

Zoonosis in xenotransplantation

Clive Patience*, Yasuhiro Takeuchi† and Robin A Weiss‡

Species barriers against microbial infection will be lowered to an unprecedented degree in xenotransplantation settings. Our knowledge about micro-organisms in donor animals is limited and it is difficult to predict the consequence of such cross-species infection.

Addresses

Chester Beatty Laboratories, The Institute of Cancer Research, 237 Fulham Road, London SW3 6JB, UK

*e-mail: clive@icr.ac.uk

†e-mail: yasuhiro@icr.ac.uk

‡e-mail: robinw@icr.ac.uk

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Abbreviations

ERV endogenous retrovirus

PERV porcine endogenous retrovirus

Introduction

There exists a world-wide shortage of human donor organs available for transplantation. The xenotransplantation of animal organs and cells is seen as a solution to alleviate this shortage and also as a therapy for other diseases. To date, most attention has focused on overcoming the immunological barriers that cause the rapid rejection of the xenografts [1]. As significant advances in overcoming the problems of xenograft rejection have recently been made, it seems reasonable to envisage that xenografts — first of cells and later of whole organs — could soon survive in immunosuppressed hosts. Indeed, phase I clinical trials have taken place with transplantation of pig islets of Langerhans cells, for diabetes, and foetal pig brain cells, for Parkinson's disease [2,3]. Consequently it is timely to consider the microbiological hazards — the potential zoonoses — of xenotransplantation and these are the subject of this review.

In principle three main questions remain to be answered regarding zoonoses. Firstly, do we know enough, at the present time, about the micro-organisms present in donor animals to proceed with xenotransplants? Secondly, if there are microbes that might cross the species barrier, either from the donor to recipient or *vice versa*, how likely is this to occur and would it trigger a human epidemic? Finally, if the organisms did cross, how likely is it that they would cause disease in humans? Evaluation of these and other risks have been raised in several recent articles [4–7]. Here we address the current state of knowledge regarding possible zoonoses, in particular regarding viruses.

Probably the most serious problem for xenotransplants concerns the transfer of latent microbes, present in the

xenograft, to the human recipient. These may not be pathogenic in their natural host yet might cause disease in humans, especially in immunosuppressed patients. In particular the possible transfer of viruses such as herpesviruses and retroviruses is a problem, due to their capacity to remain latent for long periods. Baboons and pigs are the most likely candidates to be used as sources for donor organs and cells. Both these species have a wide range of organisms known to replicate in human cells or infect humans [8*,9–12]. With the well documented array of viruses known to be present in baboons it seems unlikely that these animals will ever be the supply of clean donor organs [13]. To date the major microbiological concern over pigs has been the identification of a porcine endogenous retrovirus (PERV) that is infectious for human cells [14**,15**].

Do we know enough about the micro-organisms of donor species?

Infection resulting from the transfer of microbes along with human donor organs is a well recognised phenomenon with allotransplantation; however, zoonoses may represent a more severe risk because the species barrier is being crossed and humans may be exposed to novel infections. Whether such risks would have raised this degree of interest if it were not for the recent outbreaks of new-variant Creutzfeld–Jacob disease, Ebola virus, Sin Nombre hantavirus, H5N1 influenza virus in Hong Kong, as well as the established association of simian retroviruses with the HIV pandemic, remains a matter for speculation. Not all zoonoses, however, raise such a spectre of disease. For example, Heneine *et al.* [8*] have reported that although up to 2% of primate handlers contract simian foamy virus infections, none has developed disease nor, and perhaps more importantly, did the virus spread to sexual contacts. The risk to the human population at large of a zoonosis may be greater if an agent takes years to produce an effect, for example HIV, rather than with an organism that causes acute disease, such as the filoviruses (e.g. Marburg and Ebola).

Although it is a relatively simple case to raise donor animals free from the known exogenous pathogens, the animals may still carry unrecognized microbes even though they appear healthy. For example, within the past year, two previously unknown viruses in pigs — a ubiquitous virus related to human hepatitis E virus [16] and a new torovirus [17] — have been identified. In baboons, variants of simian foamy viruses and also T-lymphotropic viruses have recently been characterized [18,19]. It will be important to continue to identify new microbes and to develop assays to detect these potential pathogens associated with donor animals; it seems likely that new infectious agents will continue to be discovered after xenotransplantation has commenced.

How likely is it that microbes could be transferred between donor and recipient or on to the community at large?

An almost unavoidable source of potentially infectious material, that will be present in donor tissue, are endogenous retroviruses (ERVs). These form part of the normal germline DNA of their host and multiple copies have been identified, integrated in chromosomes of all vertebrates studied to date. ERVs closely resemble infectious, exogenous retroviruses and are thought to be the genetic fossils of ancient retroviral infections. Natural selection favours the survival of ERV forms that are not pathogenic to their host [20]; however, there is ample evidence that retroviruses, including ERVs, can cross the species barrier and, once across, might behave in a much more pathogenic manner. For example, gibbon ape leukaemia virus (GALV) which spreads as an ape-to-ape infection was probably derived by interspecies infection of an ERV of rodents (or possibly marsupials) from south-east Asia and it causes disease in captive gibbons. Baboon endogenous retrovirus (BaEV) is known to replicate to a high titer in human cells [21*] but its pathogenicity in experimental animals has yet to be tested.

Pig endogenous retrovirus

The first evidence that PERV can infect and replicate in human cells was reported by our group in 1997 [14**]. The virus (PERV-PK) was isolated from the pig kidney cell line PK15 and could infect human, mink and pig cells. A second virus, PERV-MP, that was also isolated from a pig kidney cell line, exhibited a different cell tropism — only infecting pig cells. The PERV family to which both of these viruses belong is most closely related to the ERV sequence PoEV (porcine endogenous retrovirus) described by Tristem *et al.* [22] and may be present at up to 50 copies per cell. Other closely related PERV sequences have also been identified [15**,23*]. Detailed sequence comparisons and infectivity studies will be needed to determine the relationship between the viruses and their host ranges. It will also be important to determine whether other, as yet undiscovered, PERV families exist. As the viral envelope is both the primary determinant of the virus host range and tends to be the most variable region of a retroviral genome, interest is focused on this region. Envelope genes of two classes of human-tropic PERV, PERV-A and PERV-B, were molecularly cloned from PERV-PK cultures [24*]. All pigs of various herds tested so far possessed multiple copies of these envelope genes and their hearts, kidneys and lymphocytes express both the genes. Yet another envelope was found in a full-length, PERV cDNA clone isolated from miniature-swine lymphocytes [23*].

Infectious PERV was originally isolated from established pig cell lines in culture. Clearly it was important to determine whether similar viruses could be released by primary pig cells. If PERV was not produced the potential microbiological hazards of these elements for xenotransplantation would be greatly diminished; however, Wilson *et al.* [15**]

have recently described the isolation of infectious PERV from primary pig cells. Lymphocytes from two pig strains were stimulated, with mitogens and inducing agents, in short-term culture and they released virus capable of replicating in pig and human cells. Whether the viruses released are a pure population from a single PERV locus or whether they arise from the activation of multiple loci remains to be determined.

If normal pig organs are xenografted into Old World primates, a rapid rejection of the organ occurs. This hyperacute rejection is mediated by antibodies, which mainly recognise α -galactosyl sugars present on the pig cells. Old World primates, including humans, develop these 'natural' antibodies since they are natural genetic 'knockouts' for the galactosyltransferase gene and develop a response due to immunogenic stimulation by bacteria which form part of the normal gut flora [25]. The adaptation of pig cells to appear less foreign to the human immune system may increase the likelihood of zoonoses. The same antibodies which cause hyperacute rejection may also protect us from interspecies infection of enveloped viruses [26,27]. As PERV particles released from pig cells carry these sugars on their envelopes, they are sensitive to complement-mediated lysis; however a single passage of the PERV through human cells renders the particles almost totally resistant to lysis by human serum [14**] due to the absence of the sugars.

In attempting to overcome hyperacute rejection, transgenic pigs are being bred that express human inhibitors of complement activation such as CD46 (membrane cofactor protein) and CD55 (decay accelerating factor) [1]. Incorporation of these proteins into HIV particles has been shown to protect HIV from inactivation by complement [28,29]. The levels of incorporation of these molecules into PERV particles and their effect on PERV complement-sensitivity remain to be determined. Human CD46 and CD55 can be utilised as cell surface receptors by measles virus and picornaviruses respectively [30–34]. Expression of CD46 in transgenic mice can protect the organs and cells from the actions of human and rat complement but also render the cells susceptible to productive measles virus replication [35]. Expression of these molecules in transgenic pigs may thus not only render the animals susceptible to infection by the human pathogens but might also provide an opportunity for pig viruses to adapt from using the porcine equivalent of CD46 and CD55 to the human forms of the molecules [36]; therefore thought should be given as to whether it may be possible to engineer the CD46 and CD55 constructs so that they retain their complement-inhibitory capacities but cannot be internalised in a manner which leads to virus replication.

What are the risks of disease due to zoonoses to the recipient and to the general population?

Ultimately the true risk of zoonoses can only be addressed by performing xenotransplantation. Before such procedures

are carried out several approaches can be taken which may provide some answers regarding these risks. For example, *in vitro* experiments can be performed that indicate what types of cell may be particularly susceptible to infection and whether the virus induces any gross changes in the cell. It is a matter of debate whether *in vitro* infection mirrors *in vivo* susceptibility, so animal models of xenotransplantation may be informative. Eventually the only decisive answers will come from the investigation of humans exposed to pig tissues in a controlled and careful manner. The scientific community, however, is already in a position to make preliminary investigations into this very scenario. There exists a relatively small number of humans who have been treated with cells or tissues from pigs for various conditions — for example, Parkinson's disease, diabetes and burns. Samples from these patients are currently being examined for signs of the persistence of porcine cells and PERV replication following the development of molecular epidemiological probes [37–39]. There are available a very limited number of individuals exposed to baboon tissues and these patients are also under investigation [37]. It should be borne in mind, however, that not all of the above patients have been on immunosuppressive regimes such as those required for transplant recipients (e.g. patients with third degree burns with temporary pig skin cover are not immunosuppressed). As a consequence they may not represent an ideal study group. Clearly no acute pathogenic infections have occurred in these patients but there may be others progressing in an asymptomatic manner which may not become apparent for some years.

The risks of zoonoses not only lie with the recipient of the transplanted organ but potentially also with contacts of the recipient. This may pose a particular problem if either the recipient, or contacts who become infected, were young. The effect of age on the outcome of infection is well documented and can be demonstrated by the transmission of oncoviruses related to PERV, such as murine leukemia virus and avian leukosis virus [21*]. Horizontal transmission results in a transient viraemia, followed by immunity and only rare incidence of leukaemia; however, if the virus is transmitted congenitally or to newborn animals, they become viraemic and are often tolerant to viral antigens. Although the animal initially grows normally, leukaemias are common and, significantly, the viraemic host can be a major source of further horizontal and vertical transmission; therefore pathogenicity tests of PERV should include neonatal animals; moreover, if xenotransplantation were to develop sufficiently to allow recipients to have children, it would be important to monitor the offspring carefully for evidence of infection by PERV.

Conclusions

Undoubtedly xenotransplantation promises great benefits to those people waiting for transplants. Balanced against the benefit of xenotransplantation to the individual recipient is the risk of the emergence of a new human pathogen. It will be of the utmost importance to ensure that stringent

screening procedures are in place if xenotransplantation is to proceed and that results of the tests are rapidly and widely made available to the scientific community and to regulatory authorities.

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