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AN APPROACH TO THE CONTROL OF DISEASE TRANSMISSION IN PIG-TO-HUMAN XENOTRANSPLANTATION

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ABSTRACT

Although several major immunologic hurdles need to be overcome, the pig is currently considered the most likely source animal of cells, tissues and organs for transplantation into humans. Concerns have been raised with regard to the potential for the transfer of infectious agents with the transplanted organ to the human recipient. This risk is perceived to be increased as it is likely that the patient will be iatrogenically immunocompromised and the organ-source pig may be
genetically engineered in such a way to render its organs particularly susceptible to infection with human viruses. Furthermore, the risk may not be restricted to the recipient, but may have consequences for the health of others in the community. The identification of porcine endogenous retroviruses and of hitherto unknown viruses have given rise to the most concern. We document here the agents we believe should be excluded from the organ-source pigs. We discuss the likelihood of achieving this aim and outline the potential means by which it may best be achieved.

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Introduction

The great limitation in the number of human donor organs available for transplantation has led to a renewed interest in overcoming the immunological problems related to xenotransplantation [I]. For a number of reasons, including microbiologic safety, non-human primates are no longer considered to be the preferred animal organ source despite their immunologic closeness to humans. Most effort is being directed to the use of the pig, for reasons that have been elucidated and discussed elsewhere . Although several major immunologic hurdles need to be overcome, the pig has many logistic advantages, one of which is its suitability for genetic manipulation, which can, for example, provide its organs with some protection against human complement [8]. The risk regarding the potential for the transfer of infectious agents to the human recipient with the transplanted tissues or organ is considered less if the pig (rather than a non-human primate) is the source (donor) animal. The very few studies carried out to try to assess this risk have been largely limited to an assessment of non-viral agents, and have demonstrated that swine [commercial specified pathogen free (SPF)] are largely free of bacteria, fungi and parasites that might be pathogenic in humans. Nevertheless, some concern remains.

With human organ donors, present technology is able to identify most serious pathogens, such as toxoplasma, hepatitis viruses, and human immunodeficiency viruses, but the presence of one or more of these agents is sometimes accepted when the patient's condition is critical. Other organisms, which are likely to cause disease that may be considered less serious, such as cytome- galovirus and Epstein-Barr virus, are commonly knowingly transferred from donor to host; to exclude them would entail excluding an unacceptably high number of potential donors. Although there will be much greater control over the microbiologic status of specially bred and housed swine, there remains an unknown risk of the transfer of a porcine infectious agent that is not seen in humans. This risk may not be restricted to the recipient, but may have consequences for public health. For example, as pig endogenous retroviruses (PoERVs) are present in the pig cell genome, their transfer with the transplant would be inevitable. This does not necessarily imply, however, that PoERVs would be expressed, propagated, transmitted and become pathogenic in humans.

With implantation of a pig organ into a human patient, many barriers to infection are bypassed. Ifan infection with a pig agent were established in a patient, further adaptation to the host may follow. Several recent examples of interspecies transmission of viruses accompanied by adapta- tion to a new host have been reported. The immunosuppression required to prevent xenograft rejection further reduces the host's resistance to infection.

For example, human antibodies directed against Galα1l-3Gal (Gal) epitopes on the pig organ are the prime initiators of antibody- mediated rejection of transplanted pig organs. Several bacteria, viruses and protozoa express Gal on their cell sufaces, and anti-Gal antibodies may form part of the body's immune defence mechanism against them. Weiss has emphasized that lysis of certain nonhuman retroviruses is triggered in humans by the binding of anti-Gal antibodies to Gal residues expressed on the viral envelope [22-24]. Many current strategies for xenotransplantation involve depletion of anti-Gal antibodies and/or suppres- sion of Gal-reactive B cells. Such modifications may remove a barrier to infection with both PoERVs and other infectious agents.

A second strategy for xenotransplantation is to develop pigs transgenic for human complement regulatory proteins, such as decay-accelerating factor (CD55), membrane cofactor protein (CD46), and CD59. This may provide opportunities for human viruses to infect the porcine organ. It has been shown, for instance, that two of these human complement regulatory proteins are receptors for human viral pathogens [21]. CD46 can function as a cell surface coreceptor for the measles virus [25] and CD55 can serve as a binding receptor for Echo and Coxsackie B viruses [26,27]. A theoretical possibility is that human viruses might adapt to replication in transgenic pig tissue. Consequently, transgenic pigs may become susceptible to human and other viruses to which they were not susceptible previously. Subsequent transmission to domestic and wild pigs could have adverse economic and environmental

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consequences. Consideration of this issue is, however, beyond the remit of this paper.

Two aspects of the potential for the transfer of infectious agents have given rise to most concern. First, the introduction of PoERVs, which, if they are found to infect human cells in vivo, might lead to malignant, immunosuppressive or other dis- ease in the transplant recipient. Secondly, the transfer of a PoERV or a hitherto unknown agent might lead to spreading beyond the transplant recipient into the human population. A compli- cating factor is the possibility of a long latent and adaptation period, extending over decades, between the introduction of a new agent and the development of pathology related to its presence.

The risk of transferring an unknown agent will, by definition, never be totally avoided, but every effort must be made to ensure that no known agent of pathogenic potential is transferred to the human recipient. A less-than-ideal microbiologic status of a brain-dead human organ donor is frequently acceptable in view of the desperate need for the organ. This standard will not be acceptable as a reference against which pig organs can be compared. The pig organ will be considered a "biological product", which must be held up to exacting standards. A definition of these standards forms the subject of this paper, which is put forward as a basis for discussion.

The authors of this paper are independent members of an advisory board (set up by Novartis Pharma AG) charged with advising on the microbiologic safety criteria to be met before clinical trials of pig organ xenotransplantation. To this end, we have assessed the topic in detail and have developed standards to minimize risk. Our mandate related specifically to xenotransplantation of the kidney and heart and the use of the liver for ex vivo perfusion. We have documented the agents we believe should be excluded from the organ-source pigs and from the donor organs. We have considered the likelihood of achieving this aim and the means by which it can be achieved.

Table 1. *Bacteria, fungi and parasites to be excluded from the organ source herd*

Bacteria

Actinobacillus equuli

Actinobacillus pleuropneumoniae

Actinobacillus suis

Actinomyces (Eubacterium) suis

Arcobacter spp.

Bacillus anthracis

Bordetella bronchiseptica

Brucella suis Campylobacter spp.

Chlamydia spp.

Clostridium spp.

Coxiella burnetti

Eperythrozoon suis

Erysipelothrix spp.

Escherichia coli (verotoxigenic)

Hemophilus parasuis

Lawsonia intracellularis

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Leptospira spp.

Listeria spp.

Multiresistant organisms:

Vancomycin· resistant enterococci

Methicillin-resistant Staphylococcus aureus

Vancomycin intermediate-resistant Staphylococcus aureus

Mycobacterium spp.

Mycoplasma spp.

Pasteurella spp.

Pseudomonas pseudomallei

Rhodococcus equi

Salmonella spp.

Serpulina hyodysenteriae

Serpulina pilosicoli

Staphylococcus hyicus

Streptococcus suis

Streptococcus spp. (only exclude types known to be pig pathogens or which result in clinical disease in the donor cohort)

Yersinia spp.

Fungi

Systemic mycoses:

Absence of systemic mycosis will be confirmed at sentinel and donor necropsy.

Dermatophytes:

Microsporum spp.
Trichophyton spp.

Parasites

Protozoa

Babesia spp.

Balantidium coli

Cryptosporidium spp.

Eimeria spp.

Entameba suis

Giardia spp.

lsospora spp.

Neospora spp.

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Sarcocystis miescheriana

Sarcocystis suihominis

Toxoplasma gondii

Trypanosoma spp. (in geographically relevant areas)

Helminths

Ascaris suum

Echinococcus granulosus

Esophagostomum spp.

Hyostrongylus rubidus

Macrocanthorhynchus hirudinaceous

Metastrongylus spp.

Stephanura dentatus

Strongyloides spp.

Taenia solium

Toxocara spp.

Trichinella spiralis

Trichostrongylus spp.

Trichuris suis

Arthropods

All arthropods to be excluded

Breeding and maintenance of the donor pig cohorts

All pigs that are destined for use as sources of organs for xenotransplantation will need to be of exceptionally high health status. It is not recommended that attempts should be made to raise pigs as gnotobiotes until they have grown large enough for organ excision. This would be impractical (because of the limited size of the isolators and the large volume of material that would need to be passed through them), counter- productive (as gnotobiotic pigs lack the gastro- intestinal flora required for normal development), and not in the best interests of the welfare of the animals. There are also benefits in characterizing source animals when they are raised in stable cohorts, which would not be possible if they were maintained separately in isolators. For example, extensive testing of representative sentinal animals from such a cohort may provide an indication of the microbiologic status of the entire group.
A founder line of SPF (transgenic or nontransgenic) pigs will be used to derive a breeding herd. Pigs destined for this herd will be obtained by gnotobiotic surgical techniques (e.g. hysterotomy or hysterectomy) from pregnant SPF foundation sows to establish a breeding herd of qualified health status. On practical and welfare grounds, our recommendation is that these pigs should be maintained under such conditions for only two to three weeks, which will ensure that the vulnerable neonates are maintained in clean conditions during the first few days of life. They will then be transferred into a separate barrier facility, with barrier air filters and strict biosecurity. Because the breeding herd is intended to be maintained over a period of

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years with recurrent introductions of new stock, it may not be possible to maintain it as free from infectious agents as will be required for the subsequent source pig cohorts (i.e. the offspring of the breeding herd).
The actual organ-source pigs may be derived in one of two ways. One is by hysterotomy, followed by an initial period in isolators under gnotobiotic conditions (as described above for the derivation of the breeder herd). However, this method generally involves euthanasia of the sow and is thus costly and can be criticized in terms of unnecessary termination of life of the sow. The alternative would be for the source pigs to be naturally farrowed and, after five days, undergo segregated early weaning (Isowean), which is known to minimize the transmission of undesirable microorganisms from the flora of the breeding herd. This method is probably prefer- able when large numbers of pigs are being bred (and will be considered the method of choice for the purposes of this paper). Whichever method is chosen for delivery and initial care, each cohort of pigs (perhaps 10 to 20 in number) will subsequently be reared in strict isolation in separate barriers away from the breeding herd. Such barrier techniques are well-established in the rearing of high-health and immunocompromised laboratory animals. Approximately one month before the excision of the organs to be transplanted, the cohort will be moved to a separate quarantine barrier room.
The breeding herd and the cohorts of source pigs will be housed in a building or barrier with tight biosecurity ensuring that no contact occurs with other animal species, including rodents, birds and arthropods. No direct contact will be allowed between one cohort and any other cohort. Positive (above atmospheric) air pressure gradients of high-efficiency particulate (HEPA) filtered air should exclude most airborne infectious agents from the animal rooms. All feed and water supplied will be sterilized. The diet will be vegetarian, with no animal proteins or any bovine products, except milk-derived lactose. Antibiotics will be excluded from the pig feed to reduce the risk of selection for antibiotic-resistant bacteria. All persons who come into contact with the breeding herd or the organ-source pigs will be required to shower and change into sterilized clothing on entering the facility and when moving from one cohort to another. The wearing of body exhaust suits to allow filtration of expired air and exclusion of shed skin scales is currently not considered to be necessary. We recommend that screening of animal caretakers should include specific assays for viral hepatitis, including hepatitis E, and broad screening of liver function, which might indicate a low-grade viral infection. Vaccination for hepatitis B would be appropiate, but vaccination for some viral infections, such as influenza, might be contraindicated as they may mask viral shedding. Decisions on such questions would be made on a case-by-case basis. All animal caretakers who may have any potentially infectious ailment will be excluded from the facility, again on a case-by-case basis. They will also be temporarily excluded if they have been in close contact with wild or domestic animals outside of the workplace.
We recommend, as a general policy, that vaccination of the pigs against bacteria and viruses should be avoided as it may be unreliable, may mask clinical signs of infection, and may impair the ability to diagnose infection by monitoring the antibody response.
In the time required to rear a newborn pig (under the conditions of barrier isolation outlined above) to the size necessary for its organs to be suitable for transplantation into an adult human (which will be several months), some organisms listed in Tables 1 and 2 (below) may become established in the pigs. However, the presence of many of these agents in the digestive or respiratory tracts is unlikely to present a problem for the use of the kidneys or heart for xeno- transplantation as long as these specific organs remain free of these agents.

Infectious agents to be excluded from the organ- source cohort pigs

The infectious agents that are considered unac- ceptable in source animals are largely those that (i)carry a risk to the pigs, (ii) are of zoonotic or potential xenozoonotic risk if transferred to an immunosuppressed human recipient of a pig organ, or (iii) indicate a breach in

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biosecurity. Detection of any of these agents would require immediate action and full investigation of animals, facilities and personnel.

BACTERIA, FUNGI AND PARASITES (TABLE 1)

It is known from experience with human allotransplantation that bacteria, fungi and parasites can cause serious infection in the graft and recipient [30-33]. With this in mind, the organisms known to infect pigs have been considered. Many of these were previously identified by Fishman [34]. It would be misleading to consider only those microorganisms known to be zoonotic. A wide variety of organisms, including environmental organisms, plant pathogens, and saprophytic fungi, can infect the immunocompromised human. Table 1 lists the bacteria, fungi and parasites that we believe should not be present in organ-source pigs. There are organisms listed here (and new ones being described) whose epidemiology is still only partially understood. The list of organisms to be excluded therefore requires regular review.

The risks of bacteria in xenotransplantation differ from those of viruses in that bacterial infections pose less risk to the population at large and are generally amenable to therapy. Never- theless, all pathogenic bacteria that cause systemic disease in pigs or affect any of the organs to be used for transplantation should be excluded. The multidrug resistant bacteria [methicillin-resistant Staphylococcus aureus (MRSA), vancomycin- intermediate-resistant Staphylococcus aureus (VISA) and vancomycin-resistant enterococcus (VRE)] should be excluded from the pig in line with most recommendations for standards for human donation.

Fungi are usually acquired from the external environment and are not thought to pose a particular risk associated with the pig organ unless the pig has a systemic mycosis. Parasites limited to the gastrointestinal tract are unlikely to pose a risk, but may indicate a break in biosecurity. Some porcine parasites, however, such as Toxoplasma gondii, could be transferred in the pig organ or tissue and must therefore be excluded from the organ-source cohort. Our recommendation is that all parasites should be screened for and excluded, while further evidence is accrued.

Routine monitoring will be necessary at intervals throughout the life of the source pig. We would recommend that it consist of physical examination and serologic testing of all piglets in the cohort soon after segregated early weaning and transfer to the rearing barrier. Serologic testing would be repeated at two months and again on transfer to the final quarantine barrier, approximately one month before organ excision. Bacteriologic screening of nasal and fecal swabs should be carried out in all cohort members every month and of tonsillar swabs at two months of age. A monthly check for parasites should be made on a pooled fecal sample. If clinically indicated, cultures of blood, nose, throat, urine and feces should be carried out in individual animals at any time. At two months and prior to approval of a cohort for human clinical use of the organs, sentinel animals will be selected. Samples for extensive bacterial and parasite serology and antigen-detection tests will be collected and full necropsies performed.

As a precaution in case the pig is in an asymptomatic bacteremic phase of an infection, blood will be drawn for culture at the time of organ excision. If a positive blood culture is obtained, then, as when such organisms are identified in a cadaveric human allograft donor, the recipients of the organs can receive the relevant anti-microbial therapy.

Should a potential pathogen be detected in one or more members of the cohort (e.g. a sentinel animal), a number of possible actions would need to be considered, the appropriate choice made, and action taken. This action might include culling (i) all the pigs in the building, (ii) the pigs in the specific contaminated barrier or pen, (iii) individual pigs, or (iv) initiating treatment of one or more pigs to eliminate the infection. Treatment should be restricted to short courses with narrow- spectrum anti-microbials. In all cases, organ procurement should be discontinued until mon- itoring confirms that the cohort or herd is infection-free.

VIRUSES (TABLE 2)

Viruses pose a particular hazard in that, although they are often species-specific, cross-species transmissions have been recorded and these can be devastating in an immunologically naive host

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population. Furthermore, most, if not all, recent examples of newly emerging viral diseases in humans, such as AIDS, pandemic influenza, hanta- and Nipah virus disease, have been elicited by cross-species transmission of their causative agents. In particular, there are examples of RNA viruses, which have a high rate of mutation, and of the simpler DNA viruses, like parvoviruses, becoming established in new hosts. In addition, few viruses are amenable to therapy so that any successful transfer of infection from source animal to human would be difficult to treat.

In addition to the swine-specific viruses, many other viruses must be rigorously excluded. Table 2 lists all of the viruses recommended for exclusion from the organ-source pigs, and are categorized on the basis of (i) being of known zoonotic potential, having the ability to replicate in human cells or having some evidence for zoonotic potential, (iii) having poor replication efficiency in humans but nevertheless with the potential to be oncogenic, (iv) if not currently defined as zoonotic, to be of wide host range, and (v) being detrimental to the health of the herd or indicating a breakdown in biosecurity. Consequently, source pigs would need to be meticulously screened for viruses in these categories. Where screening tests are not currently available, these will need to be developed. Introduction of any one of these viruses into the herd would be an indication of a breach in biosecurity and mandate closer examination or re-examination of all husbandry practices. Sound husbandry practices, with barrier rearing, should ensure that most viruses are excluded from the herd.

Exclusion may in some instances be based on geographic isolation, but in many instances diagnostic assays will be required. For instance, vesicular stomatitis does not occur in Europe but serological testing for this virus and the equine encephalitis viruses would be required in the USA where they are endemic or epizootic. In Australasia, antibody tests will need to include those for Apoi, Ibaraki, Japanese B encephalitis, and Menangle viruses. The frequency of sampling and the type of test will depend on the virus in question. The intensity of testing may change depending on circumstances, such as an outbreak of viral disease in the local area.

Daily clinical examination and periodic testing for viral infection (after segregated early weaning, at two months, and when the cohort enters the final quarantine barrier) should be carried out in all members of the cohort of pigs. Tests will include those for infectious virus, viral antigen, viral nucleic acid, and/or antibodies. There are some viral diseases of swine that are not easily amenable to detection through serologic antibody testing, necessitating the use of other methods; for instance, it is proposed to screen for porcine cytomegalovirus by polymerase chain reaction (PCR). At two months and on entry to the quarantine barrier, sentinel pigs from the cohort will be euthanized. Histopathologic examination and virus isolation studies will be performed on appropriate organs. Any evidence of viral infection will require implementation of control measures as outlined above. The documentation of any virus listed in Table 2 would require culling of the entire cohort and possibly all other cohorts, depending on the virus. Intensive investigation would need to be carried out of the remammg animal.s, and the whole facility would be put on hold.

Awareness of the possibility of detecting hitherto unknown swine viruses will always be necessary. The possibility that new pathogens will be detected from time to time is provided by the recent identification of a porcine virus related to the human hepatitis E virus, and of several new paramyxoviruses in swine, including the Menangle and Nipah viruses. No doubt further viruses will be identified, necessitating an ongoing review of emerging diseases. Each emergent virus must be the subject of a rigorous risk assessment that may require review and modification of biocontainment procedures together with research into the implications for xenotransplantation. If the virus is identified as having significance for xenotransplantation, it may be necessary to develop serologic and other tests that allow testing of the pigs to demonstrate exclusion of the virus.

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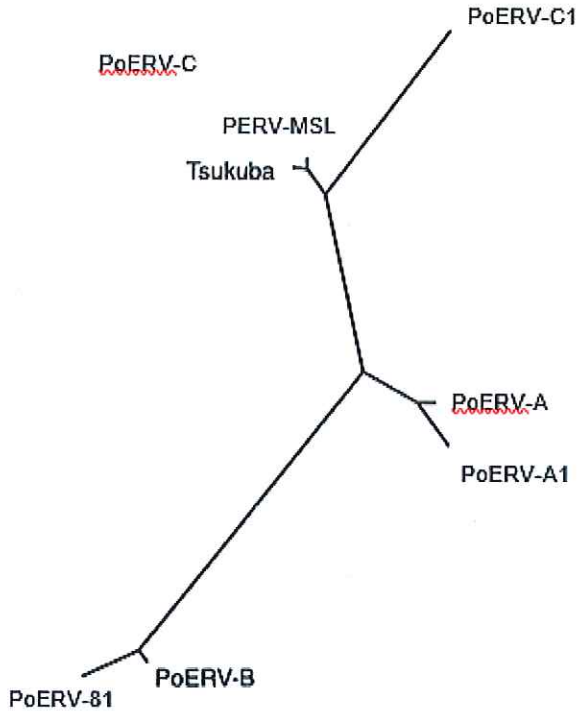

Fig. 1. Unrooted phylogenetic tree of the porcine endogenous retrovirus (PoERV) *env* sequences identified to date. Sequences corresponding to PoERV A, B and C have been shown to form subgroups by interference analysis; formal subgroup analysis of PoERVs. A1, B1 and C1 has not been conducted and their designation is provisional. Prototyope PoERV A and B were obtained from PKI 5 cells and human cells infected with PK15virus. Virus strains, known as MSL and Tsukuba, form prototypeType C isolates. Marker (0.1) indicates 10% divergence.

Endogenous retroviruses

It has been estimated that 0.1 to 1% of the DNA in the genome of humans and other vertebrates consists of retroviral genomes (proviruses) that have been incorporated over millions of years. The genomes of these endogenous retroviruses are carried as Mendelian traits in the chromo- somes, which distinguishes them from exogenous viruses acquired by infection. Many proviral elements in mammalian cells are defective, but pigs carry a group, known as porcine endogenous retroviruses

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Table 2. Viruses to be excluded from the donor cohort

Family/genus	Species	Exclude from herd/cohort	Category
Picornaviridae			
Aphthovirus	Foot and mouth disease	Yes	2,5
Enterovirus	Enterovirus 1	Yes	5
	Talfan/Teschen		
	Enterovirus (other serogroups)	Yes	5
	Swine vesicular disease	Yes	1
	Human enteroviruses	Yes	
Cardiovirus	Encephalomyocarditis	Yes	1
Hepatovirus		N/A	*
Rhinovirus	Human serotypes	Desirable but may not be possible	5
Caliciviridae			
	Vesicular exanthema	N/A (extinct in pigs)	5
	Enteric calicivirus	Yes (possibly same virus as swine hepatitis E virus)	1
	Swine hepatitis E	Yes	1
Astroviridae			
	Porcine astrovirus	Desirable but not essential	5
Togaviridae			
Alphavirus	Western encephalitis	Yes	1
	Eastern encephalitis	Yes	1
	Venezuelan encephalitis	Yes	1
	Getah	Yes	1
	Chikungunya	Yes	1
Rubivirus		N/A	*
Flaviviridae			
Flavivirus	Japanese B encephalitis	Yes	1
	Louping III/TBE complex	Yes	1
	Wesselsbron disease	Yes	1
	Apoi	Yes	2
	Dengue fever	Yes	1
	West Nile fever	Yes	1
Pestivirus	Classical swine fever (hog cholera)	Yes	5
	Bovine viral diarrhoea	Yes	5
	Border disease	Yes	5
Coronaviridae			
Coronavirus	Transmissible gastroenteritis	Yes	4, 5
	Porcine respiratory coronavirus	Yes	4, 5
	Epidemic diarrhea	Yes	4, 5
	Haemagglutinating encephalomyelitis	Yes	4, 5
Arterivirus	Porcine reproductive & respiratory disease syndrome	Yes	4, 5
Torovirus	Porcine torovirus	Desirable but not essential	5
Paramyxoviridae			
Respirovirus	Parainfluenza type 1 (Sendai)	Yes	2
	Parainfluenza 2	Yes	2*
	Parainfluenza 3	Yes	2
Morbillivirus	Not recorded in pigs but the CD46 transgene may act as coreceptor for the measles virus		*
Rubulavirus	Porcine rubulavirus (La Pied ad Michoacan)	Yes	5
Pneumovirus	Respiratory syncytial	Yes	2
Undesignated genus	Menangle	Yes	1
Undesignated genus	*Nipah*	Yes	1
Rhabdoviridae			
Vesiculovirus	*Vesicular stomatitis*	Yes	1
Lyssavirus	*Rabies*	Yes	1
Ephemerovirus		N/A	*
Filoviridae			
Filovirus		N/A	*
Bornaviridae			
Bornavirus	Bornavirus	Yes	2, 5
Orthomyxoviridae			
	Influenza A	Yes	1
	Influenza B	Yes	5
	Influenza C	Yes	5
Bunyaviridae			
Bunyavirus	Cache valley	Yes	1, 5
	Akabane	Yes	5
	Batai	Yes	1, 5
Hantavirus	Hantavirus	Yes	1, 5
Nairovirus	N/A		
Phlebovirus	N/A		
Arenaviridae			
	Lymphocytic choriomeningitis	Yes	1, 5
Reoviridae			
Orbivirus	Ibaraki	Yes	5
Coltivirus		N/A	5*
Reovirus	Reovirus 1–3	Yes	2
Rotavirus	Rotavirus A, B, C, E.	Yes	2
Birnaviridae			
	Porcine picobirnavirus	Desirable but not essential	5
Retroviridae			
Gammaretrovirus	Porcine endogenous	See text	2
Hepadnaviridae			
Hepadnavirus	Hepatitis B	N/A	*
Circovitidae			
Circovirus	Porcine circovirus	Yes (but may be difficult as may cross placenta)	5
Parvoviridae			
Parvovirus	Porcine parvovirus	Yes	4, 5
Papovaviridae			
Polyomavirus	Porcine polyomavirus	Yes	3
Papillomavirus	Porcine genital papillomavirus	Yes	3, 5
Adenoviridae			
	Porcine adenovirus serotypes 1–4	Yes	3
Herpesviridae			
Alphaherpesvirinae	Pseudorabies	Yes	2
Betaherpesvirinae	Porcine cytomegalovirus	Yes (but may be difficult as crosses placenta and becomes latent)	5
Gammaherpesvirinae	Porcine lymphotropic herpesvirus type 1	Yes (but may be difficult)	3
	Porcine lymphotropic herpesvirus type 2	Yes (but may be difficult)	3
Poxviridae			
Suipoxvirus	Swinepox	Yes	5
Orthopoxvirus	Vaccinia	N/A	2
	Cowpox	Yes	1, 5*
Parapoxvirus	Orf/pseudocowpox	N/A	1, 5*
Desoxyviridae			
Desoxyvirus	African swine fever	N/A (unless herd established in endemic region)	5

1 = Zoonotic.
2 = Replicates in human cells or weak evidence for zoonotic potential.
3 = May undergo abortive replication and may possibly be oncogenic.
4 = Belongs to a family with evidence of frequent changes in host range or pathogenicity.
5 = Undesirable as indicates a breakdown in biosecurity and/or may compromise health of the pigs.
*. Although the virus has not been recorded in pigs, it has been included for reasons such as its wide host range.
N/A=Not applicable, e.g. not reported to occur in pigs, may have been a porcine virus but not recorded in recent history, or geographically restricted.

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(PoERVs or PERVs), that often become transcriptionally active in vitro to produce virions capable of infecting cells of many species. PoERVs are members of the Gammaretrovirus (C type retrovirus) family. These viruses were first described in the 1970s. At that time, they were not shown to be capable of infecting human cells, but more recent studies have shown that certain PoERVs will infect human cells, albeit with low efficiency.
PoERVs are closely related to the gibbon ape, feline and murine leukemia viruses, which are the paradigm members of the Gammaretrovirus group. Three subgroups have been identified, PoERV-A, B and C (Fig. 1), which, by definition, use different receptors to infect cells. The complete nucleotide sequence of one subgroup of virions released from PK 1 5 cells has been obtained, as has another from a proviral clone derived from miniature swine. These sequences represent PoERV-B 1 and PoERV-C, respectively. These two viruses share 90.1% identity within gag and 96.8% identity within pol, but have distinct envelope (env) genes. Pseudotype analysis has shown that both PoERV-A and B can infect human cells, but with an efficiency about 102 to 103 lower than that of the amphotropic murine leukemia virus.

Only one human cell line (HT-1080-1) has been shown to be susceptible to PoERV-C, and it is not yet clear whether this reflects a general capacity of this subgroup to infect human cells. The porcine genome appears to carry approxi- mately I0 to 20 copies of both PoERV-A and PoERV-B. Detailed analysis has only been carried out on relatively few pigs, but it would appear that around 6 to I0 of the loci are common to many pigs. PoERV-C has a more polymorphic pattern; some pigs appear to lack the virus completely, whereas certain miniature swine have many copies (c. 50).

Both the transmission of exogenous Gamma- retroviruses and disease induction generally require active replication accompanied by a persistent plasma viremia and shedding of virus in bodily fluids. In contrast to lentivirus infections, such as those caused by human or feline immunodeficiency viruses, full recovery from Gammaretrovirus infections, such as feline leukemia virus, is not uncommon in nonimmunocompromised animals. The balance between a life-long persistent infection and recovery is almost certainly influenced by the host's cell-mediated response, but it is accom- panied by the prod uction of antiviral antibody, including neutralizing antibody [56]. With regard to the development of organ-source pigs for humans, although more is becoming known about PoERV variants and their ability to infect cells in vitro, PoERVs present a particularly difficult problem. Little is known about their potential to infect human cells in vivo and their capacity to cause disease in immunocompetent subjects, let alone in those who are iatrogenically immunocompromised.

As with other viral infections, there is greater concern that a xenograft recipient could transmit the virus through transfer of bodily fluid to an intimate contact. Transmission would be favored if the latent period before disease development is long, as is typical for Gammaretroviruses. Xenotransplantation could provide the milieu for selection of variants that may infect the human population efficiently. Genetic variation within retroviruses is favored by the lack of fidelity of reverse transcriptase. Moreover, there is the potential for recombination between the porcine retrovirus and endogenous retroviral elements within the human genome. The potential risk of PoERV can therefore be evaluated at several levels. Will virus be produced from the cells of the xenografted organ? Will the human recipient's cells be productively infected, leading to viremia? Will infectious virus be shed into the patients' bodily fluids, posing a public health hazard?

Transcription of PoERV proviruses appears to occur in many tissues in vivo, and there is recent evidence of a low-level viremia in some pigs (G. Langford et al., submitted), suggesting that production of virus particles may be expected in some patients. Some porcine cell types, including hepatocytes, do not readily express PoERVs, although others, such as blood mononuclear cells and endothelial cells, may be more readily induced to express virions in vitro. The normal lytic complement barrier to PoERVs may not operate in patients in which the transplanted pig organ is transgenic for one or more human complement regulators (e.g. decay accelerating factor), so attention must be paid to the possibility that the patient's cells will become infected. PoERVs appear to have envelope glycoproteins with a low ffinity for receptors on human cells. Furthermore, infection of human cells in vitro often results in a silent infection in which the provirus is not transcribed. In immunocompetent hosts, these features would probably lead to a failure of the virus to establish a persistent, productive infection. However, the unknown factor in xenotransplantation is the influence ofiatrogenic immunosuppression, which may

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limit the ability of the host's immune response to control the infection.

The risk assessment strategy to be adopted comprises three main areas of focus: (i) determination of the expression pattern of PoERVs in pigs used in xenotransplantation, (ii) the evalu- ation of primate models for evidence of PoERV infection following the transplantation of porcine organs or cells, and (iii) a retrospective analysis of human patients who have been exposed to viable pig tissues (e.g. ex vivo perfusion of pig livers, pig skin grafts in the treatment of burns, pig pancreatic islet transplants, etc.). It should be emphasized, however, that the latter two approaches suffer from some limitations. First, the susceptibility of non-human primates to PoERV infection may differ from that of humans. Secondly, most humans who have been exposed to viable pig tissues to date have not been severely immunocompromised, and exposure to these tissues has in many cases been relatively short. However, detailed studies on the PoERV status of persons transiently exposed to living porcine tissues have recently been published, and have been encoura- ging in being unable to identify any definite transmission of PoERVs to the recipients. In view of these findings, limited, carefully controlled trials of pig organ transplantation may be the only means of confirming whether there is a quantifiable risk of PoERV infection from xenotransplantation. We would suggest that the unequivocal detection of the PoERV genome in non-human primate or human cells, or the detection of a plasma viremia, would be cause to pause and review the development of a clinical trial of xenotransplantation using pig organs.

Prion disease

Prion diseases, sometimes known as transmissible spongiform encephalopathies (TSE), are characterized by the deposition of abnormal proteinase- resistant prion protein (which is an isoform of cellular prion protein) in the brain of animals. The only known difference between the two conformationally distinct forms of prion protein is a relative resistance of the former form to treatment with proteinase K, and this is used as a basis for diagnosis of prion diseases.

Creutzfeldt-Jakob disease (CJD), the proto- type human prion disease, occurs in three forms, namely (i) sporadic, which is of unknown etiology and occurs worldwide at a frequency of about Iin 1 million, (ii) genetic, which is linked to a mutation in the prion protein gene, and (iii) infectious, of which two types have been described for humans, namely Kuru, transmitted by ritual cannibalism, and iatrogenic CJD. This latter type has been caused, for example, by the administration of cadaveric human pituitary- derived hormones, such as growth hormone, or by the use of contaminated grafts of cadaveric human dura mater or cornea. In addition, accumulating scientific evidence indicates that a new variant CJD is caused by oral infection with bovine spongiform encephalopathy (BSE) through dietary exposure.

Susceptibility of pigs to CJD has been exam- ined by the intracerebral inoculation of brain tissue from patients with Kuru; no histopatholo- gic evidence of transmission of the disease to the pigs was observed after 5 to 6 years [64]. Pigs have been fed BSE-contaminated meat and bone meal, but no case of prion disease has been reported. Moreover, the experimental adminis- tration of BSE-infected cattle brain by the oral route has not led to the development of the disease in pigs after follow-up periods of over 6 years, whereas sheep and goats, similarly treated, have developed the disease after minimum incubation periods of 18 and 31 months, respectively. Only when an intracerebral inocula- tion of BSE-infected cattle brain was administered to pigs, combined with intravenous and intraperitoneal inoculation, has disease been documented. (The intracerebral inocula- tion of such tissues is considered to be 100000 times more effective in causing prion disease in mice than is the oral route.)

Although scrapie in sheep, the prototype animal prion disease, and BSE in cattle occur only through infection, it is possible that very rare unexplained "sporadic" cases of transmissible spongiform encephalopathy could occur in any mammalian species, including pigs. However, the breeding and maintenance of prion-free pigs should be possible if strict measures regarding the nature of feed stuffs (with no ruminant-derived products except lactose) and isolation from contact with other animals are enforced.

The risk of prion disease associated with the xenotransplantation of pig organs can therefore be considered to be extremely remote. Moreover, even if transmission of prion disease to a transplant recipient occurred, this should not pose a public health hazard. Nevertheless, monitoring of the pigs

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at intervals should include (i) a search for proteinase-resistant prion protein in the lymph nodes and brain of sentinel animals (by tests such as Western blot and ELISA), and (ii) histologic examination of the brain of sentinel animals for histopathologic changes of prion disease.

Discussion

The ability to utilize pig organs in humans would not only resolve the current crisis relating to the shortage of human cadaveric organs for purposes of transplantation but would also negate the high financial and emotional costs associated with the need for patients to wait long periods, often in an intensive care unit, until a donor organ becomes available. Although attention is currently being directed towards the risk of transfer of a xenozoonosis, this should not distract from the advantages of the use of pig organs (if scientifically feasible) which, for example, would obviate the risk of transfer of an infectious agent with a human organ]. The transfer of human cytomegalovirus and Epstein-Barr virus is common and is a major cause of morbidity and mortality in the post-transplant period. Cytomegalovirus may result not only in infection but also in an increased incidence of acute, and possibly chronic, allograft rejection. Epstein-Barr virus from the donor may play a role in the development of post-transplant lymphoproliferative disease. The transfer of many other human microorganisms, including bacteria (e.g. Mycobacterium tuberculosis, Pseudomonas aeruginosa), parasites (e.g. Toxoplasma, Strongyloides), and viruses (e.g. hepatitis B and C, human immunodeficiency viruses), have all been documented in recipients of allografts, and are a significant cause of post- transplant morbidity and mortality.

The rearing and monitoring of pigs under the conditions outlined in this paper would enable organ transplantation to be performed using donor organs with a minimized microbiologic burden and, furthermore, would also be likely to reduce the probability of transmission of porcine agents yet to be identified. With the exception of PoERVs, it should be possible to provide organs free of all known agents of potential pathogenicity in humans or swine. However, the expense and effort to do so will be considerable, particularly as some agents cross the placenta and others can be readily re-introduced into breeding herds. The major remaining risks would appear to be those related to the presence of unknown viruses and/or PoERVs. Hitherto unknown viruses may possibly remain undetected until they cause symptoms and signs of infection in the organ recipient. There should be continuing efforts to identify new infectious agents that may infect normal and/or transgenic pigs using state-of-the-art technologies. Experiments are in progress involving intense microbiologic monitoring of pigs receiving pharmacologic immunosuppression and may lead to identifica- tion of viruses not isolated from non-immuno- suppressed pigs.

Three PoERV subgroups have to date been documented. As some of these may be present in a high copy number and low zygosity, selective breeding or genetic technology to eliminate these proviruses may be difficult, if not impossible. The alternative, for xenotransplantation might be to accept their presence, and establish whether or not they are pathogenic, or might give rise to pathogenic agents, in humans. If PoERVs are found not to result in clinical problems, such as cancer or an immunodeficiency state, in the organ recipient for many years, possibly decades, then the benefit of an organ transplant to that individual patient may well outweigh the risks of such infection. The acceptability of xenotrans- plantation may then depend on an assessment of whether such retrovirus-infected patients pose a risk to their human contacts, such as family, friends and healthcare professionals. However, it should be realized that, if infection from human- to-human contact occurs initially only at a low

frequency, which is not unlikely, this assessment may prove extremely difficult.

Recipients of pig xenografts will need to be intensively monitored for PoERV infection by PCR for latent infection in blood mononuclear cells, reverse transcriptase-PCR for viral nucleic acids, product-enhanced reverse transcriptase assay to detect plasma viraemia, and serology for the detection of retroviral-specific antibody. Pre-operative patient counseling regarding the possibility of retroviral infection, and implica- tions for the individual and his/her intimate contacts, will be important. The development of anti-viral strategies, such as vaccination, passive immunotherapy, and anti-viral agents, should be investigated. Before clinical trials are initiated, contingency plans should be developed in the event that a patient is found to be infected with PoERV so that the risk to the health of the public is minimized.

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As with most medical advances, no matter how rigorous the pre-clinical testing, it may be impossible to exclude all risk, even if this is related only to hitherto unknown pig viruses and microorganisms. The ultimate decision on whether to employ any new therapeutic agent or procedure rests on an assessment of the risk- benefit ratio. In the case of xenotransplantation, this may possibly require weighing the potential risk of the emergence of a new human pathogen against the benefit of an unlimited supply of organs and tissues for patients with end-stage organ failure.

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