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Xenotransplantation: one trotter forward, one claw back

Since the volume of organ transplantations remains static each year whereas the number of patients who could benefit from such procedures increases, most patients on waiting lists die before a suitable donor is found. The supply of human organs for transplantation cannot match demand, so there is active research into acceptability of animal organs by both the human immune system and the public. For various reasons, such as size of organs, matching physiology, fecundity, and practical matters of animal husbandry, pigs are the likely donor species.

Xenografts from pigs face an immediate hurdle termed hyperacute rejection because human beings contain antibodies against a terminal α-galactose (alpha-gal) epitope on carbohydrates.[1] These antibodies bind alpha-gal on donor endothelial cells and fix complement. They thus destroy the function of these essential cells by impairing the tight junction linkage to adjacent cells, which leads to oedema and haemorrhage, and by impairing natural antithrombotic activities, which leads to thrombosis and organ ischaemia. This type of host response is part of innate immunity, more primitive than the adaptive immune responses that will face a pig organ rendered compatible with human antibodies to alpha-gal.[2] Medium-term approaches to solving the problem of hyperacute rejection may involve deletion of the pig enzyme that adds the offending terminal alpha-gal or, in the short term, expression of human control proteins to degrade activated complement. Thus, pig organs expressing CD55 (decay-accelerating factor) can survive the hyperacute rejection phase in pig–primate transplants,[3] and additional work with CD59 and CD46 is expected.

In anticipation of the move of this research to pig-human clinical trials, what infectious complications can be envisaged? First, there is the possibility that the donated organ may be susceptible to human viruses, including those that use complement-control proteins as cellular receptors—eg, enteroviruses, which use CD55,[4] and measles[5] and human herpesvirus 6, which use CD46.[6] Second, there is the possibility that porcine viruses may cross the species-barrier[7] to infect human beings (xenosis), especially since the combined components of the xenotransplant protocol both activate gene expression and decrease immune responsiveness and so seem designed to facilitate activation of viruses latent within donor tissues. A series of undesirable processes can be envisaged that would indicate progressively increasing risk of such xenosis (panel). Attention so far has focused on porcine endogenous retroviruses (PERV), endogenous genetic elements with the potential to produce infectious virions. Multiple copies are integrated into germ-line DNA and so are present in all organs. The demonstration that two pig kidney cell-lines spontaneously released C-type retroviruses generated much interest, especially when these viruses could infect not just pig but also cells of other species, including human kidney 293 cells.[8] Continued co-cultivation of these viruses broadened the range of human cells that could be infected, and after passage in human cells PERV were able to convert a replication-defective Moloney retrovirus vector to competence and to acquire resistance to human complement.

Xenosis: undesirable processes

Porcine virus:
- persists in donor animals
- is present in organs destined for donation
- enters human cells
- replicates in human cells
- persists in human recipient
- disseminates from donation site
- interacts with human viruses

Long-standing human parenteral exposure to pig biomaterials in the form of porcine insulin or porcine heart valves might provide some reassurance that porcine viruses may not be readily transmitted to human beings,[9] but the treatment processes employed (hydrochloric acid/ethanol and glutaraldehyde, respectively) would be expected to reduce infectivity, and the recipients of these products are not immuno-compromised. More recent results from patients exposed to living pig tissue, some of whom were immunocompromised, therefore represent more realistic tests of the possibility of xenosis. Remarkably, extracorporeal perfusion of human blood through pig organs or hepatocytes has been used for the treatment of various medical disorders, such as septicaemia and impotence. There are no efficacy data to support such interventions, but follow-up of the 160 recipients (36 of whom were immunocompromised) provides a test of possible PERV transmission.[10] RT-PCR was used to detect virion RNA in serum and PCR to detect pig centromeric mitochondrial DNA in peripheral-blood mononuclear cells. All the recipients were RT-PCR-negative, which indicates no evidence of virus infection. However, of the 100 patients (one of whom was immunocompromised) treated by spleen perfusion, 23 had clear evidence of microchimerism because PCR revealed pig DNA in their blood. Microchimerism may be an important component of the immunological adaptation of the donated organ to the recipient,[2] but it offers the possibility that allogeneic stimulation of microchimeric cells at various body sites might activate PERV into an infectious state.

Very recently, experiments to raise PERV antiserum in guineapigs by inoculation of virus particles showed

broad immune recognition of viral proteins, which implies transmission of active infection to this species (cited in ref 11). Meanwhile, transplantation of pig islet cells under the kidney capsule of mice with severe combined immunodeficiency (SCID) also transmitted PERV to murine cells and, after cocultivation, to human cells.[12] In both sets of experiments, there was evidence of active virus infection of these rodents, not just transmission of PERV DNA. The establishment of microchimerism, though, correlated strongly with the presence of virus infection in the SCID mice experiments.[12] These new results present opportunities to investigate in small animal models several important components of the pathophysiology of cross-species transmission of PERVs. For example, does the establishment of microchimerism facilitate production of infectious PERVs? What is the effect of immunosuppressive drugs on microchimerism and infectious PERVs? Can antiretroviral compounds be used prophylactically to prevent activation of PERV DNA or reduce PERV infectivity? Although existing antiretroviral agents have been selected for their ability to inhibit the distantly related lentivirus HIV, pharmaceutical companies should examine their libraries of related compounds to check whether any can inhibit PERVs. Can a vaccine be developed against PERVs so that prospective recipients (rodents and then human beings) can be protected against these viruses? If so, what are the correlates of protective immunity in rodents and do these translate into protection of human beings once clinical trials begin?

Although these new results provide exciting scientific research opportunities, what effect will they have on the prospects for xenotransplantation moving soon into the clinical phase of testing? Various views are likely, depending upon the perspective of the individual concerned.

- An official in a regulatory authority will have been concerned about PERV transmission before the most recent experiments, and will have insisted that surveillance for these viruses be added to the experimental clinical protocol. He/she will view the development of PERV-induced lymphomas as the worst case for the individual, yet patients undergoing transplantation already have an increased risk of lymphomas due to Epstein-Barr virus and still consent to go ahead with the procedure. The main concern will thus be the possibility of transmission of PERV virions to close contacts of the transplant recipient—ie, concerns for society as well as the individual.
- An executive of a biotechnology company will wish to decide whether to invest further in the present generation of engineered pigs or seek additional long-term investment to derive pigs devoid of PERV DNA. Although xenotransplantation is potentially a US$6 billion market,[1] the executive may see financial gains retreating into the distant future and so be reluctant to invest further.
- A transplant clinician is faced with patients not attending follow-up clinics because they have died while waiting for a transplant. A good supply of organs is needed now to combat a real and present danger, so the doctor may be impatient about the theoretical concerns for future disease raised by the new results.
- Finally, the patient, who has little chance of survival with good quality of life without this experimental treatment, may not appreciate why more attention is given to the results of work with rodents than to the results of extracorporal perfusion of human blood through pig tissue. After all, he is not a guineapig—or is he?

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THET–*Lancet* Research Award in international health

The Tropical Health Education Trust (THET) and *The Lancet* this week launch an annual research award in international health. The aim of the award, worth at least £2000, is to promote clinical work and inquiry in everyday health care in any developing-world setting. The larger wish is to assist development of capacity and capabilities of individual doctors and medical schools. We seek research papers, research letters, or case-reports that will in some way help to improve health care or develop clinical services. THET has been working since 1989 with medical schools and other training institutions in African countries to assist them to reach their goals, so that they can deliver the sort of education and training that they consider to be relevant for the people whom they serve. THET, with the Reuter Foundation, already runs an annual competititon for the best student projects in African medical schools; the THET-*Lancet* award now offers qualified health workers a similar but wider opportunity, not restricted to Africa. The submissions will be judged by a joint THET-*Lancet* awards committee. The winning entries will be published in *The Lancet*. To apply for a THET-*Lancet* research award, please send five copies of the article, together with a covering letter setting out the strengths and limitations of the work, to Richard Horton at *The Lancet* by March 31, 2001.

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