Cell. Mol. Life Sci. 65 (2008) 3399 – 3412
1420-682X/08/213399-14
DOI 10.1007/s00018-008-8498-z

Cellular and Molecular Life Sciences

Porcine endogenous retroviruses and xenotransplantation

C. A. Wilson[+]

Gene Transfer and Immunogenicity Branch, Division of Cellular and Gene Therapies, Office of Cellular, Tissues, and Gene Therapies, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Building 29B, Rm 5NN20, Bethesda, MD 20892 (USA), Fax: +1 301 827 0440, e-mail: Carolyn.wilson@fda.hhs.gov

Online First 27 September 2008

Abstract. Xenotransplantation is defined by the PHS as any procedure that involves the transplantation, implantation or infusion into a human recipient of either (a) live cells, tissues or organs from a nonhuman animal source, or (b) human body fluids, cells, tissues or organs that have had *ex vivo* contact with live nonhuman animal cells, tissues or organs (Public Health Service Guideline on Infectious Disease Issues in Xenotransplantation). Use of pigs for human xenotransplantation raises concerns about the risks of transfer of infectious agents from the pig cells to xenotransplantation recipients. The observation that the porcine germline harbors genetic loci encoding porcine endogenous retroviruses (PERVs) that are in some cases infectious for human cells has resulted in renewed scientific interest in PERVs. However, in spite of the past 10 years of investigation, the actual risk for PERV infection, replication, and pathogenic outcome in human recipients of xenotransplantation products is still undefined. (Part of a Multi-author Review)

Keywords. Porcine, retrovirus, endogenous, xenotransplantation.

Introduction and historical perspective

In 1971 a porcine kidney cell line, PK-15, was reported to spontaneously release retrovirus particles [1]. Studies over the next 10 years indicated that PERV replication was limited to porcine cells [2, 3], and attempts to identify causative associations between PERV and cancer were unsuccessful [4–7].

Nearly 20 years later, a field of clinical investigation in transplantation medicine emerged that involved the use of living non-human cells or tissues to treat human disease, termed xenotransplantation. In the 1990s the field of xenotransplantation began moving away from the use of non-human primates, towards the use of pigs as the primary source for xenotransplantation products, based on a number of considerations: 1) easier animal husbandry, 2) relatively similar anatomical size of organs, i.e. physiologic compatibility [8], and 3) the assumption that pigs would be safer from a microbiological point of view [9–11].

While the studies of the 1970s on porcine endogenous retrovirus indicated that PERVs had a narrow host range exclusive of human cells, the increased risk of porcine to human xenotransplantation warranted further study of this question using modern tools of molecular virology. Robin Weiss and colleagues used the same cell line that Armstrong used, PK-15, and demonstrated that PERV could be transmitted to human cells *in vitro* [12]. One year later, it was shown that primary porcine peripheral blood mononuclear cells, upon mitogenic stimulation, also released virus that could be transmitted to and replicate in human cells [13]. Thus PERV research returned to the mainstream, this time with new goals that included the development of the following: 1) baseline knowledge of PERV biology, replication and potential for pathogenesis to increase our understanding of the risks in porcine to human xenotransplantation; 2) methods with improved sensitivity and specificity for detecting evidence of PERV transmission in xeno-

[+] Dedicated to the memory of Dr Eda T. Bloom to honor her scientific and regulatory contributions to the field of xenotransplantation.

transplantation product recipients; and 3) means to prevent transmission or to treat disease, should it develop in xenotransplantation product recipients.

In vitro host range and replication properties of PERV

Three receptor classes of gammaretroviral PERVs have been characterized. PERV-A and -B were shown to be the human-tropic forms of PERV [14], while PERV-C enveloped vectors were shown to exhibit a pig-tropic or ecotropic host range (referring to the nomenclature used for murine leukemia virus host range variants) [15, 16]. Interference assays in which retroviral vector pseudotypes were used demonstrated that all three envelopes confer distinct host range and receptor specificities. Subsequently, it was found that expression of either of the two cDNAs encoding the receptor for PERV-A rendered resistant cells susceptible to PERV-A, but not to PERV-B or PERV-C [17] (details on the PERV-A receptor, below).

Retroviral vector pseudotypes bearing PERV-A, -B or -C envelopes can infect porcine cell lines, but only pseudotypes carrying PERV-A or -B envelopes infect human cells. However, *in vitro* infection and replication of PERV-A and -B are restricted, for the most part, to porcine or human cells [16, 18, 19].

The question of whether PERV infects non-human primate cells has been examined with conflicting results. Using retroviral vector pseudotypes, infection of primary primate fibroblasts from human, chimpanzee, gorilla and baboon was observed with pseudotypes carrying PERV-A envelopes, but not those of PERV-B or PERV-C. Using PCR for viral DNA as an endpoint, transmission was observed after coculture of lethally irradiated PERV producer cells with human, gorilla or baboon primary fibroblast cultures [20]. However, viral replication is not proven in the absence of showing transmission of infectious virus from the PCR-positive cultures. A more systematic analysis performed by Ritzhaupt et al. demonstrated that PERV infection and replication of non-human primate cells is compromised due to inefficient cell entry (approximately 100-fold decrease in unintegrated DNA formed after reverse transcription relative to human 293 cultures) and replication. While low levels of DNA and RNA could be detected in cell lines derived from rhesus macaque, African green monkey or baboon, the copy numbers remained at very low levels over the course of 12–16 weeks of culture, and no reverse-transcriptase activity could be detected during this period, suggesting that the initial limited infection of these cell lines was not followed by amplification via viral replication. While infectious virus could be rescued from the PERV DNA-positive cells when supernatant was transferred to human cell lines, no evidence of replication was observed when NHP cell lines were exposed to the same supernatants. Additional studies of primary cultures from rhesus kidney, or umbilical vein and aortic endothelial cells confirmed these negative results [21]. Therefore, it appears that PERV replication is restricted in NHP cells and may be the primary reason for the lack of detection in *in vivo* studies in non-human primates (described in more detail below).

Of more relevance to the discussion of safety risks in xenotransplantation are the infection and replication properties observed in primary human cells or established human cell lines. Using retroviral vector pseudotypes to screen an array of human cell lines, representing various tissue types, variable levels of infection by PERV-A and PERV-B pseudotypes were observed with a more limited pattern of susceptibility to replication [19]. The human embryonic kidney-derived cell line, 293, is consistently the most permissive human cell line to PERV infection and replication. In addition, infection and in some cases replication, has been observed in cell lines derived from tumors of muscle, cervix, glial, liver, and selected lymphoid or myeloid origin [16, 19, 22]. No evidence of pseudotype infection was observed in cell lines derived from human osteosarcoma, clear cell kidney carcinoma or colon carcinoma [19]. While infection and replication have been observed in primary cultures of various types of endothelial cells [22, 23], no evidence of replication has been observed in primary cultures of peripheral blood mononuclear cells [19] (summarized in Table 1). In addition, PERV infection has been observed in primary cultures of primary human endothelial cells as well as human vascular fibroblasts, as evidenced by detection of PERV *pol* sequences by RT-PCR, reverse-transcriptase activity, and infection by pseudotyped retroviruses bearing PERV-A or PERV-B envelopes. PERV infection was not detected in other primary human cells tested, human glomerular mesangial cells, bone marrow stromal cells or hematopoietic precursor cells [23]. In summary, there are clearly primary human cells that have been shown to support PERV replication, underscoring the continued need for caution in the context of exposure from xenotransplantation.

Molecular determinants of human cell tropism

As stated above, three receptor classes for PERV have been described; however only the receptor(s) for the PERV-A class has been identified and well characterized. Using a cDNA library derived from human HeLa cells expressed in a non-permissive cell line, rabbit SIRC fibroblasts, Ericsson et al. were able to

Table 1. Summary of PERV infection in primary human cells.

Tissue of origin	PERV-A	PERV-A-NIH Replic[a]	PERV-B	Reference
PHA-activated peripheral blood mononuclear cells	ND[b]	–*	ND	[19]
PHA and IL-2 activated PBMC (primary T cells)	ND	–*	ND	[19]
Human primary pulmonary, coronary, aortic, saphenous and umbilical endothelial cells	+	ND	+	[22, 23]
Human glomerular mesangial cells	+	ND	+	[23]
Human hematopoietic precursor cells	–	ND	–	[23]
Human bone marrow stromal cells	–	ND	–	[23]

[a] Represents results using replication-competent isolate; other results without asterisk were obtained using retroviral vector pseudotypes or PCR, RT-PCR or RT activity to demonstrate virus infection.
[b] ND, not done.

identify a cDNA that supported both binding of PERV SU and infection by PERV-A, but not PERV-B or PERV-C. A second cDNA with 86.5 % aa identity was identified, cloned by PCR amplification, and also shown to support PERV-A infection and binding. These two cDNAs were named HuPAR-1 and HuPAR-2 for human PERV-A receptor 1 and 2, respectively. Ericsson et al. also showed that the baboon homolog to HuPAR-1 was functional, while a cDNA homolog from murine cells was not able to support PERV-A infection [24].

The normal cellular function of HuPAR-1 or 2 is unknown, although Northern blot analysis shows that the cDNAs are ubiquitously expressed [24]. The cDNAs encoding HuPAR-1 and 2 are predicted by hydrophobicity algorithms to contain between 10 and 11 trans-membrane domains (varies depending on which algorithm), a topology suggestive of an endogenous function as a transporter – similar to receptors used by other gammaretroviruses (reviewed in [25]). However, the observation that the PAR protein is predicted to contain a DUF1011 domain that is characteristically found in G-protein-coupled receptors (GPCRs), led Ericsson et al. to hypothesize that PAR may be a GPCR, although GPCRs characterized to date all have 7 transmembrane domains, so this hypothesis is somewhat controversial and, until recently, unsupported by data. A report by Andriamampandry et al. used PCR primers based on the rat gamma-hydroxybutyrate receptor (GHB receptor) to amplify the human homolog from a cDNA library of human frontal cortex. Using these primers, two cDNAs were isolated, one that is the same as HuPAR1 and a second that is nearly identical, except for a frameshift caused by deletion of a single nucleotide resulting in a longer C-terminal tail. The investigators performed a functional analysis of these two cDNAs by expressing them in Chinese hamster ovary cells that led to the conclusion that these two cDNAs serve as receptors for GHB [26]. Unfortunately, the lack of inclusion of appropriate negative controls in all the experimental results presented makes interpretation of them impossible. In addition, as of this time, no follow-on publications have been published. Therefore, at this time, the endogenous cellular function of the PAR cDNAs has still not been proven.

Recently, a study by Mattiuzzo et al. used chimeric cDNAs between the non-permissive murine homolog and the permissive HuPAR-1, followed by site-directed mutagenesis to map the domains of HuPAR that support PERV-A infection. The proline residue at position 109 in the second putative extracellular loop of murine PAR was identified as responsible for the restriction to PERV-A infection in mouse cells. All permissive forms of PAR contain a leucine at this position. In addition, this study identified a second mechanism of restriction to PERV-A infection in rat cells. The level of expression of PAR is too low to allow for a functional interaction with PERV [27].

In addition to these detailed studies of the molecular determinants on the PERV-A receptors are the counterpart analyses to identify regions of the PERV-A envelopes critical for human cell infectivity. We showed that the regions of the PERV SU comparable to the receptor-binding domains (RBD) of other gammaretroviruses was necessary but not sufficient for PERV binding. The proline-rich region (PRR) of the envelope SU, in addition to the RBD, is required for efficient binding to permissive pig or human cells [28]. This result was intriguing in light of the prior observations of Harrison et al. that residues within the PRR correlated with increased titers of certain PERV-A isolates on human cells. Using a naturally occurring recombinant between PERV-A and PERV-C, derived from NIH mini-swine strain 14–220 (originally identified by [24]), that was shown to have a higher titer on human cell lines than PERV-A, Harrison et al. made additional chimeric envelopes between the 14–220 recombinant envelope cDNA and the 'authentic' PERV-A envelope. Retroviral vector pseudotypes carrying the 14–220/PERV-A

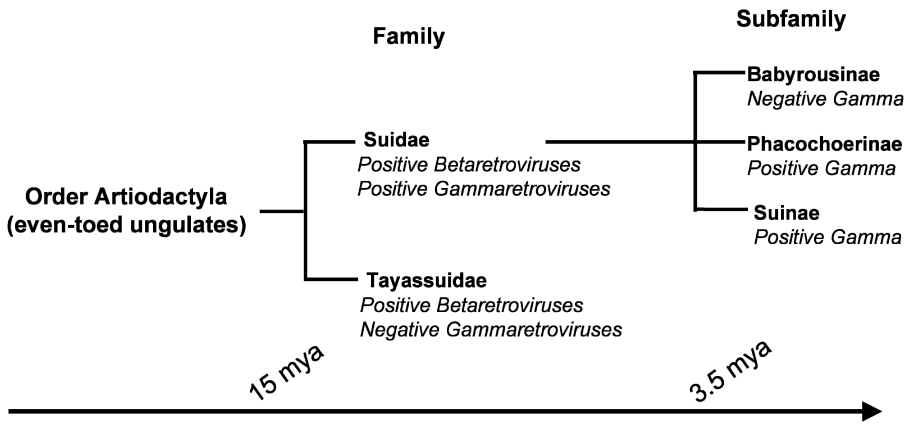

Figure 1. Schematic representation of the coevolution of the Order Arteriodactyla with PERV-related sequences detected in the DNA of modern members of the order. The figure is a schematic representation of published results [17, 34–37].

chimeric envelopes or envelopes with specific mutations introduced by site-directed mutagenesis revealed two regions that were required for the enhanced titer of 14–220 on human cells, an isoleucine at position 140 adjacent to the variable region B and the proline-rich region [29]. An additional determinant for binding and infectivity was also shown to be present in a domain C-terminal to the PRR, outside of the minimal domain required for binding [28].

One interesting twist to the analysis of factors influencing PERV cell entry is the observation that PERVs may infect PERV receptor-negative cell lines under very specific circumstances. It had previously been shown that the addition of soluble RBD of certain gammaretroviruses restores infectivity to fusion-impaired gammaretroviruses, as long as the receptor for the soluble RBD is present on the target cells [30, 31]. The ability to restore infectivity has been termed trans-activation. Lavaillette and Kabat showed that PERVs, like these other retroviruses, can also be transactivated by soluble RBDs and can infect PERV receptor-negative cells so long as the cells bear the receptor for the RBD. The authors cautioned that this observation demonstrates an increased risk for xenotransplantation recipients, since transactivation may provide a means to overcome barriers to cross-species transmission [32]. However, transactivation is less probable in a xenotransplantation product recipient, because the rescuing virus or RBD must be proximal to the non-infectious PERV, and only non-human retroviral RBDs have been shown to transactivate PERV *in vitro*.

Genetics and evolution of PERV in the porcine germline

All vertebrate species carry in their genome a repository of sequences encoding endogenous retroviruses, but they vary in diversity, copy numbers, and relative ability to replicate [33]. The endogenous retroviral sequences present in the pig genome are representative of betaretroviral and gammaretroviral genera [17, 34, 35]. Betaretroviral sequences have been detected in genomic DNA samples representing members of the families Suidae and Tayassuidae [34], suggesting that these are very ancient, presumably entering the germline of the order Artiodactyla (even-toed ungulates), suborder Suinae (pigs and peccaries) prior to the divergence of these two families approximately 15 million years ago (see Fig. 1). In contrast, the gammaretroviral sequences are relative newcomers, based on lack of detection in any members of the family Tayassuidae (New World Peccaries) or subfamily Babyrousinae. Gammaretrovirus sequences were detected in all members of the subfamilies Phacochoerinae and Suinae (representing two of three subfamilies of the family Suidae), suggesting entry of gammaretroviral sequences into the pig genome, approximately 3.5 million years ago [34, 36], correlating well with predictions based on divergence of LTR sequences present in modern day pigs [37] (Fig. 1).

To date, four groups of betaretroviral and 10 groups of gammaretroviral sequences encoding the protease/polymerase region of the viral genome have been identified in the domestic pig genome by PCR using degenerate primers [17, 34, 38]. Of these, only the sequences representing the γ1 group have been detected as proviral sequences that are replication-competent, although there is a single report of detection of full-length defective genomes representing the PERV γ2 group [39]. PCR detection of PERV-A, B and C-specific envelope sequences has been used to map entry of these variants of PERV into the pig germline. The two human tropic envelopes, PERV-A and B, have been proposed in one study to predate the pig-tropic ecotropic PERV-C envelope found in members of the genus *Sus* that diverged approximately 1.5 million years ago [35]. Using distinct primers

and DNA representing different members of the family Suidae, Patience and co-workers were able to detect PERV-C envelope sequences in all members of the *Sus* genus as well as some members of the *Potamochoerus* genus [34]. A similar distribution of PERV-A and -B envelope sequences was reported by both groups [34, 35]. The discrepancy in results is likely due to sequence variations within the primer binding domains.

The remnants of porcine endogenous retroviruses in the genomes of modern-day pigs differ between different breeds of pigs in composition, expression and ability to encode infectious virus. Initial reports by Southern blot suggested that the porcine genome carries between 50 [14] and 200 [15] copies of PERV. To understand how many of the genomic sequences may represent full-length replication-competent proviruses, authoritative characterization of the Large White pig was undertaken [40–42], revealing considerable variation in the distribution and number of PERV proviruses within this single breed.

Molecular clones encoding infectious viruses of all three PERV classes have been isolated from various sources using different types of screening methods. Infectious molecular clones of PERV-A and PERV-B have been isolated from PK-15 cells using a bacteriophage lambda library [43] or libraries derived from 293 cells infected with PK-15-derived viruses [44]. The first molecular clone of PERV-C, Tsukuba-1, was derived from unintegrated DNA isolated from swine kidney cells after exposure to supernatant from swine malignant lymphoma-derived PERV-producer cells [45]. The sequence of Tsukuba-1 was used to derive *pol* and *env* probes to screen cDNA libraries made from miniature swine peripheral blood mononuclear cells or from PK-15 cell mRNA. While the molecular clones isolated from these experiments were not shown to be replication-competent, they were the first full-length cDNA sequences reported for any PERV isolate at the time [15]. In 2006, a full-length PERV-C clone was obtained by Preuss et al. from a library prepared from miniature swine genomic DNA. Despite sharing $>99\,\%$ nucleotide identity with the previous two PERV-C cDNA clones, this molecular clone generated infectious virus after transfection into ST-IOWA cells [46].

PERV genomes have been analyzed in a variety of other pig breeds, such as Westran pigs [47], Duroc, Landrace, Large Yorkshire and crossbreed pigs [48], Chinese pigs, including Banna minipig inbreed, Wu-Zhi-Shan pig and Nei Jiang pig [49]. Similar to the studies in Large White pigs, PERV sequences in these breeds were found to be widely distributed across the genome, although differing among breeds and individual members within a breed in copy number, chromosomal distribution and frequency of full-length sequences.

Expression of PERV *in vivo* differs between pig breeds and tissues

Most endogenous retroviruses are maintained in their host genome in transcriptionally repressed states [50]. However, under certain circumstances, transcription of endogenous retroviruses may be activated. Activation cues include *in vitro* treatment with demethylating agents, growth factors or cytokines that may alter cell differentiation or activation state, or *in vivo* mechanisms such as graft vs. host disease. To examine basal expression levels across different breeds of pig, Jin et al. isolated peripheral blood leukocytes from 10 different breeds or in some cases pig hybrids from diverse geographical regions. Using primers derived from either highly conserved regions or regions specific for PERV-A, -B or -C, RNA-encoding PERV envelope was detected in all breeds examined [51]. Expression has also been observed in primary pig kidney cells [52] and cultures of porcine islets [53–55]. Large White pigs were generally positive for PERV expression in all tissues examined [53].

Primary cultures of porcine hepatocytes were assessed for conditions that would activate release of infectious virus. Nyberg et al. found PERV RNA in supernatants from cultured primary pig hepatocytes, as well as hepatocytes exposed to PHA and PMA or serum from patients with fulminant hepatic failure (a scenario typical for the clinical use of porcine hepatocytes in a bioartifical liver assist device). However, none of the culture conditions resulted in release of infectious virus [56]. Similar results were obtained when 293 cells were exposed to supernatants from or cocultured with primary pig hepatocytes with or without stimulation with PHA and PMA [57]. These experiments would not detect release of infectious PERV-C, and therefore may not be conclusive regarding the likelihood of transmission of infectious virus from porcine hepatocytes.

We isolated infectious PERV-C directly from porcine plasma, as well as porcine plasma-derived factor VIII, suggesting that *in vivo* blood or endothelium constitutively expresses PERV [58]. Indeed, infectious PERV has been transmitted from cultured primary porcine endothelial cells to human cells in the absence of any special activation conditions [59, 60]. In contrast, increased reverse-transcriptase (RT) activity was observed in primary pig endothelial cells only after activation with tumor necrosis factor-alpha, interferon-gamma or lipoplysaccharide [60].

A recent publication showed evidence of PERV RNA expression in melanomas isolated from Munich miniature swine (MMS) of the strain Troll. However, the normal skin samples from matched animals were also positive for PERV RNA, although at levels that were approximately 5–10 times lower than those observed in the melanoma samples [61]. The investigation did not analyze whether PERV RNA expression correlated with expression of infectious virus. The fact that RNA was also detected in normal skin indicates that there is basal PERV expression, suggesting either the dysregulated gene expression that often accompanies the tumor phenotype causes an increase in PERV expression or that increased PERV expression may have been involved in the tumorigenic process.

PERV recombination

The co-culture of human 293 cells with activated primary porcine peripheral blood mononuclear cells from NIH mini-pigs provides a means to isolate human-tropic replication competent PERVs (HTRCs) [13, 62]. Reports of the sequences isolated in these experiments showed that the HTRCs appeared to be recombinants between PERV-A and PERV-C proviral sequences [19, 62]. The repeated isolation of PERV-A/C recombinants prompts the question whether the recombinant proviruses exist in the NIH mini-pig genome or whether they occur only upon activation and co-culture of the mini-pig cells with human cells (i.e., upon reverse transcription of a PERV-A and PERV-C genome co-packaged in a single particle). Initial reports that the A/C recombinant sequences were absent from the mini-pig genome [63, 64] suggested that recombination events occurred after expression of the PERV genomic loci and that the PERV-A/C recombinants only exist as exogenous viruses, not endogenous genomic sequences. However, a recent report challenges that conclusion. Four HTRC-positive NIH mini-pigs were positive for the PERV-A/C recombinant as detected by PCR of genomic DNA, and the A/C recombinant was confirmed by sequencing. In contrast, no sequences were amplified from the four HTRC-negative animals tested [65].

Whether the A/C recombinants are endogenous or exogenous, it appears that the acquisition of some portion of the PERV-C envelope sequences correlates with increased HTRC titers. Bartosch et al. demonstrated that at least one PERV-A/C HTRC isolated from NIH mini-pig PBMCs (originally described in [62]) had consistently higher titers than a 'classical' PERV-A isolate on every cell line that was tested (human 293, mink Mv-1-Lu and cat CRFK). Pseudotypes bearing PERV-A/C recombinant envelopes (PERV-A-14/220) also had approximately 10-fold or greater increased titer compared to pseudotypes bearing PERV-A envelopes, demonstrating that increased replication correlates with changes in the envelope sequence. This was confirmed by making recombinant and mutant envelopes that mapped the sequences responsible for the enhanced titers to the envelope gene [29, 66].

In addition to the risks posed by recombination between PERV genomes generating HTRCs, there are theoretical risks associated with generation of novel retroviruses with expanded or improved tropism for human cells by recombination between PERV and human endogenous retroviruses (HERVs) in human cells. Suling et al. developed sensitive PCR assays for detection of each of four different HERVs that are known to be expressed in 293 cells and then analyzed sucrose gradient purified virions released from PERV-infected 293 cells for each of these RNAs in order to identify the rate of copackaging of HERV and PERV RNA. No HERV RNAs were detected in these assays. Based on the sensitivity of the PCR assays used, the authors calculated that the likelihood of HERV co-packaging in PERV particles was at best $< 1:50\,000$ (for the betaretrovirus HERV-K, the least-sensitive assay) and $< 1:3.9\times10^{6}$ for the other HERVs tested (gammaretroviruses HERV-W, E, R and ERV-9) [67]. While these findings do not eliminate the possibility that recombination between PERV and HERVs may occur, they suggest it occurs at a very low frequency.

Animal models for assessing PERV replication and pathogenesis

While PERV infects and replicates in human cells *in vitro*, the risk to human recipients of xenotransplantation products is still unknown. *In vitro* studies have been useful to identify the molecular determinants of human cell tropism, but have not revealed any obvious pathogenic effects. For example, after serial passage of PERV-infected human 293 cells for 6 months, no obvious phenotypic changes in morphology or cell growth properties were observed [68]. However, a more recent study analyzing the integration site preferences for PERV demonstrated that like oncogenic murine leukemia viruses, PERV integration in human cells is biased towards transcriptionally active regions, with a strong preference towards the transcription start site and CpG islands [69], suggesting the potential for PERV-mediated oncogenesis via insertional mutagenesis. Recent reports of leukemias in children participating in gene therapy clinical trials

using replication-defective murine leukemia virus-based vectors show that in principle non-human gammaretroviruses can be oncogenic in humans [70, 71]. In order to understand whether PERV may be tumorigenic, many investigators have attempted to establish a permissive animal model that supports PERV replication.

Initial attempts to identify a permissive animal model were focused on rodents. Based on unpublished data presented at a public Advisory Committee meeting of the FDA Subcommittee on Xenotransplantation (13 January, 2000, agenda and transcripts available http://www.fda.gov/cber/advisory/ctgt/ctgtmain.htm), we examined whether guinea pigs would provide a permissive animal model for PERV replication. We concluded that guinea pigs are not a permissive model, based on the ability to detect transient low-level PERV DNA in guinea pig cells, accompanied by development of anti-PERV antibodies [72]. Similar results were observed with rats or guinea pigs after PERV was inoculated by either intra-muscular or intra-peritoneal routes [73]. Two subsequent reports showed that implantation of porcine pancreatic islets into NOD/SCID (non-obese diabetic, severe combined immunodeficiency) mice resulted in PERV infection in tissue compartments that also showed evidence of microchimerism for pig cells [55, 74]. However, the observation that PERV was transmitted to murine tissues in NOD/SCID mice was not reproduced in a different animal model of diabetes [75]. While this apparent difference was puzzling at the time, later studies showed that PERV infection of murine tissues may have been mediated by the ability of endogenous xenotropic murine leukemia virus (X-MuLV) envelopes to pseudotype PERV [76].

PERV replication was also tested in humanized SCID mice. While PERV RNA and DNA were detected, there was no evidence of replication [77]. The result was not completely surprising in view of prior observations that suggested human PBMCs are not permissive for PERV replication *in vitro* [19]. However, McKane et al. report transmission of PERV to human PBLs after transplantation of porcine aortic endothelial cells or porcine islets in SCID mice [78, 79].

In addition to the complication presented by X-MuLV pseudotyping, Ericsson et al. demonstrated that the murine ortholog of the PERV-A receptor is non-functional for PERV-A entry [24], and therefore mice are not likely to provide a permissive model. To address both of these problems, Martina et al. introduced the human PERV-A receptor (HuPAR-2) into the germline of a strain of mouse known to lack X-MuLVs, FVB/Nj mice. The HuPAR-2 transgenic mice were inoculated intraperitoneally and intravenously with cell-free PERV-containing supernatants. Increasingly higher copy numbers of PERV RNA and DNA were detected over the period of 8 weeks post-inoculation, providing indirect evidence of PERV replication. A subsequent decrease in PERV RNA and DNA observed after 12 weeks correlated with detection of neutralizing anti-PERV antibodies. PERV replication was confirmed by detection of PERV gag protein by immunoblot and confocal microscopy. In addition, supernatants from primary kidney cell cultures established from infected animals were able to transmit replication-competent PERV to human 293 cells *in vitro*, confirming PERV replication in the mouse/HuPAR-2 transgenic mice [80]. To date, this is the most promising animal model for *in vivo* studies of PERV.

Attempts to develop a large animal model have not met with success. Popp et al. transplanted pig proislets into thymectomized fetal lambs to see whether introduction of the virus prior to development of immunocompetence would allow viral replication. While evidence for transmission of PERV was found, DNA was only detected in a few lambs (4/12 liver samples and 2/12 spleen samples), with no detectable PERV RNA in any samples. The absence of PERV DNA in the period following day 23, once the lambs gained immunocompetence (approximately 21–23 days post-transplant), suggests transient PERV infection in the absence of replication [81].

Several investigators have attempted to infect baboon, rhesus macaque, bonnet macaques and pig-tailed monkeys with PERV to establish non-human primate models to study PERV replication. A variety of immunosuppressive or immunoevasive strategies did not result in evidence of PERV transmission or replication [82–85]. In consideration of *in vitro* studies showing that primary cultures and established cell lines from baboon, rhesus macaque and African green monkey were all non-permissive for PERV replication, the lack of replication *in vivo* is not unexpected [21].

Xenotransplantation: clinical protocols

Methods used for detection of evidence of PERV transmission

Most commonly, PCR-based methods have been used to detect PERV-specific nucleic acid sequences in human peripheral blood mononuclear samples and plasma samples. Interpretation of a positive result is complicated by the possible presence of porcine cells in the recipient, commonly referred to as microchimerism. To distinguish between detection of PERV DNA as a consequence of PERV presence in the pig

genome compared to PERV transmission to human cells, assays are applied that normalize the PERV signal to some other multi-copy gene present in pig cells. For example, in the study of Paradis et al. the average copy number per cell of porcine centromere or mitochondrial DNA were compared to the average copy number per cell of PERV in a cohort of pig samples tested, so that a ratio of the PERV: multi-copy pig sequence could be used to interpret a sample result as either microchimerism or evidence of infection [86]. While peripheral blood mononuclear cells or plasma represent easily obtained, relatively non-invasive samples for analysis of PERV, they may not be the most clinically relevant samples. To date, no significant evidence of infection of human PBMCs has been obtained [19]. However, that does not rule out the ability of PERV to infect precursor cells in the bone marrow, for example, that upon differentiation would then be found in the peripheral blood and could be detected.

The second method used to obtain indirect evidence of transmission is detection of anti-PERV antibodies as. Most reports have used Western blot analysis against either PERV-infected 293 cell lysates [87], lysates of purified virions or recombinant p30 [88]. In addition, Xu et al. have developed an ELISA detection method using recombinant gag p30 [89]. Some have included analysis for anti-PERV neutralizing antibodies as a measure of serologic response in recipients [90]. A major problem inherent in all these methods is the lack of a positive control, in the absence of any documented human seroconversions.

Data from xenotransplantation product recipients

Exposure of xenotransplantation product recipients to pig cells will represent a spectrum of exposures ranging from transplantation of pig cells directly, to transplantation of cells within a device, to extracorporeal exposure of pig cells with or without devices. The devices used may provide some barrier to transmission of infectious agents.

PERV infection of human cells *in vitro*, suggesting risks of cross-species transmission, prompted investigators to perform retrospective studies to determine whether subjects who had previously participated in clinical xenotransplantation trials may have evidence of PERV transmission. One of the more comprehensive studies of this type was coordinated by Novartis and included samples from a total of 160 subjects who received any one of a variety of exposures to porcine cells: 1) 100 subjects who had been treated by extracorporeal perfusion through porcine spleens from slaughterhouse pigs; 2) 28 subjects who had been treated by extracorporeal perfusion for liver failure with a device that was seeded with porcine heptatocytes; 3) 15 subjects who received pig skin grafts for treatment of burns; 4) 14 subjects who received porcine pancreatic islet cell transplants; 20 subjects received extracorporeal pig kidney perfusion; and 1 subject who underwent extracorporeal perfusion through a pig liver. The subjects represented the full range of exposures to living porcine cells and duration of exposures varied from less than 1 h (splenic perfusion) to 460 days (skin graft). Since the survey was retrospective, a significant period of time had passed since most subjects had been exposed to the pig cells (average duration since treatments ranged from approximately 2 to 12 years). All samples were analyzed by both PCR and serologic assays, and each was performed independently by two different laboratories in order to provide confirmatory data of all results. While some samples of DNA were insufficient to allow interpretation of a negative result, those samples that did yield positive results by PERV-specific PCR were also positive for pig centromeric DNA, and the ratios of the two sequences detected were at a level to suggest microchimerism rather than evidence of transmission to human cells. Two samples tested positive for anti-PERV antibodies by one of the testing laboratories but were negative by the other testing laboratory, leaving open the question of whether the positive represents an artifact [86]. While this study provided encouraging data regarding the risk of PERV transmission, there were obvious challenges to strong conclusions because of the nature of the samples (i.e., the absence of pre-treatment samples, and the long duration between treatment and analysis) and the drawbacks to the testing methods (see above).

While fixed or acellular heart valves are not considered xenotransplantation products because they do not retain living cells, it is worth mentioning them here because they are commonly used in valve replacement surgeries. No PERV DNA was detected in either the valves themselves or in subjects treated with either commercially available glutaraldehyde-fixed porcine valves or acellular porcine aortic valves [91, 92].

Next on the spectrum of risk associated with porcine products are those exposures that are extracorporeal. A limited study of two patients who were treated by extracorporeal perfusion through pig kidneys showed no evidence of transmission when examined immediately after the procedure, 7 days, and 2 and 3 years later. Assays in this study included PCR for PERV and pig-specific genes and analysis for anti-PERV neutralizing antibodies [90]. No evidence of PERV transmission was observed in a different study of two subjects that were exposed to extracorporeal perfusion through porcine livers from pigs transgenic for the human complement regulatory proteins, CD55

and CD59. This study analyzed PERV DNA by PCR in PBMC at multiple time points post-perfusion (days 30–300 in patient 1 and days 30–120 in patient 2) [93]. While both subjects developed strong anti-pig antibody responses, no anti-PERV antibodies could be detected against recombinant PERV gag by ELISA [89]. In addition to monitoring the study subjects, the healthcare workers who were exposed to the porcine livers or the body fluids of the study subjects were also retrospectively analyzed by PCR and Western-blot analysis for evidence of PERV transmission. All samples were negative, suggesting low risks to healthcare workers who use standard precautions to prevent transmission of other infectious agents in the healthcare setting [94].

More typical of extracorporeal exposures to pig tissues or cells are the bioartificial liver (BAL) assist devices. In contrast to extracorporeal perfusion through a pig organ, these devices typically include a semi-permeable barrier between the porcine hepatocytes and the human blood or plasma that is perfused through the device. Nyberg et al. analyzed whether hollow fibers typically used in BAL are sufficient to prevent PERV transmission. Using various pore sizes typical of the BAL, membranes with 200 nM pores allowed transfer of infectious virus across the membrane, while pore sizes of 400 kD or 70 kD allowed transfer of viral RNA, but no infectious virus [95]. Likewise, a study of a BAL with a 100-kD pore size showed no transmission of PERV across the membrane [57]. Five subjects with acute liver failure were treated with the BAL with 100-kD pores. PBMC collected prior to BAL exposure and 6 h after exposure were all negative for PERV RNA and DNA sequences, as well as for porcine mitochondrial DNA [57]. While the study demonstrates that the pig cells did not cross the barrier, the lack of detectable microchimerism suggests that the time at which the sample was taken was too short after the exposure to definitively rule out evidence of PERV transmission to recipients. A retrospective study of 28 patients treated with an extracorporeal BAL also proved negative for PERV transmission using PCR for PERV DNA in PBMC collected up to 5 years post-exposure [96].

Another area of clinical investigation has been the transplantation of porcine islets to treat type I diabetes. No evidence of PERV transmission was found in a retrospective study of 10 patients who had received porcine fetal islets (400 million to 2 billion cells) anywhere from 4–7 years prior to the analysis for PERV. Analyses included PCR-enhanced reverse transcriptase assay and RT-PCR for PERV RNA in sera, PCR for PERV and pig-sequences in DNA of peripheral blood lymphocytes, as well as Western blots using lysates from PERV-infected 293 cells for anti-PERV antibodies [87]. More limited studies were conducted involving patients that had been implanted with pig islets encapsulated in alginate that tested negative for PERV sequences in their PBMC (DNA PCR) or plasma (RT-PCR) [97, 98]. Finally, no PERV DNA was detected in PBMCs in a clinical trial with the highest risk of exposure wherein 12 patients with Parkinson's disease were implanted with embryonic porcine ventral mesencephalic tissue combined with immunosuppressive therapies [99].

Strategies to prevent PERV transmission or to treat PERV infection

A variety of strategies are being examined to address the risk to the recipients of xenotransplantation products in the event of PERV transmission. One obvious strategy is to determine whether existing antiretroviral reverse transcriptase or protease inhibitors are effective against PERV. As expected, PERV is resistant to protease inhibitors that were developed for inhibiting HIV protease; however, a screen of 5 RT-inhibitors identified zidovudine as a candidate for clinical use; the IC50 of zidovudine was only threefold higher for PERV relative to HIV-1 [100]. Similar results were reported by others [101–103]. More recently, a series of 10 acyclic nucleoside phosphonates shown to effectively treat HIV-1 infection were studied for anti-PERV activity. Again zidovudine was the most potent against PERV, but two of the acyclic nucleoside phosphonates were shown to have promising levels of anti-PERV activity [104].

The identification of targets for inhibition of PERV expression by inhibitory RNA (RNAi) is another area of active investigation. While several targets have been identified that lead to decreases in PERV RNA and protein expression, none have been shown to inhibit PERV infectivity beyond 80–90 % [105–107], even when using lentiviral vectors to stably express the short hairpin RNAs [107]. Although a recent report generating transgenic pigs expressing PERV-specific shRNAs has shown decreased PERV RNA expression (80–95 % inhibition) [107], the authors have not yet examined whether this correlates with a decrease in infectious PERV, a critical element to demonstrate whether the genetic modification will reduce the risk of transmission.

A third area of active investigation is the development of antibodies that inhibit PERV infection or identification of the components of the human immune response that would prevent PERV infection. As an example of the former, Dekker et al. generated an intracellular single-domain antibody again the p15

matrix protein from llama and showed that expression of this antibody chain in PK15 cells led to reduced but detectable release of PERV-A and PERV-B-specific RNA into the supernatant [108]. Since the authors did not determine whether the inhibition was sufficient to prevent release of infectious virus, clinical feasibility remains undetermined.

Studies of naturally occurring immune responses that are effective against PERV have identified both humoral and cell-mediated immune responses. Simeonovic et al. showed that immunization of mice with PERV-expressing cells or virions accelerated rejection of pig thyroid xenografts in a mouse model and that the rejection was CD4 T-cell-dependent and restricted to PERV-expressing cellular xenografts [109]. A second study evaluating a series of envelope-derived peptides identified one (KLFSLIQGA, aa 303) as immunodominant in a CD8+ CTL line generated from PBL from an HLA-A2+ donor stimulated with PERV-infected 293 cells [110].

Alpha-1,3-galactosyltransferases are present in pig cells but not in human cells. PERVs carry the alpha-1,3-galactosyl (alpha-gal) residue on their envelope glycoproteins. Chronic exposure to alpha-gal residues results in up to 1 % of circulating antibodies in human serum being directed against this residue. Indeed, it has been demonstrated that anti-alpha-gal antibodies provide a potent mechanism to neutralize a variety of other retroviruses [111–113], so it seemed reasonable to expect that they would also neutralize PERV. McKane found that anti-alpha-gal antibodies could prevent PERV infection in an *in vivo* SCID model repopulated with human PBL [78, 79], suggesting the potent role these antibodies have in protecting against PERV transmission. Indeed, these results highlight the increased risk posed by genetically modified pigs that no longer express the alpha-1,3-galactosyltransferases (see next section).

Influence of genetic modifications of pigs on risks of PERV transmission

A major challenge to the clinical success of pig to human organ transplantation is the prevention of immune rejection. Certain genetic modifications of pig have been introduced to modulate the human anti-pig immune response with the intention of prolonging porcine xenograft survival. Unfortunately, these modifications also often relieve a natural barrier to PERV transmission (discussed in [114]). Two specific examples follow.

Transgenic pigs carrying the human complement regulatory proteins CD55 and CD59 were derived, shown to prevent complement-mediated damage to transplanted pig organs in a non-human primate model [115] and used as source animals in a clinical trial using extracorporeal perfusion through a transgenic pig liver [93]. Subsequently, we have performed *in vitro* studies that show expression of human CD59 on pig cells results in production of PERV virions that incorporate CD59 and that these virions are resistant to complement-mediated lysis, but not to neutralization [116]. The observed difference in susceptibility to lysis compared to neutralization is likely due to the stage that CD59 acts on the complement pathway. CD59 is important for formation of the membrane attack complex, which is not critical for neutralization. In contrast, similar studies performed on PERV harvested from human CD55-positive swine testis cells showed protection from neutralization by human serum [117]. Since CD55 acts to inhibit the deposition of complement on virions, it is not surprising that CD55 would protect from complement inactivation. Together, the two studies show that expression of human CD55 and CD59 in pigs may increase the risk of PERV transmission by overcoming two natural barriers.

The generation of alpha-1,3-galactosyltransferase knockout pigs is the second modification that may alter natural protection to cross-species PERV transmission [118]. This modifying enzyme, which is not present in human or Old World non-human primates, adds alpha-1,3-galactosyl residues to glycoproteins. Chronic exposure to this residue in humans and non-human primates leads to high levels of circulating antibodies and upon transplantation of pig organs into Old World primates leads to high levels of circulating antibodies. Furthermore, transplantation of pig organs into Old World primates causes hyperacute rejection of the organ within minutes (reviewed in [119]). However, like the human complement regulatory proteins, the antibodies to alpha-1,3-galactosyl residues provide protection through complement-mediated lysis of retroviruses that are produced in cells expressing the alpha-gal modifying enzyme [111–113, 120]. Therefore, it was not surprising to find that PERV produced in pig cells that are deficient for alpha-1,3-galactosyltransferase are resistant to complement inactivation or neutralization [121].

Conclusion

Research on PERV has increased our knowledge of the *in vitro* host range, determinants of human tropism, genetic load in pigs and pig tissue-specific expression patterns. However, the challenge in developing a permissive animal model has delayed our understanding of *in vivo* replication, potential for

pathogenicity, and development of effective anti-viral or vaccines strategies to prevent transmission. In addition, while retrospective analyses in humans exposed to pigs [122] or porcine xenotransplantation products have provided reassuring data, the risk of transmission will proportionally increase with improvements that allow longer-term survival of porcine cells in xenotransplantation product recipients. Genetic modifications designed to enhance the efficacy of a xenotransplantation product may inadvertently increase the risk of PERV transmission. In the face of still uncertain risks, efforts should continue in the area of identifying a means to prevent transmission or treat infection, development of improved animal models and improved assays to monitor for PERV transmission in xenotransplantation product recipients.

Acknowledgements. While every effort was made to make this a comprehensive review, space limitations dictated that some relevant publications were omitted. PERV-related research performed in my laboratory has been supported by the FDA/Center for Biologics Evaluation and Research or by a Collaborative Research and Development Agreement with the Scripps Research Laboratory. I thank Andrew Byrnes and Takele Argaw for critical reading of the manuscript.

1 Armstrong, J. A., Porterfield, J. S. and de Madrid, A. T. (1971) C-type virus particles in pig kidney cell lines. J. Gen. Virol. 10, 195–198.
2 Lieber, M. M., Sherr, C. J., Benveniste, R. E. and Todaro, G. J. (1975) Biologic and immunologic properties of porcine type C viruses. Virology 66, 616–619.
3 Todaro, G. J., Benveniste, R. E., Lieber, M. M. and Sherr, C. J. (1974) Characterization of a type C virus released from the porcine cell line PK(15). Virology 58, 65–74.
4 Busse, C., Marschall, H. J. and Moennig, V. (1978) Further investigations on the porcine lymphoma C-type particle (PLCP) and the possible biological significance of the virus in pigs. Ann. Rech. Vet. 9, 651–658.
5 Ref. removed in proof.
6 Moennig, V., Frank, H., Hunsmann, G., Ohms, P., Schwarz, H. and Schafer, W. (1974) C-type particles produced by a permanent cell line from a leukemic pig. II. Physical, chemical, and serological characterization of the particles. Virology 57, 179–188.
7 Strandstrom, H., Veijalainen, P., Moennig, V., Hunsmann, G., Schwarz, H. and Schafer, W. (1974) C-type particles produced by a permanent cell line from a leukemic pig. I. Origin and properties of the host cells and some evidence for the occurrence of C-type-like particles. Virology 57, 175–178.
8 Ibrahim, Z., Busch, J., Awwad, M., Wagner, R., Wells, K. and Cooper, D. K. (2006) Selected physiologic compatibilities and incompatibilities between human and porcine organ systems. Xenotransplantation 13, 488–499.
9 Allan, J. S. (1998) The risk of using baboons as transplant donors. Exogenous and endogenous viruses. Ann. N. Y. Acad. Sci. 862, 87–99.
10 Fishman, J. A. (1998) Infection and xenotransplantation. Developing strategies to minimize risk. Ann. N. Y. Acad. Sci. 862, 52–66.
11 Boneva, R. S., Folks, T. M. and Chapman, L. E. (2001) Infectious disease issues in xenotransplantation. Clin. Microbiol. Rev. 14, 1–14.
12 Patience, C., Takeuchi, Y. and Weiss, R. A. (1997) Infection of human cells by an endogenous retrovirus of pigs. Nat. Med. 3, 282–286.
13 Wilson, C., Wong, S., Muller, J., Davidson, C., Rose, T. and Burd, P. (1998) Type C retrovirus released from porcine primary peripheral blood mononuclear cells infects human cells. J. Virol. 72, 3082–3087.
14 Le Tissier, P., Stoye, J. P., Takeuchi, Y., Patience, C. and Weiss, R. A. (1997) Two sets of human-tropic pig retroviruses. Nature 389, 681–682.
15 Akiyoshi, D. E., Denaro, M., Zhu, H., Greenstein, J. L., Banerjee, P. and Fishman, J. A. (1998) Identification of a full-length cDNA for an endogenous retrovirus of miniature swine. J. Virol. 72, 4503–4507.
16 Takeuchi, Y., Patience, C., Magre, S., Weiss, R. A., Banerjee, P. T., Tissier, P. L. and Stoye, J. P. (1998) Host range and interference studies of three classes of pig endogenous retrovirus. J. Virol. 72, 9986–9991.
17 Ericsson, T., Oldmixon, B., Blomberg, J., Rosa, M., Patience, C. and Andersson, G. (2001) Identification of novel porcine endogenous betaretrovirus sequences in miniature swine. J. Virol. 75, 2765–2770.
18 Specke, V., Plesker, R., Coulibaly, C., Boller, K. and Denner, J. (2002) Productive infection of a mink cell line with porcine endogenous retroviruses (PERVs) but lack of transmission tominks in vivo. Arch. Virol. 147, 305–319.
19 Wilson, C. A., Wong, S., Brocklin, M. V. and Federspiel, M. J. (2000) Extended analysis of the in vitro tropism of porcine endogenous retrovirus. J. Virol. 74, 49–56.
20 Blusch, J. H., Patience, C., Takeuchi, Y., Templin, C., Roos, C., von der Helm, K., Steinhoff, G. and Martin, U. (2000) Infection of nonhuman primate cells by pig endogenous retrovirus. J. Virol. 74, 7687–7690.
21 Ritzhaupt, A., Van Der Laan, L. J., Salomon, D. R. and Wilson, C. A. (2002) Porcine endogenous retrovirus infects but does not replicate in nonhuman primate primary cells and cell lines. J. Virol. 76, 11312–11320.
22 Specke, V., Rubant, S. and Denner, J. (2001) Productive infection of human primary cells and cell lines with porcine endogenous retroviruses. Virology 285, 177–180.
23 Martin, U., Winkler, M. E., Id, M., Radeke, H., Arseniev, L., Takeuchi, Y., Simon, A. R., Patience, C., Haverich, A., and Steinhoff, G. (2000) Productive infection of primary human endothelial cells by pig endogenous retrovirus (PERV) Xenotransplantation 7, 138–142.
24 Ericsson, T. A. , Takeuchi, Y., Templin, C., Quinn, G., Farhadian, S. F., Wood, J. C., Oldmixon, B. A., Suling, K. M., Ishii, J. K., Kitagawa, Y. et al. (2003) Identification of receptors for pig endogenous retrovirus. Proc. Natl. Acad. Sci. USA 100, 6759–6764.
25 Overbaugh, J., Miller, A. D. and Eiden, M. V. (2001) Receptors and entry cofactors for retroviruses include single and multiple transmembrane-spanning proteins as well as newly described glycophosphatidylinositol-anchored and secreted proteins. Microbiol. Mol. Biol. Rev. 65, 371–389.
26 Andriamampandry, C., Taleb, O., Kemmel, V., Humbert, J. P., Aunis, D. and Maitre, M. (2007) Cloning and functional characterization of a gamma-hydroxybutyrate receptor identified in the human brain. FASEB J. 21, 885–895.
27 Mattiuzzo, G., Matouskova, M. and Takeuchi, Y. (2007) Differential resistance to cell entry by porcine endogenous retrovirus subgroup A in rodent species. Retrovirology 4, 93.
28 Gemeniano, M., Mpanju, O., Salomon, D. R., Eiden, M. V. and Wilson, C. A. (2006) The infectivity and host range of the ecotropic porcine endogenous retrovirus, PERV-C, is modulated by residues in the C-terminal region of its surface envelope protein. Virology 346, 108–117.
29 Harrison, I., Takeuchi, Y., Bartosch, B. and Stoye, J. P. (2004) Determinants of high titer in recombinant porcine endogenous retroviruses. J. Virol. 78, 13871–13879.
30 LaVillette, D., Ruggieri, A., Russell, S. J. and Cosset, F.-L. (2000) Activation of a cell entry pathway common to type C

mammalian retroviruses by soluble envelope fragments. J. Virol. 74, 295–304.
31 Barnett, A. L. and Cunningham, J. M. (2001) Receptor binding transforms the surface subunit of the mammalian C-type retrovirus envelope protein from an inhibitor to an activator of fusion. J. Virol. 75, 9096–9105.
32 Lavillette, D. and Kabat, D. (2004) Porcine endogenous retroviruses infect cells lacking cognate receptors by an alternative pathway: implications for retrovirus evolution and xenotransplantation. J. Virol. 78, 8868–8877.
33 Martin, J., Herniou, E., Cook, J., O'Neill, R. W. and Tristem, M. (1999) Interclass transmission and phyletic host tracking in murine leukemia virus-related retroviruses. J. Virol. 73, 2442–2449.
34 Patience, C., Switzer, W. M., Takeuchi, Y., Griffiths, D. J., Goward, M. E., Heneine, W., Stoye, J. P. and Weiss, R. A. (2001) Multiple groups of novel retroviral genomes in pigs and related species. J. Virol. 75, 2771–2775.
35 Niebert, M. and Tonjes, R. R. (2005) Evolutionary spread and recombination of porcine endogenous retroviruses in the suiformes. J. Virol. 79, 649–654.
36 Niebert, M. and Tonjes, R. R. (2003) Analyses of prevalence and polymorphisms of six replication-competent and chromosomally assigned porcine endogenous retroviruses in individual pigs and pig subspecies. Virology 313, 427–434.
37 Tonjes, R. R. and Niebert, M. (2003) Relative age of proviral porcine endogenous retrovirus sequences in Sus scrofa based on the molecular clock hypothesis. J. Virol. 77, 12363–12368.
38 Klymiuk, N., Muller, M., Brem, G. and Aigner, B. (2002) Characterization of porcine endogenous retrovirus gamma pro-pol nucleotide sequences. J. Virol. 76, 11738–11743.
39 Klymiuk, N., Muller, M., Brem, G. and Aigner, B. (2006) Phylogeny, recombination and expression of porcine endogenous retrovirus gamma2 nucleotide sequences. J. Gen. Virol. 87, 977–986.
40 Bosch, S., Arnauld, C. and Jestin, A. (2000) Study of full-length porcine endogenous retrovirus genomes with envelope gene polymorphism in a specific-pathogen-free large white swine herd. J. Virol. 74, 8575–8581.
41 Herring, C. Quinn, G., Bower, R., Parsons, N., Logan, N. A., Brawley, A., Elsome, K., Whittam, A., Fernandez-Suarez, X. M., Cunningham, D. et al. (2001) Mapping full-length porcine endogenous retroviruses in a Large White pig. J. Virol. 75, 12252–12265.
42 Rogel-Gaillard, C., Bourgeaux, N., Billault, A., Vaiman, M. and Chardon, P. (1999) Construction of a swine BAC library: application to the characterization and mapping of porcine type C endoviral elements. Cytogenet. Cell Genet. 85, 205–211.
43 Czauderna, F., Fischer, N., Boller, K., Kurth, R. and Tonjes, R. R. (2000) Establishment and characterization of molecular clones of porcine endogenous retroviruses replicating on human cells. J. Virol. 74, 4028–4038.
44 Krach, U., Fischer, N., Czauderna, F. and Tonjes, R. R. (2001) Comparison of replication-competent molecular clones of porcine endogenous retrovirus class A and class B derived from pig and human cells. J. Virol. 75, 5465–5472.
45 Suzuka, I., Shimizu, N., Sekiguchi, K., Hoshino, H., Kodama, M. and Shimotohno, K. (1986) Molecular cloning of unintegrated closed circular DNA of porcine retrovirus. FEBS Lett. 198, 339–343.
46 Preuss, T., Fischer, N., Boller, K. and Tonjes, R. R. (2006) Isolation and characterization of an infectious replication-competent molecular clone of ecotropic porcine endogenous retrovirus class C. J. Virol. 80, 10258–10261.
47 Lee, J.-H., Webb, G. C., Allen, R. D.M. and Moran, C. (2002) Characterizing and mapping porcine endogenous retroviruses in Westran pigs. J. Virol. 76, 5548–5556.
48 Edamura, K., Nasu, K., Iwami, Y., Nishimura, R., Ogawa, H., Sasaki, N. and Ohgawara, H. (2004) Prevalence of porcine endogenous retrovirus in domestic pigs in Japan and its potential infection in dogs xenotransplanted with porcine pancreatic islet cells. J. Vet. Med. Sci. 66, 129–135.
49 Li, Z., Ping, Y., Shengfu, L., Hong, B., Youping, L., Yangzhi, Z. and Jingqiu, C. (2004) Phylogenetic relationship of porcine endogenous retrovirus (PERV) in Chinese pigs with some type C retroviruses. Virus Res. 105, 167–173.
50 Boeke, J. D. and Stoye, J. P. (1997) Retrotransposons, endogenous retroviruses, and the evolution of retroelements. In: Retroviruses, pp. 343–436, Coffin, J. M., Hughes, S. H. and Varmus, H. E. (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
51 Jin, H., Inoshima, Y., Wu, D., Morooka, A. and Sentsui, H. (2000) Expression of porcine endogenous retrovirus in peripheral blood leukocytes from ten different breeds. Transpl. Infect. Dis. 2, 11–14.
52 Martignat, L., Sai, P. and Jestin, A. (1998) Detection of porcine endogenous retrovirus: possible involvement in pig islet xenotransplantation. Diabetes Metab. 24, 434–441.
53 Clemenceau, B., Lalain, S., Martignat, L. and Sai, P. (1999) Porcine endogenous retroviral mRNAs in pancreas and a panel of tissues from specific pathogen-free pigs. Diabetes Metab. 25, 518–525.
54 Schmidt, P., Forsman, A., Andersson, G., Blomberg, J. and Korsgren, O. (2005) Pig islet xenotransplantation: activation of porcine endogenous retrovirus in the immediate post-transplantation period. Xenotransplantation 12, 450–456.
55 Van der Laan, L. J., Lockey, C., Griffeth, B. C., Frasier, F. S., Wilson, C. A., Onions, D. E., Hering, B. J., Long, Z., Otto, E., Torbett, B. E. et al. (2000) Infection by porcine endogenous retrovirus after islet xenotransplantation in SCID mice. Nature 407, 90–94.
56 Nyberg, S. L., Hibbs, J. R., Hardin, J. A., Germer, J. J., Platt, J. L., Paya, C. V. and Wiesner, R. H. (2000) Influence of human fulminant hepatic failure sera on endogenous retroviral expression in pig hepatocytes. Liver Transplant. 6, 76–84.
57 Kuddus, R., Patzer, J. F., 2nd, Lopez, R., Mazariegos, G. V., Meighen, B., Kramer, D. J. and Rao, A. S. (2002) Clinical and laboratory evaluation of the safety of a bioartificial liver assist device for potential transmission of porcine endogenous retrovirus. Transplantation 73, 420–429.
58 Takefman, D. M., Wong, S., Maudru, T., Peden, K. and Wilson, C. A. (2001) Detection and characterization of porcine endogenous retrovirus in porcine plasma and porcine factor VIII. J. Virol. 75, 4551–4557.
59 Martin, U., Kiessig, V., Blusch, J., Haverich, A., von der Helm, K., Herden, T. and Steinhoff, G. (1998) Expression of pig endogenous retrovirus by primary porcine endothelial cells and infection of human cells. Lancet 352, 692–694.
60 Cunningham, D. A., Dos Santos Cruz, G. J., Fernandez-Suarez, X. M., Whittam, A. J., Herring, C., Copeman, L., Richards, A. and Langford, G. A. (2004) Activation of primary porcine endothelial cells induces release of porcine endogenous retroviruses. Transplantation 77, 1071–1079.
61 Dieckhoff, B., Puhlmann, J., Buscher, K., Harner-Marx, A., Herbach, N., Bannert, N., Wanke, R., Kurth, R., and Denner, J. (2007) Expression of porcine endogenous retroviruses (PERVs) in melanomas of Munich miniature swine (MMS) Troll. Vet. Microbiol. 123, 53–68.
62 Oldmixon, B. A., Wood, J. C., Ericsson, T. A., Wilson, C. A., White-Scharf, M. E., Andersson, G., Greenstein, J. L., Schuurman, H-J., and Patience, C. (2002) Porcine endogenous retrovirus transmission characteristics of an inbred herd of miniature swine. J. Virol. 76, 3045.
63 Scobie, L., Taylor, S., Wood, J. C., Suling, K. M., Quinn, G., Meikle, S., Patience, C., Schuurman, H.-J., and Onions, D. E. (2004) Absence of replication-competent human-tropic porcine endogenous retroviruses in the germ line DNA of inbred miniature Swine. J. Virol. 78, 2502–2509.
64 Wood, J. C., Quinn, G., Suling, K. M., Oldmixon, B. A., Van Tine, B. A., Cina, R., Arn, S., Huang, C. A., Scobie, L., Onions, D. E. et al. (2004) Identification of exogenous forms

of human-tropic porcine endogenous retrovirus in miniature Swine. J. Virol. 78, 2494–2501.
65 Martin, S. I., Wilkinson, R. and Fishman, J. A. (2006) Genomic presence of recombinant porcine endogenous retrovirus in transmitting miniature swine. Virol. J. 3, 91.
66 Bartosch, B., Stefanidis, D., Myers, R., Weiss, R., Patience, C. and Takeuchi, Y. (2004) Evidence and consequence of porcine endogenous retrovirus recombination. J. Virol. 78, 13880–13890.
67 Suling, K., Quinn, G., Wood, J. and Patience, C. (2003) Packaging of human endogenous retrovirus sequences is undetectable in porcine endogenous retrovirus particles produced from human cells. Virology 312, 330–336.
68 Yu, P., Zhang, L., Li, S. F., Li, Y. P., Cheng, J. Q., Lu, Y. R. and Bu, H. (2005) Long-term effects on HEK-293 cell line after co-culture with porcine endogenous retrovirus. Transpl. Proc. 37, 496–499.
69 Moalic, Y., Blanchard, Y., Felix, H. and Jestin, A. (2006) Porcine endogenous retrovirus integration sites in the human genome: features in common with those of murine leukemia virus. J. Virol. 80, 10980–10988.
70 Hacein-Bey-Abina, S., von Kalle, C., Schmidt, M., Le Deist, F., Wulffraat, N., McIntyre, E., Radford, I., Villeval, J. L., Fraser, C. C., Cavazzano-Calvo, M. et al. (2003) A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. N. Engl. J. Med. 348, 255–256.
71 Hacein-Bey-Abina, S., von Kalle, C., Schmidt, M., McCormack, M. P., Wulffraat, N., Leboulch, P., Lim, A., Osborne, C. S., Pawliuk, R., Morillon, E. et al. (2003) LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science 302, 415–419.
72 Argaw, T., Colon-Moran, W. and Wilson, C. A. (2004) Limited infection without evidence of replication by porcine endogenous retrovirus in guinea pigs. J. Gen. Virol. 85, 15–19.
73 Specke, V., Tacke, S. J., Boller, K., Schwendemann, J. and Denner, J. (2001) Porcine endogenous retroviruses: in vitro host range and attempts to establish small animal models. J. Gen. Virol. 82, 837–844.
74 Deng, Y.-M., Tuch, B. E. and Rawlinson, W. D. (2000) Transmission of porcine endogenous retroviruses in severe combined immunodeficient mice xenotransplanted with fetal porcine pancreatic cells. Transplantation 70, 1010–1016.
75 Goto, M., Maeda, A., Elfman, L., Suling, K. M., Wood, J. C., Patience, C., Groth, C. G. and Wennberg, L. (2004) No transmission of porcine endogenous retrovirus after transplantation of adult porcine islets into diabetic nude mice and immunosuppressed rats. Xenotransplantation 11, 340–346.
76 Martina, Y., Kurian, S., Cherqui, S., Evanoff, G., Wilson, C. and Salomon, D. R. (2005) Pseudotyping of porcine endogenous retrovirus by xenotropic murine leukemia virus in a pig islet xenotransplantation model. Am. J. Transplant. 5, 1837–1847.
77 Kuddus, R. H., Metes, D. M., Nalesnik, M. A., Logar, A. J., Rao, A. S. and Fung, J. J. (2004) Porcine cell microchimerism but lack of productive porcine endogenous retrovirus (PERV) infection in naive and humanized SCID-beige mice treated with porcine peripheral blood mononuclear cells. Transpl. Immunol. 13, 15–24.
78 McKane, B. W., Ramachandran, S., Yang, J., Xu, X. C. and Mohanakumar, T. (2003) Xenoreactive anti-Galalpha(1,3)-Gal antibodies prevent porcine endogenous retrovirus infection of human in vivo. Hum. Immunol. 64, 708–717.
79 McKane, B. W., Ramachandran, S., Xu, X. C., Olack, B. J., Chapman, W. C. and Mohanakumar, T. (2004) Natural antibodies prevent in vivo transmission of porcine islet-derived endogenous retrovirus to human cells. Cell Transplant. 13, 137–143.
80 Martina, Y., Marcucci, K. T., Cherqui, S., Szabo, A., Drysdale, T., Drinivisan, U., Wilson, C. A., Patience, C. and Salomon, D. R. (2006) Mice transgenic for a human porcine endogenous retrovirus receptor are susceptible to productive viral infection. J. Virol. 80, 3135–3146.
81 Popp, S. K., Mann, D. A., Milburn, P. J., Gibbs, A. J., McCullagh, P. J., Wilson, J. D., Tonjes, R. R. and Simeonovic, C. J. (2007) Transient transmission of porcine endogenous retrovirus to fetal lambs after pig islet tissue xenotransplantation. Immunol. Cell Biol. 85, 238–248.
82 Switzer, W. M., Michler, R. E., Shanmugam, V., Matthews, A., Hussain, A. I., Wright, A., Sandstrom, P., Chapman, L. E., Weber, C., Safley, S. et al. (2001) Lack of cross-species transmission of porcine endogenous retrovirus infection to nonhuman primate recipients of porcine cells, tissues, or organs. Transplantation 71, 959–969.
83 Loss, M., Arends, H., Winkler, M., Przemeck, M., Steinhoff, G., Rensing, S., Kaup, F.-J., Hedrich, H. J., Winkler, M. E. and Martin, U. (2001) Analysis of potential porcine endogenous retrovirus (PERV) transmission in a whole-organ xenotransplantation model without interfering microchimerism. Transplant. Int. 14, 31–37.
84 Simon, A. R., Templin, C., Schroder, C., Laaff, G., Tessmann, R., Winkler, M. E., Tacke, S., Denner, J., Lapin, B., Chikobava, M. et al. (2003) No evidence for productive PERV infection of baboon cells in in vivo infection model. Ann. Transplant. 8, 24–34.
85 Moscoso, I., Hermida-Prieto, M., Manez, R., Lopez-Pelaez, E., Centeno, A., Diaz, T. M. and Domenech, N. (2005) Lack of cross-species transmission of porcine endogenous retrovirus in pig-to-baboon xenotransplantation with sustained depletion of anti-alphagal antibodies. Transplantation 79, 777–782.
86 Paradis, K., Langford, G., Long, Z., Heneine, W., Sandstrom, P., Switzer, W., Chapman, L., Lockey, C., Onions, D., The XEN 111 Study Group and Otto, E. (1999) Search for cross-species transmission of porcine endogenous retrovirus in patients treated with living pig tissue. Science 285, 1236–1241.
87 Heneine, W. , Tibell, A., Switzer, W. M., Sandstrom, P., Rosales, G. V., Mathews, A., Korsgren, O., Chapman, L. E., Folks, T. M., and Groth, C. G. (1998) No evidence of infection with porcine endogenous retrovirus in recipients of porcine islet-cell xenografts. Lancet 352, 695–699.
88 Matthews, A. L., Brown, J., Switzer, W., Folks, T. M., Heneine, W. and Sandstrom, P. A. (1999) Development and validation of a western immunoblot assay for detection of antibodies to porcine endogenous retrovirus. Transplantation 67, 939–943.
89 Xu, H., Okabe, J., Cui, C., Huang, L., Wei, Y. Y., Wan, H., Lei, Y., Logan, J. S., Levy, M. F., and Byrne, G. W. (2003) Serologic analysis of anti-porcine endogenous retroviruses immune responses in humans after ex vivo transgenic pig liver perfusion. ASAIO J. 2003, 407–416.
90 Patience, C., Patton, G. S., Takeuchi, Y., Weiss, R. A., McClure, M. O., Rydberg, L. and Breimer, M. E. (1998) No evidence of pig DNA or retroviral infection in patients with short-term extracorporeal connection to pig kidneys. Lancet 352, 699–701.
91 Gu, C., Wei, X., Wang, Y., Chen, Y., Liu, J., Wang, H., Sun, G. and Yi, D. (2008) No infection with porcine endogenous retrovirus in recipients of acellular porcine aortic valves: a two-year study. Xenotransplantation 15, 121–128.
92 Moza, A. K., Mertsching, H., Herden, T., Bader, A., Haverich, A. (2001) Heart valves from pigs and the porcine endogenous retrovirus: experimental and clinical data to assess the probability of porcine endogenous retrovrus infection in human subjects. J. Thorac. Cardiovasc. Surg. 121, 697–701.
93 Levy, M. F., Crippin, J., Sutton, S., Netto, G., McCormack, J., Curiel, T., Goldstein, R. M., Newman, J. T., Gonwa, T. A., Banchereau, J. et al. (2000) Liver allotransplantation after extracorporeal hepatic support with transgenic (hCD55/hCD59) porcine livers: clinical results and lack of pig-to-human transmission of the porcine endogenous retrovirus. Transplantation 69, 272–280.

94 Levy, M. F., Argaw, T., Wilson, C. A., Brooks, J., Sandstrom, P., Merks, H., Logan, J. and Klintmalm, G. (2007) No evidence of PERV infection in healthcare workers exposed to transgenic porcine liver extracorporeal support. Xenotransplantation 14, 309–315.
95 Nyberg, S. L., Hibbs, J. R., Hardin, J. A., Germer, J. J. and Persing, D. H. (1999) Transfer of porcine endogenous retrovirus across hollow fiber membranes. Transplantation 57, 1251–1255.
96 Pitkin, Z. and Mullon, C. (1999) Evidence of absence of porcine endogenous retrovirus (PERV) infection in patients treated with a bioartificial liver support system. Artificial Organ 32, 829–833.
97 Elliott, R. B., Escobar, L., Garkavenko, O., Croxon, M. C., Schroeder, B. A., McGregor, M., Ferguson, G., Beckman, N., and Fersuson, S. (2000) No evidence of infection with porcine endogenous retrovirus in recipients of encapsulated porcine islet xenografts. Cell Transplant. 9, 895–901.
98 Elliott, R. B., Escobar, L., Tan, P. L., Muzina, M., Zwain, S. and Buchanan, C. (2007) Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation. Xenotransplantation 14, 157–161.
99 Schumacher, J. M., Ellias, S. A., Palmer, E. P., Kott, H. S., Dinsmore, J., Dempsey, P. K., Fischman, A. J., Thomas, C., Feldman, R. G., Kassissieh, S. et al. (2000) Transplantation of embryonic porcine mesencephalic tissue in patients with PD. Neurology 54, 1042–1050.
100 Qari, S. H., Magre, S., Garcia-Lerma, J. G., Hussain, A. I., Takeuchi, Y., Patience, C., Weiss, R. A. and Heneine, W. (2001) Susceptibility of the porcine endogenous retrovirus to reverse transcriptase and protease inhibitors. J. Virol. 75, 1048–1053.
101 Powell, S. K., Gates, M. E., Langford, G., Gu, M. L., Lockey, C., Long, Z. and Otto, E. (2000) Antiretroviral agents inhibit infection of human cells by porcine endogenous retroviruses. Antimicrob. Agents Chemother. 44, 3432–3433.
102 Wilhelm, M., Fishman, J. A., Pontikis, R., Aubertin, A. M. and Wilhelm, F. X. (2002) Susceptibility of recombinant porcine endogenous retrovirus reverse transcriptase to nucleoside and non-nucleoside inhibitors. Cell. Mol. Life Sci. 59, 2184–2190.
103 Stephan, O., Schwendemann, J., Specke, V., Tacke, S. J., Boller, K. and Denner, J. (2001) Porcine endogenous retroviruses (PERVs): generation of specific antibodies, development of an immunoperoxidase assay (IPA) and inhibition by AZT. Xenotransplantation 8, 310–316.
104 Shi, M., Wang, X., De Clercq, E., Takao, S. and Baba, M. (2007) Selective inhibition of porcine endogenous retrovirus replication in human cells by acyclic nucleoside phosphonates. Antimicrob. Agents Chemother. 51, 2600–2604.
105 Dieckhoff, B., Karlas, A., Petersen, B., Kues, W. A., Kurth, R., Niemann, H. and Denner, J. (2007) Transmission of porcine endogenous retroviruses (PERVs): animal models and inhibition by RNA interference. Xenotransplantation 14, 372–373.
106 Miyagawa, S., Nakatsu, S., Nakagawa, T., Kondo, A., Matsunami, K., Hazama, K., Yamada, J., Tomonaga, K., Miyazawa, T., and Shirakura, R. (2005) Prevention of PERV infections in pig to human xenotransplantation by the RNA interference silences gene. J. Biochem. 137, 503–508.
107 Dieckhoff, B., Karlas, A., Hofmann, A., Kues, W. A., Petersen, B., Pfeifer, A., Niemann, H., Kurth, R., and Denner, J. (2007) Inhibition of porcine endogenous retroviruses (PERVs) in primary porcine cells by RNA interference using lentiviral vectors. Arch. Virol. 152, 629–634.
108 Dekker, S., Toussaint, W., Panayotou, G., de Wit, T., Visser, P., Grosveld, F. and Drabek, D. (2003) Intracellularly expressed single-domain antibody against p15 matrix protein prevents the production of porcine retroviruses. J. Virol. 77, 12132–12139.
109 Simeonovic, C. J., Ziolkowski, A. F., Popp, S. K., Milburn, P. J., Lynch, C. A., Hamilton, P., Harris, K., Brown, D. J., Bain, S. A., Wilson, J. D. et al. (2005) Porcine endogenous retrovirus encodes xenoantigens involved in porcine cellular xenograft rejection by mice. Transplantation 79, 1674–1682.
110 Ramachandran, S., Jaramillo, A., Xu, X. C., McKane, B. W., Chapman, W. C. and Mohanakumar, T. (2004) Human immune responses to porcine endogenous retrovirus-derived peptides presented naturally in the context of porcine and human major histocompatibility complex class I molecules: implications in xenotransplantation of porcine organs. Transplantation 77, 1580–1588.
111 Rother, R. P., Fodor, W. L., Springhorn, J. P., Birks, C. W., Setter, E., Sandrin, M. S., Squinto, S. P. and Rollins, S. A. (1995) A novel mechanism of retrovirus inactivation in human serum mediated by anti-alpha-galactosyl natural antibody. J. Exp. Med. 182, 1345–1355.
112 Takeuchi, Y., Porter, C. D., Strahan, K. M., Preece, A. F., Gustafsson, K., Cosset, F.-L., Weiss, R. A. and Collins, M. K.L. (1996) Sensitization of cells and retroviruses to human serum by (alph 1-3) galactosyltransferase. Nature 379, 85–88.
113 Takeuchi, Y., Liong, S.-H., Bieniasz, P. D., Jager, U., Porter, C. D., Friedmann, T., McClure, M. O. and Weiss, R. A. (1997) Sensitization of rhabdo-, lenti-, and spumaviruses to human serum by galactosyl(alpha1-3)galactosylation. J. Virol. 71, 6174–6178.
114 Chapman, L. E. and Wilson, C. A. (2003) Commentary: Implications of the advent of homozygous a l, 3-galactosyltransferase gene deficient pigs on transmission of infectious agents. Xenotransplantation 10, 287–288.
115 Byrne, G. W., McCurry, K. R., Martin, M. J., McClellan, S. M., Platt, J. L. and Logan, J. S. (1997) Transgenic pigs expressing human CD59 and decay-accelerating factor produce an intrinsic barrier to complement-mediated damage. Transplantation 63, 149–155.
116 Takefman, D. M., Spear, G. T., Saifuddin, M. and Wilson, C. A. (2002) Human CD59 incorporation into porcine endogenous retrovirus particles: implications for the use of transgenic pigs for xenotransplantation. J. Virol. 76, 1999–2002.
117 Magre, S., Takeuchi, Y., Langford, G., Richards, A., Patience, C. and Weiss, R. (2004) Reduced sensitivity to human serum inactivation of enveloped viruses produced by pig cells transgenic for human CD55 or deficient for the galactosyl-alpha(1–3) galactosyl epitope. J. Virol. 78, 5812–5819.
118 Lai, L., Kolber-Simonds, D., Park, K. W., Cheong, H. T., Greenstein, J. L., Im, G. S., Samuel, M., Bonk, A., Rieke, A., Day, B. N. et al. (2002) Production of alpha-1,3-galactosyltransferase knockout pigs by nuclear transfer cloning. Science 295, 1089–1092.
119 Cooper, D. K.C., Gollackner, B. and Sachs, D. H. (2002) Will the pig solve the transplantation backlog? Ann. Rev. Med. 53, 133–147.
120 Takeuchi, Y., Cosset, F.-L., Lachmann, P. J., Okada, H., Weiss, R. A. and Collins, M. K.L. (1994) Type C retrovirus inactivation by human complement is determined by both the viral genome and the producer cell. J. Virol. 68, 8001–8007.
121 Quinn, G., Wood, J. C., Ryan, D. J., Suling, K. M., Moran, K. M., Kolber-Simonds, D. L., Greenstin, J. L., Schuurmann, J. L., Hawley, R. J. and Patience, C. (2004) Porcine endogenous retrovirus transmission characteristics of galactose alpha1-3 galactose-deficient pig cells. J. Virol. 78, 5805–5811.
122 Hermida-Prieto, M., Domenech, N., Moscoso, I., Diaz, T., Ishii, J., Salomon, D. R. and Manez, R. (2007) Lack of cross-species transmission of porcine endogenous retrovirus (PERV) to transplant recipients and abattoir workers in contact with pigs. Transplantation 84, 548–550.