Xenotransplantation, Xenogeneic Infections, Biotechnology, and Public Health

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OUTLINE

ABSTRACT

Xenotransplantation is the attempt to use living biological material from nonhuman animal species in humans for therapeutic purposes. Clinical trials and preclinical studies have suggested that living cells and tissue from other species have the potential to be used in humans to ameliorate disease. However, the potential for successful xenotransplantation to cure human disease is coupled with the risk that therapeutic use of living nonhuman cells in humans may also serve to introduce xenogeneic infections of unpredictable significance. Animal husbandry practices and xenotransplantation product preparation may eliminate most exogenous infectious agents prior to transplantation. However, endogenous retroviruses are present in the genomes of all mammalian cells, have an inadequately defined ability to infect human cells, and have generated public health concern. The history of xenotransplantation, the implications for public health, the global consensus on public safeguards necessary to accompany clinical trials, and the future direction of xenotransplantation are discussed in the context of public health. ***Mt Sinai J Med 76:435–441, 2009.*** © *2009 Mount Sinai School of Medicine*

Key Words: porcine endogenous virus, public health, xenogeneic infections, xenotransplantation.

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Xenotransplantation can be briefly described as an attempt to use living biological material from nonhuman animals in humans for therapeutic benefit. The US Public Health Service defines xenotransplantation more formally as the transplantation, implantation, or infusion into a human recipient of either (1) live cells, tissues, or organs from a nonhuman animal source or (2) human body fluids, cells, tissues, or organs that have had ex vivo contact with live nonhuman animal cells, tissues, or organs.[1]

Xenotransplantation may sound like an unlikely event. However, in vitro fertilization, a practice by which infertile couples are often able to bear children via the removal of eggs and sperm from the intended parents, fertilization of the eggs in a laboratory, growth of those fertilized eggs to a multicell stage over 3 to 5 days, and implantation of the eggs into the mother's uterus, is no longer an unlikely event. In the 1990s, the substrate used in the laboratory to support the development of a fertilized egg into the multicell stage was frequently a cell line of nonhuman origin. Thus, the multicell stage fertilization product implanted into the mother's uterus and ultimately the resulting infant was, by US Public Health Service definition, a xenotransplantation product. Additionally, hundreds of patients have been treated with investigational xenotransplantation products intended (1) to sustain patients suffering hepatic failure until a liver is available for transplant (hemoperfusion through

Published online in Wiley InterScience (www.interscience.wiley.com).
DOI:10.1002/msj.20131

a porcine liver or hepatocytes), (2) to decrease the dependence of diabetics on insulin (porcine pancreatic cell implants), or (3) to improve functions in patients with Parkinson's disease (implantation of porcine neurological cells) or other functions.[2,3]

WHY IS XENOTRANSPLANTATION A PUBLIC HEALTH CONCERN?

Rationale for Xenotransplantation

As transplantation surgery has become more technically proficient, the factor limiting transplant patient survival has ceased to be the technical complexity of the surgery and has instead become the limited availability of donor organs appropriate for transplantation. According to the United Network for Organ Sharing Web site, on June 3, 2009 at 4:45 PM, 102,010 people were candidates for the organ transplantation waiting list. However, between January 1 and May 29, 2009, only 2304 organ donors had become available. Looking specifically at heart transplantation, we find that between July 1, 2007 and June 30, 2008, only 12% of heart transplant recipients failed to survive the first year after surgery. During that same period, 18% of the people who were both in need of a heart transplant and on the waiting list died while awaiting transplantation. Thus, as a result of the mismatch between the demand for and supply of donor organs for transplantation, the greatest risk of dying due to heart transplantation today is attributable to the scarcity of transplantable organs rather than to the risk of either surgery or posttransplantation organ rejection.[4]

The shortage of donor organ availability first drove interest in exploring whether organs from nonhuman animals could be adapted for transplantation into humans. Xenotransplantation has been envisioned as a source of spare-part organs to be transplanted as a definitive cure for end organ failure. However, xenotransplantation has also been envisioned as a bridge therapy: an interim therapeutic step intended only to sustain the patient long enough to allow a compatible human donor organ to become available.

The disparity between the demand for transplantation generated by organ failure and the supply of donated human organs was the original driving force behind efforts to develop xenotransplantation. However, a second driving force emerged after it was recognized that differences in species susceptibility to specific infections might be exploited to human advantage. Two early landmark experiments demonstrated the application of this concept.

When Dr. Thomas Starzl, a pioneering transplant surgeon in Pittsburgh, learned that baboons were refractory to infection with either hepatitis B or human immunodeficiency virus (HIV), he wondered if this species difference in susceptibility could be exploited to the advantage of HIV-infected patients who were dying of liver failure due to hepatitis B virus infection. In 1992, following advances in the ability to control both cellular and humoral components of xenograft rejection in vitro, Dr. Starzl and his colleagues attempted to transplant a baboon liver into a 35-year-old HIV-infected man with hepatitis B virus–associated chronic active hepatitis. The patient survived with little evidence of rejection, and products of hepatic synthesis became those of the baboon liver without evidence of an obvious adverse impact. The patient died on day 70 after transplantation because of a cerebral and subarachnoid hemorrhage caused by an angioinvasive *Aspergillus* infection. Although Dr. Starzl did not repeat this experiment, he concluded that this experiment had demonstrated the feasibility of controlling the rejection of baboon livers transplanted into human recipients.[5] Conceptually, he had ushered in a new era of thought about xenotransplantation.

On December 14, 1995, a 38-year-old activist who had been infected with HIV for more than 15 years underwent a controversial experiment. His bone marrow was suppressed (not ablated) by sublethal doses of radiation and chemotherapeutic drugs, after which he received an infusion of stem cells and facilitator cells procured from the bone marrow of a baboon. Facilitator cells, discovered by Dr. Suzanne Ildstad, another Pittsburgh surgeon, appear to allow stem cells to proliferate in other species without producing graft-versus-host disease.[6] This experimental attempt to reconstitute chimeric functional bone marrow in a patient with acquired immune deficiency syndrome (AIDS) was another conceptualization of how the species differences in susceptibility to infection could be exploited to human health advantage. This experiment occurred prior to the advent of cocktail antiretroviral therapy at a point when the standard treatment approaches were not working adequately and the AIDS activist community was increasingly convinced that radical new approaches were necessary. In this atmosphere of desperation, a US Food and Drug Administration advisory committee debated and then voted to allow this controversial human experiment that might actually foreshorten rather than prolong the patient's life. The experiment went forward, and the patient survived and improved for reasons that are unclear, as the baboon bone marrow cells were not identifiable in his bone marrow beyond the first month.[7]

DOI:10.1002/MSJ

These 2 experiments illustrate a transition in the conceptual nature of xenotransplantation. The first concept, that whole animal organs were anticipated to serve as spare-part replacements for failed human organs, was driven by the inability of the supply of human organs to keep pace with the ability of technological advances in transplantation to save lives. Increasingly since 1990, the development of xenotransplantation applications has been influenced by the recognition that unique properties of therapeutic materials originating from nonhuman species may be exploited to the advantage of human health. Increasingly, the products are cellular rather than whole organs. Problems with immune rejection and the absence of preclinical data meeting specific recommendations for the survival of xenogeneic organs in nonhuman primates prior to clinical use in humans further influence preferential interest in cellular transplantation versus organ transplantation. Recent success with islet allotransplantation for diabetes is driving renewed interest in using porcine islets because of a lack of a sufficient supply of human islets for allotransplantation. The transplantation of cellular products likely represents the most viable near future of xenotransplantation.

Why Are Public Health Professionals Interested in Xenotransplantation?

Xenotransplantation first came to the compelling attention of public health authorities in the mid-1990s, around the time of the previously described experiments by Dr. Starzl and Dr. Ildstad.[8] The combination of advances in the control of immune rejection and the engineering of transgenic pigs that contained certain human genes anticipated to improve porcine xenotransplantation product survival in human recipients led to great enthusiasm for the rapid movement of xenotransplantation applications into human clinical trials. The absence of any precedent for regulatory policy for xenotransplantation clinical trials was of pressing concern to the public health authorities charged with safety oversight.

Although Dr. Starzl, Dr. Ilstad, and their colleagues were focused on the potential to transform the health of individuals, the public health focus was on the protection of community health. Some xenotransplantation proposals could provide great societal benefit if successful. For example, in 2005–2006, the crude prevalence of total diabetes in US residents 20 years old or older was 12.9%.[9] For decades before human insulin became available, diabetes was managed through the intermittent injection of porcine insulin. Imagine the impact if diabetes could be functionally cured through the infusion of functioning porcine pancreatic islet cells. One modeling study estimated the health economic impact of maintaining glycosylated hemoglobin values in all US patients with currently uncontrolled type 1 or type 2 diabetes mellitus at the American Diabetes Association standard of 7.0% and at the American Association of Clinical Endocrinologists target of 6.5%. This analysis, run from a societal perspective over a 10-year time horizon, estimated that achieving this level of maintenance could achieve total direct medical cost savings of 35 to 50 billion US dollars, respectively, over 10 years. When indirect cost savings were included, the total savings increased to 50 to 72 billion US dollars, which corresponded to 4% to 6% of the total annual US health care costs of 1.3 trillion US dollars.[10] This analysis estimated only financial savings and did not address quality of life issues or years of productive life reclaimed.

Although xenotransplantation's potential for positive benefits to individuals and society was a primary driver for the clinical research community, the attention of the public health community was captured more by concern for the potential for unintended negative consequences. Xenotransplantation is intended to benefit the health of individuals by replacing nonfunctioning or malfunctioning human cells, tissues, or organs with functioning nonhuman animals cells, tissue, or organs. However, the implantation, transplantation, or infusion of living nonhuman tissue into humans for therapeutic purposes has an associated potential to also transfer infections across species lines into humans. Because xenotransplantation applications breech normal host defenses and are frequently accompanied by pharmacological immune suppression, xenotransplantation may be ideally suited to have the unintended consequence of introducing new infections into the human recipients. The potential for implanted living nonhuman animal cells to also transfer infections across species lines into the human population (xenozoonosis or xenogeneic infections) was a major concern. Zoonotic infections occur in nature and produce disease in individuals and epidemics in human populations. Examples of zoonotic epidemics include the 1993 hantavirus pulmonary syndrome outbreak in the American southwest[11] and the epidemic of encephalitis that followed the introduction of West Nile virus into New York in 1999.[12] Zoonotic infection of humans by avian influenza viruses caused the largest recorded pandemic in human history, the Influenza Pandemic of 1918.[13] AIDS, now understood to have resulted from the introduction of a simian immunodeficiency virus infection across species lines into humans,[14] has gone through all these stages and is no longer a zoonosis, an epidemic, or a pandemic.

DOI:10.1002/MSJ

AIDS now is simply an endemic infection affecting all human populations throughout the world, and as much as any single force in this century, it is reshaping the world.

This then is the primary basis for public health interest in xenotransplantation and related biotechnologies. The potential for xenotransplantation to benefit individual patients is inevitably linked to a potential to introduce harm to human populations through the unintended introduction of xenogeneic infections. Because infectious diseases do not respect geopolitical boundaries, a xenotransplantation clinical trial anywhere that is not accompanied by adequate public safeguards is a concern to the global community.

XENOTRANSPLANTATION AND PUBLIC HEALTH IN 1995

In 1995, at the request of Phil Lee, the Assistant Secretary for Health of the Department of Health and Human Services, federal agencies first began to examine xenotransplantation as a public health issue. At that time, the potential for new biotechnical approaches to alleviate human suffering by the use of living biological material from nonhuman animals was recognized. These experimental approaches had the exciting potential to have an unprecedented positive impact on a broad spectrum of human disease. However, these approaches also carry an unquantifiable and probably small but still existent risk of unintended negative side effects via the introduction of xenogeneic infections into the human population.

Recognition of the potential for xenotransplantation to have a negative public health impact due to xenogeneic infections inspired collaborative efforts by the academic, clinical, industrial, research, and public health communities to identify ways to develop this promising biotechnology while adequately safeguarding the health of the larger community. The first public health priority was the development of an international consensus on the public safeguards necessary to allow xenotransplantation clinical trials to proceed with public confidence. Once consensus was achieved, it was implemented through the development of appropriate public health policy translated into regulatory practice. Simultaneous research efforts explored clinical interventions and bioengineering approaches that might increase the safety of clinical trials. The stakeholder communities also undertook collaborative basic science research to better define fundamental understandings of both the risk and potential of xenotransplantation.

Public Health Guidance

Public health guidance documents are available from national health authorities in most nations in which clinical xenotransplantation trials have been undertaken or considered, including the United States, Canada, multiple European countries, the European Union, and Australia, and from multinational organizations such as the World Health Organization and the Organization for Economic Cooperation and Development.[1,15] These various guidances are interpretations of a single global consensus.

The US Public Health Service and other guidances build on a foundation that requires xenotransplantation product source animals to originate from closed colonies of purpose-bred animals with husbandry practices that limit and define the lifelong exposures of these animals. These guidances emphasize the importance of pretransplantation screening of source animals and herds to identify and eliminate problematic infectious agents prior to the development of xenotransplantation products and of posttransplantation surveillance of xenotransplantation recipients to identify and contain any xenogeneic infections. Posttransplantation surveillance is necessary to identify infectious agents that (1) were transplanted because they were not known to exist at the time of transplant screening (eg, severe acute respiratory syndrome–associated coronavirus prior to 2003), (2) were known to exist but could not be detected because of inadequate diagnostic tools (eg, prions), or (3) were known to be present in the source animal and could not be removed (eg, endogenous retroviruses).

Endogenous Retroviruses

Retroviruses are RNA viruses that replicate by transcribing viral RNA into DNA by reverse transcription.[16] Proviral DNA is integrated into the host cell genome and replicated with the host cell DNA. Exogenous retroviruses exist as independent cell invaders that are transmitted horizontally by infection. The most familiar example is HIV.

However, when retroviruses become integrated into the host cell genome within a germ cell, they can then be transmitted vertically by inheritance through the germline DNA. These endogenous retroviruses exist as proviral DNA integrated into the germlines of all mammals, including the genomes of humans and all species considered as source animals for xenotransplantation. Endogenous retroviruses represent a sort of fossil remnant of what are presumed to have once been exogenous retroviruses that integrated into the host germline eons ago and remain as an inherited part of the genetic structure of every cell

DOI:10.1002/MSJ

of the species today. These endogenous retroviruses may express infectious viruses but do not cause disease in the host species. However, many endogenous retroviruses are xenotropic; this means that they are able to infect cells from other species. Endogenous retroviruses of pigs and baboons are able to infect human cells in vitro. Thus, living animal tissue that is apparently devoid of exogenous infectious agents nonetheless retains an innate infectious potential due to the presence of endogenous retroviruses.

C-type particles (crescent-shaped formations on the membranes of cells associated with the budding of C-type retroviruses) expressed from a variety of porcine cell lines were identified during the 1970s and 1980s and characterized as endogenous retroviruses capable of infecting ST-Iowa cells (a cell line derived from *Sus scrofa*) in vitro. After 1995, concerns about endogenous retroviruses in xenotransplantation products inspired studies that defined infectivity and host ranges for porcine endogenous retrovirus (PERV).[17,18] PERV is expressed from multiple porcine cell lines and primary tissues. Of 3 identified variants, 2 (PERV-A and PERV-B) productively infect multiple human cell lines, although human peripheral blood mononuclear cells appear resistant to productive infection. Both the murine leukemia virus and feline leukemia virus share more than 60% homology with PERV. This homology has been exploited to develop serologic assays for PERV, and the recognized characteristics of these viruses have been used as a basis for reasoning by analogy about how PERV may behave biologically.[2,3,13,18]

In response to these findings, on October 16, 1997, the US Food and Drug Administration placed all xenotransplantation trials using porcine products in the United States on clinical hold. Reimplementation of clinical trials required the development of assays for detection of infectious PERV in xenotransplantation products, implementation of surveillance for PERV infections in recipients, and development of informed consent documents adequately informing clinical trial participants of potential risks associated with the presence of PERV in porcine xenotransplantation products.[19] In 1999, in recognition of a global public health consensus, the US Food and Drug Administration issued additional guidances that preclude the use of nonhuman primates as source animals for xenotransplantation products and that defer xenotransplantation recipients from the donation of blood and other biological materials as precautionary measures.[20]

New tools were necessary to enable laboratory surveillance for endogenous retrovirus infection. Because PERV DNA is a normal part of the genome of every porcine cell, polymerase chain reaction (PCR) identification of PERV DNA will be inevitable whenever transplanted porcine cells are present. The ability to discriminate PERV infection from the presence of PERV DNA–containing porcine cells in a xenotransplantation product recipient requires additional testing. Most approaches combine a PCR assay for PERV DNA with a PCR assay for a marker of porcine (or other source animal) DNA. Although these assays frequently identify source animal mitochondrial DNA, other repetitive sequences such as centromeric sequences have also been used.[21–25] The presence of PERV DNA in the absence of porcine mitochondrial DNA implies PERV infection, whereas in the presence of porcine mitochondrial DNA, it simply implies that not all xenogeneic cells have been rejected by the recipient.[21–25] This basic approach is further refined with quantitative tests that assess the relative abundance of PERV DNA and host mitochondrial DNA in biological material from recipients with respect to the ratio that existed in source animal cells prior to transplantation. These, combined with reverse-transcriptase PCR tests that identify PERV-specific RNA evidence of viral expression, Amp-RT or similar assays that identify generic reverse-transcriptase activity, and western blot assays that identify seroreactivity against homologous retroviruses, compose the basic armamentarium for laboratory surveillance for PERV infection in porcine xenotransplant recipients.[21–25]

An early series of retrospective studies failed to identify evidence of PERV infection in recipients of porcine xenotransplantation products. Two landmark negative studies were published in *Lancet* in 1998. Patience *et al.*[23] found no evidence of PERV infection in 2 patients who had experienced short-term extracorporeal perfusion of their blood through pig kidneys in the absence of immunosuppression. Heneine *et al.*[24] did not identify PERV infection in 10 immunosuppressed diabetic recipients of human kidney transplants who also received fetal porcine islet cells either infused into the portal vein or inserted under the capsule of the kidney allograft; porcine cells had persisted for up to 6 months following transplantation. In 1999, the negative results of a global collaboration by the xenotransplantation research community were published.[25] This collaboration studied a large number of patients ($n = 160$) who were exposed to porcine xenotransplantation products through a wide variety of methods. PERV infection was sought through double-blinded testing in 2 laboratories using independently developed assays. Microchimeric porcine cells were identified in the peripheral blood of 23 recipients up to 8.5 years after exposure ended. This finding, while

DOI:10.1002/MSJ

unexpected, is consistent with evidence of Y chromosome–containing microchimeric cells in the peripheral blood of women decades after they gave birth to male offspring.[26] In the context of xenotransplantation, this finding signifies that transient exposure to xenotransplantation products may result in persistent exposure to risk of PERV infection.

2009: WHAT IS THE FUTURE OF XENOTRANSPLANTATION?

Since the dialogue between public health and xenotransplantation began in the mid-1990s, much effort has been applied to studies attempting to elucidate aspects of xenotransplantation relevant to infectious risk.[27] The development of a global agreement on public safeguards that should accompany xenotransplantation clinical trials was a significant advance. A growing body of evidence has failed to identify PERV infection in xenotransplantation product recipients, although recent advances intended to overcome immune rejection (ie, the development of alpha-gal knockout source pigs) may inadvertently increase the risk of PERV transmission.[28] Modifications of xenotransplantation product bioengineering have been shown to diminish the release of PERV virions in vitro; this observation suggests that such modifications may reduce a recipient's risk of exposure to infectious PERV in vivo.[29] Other in vitro experiments suggest that transient use of antiretrovirals peri-transplant may increase selective pressure against persistent infection.[30] The recognized homology between PERV and feline leukemia virus suggests that effective vaccination may be possible, although other observations suggest that vaccines which protect against PERV infection may also contribute to the rejection of porcine xenotransplantation products.[31] Although much remains to be explored in all these areas, findings to date endorse the decision by regulatory authorities to allow xenotransplantation clinical trials to proceed with public safeguards in place and diminish, but do not eliminate, concern about the unintended introduction of new infections into the human population as a byproduct of efforts to cure individual disease.

The potential for xenotransplantation to introduce xenogeneic infections remains a concern but is no longer the rate-limiting step in advancement of the field of xenotransplantation. Whether xenotransplantation will deliver on the promise raised by early visions will depend more on whether basic research can overcome the remaining immune barriers to long-term survival of xenotransplantation products in vivo, on discoveries about the adequacy of xenoproduct physiological function in humans, and on whether advances in the related fields of cloning and regenerative medicine outpace the rate of discovery in xenotransplantation to the point of irrelevancy.

ACKNOWLEDGMENT

This article is an adaptation of a lecture given by the author at Mount Sinai School of Medicine for the Master of Public Health program "Special Topics in Zoonosis" on April 10, 2008.

The findings and conclusions in this presentation represent the views of the author alone and not those of the Centers for Disease Control and Prevention, the US Public Health Service, the US Government, or any other entity.

DISCLOSURES

Potential conflict of interest: Nothing to report.

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DOI:10.1002/MSJ