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Possible Transmission of Zoonoses in Xenotransplantation: Porcine Endogenous Retroviruses (PERVs) from an Immunological Point of View

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Introduction

Successful xenotransplantation of cells, tissues or solid organs would offer a much needed alternative for the increasing number of patients waiting for an allograft. A prerequisite for this ever becoming a reality is of course avoiding the xenograft being rejected, and from an immunological point of view, the most suitable donor source are found among the old world primates (e.g. apes and baboons). However, when also ethical, practical and physiological considerations are taken into account, a variety of factors are working in favour of the pig as the more suitable source species of choice. The pig is by far more adapted to large scale farming, and it will be more acceptable for the general public that a domesticated species otherwise used for meat production, is also a source for organ harvest. In addition, pigs have a short generation time, are available in controlled and homogenous breeds, and during the past decade elaborate methods for genetic manipulation of pigs have been developed. The size of the pig, in particular the minature swine, is also an advantage compared to the smaller primates. Last, but not least, the risk of carrying potential human pathogens is a major concern in xenotransplantation whatever species may be the source. However, this risk is considered greater with primates than with pigs (42).

Although most, if not all, the known infectous agents found in pigs can be eliminated, porcine endogenous retroviruses (PERVs) represent a unique concern. Like in all animals, the pig genome contains many loci coding for endogenous retroviruses (ERVs). These viruses are by definition inherited and may for that reason be particularly difficult to remove. In e.g. humans none of the ERVs have been shown to be replication competent, but in the case of pigs, it has been known for several decades that PERV particles are released from a variety of pig celllines (2, 19). Ever since it was recognized by Patience and co-workers in 1997 that PERV could also be transmitted to human cells *in vitro,* there has been a vivid debate regarding the safety of clinical xenotransplantation (29). It is recognized that some retroviruses, which cause harmless infections in their natural host, can lead to severe disease when transmitted to other species (42). With extensive use of pig tissue in transplantation the main concern has been that uncontrollable viral infections may be

created with a risk of jeopardizing the health of not only the patients, but in the worst case, the whole non-transplanted population. As a result, clinical trials have been strictly regulated in most countries, and today most health authorities favor a precautionary approach awaiting further research.

PERV release of pig cells and *in vitro* transmission to human cells

Three classes of replication-competent PERVs, gammaretroviruses PERV-A, -B and -C, have been identified in the pig genome (18, 39). Of these mainly PERV-A and -B seem to have tropism for human cells (39). Replication competent PERVs were first identified from immortalized pig cell lines. Since then, functional PERVs have been isolated from a variety of primary cell cultures including endothelial cells and PBMCs and found to be able to infect different human cells (21, 29, 33, 43, 44). The majority of these *in vitro* PERV transmission studies were conducted on immortalized human cell lines. Although such reports exist (23, 33), it appears that the infection of primary cell cultures is more difficult to achieve.

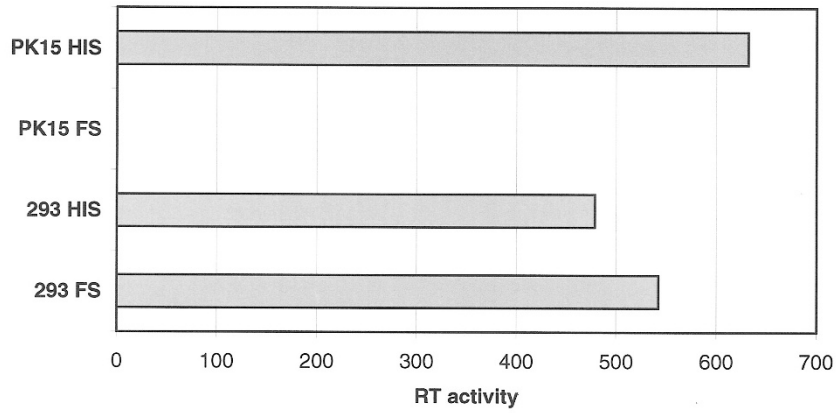
An analysis of PBMCs taken from a set of pigs from different breeds, indicates that the release of PERV particles varies, not only between breeds, but also between individuals within the same breed (37). In addition, PERV production may depend on the tissue selected for transplantation (8). Interestingly, there is a recent report of a strain of miniature swine that consistently does not transmit PERV to human cells *in vitro* (25). Depending on the pig cell type to be engrafted, different properties of the released PERVs might also be expected. In humans, an important way of inactivating retroviruses from non-primate mammals are through preformed antibodies directed against Galα(1,3)Gal sugars on the virus envelope (40). Adult porcine islets do not express these sugars, and as a consequence, any PERVs released from such cells would presumably escape this defence mechanism (35).

While it is recognized that the titre of PERV produced in pig cells is generally rather low compared to many other retroviruses (4), it is well known that the expression of many retroviruses can be induced by different chemical and biological agents, such as cytokines and steroid hormones (15, 17). It is therefore likely that in a transplantation situation the PERV production is influenced by the immunological response in the patient and possibly also directly by the immunosuppresive agents.

Infection of human cells *in vivo*

Because the pig is a domesticated species that has been living close to humans for several thousands years, it might be argued that if PERV transmission to humans is more than a theoretical possibility, it would already have taken place. However, clinical xenotransplantation represents a new setting where several of the natural immunological defence barriers against retroviruses are overcome. In a transplantation situation there are no mechanical barriers to infection by microbes, i.e. the skin and mucosal layers in the gastrointestinal tract and the lungs. Further, the various protocols needed to suppress the immune system, will also hamper the cellular and humoral immune defence. In addition, as discussed below, genetic manipulation of the donor tissue may further increase the risk of PERV particles escaping the immune system.

If ultimately PERV is transmitted to adjacent human cells, the production of virus particles will possibly be altered. Upon serial passaging in human cell lines, significant increases in viral titer and also production of PERVs with higher tropism for human cells *in vitro* have been demonstrated (33, 44). In addition, such viruses are adapted to escape some of the natu-



F i g u r e 1. PERVs produced in infected human cells escape complement mediated virolysis in human serum. Remaining intact PERV virions, as measured by RT activity, derived from a porcine cell line (PK15) or an infected human cell line (293) after incubation with fresh (FS) or heat-inactivated (HIS) human AB-serum in 30 min at 37°C.

ral immunological barriers against retroviruses (29) (Fig 1). In the worst case a primary infection could lead to an increased titer of virolysis resistant virions with high tropism for human cells, resulting in an escalating systemic infection in the patient.

Before PERV was ever considered a risk factor, many patients had already been exposed to pig tissue, mainly in trials evaluating the effect of different cell therapies. Since then, much effort has been made in developing reliable diagnostic tools able to detect the known human tropic PERVs, but also to discriminate between an actual PERV infection and merely remaining pig cells (microchimerism) in the recipient. Using such techniques, several retrospective studies have been undertaken investigating the possible virus transmission to such patients, but so far none of them have provided evidence for any PERV infection (12, 13, 14, 27, 28, 30). This is indeed reassuring data, but one has to bear in mind, that in most cases the pig cells in these patients survived only for a short period of time, immunosuppression was relatively mild and did not include any systemic complement inhibition, and in no case were the patients treated with grafts derived from genetically modified pigs.

PERV transmission in animal models

One matter complicating the study of *in vivo* transmission of PERV is that the virus is likely to have different tropisms depending on the species, making results from animal experiments difficult to translate to the clinical situation. PERV transmission into non-human primates, arguably the most relevant species, has only been reported from *in vitro* studies (5), but the *in vivo* data from the studies published to date suffer from many of the same limitations as the retrospective studies involving human subjects (20, 22, 36).

With respect to the potential large-scale rodent and other small animal models, their relevance to clinical xenotransplantion remains controversial. Unlike humans and other old world primates, rodents and other mammals express Gala(1,3)Gal sugars and therefore lack natural

antibodies directed against PERVs expressing this epitope. PERV receptors have been demonstrated in both rat and mouse cell lines (39). *In vivo* transmission to rats have so far not been described while recent papers reported PERV transmission to mouse and also implanted human cells following transplantation of porcine islets to athymic (*nu/nu*) or SCID mice (7, 11, 41). The possibility of the murine endogenous retrovirus influencing PERV infectivity in these animals, as well as the fact that these mice are incapable of mounting any cellular or humoral response, make these data difficult to relate to the pig-to-human situation. In addition, several of the commonly used mouse strains are known to have defective complement systems. Taken together, these circumstances propose that mice, immune deficient or not, may be more susceptible than humans to PERV infection. Viral load should also be a critical factor influencing transmission. However, rodent models may still be of importance when investigating the *in vivo* induction of PERV expression during inflammation, rejection or under the influence of different immunosuppressive agents.

PERV and genetically modified animals

In non-human primates as well as in humans, the most vigorous and rapid immunological reaction to vascularized pig xenografts is the hyperacute rejection (HAR) which occurs within minutes after transplantation. Although xenograft rejection can still be mediated by other more slow-acting immunological processes, preventing HAR would certainly be regarded as an important first step towards achieving long-term survival of vascularized xenografts. HAR is initiated by an immediate deposition of preformed xenoantibodies on the vessel walls of the engrafted organ. These antibodies, which are primarily directed against Galα(1,3)Gal sugar epitopes expressed on the endothelium of the porcine graft, will in turn cause classical pathway activation of the complement cascade and lead to extensive thrombosis and early graft failure. As a consequence, two main approaches have been applied to prevent HAR, those that inhibit activation of complement, and those that reduce the deposition of xenoreactive antibodies. In the case of non-vascularized xenografts, such as pancreatic islets, hepatocytes or neural cells, the need for preventing HAR will be of less importance. However, in the case of islets which are infused into the portal vein, protection against complement activation in the recipient still appears to be neccessary (3).

Substantial effort has been made in developing genetically modified pigs, and some progress has been made using transgenic pigs expressing high levels of human regulators of complement activation (RCAs), including DAF, MCP and CD59 on their endothelium (1, 6, 9). The cloning of GGTA1-knockout pigs lacking the expression of Galα(1,3)Gal sugar, has recently been reported and has given new hope with respect to eliminating HAR as the major obstacle to successful clinical xenotransplantation (16). Unfortunately, such measures taken in preventing graft rejection will most probably eliminate some of the natural immunological barriers against retroviruses (40). When budding from host cell plasma membranes, the PERV particles incorporate part of the cell membrane, including any membrane-associated proteins and Galα(1,3)Gal-positive glycoproteins. As a result, PERV particles deriving from transgenic pigs expressing RCAs, will have an innate defence against complement-mediated lysis. In parallell, viruses deriving from pigs lacking the Galα(1,3)Gal epitope will not be targets for the preformed Galα(1,3)Gal reactive natural antibodies present in the human blood. To what extent such modifications are sufficient to create more infectious PERV viruses is uncertain. In a recent study where human CD59 was incorpo-

rated into PERV, it was demonstrated that, while complement-mediated lysis of these particles was indeed reduced, the same viruses were incapable of infecting human cells after incubation with human serum (38). Results from experiments on PERVs isolated from the Galα(1,3)Gal knock-outs, are expected in the near future.

In other words the PERVs produced in such genetically modified pigs would share many of the features with those that are produced in a human cell, and will theoretically have a much higher viability in a human recipient. It will be neccessary to evaluate every genetically modified pig strain independently, since the outcome of combined genetic modifications with regard to PERV infectivity will be impossible to predict.

How to avoid PERV?

It is conceivable that the greatest risk of PERV infection will be at the time of transplantation, when inflammation and other immunological processes are likely to increase retroviral transcription, and the induction therapies suppressing complement activation and the humoral defence will reduce PERV virolysis. In this perspective, antiviral therapy at an initial stage in clinical xenotransplantation could be a valuable strategy to reduce the risk of PERV transmission. However, with the exception of azidothymidine (AZT), none of the RT- and protease inhibitors used in the clinic today have been shown to be effective against PERV (31).

Ultimately, the best option for eliminating the risk of PERV transmission would be to create pig breeds devoid of replication competent viruses. Initially, assessments argued that this would be impossible. With increasing knowledge of the complexity of PERV loci in the pig genome it has become clear that the majority of loci include deletions and mutations rendering them uncapable of encoding replicant-competent human-tropic viruses. Although the pig genome remains to be fully characterized, and unknown functional PERV loci may still be discovered, today only a handful of PERV loci have been identified that are able to produce replication competent viruses (10, 18, 24). It may in fact prove possible by means of selective breeding and knock-out techniques to eliminate these loci, and this would no doubt dramatically decrease the risk of PERV transmission. It is, however, still to early to determine whether this can be achieved.

An additional risk, albeit low, that may prove difficult to avoid with current technologies, is the potential that two defective PERV RNAs may be packaged in a particle, and recombination may lead to the creation of a functional retrovirus. Thus, even when known functional PERV loci have been removed, new infectious PERVs could be created by complementation and recombination between two defective genomes (26, 32, 34). As an interesting comparison, a similar risk is taken daily by researchers and animal caretakers who work with immune deficient mice implanted with human or porcine tissue. In these animals such recombination between defective endogenous retroviruses from the different species could also, theoretically, create an infectious new type of virus.

Pathogenicity of PERV? Risk assessment

The ultimate question concerning the potential pathogenicity of PERVs is whether PERV transmission to human cells would pose a real threat to the health of the graft recipient or even the general public. Although PERVs have not been shown to be pathogenic in pigs, it is at this stage very difficult to estimate their potential effects in humans. The only qualified prediction would be that their mere ability to infect and replicate in human cells could lead to oncogenicity, especially in heavily immunosuppressed patients.

However, the risk-benefit estimate will be in favor of xenotransplantation from the point of view of a patient with an end-stage organ disease. Highly sensitive methods for detection of replication-competent PERVs are available that could be used to carefully monitor xenotransplant recipients. The main issue to be addressed is whether PERV poses a potential threat to the non-immunosuppressed population, and in that perspective the risk of PERV transmission will be considerably lower.

Accumulated evidence from basic research over the recent years indicates that PERV may not pose as big a threat as was initially feared. Since succesful xenotransplantation would provide a tremendous advance in the therapy of end-stage organ failure and cell replacement, there is an apparent risk that too much caution in the long run, delaying important findings, will deprive the patients of an attractive alternative. Since animal models have obvious limitations, it will sooner or later be neccessary to proceed to clinical trials. Further studies of the infectious potential of PERVs produced from genetically modified pigs are warranted as this is likely to be the type of viruses that xenograft recipients will be exposed to. There is reason to believe that the present precautionary standpoint in the PERV issue may be changed towards a more open view, that in the end will allow for new carefully monitored clinical trials in the future in Europe as already allowed in the United States.

References

1. *Adams DH, Kadner A, Chen RH, Farivar RS:* Human membrane cofactor protein (MCP, CD 46) protects transgenic pig hearts from hyperacute rejection in primates. Xenotransplantation 2001, 36.
2. *Armstrong JA, Porterfield JS, De Madrid AT:* C-type virus particles in pig kidney cell lines. J. Gen. Virol. 1971, *10,* 195-198.
3. *Bennet W, Sundberg B, Lundgren T, Tibell A, Groth CG, Richards A, White DJ, Elgue G, Larsson R, Nilsson B, Korsgren O:* Damage to porcine islets of Langerhans after exposure to human blood in vitro, or after intraportal transplantation to cynomologus monkeys: protective effects of sCR1 and heparin. Transplantation 2000, *69,* 711-719.
4. *Blusch JH, Patience C, Martin U:* Pig endogenous retroviruses and xenotransplantation. Xenotransplantation 2002, *9,* 242-251.
5. *Blusch JH, Patience C, Takeuchi Y, Templin C, Roos C, Von Der Helm K, Steinhoff G, Martin U:* Infection of nonhuman primate cells by pig endogenous retrovirus. J. Virol. 2000, *74,* 7687-7690.
6. *Byrne GW, McCurry KR, Martin MJ, McClellan SM, Platt JL, Logan JS:* Transgenic pigs expressing human CD59 and decay-accelerating factor produce an intrinsic barrier to complement-mediated damage. Transplantation 1997, *63,* 149-155.
7. *Clemenceau B, Jegou D, Martignat L, Sai P:* PERV infection of mouse and human cells by SPF pig islets in nude mice. Diabetologia 2002, *45,* 914-923.
8. *Clemenceau B, Lalain S, Martignat L, Sai P:* Porcine endogenous retroviral mRNAs in pancreas and a panel of tissues from specific pathogen-free pigs. Diabetes Metab. 1999, *25,* 518-525.
9. *Cozzi E, Tucker AW, Langford GA, Pino-Chavez G, Wright L, O'Connell MJ, Young VJ, Lancaster R, McLaughlin M, Hunt K, Bordin MC, White DJ:* Characterization of pigs transgenic for human decay-accelerating factor. Transplantation 1997, *64,* 1383-1392.
10. *Czauderna F, Fischer N, Boller K, Kurth R, Tonjes RR:* Establishment and characterization of molecular clones of porcine endogenous retroviruses replicating on human cells. J. Virol. 2000, *74,* 4028-4038.
11. *Deng YM, Tuch BE, Rawlinson WD.* Transmission of porcine endogenous retroviruses in severe combined immunodeficient mice xenotransplanted with fetal porcine pancreatic cells. Transplantation 2000, *70,* 1010-1016.
12. *Dinsmore JH, Manhart C, Raineri R, Jacoby DB, Moore A:* No evidence for infection of human cells with porcine endogenous retrovirus (PERV) after exposure to porcine fetal neuronal cells. Transplantation 2000, *70,* 1382-1389.

13. *Elliott RB, Escobar L, Garkavenko O, Croxson MC, Schroeder BA, McGregor M, Ferguson G, Beckman N, Ferguson S:* No evidence of infection with porcine endogenous retrovirus in recipients of encapsulated porcine islet xenografts. Cell Transplant. 2000, *9,* 895-901.
14. *Heneine W, Tibell A, Switzer WM, Sandstrom P, Rosales GV, Mathews A, Korsgren O, Chapman LE, Folks TM, Groth CG:* No evidence of infection with porcine endogenous retrovirus in recipients of porcine islet-cell xenografts. Lancet 1998, *352,* 695-699.
15. *Khan AS, Muller J, Sears JF:* Early detection of endogenous retroviruses in chemically induced mouse cells. Virus Res. 2001, *79,* 39-45.
16. *Lai L, Kolber-Simonds D, Park KW, Cheong HT, Greenstein JL, Im GS, Samuel M, Bonk A, Rieke A, Day BN, Murphy CN, Carter DB, Hawley RJ, Prather RS:* Production of alpha-1,3-galactosyltransferase knockout pigs by nuclear transfer cloning. Science 2002, *295,* 1089-1092.
17. *Larsson E, Venables PJ, Andersson AC, Fan W, Rigby S, Botling J, Oberg F, Cohen M, Nilsson K:* Expression of the endogenous retrovirus ERV3 (HERV-R) during induced monocytic differentiation in the U-937 cell line. Int. J. Cancer 1996, *67,* 451-456.
18. *Le Tissier P, Stoye JP, Takeuchi Y, Patience C, Weiss RA:* Two sets of human-tropic pig retrovirus. Nature 1997, *389,* 681-682.
19. *Lieber MM, Sherr CJ, Benveniste RE, Todaro GJ:* Biologic and immunologic properties of porcine type C viruses. Virology 1975, 66, 616-619.
20. *Loss M, Arends H, Winkler M, Przemeck M, Steinhoff G, Rensing S, Kaup FJ, Hedrich HJ, Winkler ME, Martin U:* Analysis of potential porcine endogenous retrovirus (PERV) transmission in a whole-organ xenotransplantation model without interfering microchimerism. Transpl. Int. 2001, *14,* 31-37.
21. *Martin U, Kiessig V, Blusch JH, Haverich A, von der Helm K, Herden T, Steinhoff G:* Expression of pig endogenous retrovirus by primary porcine endothelial cells and infection of human cells. Lancet 1998, *352,* 692-694.
22. *Martin U, Steinhoff G, Kiessig V, Chikobava M, Anssar M, Morschheuser T, Lapin B, Haverich A:* Porcine endogenous retrovirus (PERV) was not transmitted from transplanted porcine endothelial cells to baboons in vivo. Transpl. Int. 1998, *11,* 247-251.
23. *Martin U, Winkler ME, Id M, Radeke H, Arseniev L, Takeuchi Y, Simon AR, Patience C, Haverich A, Steinhoff G:* Productive infection of primary human endothelial cells by pig endogenous retrovirus (PERV). Xenotransplantation 2000, *7,* 138-142.
24. *Niebert M, Rogel-Gaillard C, Chardon P, Tonjes RR:* Characterization of chromosomally assigned replication-competent gamma porcine endogenous retroviruses derived from a large white pig and expression in human cells. J. Virol. 2002, *76,* 2714-2720.
25. *Oldmixon BA, Wood JC, Ericsson TA, Wilson CA, White-Scharf ME, Andersson G, Greenstein JL, Schuurman HJ, Patience C:* Porcine endogenous retrovirus transmission characteristics of an inbred herd of miniature Swine. J. Virol. 2002, *76,* 3045-3048.
26. *Overbaugh J, Riedel N, Hoover EA, Mullins JI:* Transduction of endogenous envelope genes by feline leukaemia virus in vitro. Nature (Lond.) 1988, *332,* 731-734.
27. *Paradis K, Langford G, Long Z, Heneine W, Sandstrom P, Switzer WM, Chapman LE, Lockey C, Onions D, Otto E:* Search for cross-species transmission of porcine endogenous retrovirus in patients treated with living pig tissue. Science 1999, *285,* 1236-1241.
28. *Patience C, Patton GS, Takeuchi Y, Weiss RA, McClure MO, Rydberg L, Breimer ME:* No evidence of pig DNA or retroviral infection in patients with short- term extracorporeal connection to pig kidneys. Lancet 1998, *352,* 699-701.
29. *Patience C, Takeuchi Y, Weiss RA:* Infection of human cells by an endogenous retrovirus of pigs. Nat Med 1997, *3,* 282-286.
30. *Pitkin Z, Mullon C:* Evidence of absence of porcine endogenous retrovirus (PERV) infection in patients treated with a bioartificial liver support system. Artif. Organs 1999, *23,* 829-833.
31. *Qari SH, Magre S, Garcia-Lerma JG, Hussain AI, Takeuchi Y, Patience C, Weiss RA, Heneine W:* Susceptibility of the porcine endogenous retrovirus to reverse transcriptase and protease inhibitors. J. Virol. 2001, *75,* 1048-1053.
32. *Rowe WP:* Deformed whiskers in mice infected with certain exogenous murine leukemia viruses. Science 1983, *221,* 562-564.
33. *Specke V, Tacke SJ, Boller K, Schwendemann J, Denner J:* Porcine endogenous retroviruses: in vitro host range and attempts to establish small animal models. J. Gen. Virol. 2001, *82,* 837-844.

34. *Stewart MA, Warnock M, Wheeler A, Wilkie N, Mullins JI, Onions DE, Neil JC:* Nucleotide sequences of a feline leukemia virus subgroup A envelope gene and long terminal repeat and evidence for the recombinational origin of subgroup B viruses. J. Virol. 1986, *58,* 825-834.
35. *Strokan V, Bennet W, Molne J, Korsgren O, Breimer ME:* Distribution of the Galalpha1-3Gal antigen in cultured adult and fetal porcine pancreatic islet cells: an immunoelectron microscopic study. Transplantation 2000, *70,* 846-851.
36. *Switzer WM, Michler RE, Shanmugam V, Matthews A, Hussain AI, Wright A, Sandstrom P, Chapman LE, Weber C, Safley S, Denny RR, Navarro A, Evans V, Norin AJ, Kwiatkowski P, Heneine W:* Lack of cross-species transmission of porcine endogenous retrovirus infection to nonhuman primate recipients of porcine cells, tissues, or organs. Transplantation 2001, *71,* 959-965.
37. *Tacke SJ, Kurth R, Denner J:* Porcine endogenous retroviruses inhibit human immune cell function: risk for xenotransplantation? Virology 2000, *268,* 87-93.
38. *Takefman DM, Spear GT, Saifuddin M, Wilson CA:* Human CD59 Incorporation into porcine endogenous retrovirus particles: implications for the use of transgenic pigs for xenotransplantation. J. Virol. 2002, *76,* 1999-2002.
39. *Takeuchi Y, Patience C, Magre S, Weiss RA, Banerjee PT, Le Tissier P, Stoye JP:* Host range and interference studies of three classes of pig endogenous retrovirus. J. Virol. 1998, *72,* 9986-9991.
40. *Takeuchi Y, Porter CD, Strahan KM, Preece AF, Gustafsson K, Cosset FL, Weiss RA, Collins MK:* Sensitization of cells and retroviruses to human serum by (alpha 1-3) galactosyltransferase. Nature (Lond.) 1996, *379,* 85-88.
41. *van der Laan LJ, Lockey C, Griffeth BC, Frasier FS, Wilson CA, Onions DE, et al.* Infection by porcine endogenous retrovirus after islet xenotransplantation in SCID mice. Nature (Lond.) 2000, *407,* 90-94.
42. *Weiss RA:* Retroviral zoonoses. Nature Med. 1998, *4,* 391-392.
43. *Wilson CA, Wong S, Muller J, Davidson CE, Rose TM, Burd P:* Type C retrovirus released from porcine primary peripheral blood mononuclear cells infects human cells. J. Virol. 1998, *72,* 3082-3087.
44. *Wilson CA, Wong S, VanBrocklin M, Federspiel MJ:* Extended analysis of the in vitro tropism of porcine endogenous retrovirus. J. Virol. 2000, *74,* 49-56.