## The process of discovery

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The process of discovery
Jay A. Fishman

Questions about xenotransplantation are often posed philosophically, in terms of right and wrong. This approach contrasts with the rest of evidence-based biomedicine, which rarely provides absolute answers to questions of merit. Should xenotransplants be performed? Do the potential benefits outweigh the theoretical risks? In general, such questions are examined in the light of current biological knowledge, and are subject to revision over time. As our database develops, hypotheses are formulated. Certainty does not exist. Thus, when allotransplantation was introduced, knowledge of the infectious risks associated with immune suppression was rudimentary. Data about infection in allotransplantation has grown rapidly with basic science and clinical experience (1). This relatively recent information was central to the discussions on the risk to the individual (potentially great) and to society (thought to be small) when the transplantation of baboon marrow into a human with AIDS was reviewed for the Public Health Service in the United States. Those discussions were coloured by passions about science, AIDS and individual rights, but they resulted in a far safer experiment in terms of risks of infection. They were also of benefit for more general considerations of clinical xenotransplantation.

The term “xenosis” was coined to reflect not only the experience in allotransplantation, but also the unique epidemiological aspects of interspecies transplantation – the potential transfer into the general human population of novel or unknown pathogens derived from animal donors (2–6). In terms of basic science, the search for such novel organisms has already had a beneficial effect on studies of the microbiology of transplantation, for example the isolation and sequencing of the first full-length porcine endogenous retrovirus (7) and to virological studies which suggest the ability of such organisms to infect human cells in vitro (8–10). Such data have been used by the US Public Health Service, and by corporate interests, to develop testing strategies for pig-raised as potential xenograft donors. Such studies and discussions have also made it clearer to most investigators that infection is a critical consideration in the development of clinical xenotransplantation.

Further studies are likely to find additional new, and possibly pathogenic, organisms in xenograft donor species, and the potential for human infection can be assessed for each. This discovery process adds to our understanding of the risks of xenotransplantation, and of the microbiology of immunocompromised hosts in general. It is a part of the evolution of the science of xenotransplantation. 

Microbiological guidelines for xenotransplantation should therefore be devised, to indicate what is currently known about infection for such procedures, and serve as a basis for future scientific investigations.

Benefits of xenotransplantation

The benefits of xenotransplantation must be seen in the light of the limitations of currently available technologies. Many patients die while awaiting cadaver donor organs. Cadaveric donors provide life-saving opportunities for a small number of fortunate recipients, but the organs have often been subjected to hypoperfusion, infectious contamination, or injury during surgical procurement. All of these are problems which may be eliminated by the use of planned xenotransplants. Allograft recipients may suffer from chronic infection or debilitation but these problems could be reduced if elective transplantation could be scheduled, as it is currently for living donor transplants. In addition to their potentially unlimited availability and correct size, xenogenic organs may be resistant to infection with viral pathogens of humans, including HIV (1 and 2), HTLV, hepatitis viruses, and herpes viruses – including human cytomegalovirus (CMV), which has been shown in our laboratory to be unable to infect porcine cells in vitro (11). Further, human hepatitis B virus does not appear to infect baboon in vivo (12). While such protection is unlikely to be observed for all potential pathogens, it may provide a significant advantage to patients with organ failure due to viral infection.

Assessing the risks

The central goal of infectious disease physicians in transplantation is disease prevention, given the poor clinical response of the immunocompromised host to established infections. What types of organisms should we be concerned about in xenotransplantation? As in the case of allotransplantation, the following points are fundamental to the assessment of the risks of infection in xenograft recipients.

• All organisms are a potential cause of infection in any species, but the organisms most likely to cause infection are the ones that are similar or identical to those that do so in the immunosuppressed human allograft recipient, in addition to species-specific organisms not associated with human tissues.

• The risk of infection is a direct function of the overall level of immune suppression needed to maintain allograft function and the nature and intensity of the epidemiological exposure of the recipient. The minimization of immune suppression (for instance by tolerance induction strategies such as bone marrow transplantation) may significantly reduce the risk of infection if it does not increase the exposure of the recipient to donor-derived organisms or reduce the immune response to such organisms.

• The manifestations of infection in the xenograft recipient will also be modified by the type (such
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as corticosteroids, antilymphocyte therapy or cyclosporin), intensity and duration of the immune suppression needed to sustain organ function, and by the clinical status of the recipient following transplantation.

A number of additional factors conspire to increase the risk of infection in xenotransplantation:

• The xenograft itself serves as a nidus or "culture plate" from which such organisms can spread in the human host without needing a "vector" to achieve disease transmission;

• clinical laboratory tests for most organisms derived from non-human species (e.g., antibodies, molecular probes, culture systems for species-specific organisms, or serological tests for human antibodies against animal pathogens) are not generally available;

• the migration of cells from the graft to other sites in the host may carry cell-associated infection throughout the host;

• clinical syndromes caused by new pathogens are not necessarily recognizable;

• little is known about the behaviour of potential pathogens from the donor species in humans;

• graft rejection and immune suppression are strong stimuli for the activation of many organisms from latency;

• genetic recombination, mutation, or interactions between exogenous and/or endogenous organisms may mask or alter the common manifestations of infection;

• the absence of pre-existing immunity in the recipient to novel, animal-derived organisms may render the host more susceptible to infection;

• species disparity of histocompatibility antigens may be associated with diminished cellular immune function by the host against organisms within the xenograft (4).

Which organisms should we fear?

The organisms of greatest concern to the general public are those which spread easily between immunocompetent individuals, which can attach to and enter human cells, which can replicate within these cells or tissues, and which can spread with few clinical signs or symptoms. T he ideal "stealth organism" takes on surface antigens from the host so as to reduce the capacity of the host's immune system to attack the pathogen. T he organism must also cause disease (injury) directly or must develop pathogenic characteristics in the xenograft recipient so as to justify concern about its spread in the general population.

T he endogenous retroviruses, like the beta-herpesviruses, are almost perfect transplantation pathogens. T he endogenous retroviruses are carried in the genome of every cell, acquire host antigens while budding off from infected host cells, and require cell-mediated immunity for clearance, which is diminished by immune suppression of the transplant recipient. H owever, there is no clear association of the porcine retroviruses, as yet, with clinical disease, infection of normal or immunocompromised individuals, or spread between individuals. All retroviral assays to date (fortunately largely based on anecdotal reports) have been negative in terms of infection of humans exposed to porcine tissues (12–14). Preclinical studies of xenotransplantation will be important in defining the risk of spread of infection between species and the factors which control viral replication. H owever, preclinical models (e.g., pig-to-primate) may not predict the infectivity or all the manifestations of disease which may occur in human xenograft recipients.

T he available data have not demonstrated risks which should impede measured progress towards further studies of clinical xenotransplantation. H owever, further microbiological studies of xenograft tissues or cells from human recipients may be very revealing, and of critical importance. To this end, new molecular or antigen detection assays for known organisms of donor species are needed. Each approved clinical trial must also be designed to optimize the possible recovery of novel organisms and should not be limited to searching for previously identified organisms. T herefore, biopsies and blood samples must be collected from xenograft donors and recipients not only for archiving but for use in an active programme of microbiological investigation, including the search for unknown organisms (for instance by representational difference analysis) and studies of the biology of such potential pathogens. It seems likely that new organisms will be discovered in association with the transfer of animal tissues into immunocompromised or otherwise modified human hosts, just as novel infections have been described in association with the immune deficits of AIDS and cancer chemotherapy. Rather than being guided by fear of the unknown, we must not squander a unique opportunity for discovery. ■

Xenotransplantation in Sweden

C.G. Groth1 & M. E. Breimer2

Several Swedish research groups are working actively in the field of xenotransplantation. Recently, these efforts have resulted in two clinical pilot trials: 10 diabetic patients have been transplanted with porcine pancreatic islets, and two patients have had pig kidneys connected to their blood circulation and perfused extracorporeally.

Transplantation of porcine islets

Transplantation of isolated pancreatic islets offers a simple and safe method to provide the diabetic patient with insulin-producing tissue. If such transplantation is to be widely applied, the supply of human pancreases will not suffice. Transplantation of pig islets could then serve as an alternative. Porcine insulin differs from human insulin only in regard to one amino acid, and glucose homeostasis and the regulation of insulin secretion are similar in pig and man. Furthermore, porcine insulin has been used for decades to treat diabetic patients. Since the transplanted islets will become vascularized by host vessels, hyperacute rejection should not occur.

Fetal porcine islets can be prepared in large quantities by tissue digestion and culture. After transplantation, the fetal cells mature and differentiate into insulin-producing cells. Microbiological screening revealed no infectious agents in the material, and, when the material was injected intraperitoneally in dogs, no adverse effects were noted.

On the basis of the above findings, a clinical pilot trial was conducted in the years 1990–1993 in 10 diabetic renal transplant patients. All patients were given immunosuppressive treatment because of their renal transplant (1). Eight patients who had previously undergone kidney transplantation had the porcine islets injected into the portal vein. Four of the patients excreted small amounts of porcine C-peptide for 100–400 days after transplantation, indicating porcine insulin production. In two patients, the islets were placed under the capsule of their renal graft just after the graft had been revascularized. In one of the patients, a kidney biopsy specimen taken three weeks after transplantation revealed morphologically intact epithelial cells under the kidney capsule. These cells stained positively for insulin and glucagon. Ultrastructural and immunocytochemical features were typical of pancreatic islet cells; the appearance of the cells indicated that they were viable (2).

All patients had preformed xenobodies against the Gal 1,3Gal, an antigen epitope present on porcine but not on human cells. After transplantation there was a pronounced increase in the antibody titres (3). The finding that the porcine islets did not function at all in some patients, and only for a limited time in the others, probably indicates rejection. The role of the xenobodies in this process remains obscure.

The patients derived no clinical benefit from the transplantation in that their insulin requirements remained unaffected. A larger and longer-lasting insulin production will be required to accomplish a clinical benefit. Recently, we have isolated adult pig islets which provide immediate function. When such islets were transplanted into unmodified rats, rejection occurred in 4–5 days. However, when the rats were treated with novel immunosuppressive drug regimens, rejection was prevented for several weeks (4). These encouraging findings provide the basis for further trials with pig-to-human islet transplantation.

Extracorporeal (ex vivo) perfusion of pig kidneys in two patients

Removal of the preformed xenobodies by immunoadsorption, or plasmapheresis should facilitate xenograft survival. In order to test this concept, we performed a clinical trial in 1995 where pig kidneys were extracorporeally connected to the blood circulation of two volunteer dialysis patients. The procedure was similar to an ordinary dialysis, except that the dialysis filter was replaced by a pig kidney. Prior to the procedure, the patients underwent plasmapheresis with a subsequent reduction in xenobody titres. No immunosuppressive drugs were given (5–7).

Initially both the pig kidneys perfused well, and produced urine. However, after 65 minutes of perfusion, the first kidney underwent rejection as evidenced by discoloration and a reduction in blood flow, and the experiment was discontinued. The second patient developed symptoms of anaphylaxis after 15 minutes of perfusion. The pig kidney appeared normal at this time but the perfusion was terminated for safety reasons. Histopathological examination of the first kidney confirmed the diagnosis of rejection.