Xenotransplantation: assessing the unknowns

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There has been much discussion recently in both the scientific literature and the popular media about the potential benefits and risks of proceeding with clinical xenotransplantation trials. The idea of using animal cells, tissues or organs for human transplantation is not new, but several factors have combined to rekindle interest in xenotransplantation. These include scientific and technological advances that improve prospects for clinical success, a chronic and escalating shortage of human organs for transplantation, recognition that the use of certain human tissues (e.g., those from fetal sources) is not likely to be sanctioned and significant commitment within the biotechnology industry to developing safe and practical strategies. Various species, particularly nonhuman primates and pigs, have been considered as potential xenograft sources. Pigs are now generally favoured, in view of ethical concerns about using primates and given the fact that pigs can be bred more easily and, potentially, in pathogen-free herds.

Despite the obvious benefits of a potentially unlimited supply of organs and tissues, xenotransplantation has given rise to certain misgivings. Some of these are related to religious belief, concern for animal welfare and issues of informed patient consent. Others involve questions of medical and scientific feasibility and concerns about the possibility that xenotransplantation may enhance the emergence of novel infectious diseases. There is also discussion about the degree of regulation and monitoring that would be needed in this new field. Risk assessment must be based on sound scientific and medical evidence. But since there have been relatively few clinical trials of xenotransplantation, such evidence is sparse. Thus the dilemma: Should investigators proceed with further trials and thus generate data that will determine whether this promising technology carries real rather than merely theoretical risks? Can we devise effective monitoring and risk management strategies and establish contingency plans that will ensure the safety of patients and the public once trials have begun? The prevailing opinion in the US is that appropriate and sufficient controls can be put in place. At a meeting sponsored in January of this year by the US Public Health Service, both the US Food and Drug Administration and the US Centers for Disease Control and Prevention outlined plans for the careful scrutiny of clinical trials and for the establishment of a national xenotransplantation registry. Although a number of eminent scientists have called for a moratorium on human xenotransplantation, the US Public Health Service did not feel this was warranted.

The main practical obstacle to successful xenotransplantation of solid organs is immunological rejection of the graft; such rejection can be hyperacute, delayed or mediated by T lymphocytes. Many antigens can stimulate an immune response in discordant xenografts (e.g., from pigs to humans), but the main culprit in hyperacute rejection is the carbohydrate α-galactosyl (Gal) epitope present on endothelial cells in the xenograft's vasculature. Humans and other Old World primates differ from other mammals in lacking the Gal epitope. But humans have developed “naturally occurring” anti-Gal antibodies in response to Gal antigen present on normal bacteria in the gut; these antibodies are present in high concentration in human blood. As a result, the endothelial cells of the xenograft are almost immediately destroyed by human anti-Gal antibodies in a complement-mediated process that is essentially irreversible once initiated (see Fig. 1).

Because human anti-Gal antibodies represent about 1% of total circulating immunoglobulin G, and because a single endothelial cell can express millions of
Gal epitopes, it is difficult to eliminate this reaction. Nonetheless, a number of strategies to address the problem are being pursued experimentally. Some of these involve removing or neutralizing human anti-Gal antibodies before transplantation, eliminating Gal expression on xenograft cells by genetic knock-out, and inducing immunologic tolerance to the Gal antigen. Transgenic modifications are also being investigated, most notably the introduction into pigs of human proteins that inhibit complement-mediated cell lysis.

The use of nonvascularized grafts minimizes the occurrence of hyperacute rejection. Other factors, such as the location of the implant, may also reduce the likelihood of rejection. For example, promising clinical outcomes have resulted from the implantation of neural cells from fetal pigs into the brains of patients with Parkinson’s disease. In fact, the first double-blind study to examine this treatment has recently been approved in the US. Trials now in progress are also investigating the transplantation of pig islet cells, with or without encapsulation, into diabetic patients and of calf adrenal cells into the spinal cords of cancer patients (for the treatment of severe pain). The extracorporeal perfusion of blood through pig liver tissue as an interim measure while the patient awaits allotransplantation, or the use of a bioartificial liver containing pig hepatocytes, may also provide therapeutic options in the future for patients with acute organ failure.

Despite encouraging preliminary results, there is great caution about proceeding. The reason most often cited is fear of launching an epidemic precipitated by the transmission of an (as yet) unidentified animal pathogen. Although the risk of such “xenozoonoses” is remote, it is not zero. Concern about this possibility focuses on 2 targets: known and unknown pathogens. Many infectious agents of animal origin are also potential human pathogens, and history demonstrates that virulence of a pathogen may be increased if it adapts to infect a species other than the natural host. Examples of cross-species infection are well documented in the animal world; influenza, hantavirus infection, Ebola hemorrhagic fever and, most probably, HIV/AIDS are powerful examples of the same phenomenon manifested in human disease.

![Diagram of hyperacute rejection in solid organ xenografts](image-url)

Fig 1: Hyperacute rejection in solid organ xenografts. Endothelial cells line normal blood vessels and maintain a balance between anticoagulant and procoagulant activities. In a xenograft, naturally occurring host anti-Gal immunoglobulin G (IgG) antibodies bind to Gal epitopes expressed on the endothelial cell surface of the graft. This activates the classical pathway of complement (C) via binding of C1, leading to the formation of complement effector molecules (C5a and C5b–9), which results in endothelial cell activation, increased vascular permeability, thrombus formation and vascular destruction.
The potential for xenografts to contain endogenous retroviruses is at least a theoretical concern. Most mammals harbour many inactive endogenous “proviruses” in their germline DNA. These appear to have evolved harmlessly in tandem with their natural host species, simply by being passed along to successive generations. However, they have the potential to become biologically active, for example after genetic recombination with viral sequences in the xenograft recipient. Such events are known to lead to outcomes such as oncogene activation, modified virulence and altered transmissibility. In fact, because xenograft recipients will probably be immunosuppressed to at least the same degree as allograft recipients, such occurrences may be especially likely in these patients. Although the presence of endogenous retroviruses in general is widely acknowledged, their specific identities remain largely unknown. This poses unique challenges for recognizing novel infectious disease syndromes and for developing assays, vaccines and public health strategies.

The risk of xenozoonoses has led to opposition to the use of nonhuman primates such as baboons as source animals, despite their better immunological compatibility with humans. Xenografts from nonprimate sources are generally viewed as safer, but the possibility of retroviral infection was highlighted last year when it was shown that known pig endogenous retroviruses (PERVs) were able to infect human cells in vitro. Proponents of the use of pigs as a source for xenografts are optimistic that PERVs can be removed from herds by selective breeding and that other infectious agents can be controlled by rigorous animal husbandry practices. Others feel that this would be a truly formidable task.

The critical issue of xenozoonoses and public health is further clouded by the fact that it is difficult to assess “acceptable risk” when certain questions remain unanswered. First, is it probable that endogenous retroviruses or other pathogens from the donor animal would infect human cells? Second, would these agents replicate in human cells in vivo? Third, if replication occurred, would this infection cause disease? And, fourth, could the disease be transmitted to other humans? Although most infectious disease experts would likely answer Yes to the first question, there is certainly no consensus on the rest. There is agreement, however, that research is desperately needed to shed light on these issues.

The potential and inadvertent introduction of new pathogens via xenotransplantation would respect no national boundaries. After all, pathogens do not carry passports, and decisions to proceed with xenotransplantation in one country may spur patients to travel to those jurisdictions for treatment. International cooperation in this developing area is both necessary and prudent. Recent meetings on xenotransplantation have been organized by the World Health Organization, the Organization for Economic Cooperation and Development and the US Food and Drug Administration; Canada has been an active participant at these meetings.

In Canada, xenografts are regarded as therapeutic products and meet the definition of a drug under the Food and Drugs Act and Regulations. Health Canada’s Therapeutic Products Directorate is responsible for the regulation of xenografts to ensure their safety, efficacy and quality before they are brought into use. Preclinical research is being carried out to study the suitability of animal organs and tissues for human transplantation. Given that this is a new technology, specific guidelines are being drafted to ensure that appropriate safety standards and the necessary level of scrutiny are applied to any clinical trials. Furthermore, in view of the unique societal implications of xenotransplantation, input from various stakeholders and the public has been sought.

Participants at a National Forum on Xenotransplantation sponsored by Health Canada and held in Ottawa in November 1997 agreed that international cooperation was needed in this emerging field, that stakeholders and the public should be given ample opportunity to present their views, and that the process of regulatory decision-making should be transparent. The publication of a report on the forum by Health Canada later this year will contribute to the continued exploration of these issues.

References


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