PERSPECTIVES: BIOMEDICINE

Xenografts and Retroviruses

Robin A. Weiss

"Uncertain peril and certain promise" was how Joshua Lederberg described the new recombinant DNA technology when molecular biologists met in 1975 to impose a short-lived moratorium on genetic engineering. The same epithet applies today to xenotransplantation, the grafting of animal cells and tissues into humans (*1*). Xenotransplantation, too, has led to calls for a moratorium (*2*) so that the ethical issues involved (*3*) and the hazards of cross-species infection can be publicly debated. Some reassurance on safety is provided on page 1236 of this issue (*4*) by a consortium of investigators from Novartis and the Centers for Disease Control and Prevention (CDC). Paradis *et al.* report that infection by porcine endogenous retrovirus (PERV)—one potential risk of transplanting patients with pig tissues—does not appear to have occurred in any of 160 persons exposed to living pig cells (*4*).

The "certain promise" is that xenotransplantation could provide a ready supply of cells, tissues, and organs to treat a variety of serious human conditions. The "uncertain perils" are to what extent the animal cells or tissue will perform properly in the human host, whether immunological rejection can be overcome, and whether harmful zoonoses (animal-to-human infections) can be prevented (*1, 3*). Despite the greater immunological barrier, pigs are favored over primates as a source of transplant tissue for a variety of practical, ethical, and safety reasons. Among the many microbes harbored by pigs, PERV has aroused particular concern. Animal endogenous retroviruses are integrated proviral DNA genomes inherited in a Mendelian manner. At least 50 copies of PERV exist in pig chromosomes and PERV cannot be eliminated by pathogen-free, closed breeding of pigs. Some PERV genomes have given rise to human-tropic PERV strains in culture (*5, 6*).

Fetal pig nerve cells have been transplanted into patients' brains in an attempt to slow down neurodegeneration in Parkinson's and Huntington's diseases (*7*) and to treat epilepsy. A recent report on 24 such patients indicated no evidence of subsequent PERV infection in the blood (*8*). Last year, lack of PERV infection was reported in 10 diabetic patients transplanted with pig pancreatic islet cells secreting insulin (*9*) and in two renal dialysis patients whose blood was extracorporeally perfused through pig kidneys (*10*). Eight of the diabetics and both of the dialysis patients were reanalyzed in the present 160-patient study (*4*), which used similar PCR and RT-PCR amplification methods to detect viral genomes, and Western blotting to detect viral antigens in serum (see the table).

The lack of evidence for PERV infection will encourage biotechnology companies, physicians, and surgeons to explore pig tissue treatments further. For example (as reported for one patient by Paradis *et al.*), circulation of human blood across porcine hepatocytes in culture is under investigation as a treatment for patients with acute liver failure (*4*). Of course, a major goal is to make up for the shortage of available human organs for transplantation by supplying pig kidneys and hearts instead. But hyperacute rejection and acute vascular rejection present a major hurdle to whole-organ xenotransplantation (*1, 3*). Rejection of pig organs is triggered by natural human antibodies that recognize carbohydrate "xeno-antigens"—mainly α (1-3) galactose—expressed on pig endothelial cells that line blood vessels (*11*). Several biotechnology companies are attempting to block complement-mediated immune attack of xenografts by breeding transgenic pigs that express human proteins (*1*). But an unfortunate corollary of such genetically modified pigs may be that the porcine viruses they carry may more readily infect humans (*12*).

DETECTION OF PERV IN HUMAN TISSUE AND SERUM

Reverse transcriptase (RT) activity	(*4, 9*)
Product-enhanced (amplified) RT assays detect <100 particles ml^{-1} but are not PERV-specific.	
Viral genome sequences	(*4, 8–10*)
PERV-specific amplification of proviral DNA in cells by polymerase chain reaction (PCR) detects <10 PERV genomes with high specificity.	
RT-PCR, DNA synthesis, and amplification from viral RNA in plasma detects <50 PERV genomes ml^{-1} with high specificity.	(*4, 9*)
Antiviral antibodies	
Western blot detects recombinant Gag viral antigen, whole virus, or infected cell lysate.	(*4, 9*)
Enzyme-linked immunosorbent assay (ELISA) detects Gag antigen or whole virus. Expected to be more sensitive though slightly less specific than Western blot.	
Neutralization of virus distinguishes between envelope variants of infectious PERV (*6*) but probably less sensitive than ELISA or Western blot.	(*10*)

The most striking scientific finding reported by Paradis *et al.* is the apparent long-term survival of porcine cells in the blood of 23 of 100 patients in St. Petersburg, Russia, whose blood had been perfused through pig spleens for 1 hour up to 8 years previously. Survival of transplanted donor cells in the recipient is known as microchimerism, and it can confound the sensitive detection of PERV infection. In fact, the Novartis/CDC study only examined microchimerism in those patients who first yielded a PERV-positive result; in other words, the 50 copies of retroviral DNA naturally resident in the pig genome were used as first-line detection of pig cell microchimerism. Extremely low numbers of pig cells (<1 per 100,000 human cells) were estimated to be present in the human samples. However, only 4 of the 23 samples tested by both Novartis and CDC were PERV-positive, which raises the question of PCR contamination (the bane of all highly sensitive forensic or diagnostic DNA amplification tests). The long-term survival of pig cells detected as pig DNA in the patients' circulation is surprising in view of a report on pig-to-baboon microchimerism indicating that the pig cells become rapidly sequestered from the circulating blood (*13*).

Paradis *et al.* do not state the ailments for which the Russian patients were subjected to "immunotherapy" by spleen extracorporeal perfusion. One wonders whether the physicians conducting this procedure ever stop to consider a risk-benefit analysis of passing their patients' blood through the spleens of farm pigs fresh from the abattoir and whether they inform the patients of the risk of infection. Nevertheless, the Russian data provide us with evidence for a lack of PERV infection despite apparent long-term survival of pig viruses in the human body. It will surely be most important to test these patients for evidence of infection by other prevalent systemic pig viruses such as porcine parvovirus and porcine circovirus, which are unlikely to be wholly excluded from closed breeding herds.

A chain of events required for PERV to pose a threat to public health has been proposed (*14*). PERV is present in the pigs being bred for xenotransplantation, and human-tropic PERV is expressed in many cells and tissues (*5, 6*), though apparently not in fetal brain cells (*8*). Thus far, PERV has not infected patients exposed to porcine tissues (*4, 8–10*), but if this were to occur,

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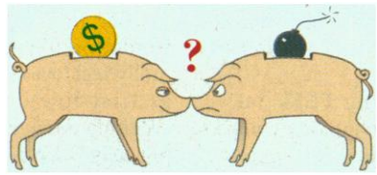
we would need to investigate whether PERV caused disease and whether it could be transmitted to other individuals. To address the risks of infection, the U.S. Food and Drug Administration (FDA) established an Advisory Panel on Xenotransplantation. And in 1997, the British government, still reeling from the evidence that bovine spongiform encephalopathy ("mad cow disease") had spread to humans, moved quickly to set up the UK Xenotransplantation Interim Regulatory Authority (UKXIRA).

With the latest reports (*4, 8*) on the lack of evidence for PERV infection in vivo, the keenest advocates of xenotransplantation may mutter that the concern over the risk of PERV infection unnecessarily delayed progress in the field. The Novartis/CDC teams, however, conclude that only cautious progress in closely monitored, prospective clinical trials will help in "assessing the safety and efficacy of using porcine cells, tissues, or organs therapeutically in humans" (*4*). Both the FDA and UKXIRA take this attitude and appear ready to approve, in principle, small-scale human trials of porcine cellular therapy.



Whereas the endogenous retroviruses in our house guests (cats and mice) have not naturally been transmitted to humans, we have known for more than 20 years that human tumor xenografts grown in immunosuppressed animals sometimes become infected (*15*). PERV, however, does not proliferate as readily in human cells as human-tropic feline and murine endogenous retroviruses (*5, 6*). But the possibility remains that, say, one among 1000 xenograft recipients may become infected by PERV or by a virus resulting from recombination between PERV and human retroviral sequences.

The concern, then, will be the potential for onward transmission from the rare, infected transplant recipient to his or her contacts. Lest we dismiss this notion as ridiculous, we should bear in mind that HIV-1 began as a zoonosis, probably from chimpanzees, and that the worldwide pandemic of the major HIV-1 subgroups may be attributable to a single cross-species event (*16*). Neither can we be sure that AIDS did not have an iatrogenic (medically caused) origin, if chimpanzee kidneys were used in Africa to propagate certain batches of poliovirus vaccine (*17*).

Although the public may demand evidence of no risk, retrospective epidemiological surveys can at best provide no evidence of risk, which is a rather different matter. We should heed the Hippocratic precautionary principle—"at least do no harm." Yet no new medical procedure can be deemed entirely safe, so we need to balance risk with benefit, for the patient and for the human population.

For the individual transplant recipient, the real promise seems to be greater than uncertain peril. Indeed, one of the potential advantages of xenotransplantation over allotransplantation (person-to-person grafts) is that pathogen-free pigs might pose a lesser threat of infection than a graft from an unknown human donor. After all, many cases of life-threatening infections have been transmitted by human transplantation and transfusion: HIV, hepatitis B and C viruses, various herpesviruses, tuberculosis, and Creutzfeldt-Jakob disease.

For the community at large, the risk-benefit equation is much more difficult to quantify. It took more than 20 years for HIV-1 to spread out of Africa, and it is only after 55 years of individual benefit from antibiotics that we are facing the public health threat of multidrug-resistant microbes. The ethical and technical problems of maintaining vigilance over xenotransplantation should not be underestimated.

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PERSPECTIVES: PLANT BIOLOGY

Taking Transgenic Plants with a Pinch of Salt

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About one-third of the world's irrigated land is unsuitable for growing crops because of contamination with high levels of salt. Currently more arable land is lost through salinity than is gained through the clearing of valuable forests. Most trees and crop plants are highly sensitive to salty conditions, experiencing a water deficit because of osmotic stress and biochemical perturbations due to the influx of sodium ions (Na^+). Varieties of a single plant species, such as barley or tomato, exhibit a high degree of variation in salt tolerance (*1*). This suggests that only a few mutations in several key transporter or regulatory proteins could confer salt tolerance on salt-sensitive plants. For example, the differing ability of two species of *Plantago* to withstand salty soil is due to the presence of the sodium/proton (Na^+/H^+) antiport protein in salt-tolerant *P. maritima* and its absence in salt-sensitive *P. media* (*2*).

Twenty years after Epstein and his colleagues proposed genetically engineering salt-resistant crops (*1*), Apse *et al.* now report on page 1256 that they have engineered salt-tolerant *Arabidopsis* by overexpressing a single endogenous gene (*AtNHX1*) encoding a Na^+/H^+ antiport protein (*3*). With this strategy, it should be possible to engineer a whole spectrum of salt-tolerant crop plants, enabling them to be irrigated with seawater or water of marginal quality. Public acceptance of genetically engineered salt-tolerant plants (which could enable crops to thrive in salty environments, thus saving further deforestation in the quest for more arable land) is likely to be greater than for plants engineered to be pesticide- or herbicide-resistant.

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