DNA wrapped in a coat of fatty molecules known as liposomes.

More than 700 gene therapy studies have been performed since the first trials began in the United States in 1990. However, gene therapy is still in its early days in terms of development, and most of the studies conducted to date have been Phase I studies that investigate safety rather than efficacy. Less than 1% of gene therapy trials in the United States have progressed to Phase III studies, which test for effectiveness by using large numbers of subjects. No human gene therapy product has yet been approved by the FDA to be marketed for medical use. The field of gene therapy is more advanced than xenotransplantation, but both are still considered experimental procedures.

Gene therapy has shown some promise the treatment of X-linked severe combined immunodeficiency, however there have been serious complications of leukemia in some cases.[95,96] It has also been successful in the treatment of a form of immune deficiency known as adenosine deaminase deficiency.[97,98] Other potential applications of gene therapy include additional genetic hematopoeitic deficiencies, hemophilia, heart disease, cancer, cystic fibrosis, and organ transplantation. In organ transplantation, gene therapy approaches could one day be useful in preventing transplant rejection, inducing tolerance, prolonging graft survival, and ameliorating some of the problems associated with systemic immunosuppression.[99] In this context, gene therapy could serve as a complement to xenotransplantation. However, although gene therapy could replace the need for xenotransplantation for some diseases (e.g.,

Huntington's disease, cystic fibrosis), it could not correct all of the end-organ failure states for which xenotransplantation has potential.

Stem Cell Therapy

Stem cells are unspecialized cells that have the ability to self-renew, divide repeatedly, and develop into different specialized cell types. Stem cells offer the possibility of a renewable source of replacement cells and tissues to treat a variety of diseases and disorders involving tissue destruction or cellular injury and dysfunction.[100,101,102] Two types of stem cells are being studied: (1) adult stem cells, which can be recovered from tissues and blood and are multipotent and potentially useful for autologous adult stem cell transplants; and (2) embryonic stem cells, which are derived from 5-day-old blastocysts of human embryos and are pluripotent because they have the capacity to give rise to all of the various differentiated cells of the body.

Stem cells, whether adult or embryonic, have potential applications for almost every realm of medicine, including the treatment of Parkinson's and Alzheimer's diseases, spinal cord injury, stroke, burns, heart disease, osteoarthritis, and rheumatoid arthritis. Early-stage experiments are under way with pancreatic, neural, muscular, hepatic, cardiac, and kidney adult stem cells. One example is adult human pancreatic stem cells extracted from a pancreas and grown in the laboratory in the presence of the hormone glucagon-like peptide-1 (GLP-1). The pancreatic stem cells transform and mature into insulin-secreting cells. Although this approach is far from being ready for clinical application, it could ultimately benefit patients whose islet cells have stopped producing insulin.[103]

Closer to reality are clinical trials with adult stem cells to treat heart failure; however, the safety and efficacy of this approach is unknown, and the ability of bone marrow stem cells to differentiate into other cell types, such as heart muscle cells, is still controversial.[104] Additional research will yield more efficient methods of isolating and purifying stem cells and growing them in culture, as well as a better understanding of the characteristics of stem cells, the molecular signals that direct their differentiation and transdifferentiation into specialized cells and tissues, and the long-term survival, fate, and function of transplanted cells and tissues generated from stem cells. From this perspective, stem cell therapies offer hope for treating a multitude of clinical diseases and have several advantages and disadvantages when compared with xenotransplantation. Thus, with more research stem cells may have considerable potential for cellular replacement and repair, but its potential for whole-organ replacement is currently unknown.

Artificial Organs

Although left ventricular assist devices can improve cardiac function in patients with isolated left-sided heart failure, many patients have biventricular failure for which a total artificial heart or a transplant could provide the only replacement. An artificial heart has been developed and evaluated in a small group of patients. This device (Abiocor) is a totally implantable, mechanical replacement heart.. Two of seven recipients lived for more than 3 months with the artificial heart. At present, the device is limited to patients with end-stage congestive heart failure whose other vital organs remain viable. Several obstacles remain to the more widespread

use of such devices. The exact costs of artificial organs and the ongoing patient care and rehabilitation associated with their use are yet to be determined. Despite the very large public investment that has been made in the development of artificial hearts over a period of 40 years, the potential for the success of these technologies in terms of improving quality of life and longevity are still unknown.

FINDINGS AND RECOMMENDATIONS

The SACX makes the following recommendations for pursuing xenotransplantation as a strategy for treating a variety of medical disorders:

1. Continue to evaluate pigs as a suitable source animal for xenotransplantation. Due to heightened risks and ethical concerns apparent with nonhuman primates, these animals should not be considered as source animals for xenotransplantation. The establishment of closed colonies of pigs will ultimately be needed to raise animals for clinical trials.

2. Support existing federal guidelines on source animals for xenotransplantation.

3. Further development of diagnostic tools, including antibody and nucleic acid-based assays, to detect known and unrecognized porcine pathogens that might pose a risk to humans should be supported. Continue research on the risks of zoonotic infection in xenotransplantation recipients and gauging the potential for new emerging diseases is needed.

4. Initiate research studies that will use the new tools of molecular biology and genetics to reveal physiologic and immunologic incompatibilities between source animals and humans.

5. Develop facilities where pig–to–non-human primate models could be used to gauge the efficacy of xenotransplantation of pig organs, tissues, and cells to humans.

6. Encourage scientists from diverse disciplines to apply their expertise in the discovery of solutions for successful xenotransplantation.

7. Establish repositories in which reagents, genetically modified pigs, and other valuable materials can be maintained and distributed to researchers and laboratories engaged in xenotransplantation research.

8. Build government-industrial-academic partnerships that ensure the sharing of reagents and research animals.

9. Provide counseling to industry early in their development of xenotransplantation products on issues related to compliance with federal regulatory and safety issues.

10. The problem of broad liability for the consequences of possible zoonotic infections is perceived by some as a deterrent to participation by industry in xenotransplantation research. Investigate this issue and identify solutions.

11. Periodically re-evaluate federal guidelines on xenotransplantation and institute a system of review and oversight of regulations.

12. Investigate the scope of xenotransplantation in countries lacking stringent oversight and the extent of risks posed by entry into the United States of persons receiving xenotransplants in such countries. Appropriate federal agencies should consider the need for adjustments to immigration policy and questionnaires to protect the public health.

13. Educate U.S. residents about the risks of unregulated xenotransplantation procedures and discourage their participation in those lacking regulatory oversight as stringent as that in the United States.

14. Work closely with international health agencies to promote regulations and guidelines for xenotransplantation that are as rigorous as those developed by the PHS and assist other countries in implementing them.

GLOSSARY

Galactose(a1-3)galactose (Gala1-3Gal) (aGal): A carbohydrate that is attached to numerous proteins and lipids on cell surfaces and is expressed by most animal species other than humans and Old World monkeys.

Allogenic: Involving, derived from, or being individuals of the same species that are sufficiently unlike genetically to cause an immune response.

Allotransplantation: Transplantation between genetically different individuals.

Antigen: A substance (usually a protein or carbohydrate) recognized by the immune system and capable of stimulating an immune response.

Bacteremia: The usually transient presence of bacteria in the blood.

Closed colony: A group of animals that have been raised separately from other animals in order to maintain the genetic integrity of members of the group and to prevent transmission of infectious organisms.

Collagen: An insoluble fibrous protein of vertebrates that is the chief constituent of the fibrils of connective tissue.

Complement: A complex group of blood proteins that are activated in a cascade to form structures capable of lysing cell membranes of microorganisms. In certain situations, uncontrolled activation of complement can also damage host cell membranes. Certain complement proteins bind antibody-antigen complexes and facilitate their removal by activating complement receptors of phagocytic cells.

Cryopreserved: Preservation by subjection to very low temperatures.

Down-regulation: The process of reducing or suppressing a response to a stimulus; specifically, reduction in a cellular response to a molecule (e.g., insulin) due to a decrease in the number of receptors on the cell surface.

Encapsulation: The process of surrounding, encasing, or protecting in or as if in a capsule.

Endogenous: Caused by factors within the body or arising from internal structural or functional causes.

Exogenous: Caused by factors outside the body or arising from external causes.

Extracorporeal: Occurring or based outside the living body.

Gene knockout: Having all or part of a gene eliminated or inactivated by genetic engineering.

Gene therapy: The insertion of genes into cells to replace defective genes in the treatment of genetic disorders or to provide a specialized disease-fighting function (as the destruction of tumor cells). Vectors such as those derived from altered, nonreplicating viruses, are often used for the insertion.

Genetic engineering: A group of applied techniques of genetics and biotechnology used to separate and join together genetic material, particularly DNA, from one or more species and to introduce the result into an organism in order to change one or more of its characteristics.

Gnotobiotic: A controlled environment containing only one or a few kinds of microorganisms.

Growth factor: A biologic substance that promotes growth, particularly cellular growth.

Hepatocyte: A parenchymal cell of the liver.

Heterotopic: Alongside or adjacent; a heterotopic transplant is one in which a donor organ is placed into a recipient at a location other than that of the native organ.

Immunosuppression: Suppression (as by drugs) of natural immune responses.

Islet cell: An insulin-producing cell of the pancreas.

Latency: The state or period of living or developing in a host without producing symptoms.

Neovascularize: To provide with a new supply of blood vessels.

Orthotopic: In place of; an orthotopic transplant is one in which a donor organ replaces a recipient's organ in its original location.

Porcine endogenous virus (PERV): A virus that is found in all swine and is contained within the pig genome.

Porcine: Of or derived from swine (pigs).

Rejection: An immune response in which foreign tissue (such as a skin graft or transplanted organ) is attacked by immune system components (such as antibodies, T-cells, and macrophages) of the recipient organism.

Simian: Pertaining to an ape or monkey.

Stem cell: An unspecialized cell that gives rise to differentiated cells.

Thymus: A gland of largely lymphoid tissue that serves as the site where T cells develop in cell-mediated immunity.

Tolerance: The capacity of the body to endure or become less responsive to a substance (as a drug) or a physiological insult with repeated use or exposure. Immune tolerance is the ability of the immune system to specifically accept donor organs, cells, or tissues without requiring therapies that generally suppress the immune response.

Transgenic engineering: A technique to produce an organism or cell of one species into which one or more genes of other species have been incorporated (e.g., transgenic mice).

Xenogeneic: Derived from, originating in, or being a member of another species.

Xenotransplantation: According to the U.S. Public Health Service, "Any procedure that involves the transplantation, implantation, or infusion into a human recipient of either (a) live cells, tissues, or organs from a non-human animal source or (b) human body fluids, cells, tissues, or organs that have had ex vivo contact with live non-human animal cells, tissues, or organs." (See footnote 1.)

Zoonotic: A disease communicable from animals to humans under natural conditions.

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