Endogenous retroviruses and xenotransplantation

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Introduction

The hallmark of the retrovirus family is its replication strategy, which includes reverse transcription of virion RNA into linear double-stranded DNA and subsequent integration of this DNA into the host genome. Retroviral RNA is linear, single-stranded, non-segmented and approximately 7 to 12 kb in length. Based on their genome organisation, retroviruses are divided into two categories: simple and complex (Figure 1) [19].

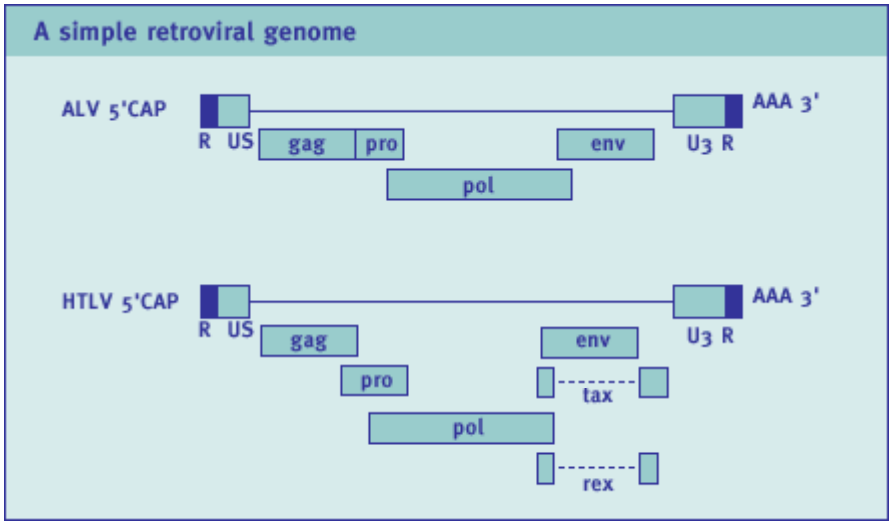

Figure 1. The upper portion of the figure represents a simple retroviral genome, that of avian leucosis virus (ALV). The genetic map of the virus reveals four major coding regions: *gag*, *pro*, *pol*, and *env*. Different open reading frames are indicated by vertical displacement of the coding region. The *pro* gene is encoded in the *gag* reading frame. The terminal non-coding sequences include two direct repeats (R), a U5 (5' unique) and a U3 (3' unique) sequence. The lower portion of the figure is a complex retroviral genome. The genetic map of human T-cell leukemia virus (HTLV) contains *tax* and *rex* encoded in regions joined by RNA splicing (dashed line). In this case, *gag*, *pro* and *pol* are all in different reading frames.

In general, simple retroviruses carry four coding domains: 1) *gag*, which encodes the internal virion proteins that form the capsid and nucleoprotein structures; 2) *pro*, which directs the synthesis of virion protease; 3) *pol*, contains genomic information of reverse transcriptase and integrase; and 4) *env*, which directs the synthesis of viral envelope proteins that include surface (SU) and transmembrane (TM) components (Figure 2) [19].

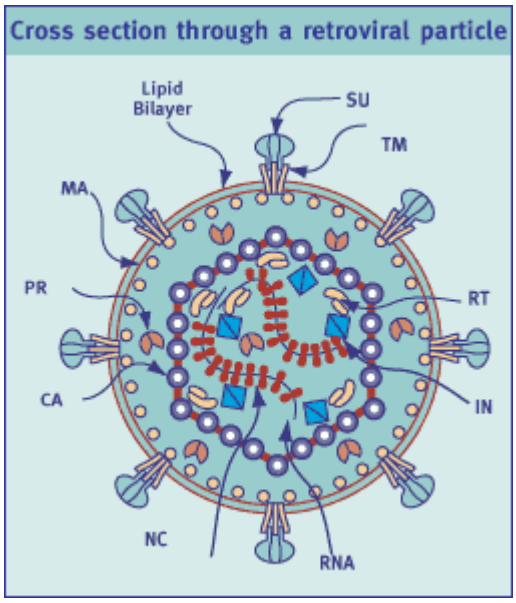

Figure 2. Schematic cross-section of a retroviral particle. The viral envelope is formed by a cell-derived lipid bilayer, into which proteins encoded by the *env* region of the viral genome are inserted. These proteins consist of the transmembrane (TM) and the surface (SU) components linked together by disulfide bonds. The gag region of the viral genome encodes internal non-glycosylated structural proteins: the matrix (MA) protein, capsid (CA) protein and nucleocapsid (NC) protein. Major products of the *pol*-encoding region are reverse transcriptase (RT) and integrase (IN). Protease (PR) is derived from the pro gene.

Besides these four domains, complex retroviruses usually contain coding information for regulatory proteins of virus expression, like additional *rex* and *tax* genes of HTLV (Figure 1). Based upon evolutionary distance, retroviruses are divided into seven groups (or genera) (Table 1). Lentivirus, HTLV-BLV and spumavirus are complex viruses; the other groups, which represent all oncogenic viruses except the HTLV-BLV group, belong to the simple virus category [19].

Classification of retroviruses			
Genus	**Example**	**Virion morphology**	**Genome**
Avian sarcoma and leucosis viral group	Rous sarcoma virus	central spherical core "C particles"	simple
Mammalian B-type viral group	Mouse mammary tumour virus	eccentric spherical core "B particles"	simple
Murine leukemia related viral group	Moloney murine leukemia virus	central spherical core "C particle"	simple
Human T-cell leukemia-bovine leukemia viral group	Human T-cell leukemia virus	Central spherical core	complex
D-type viral group	Mason-Pfizer monkey virus	cylindrical core "D particle"	simple
Lentiviruses	Human immuno-deficiency virus	cone-shaped core	complex
Spumaviruses	Human foamy virus	central spherical core	complex

Table 1. Classification of Retroviruses [19]

Characteristics of endogenous retroviruses

One unique characteristic of retroviral life is the integration of viral genome into the chromosome of host cells, forming the provirus. Once integrated into a host germ cell chromosome, exogenous retrovirus can be inherited in Mendelian fashion and persist for multiple generations, and are known as endogenous retroviruses. So far, endogenous retroviruses have been found in all vertebrate genomes investigated, and they all belong to the simple retrovirus category. No endogenous lenti-, spuma-, or HTLV-like viruses have been identified. In the human genome, endogenous retroviruses make up as much as one per cent of genomic DNA, while in other mammalian species they can constitute between 0.5 and 2 per cent of the host genome. It would seem that the products of reverse transcription have played a major part in shaping the eukaryotic genome [16].

Dozens of complete endogenous retroviral sequences have been characterised that could potentially encode functional retroviral proteins, although most are interrupted by stop mutations and therefore cannot be translated. Some endogenous retroviruses, however, are not expressed. Indeed, retroviral mRNA and protein can be expressed and viral particles formed in some tissues in several mammalian species, for instance the human endogenous retrovirus K family (HERV-

K) in man [72], baboon endogenous virus (BaEV) in baboons, porcine endogenous retrovirus (PERV) in pigs and RD114 in cats [49, 50, 62]. Thus, the viral transcription, expression and particle formation arising from a retrovirus transmitted through the germ-line could be defined as a semi-endogenous phase. In man and most other mammalian species, endogenous retroviral particles cannot re-infect cells, although some inbred mice strains contain endogenous retroviruses that can re-infect their own cells.

Possible biological functions of retroviruses

Owing to the fact that endogenous retroviral sequences constitute 0.5 to 2 per cent of the mammalian host genome, only a small proportion have been sequenced and analysed in detail. Based on those few results, most retroviral elements are generally considered to be the by-product of host evolution and the 'junk' DNA because they contain no open reading frame capable of encoding protein. Consequently, these viral sequences may have no functional effect on the host and instead only reflect the evolutionary track of their host [16].

Host protection against superinfection by exogenous virus

Apart from the unexpressed 'junk' DNA, some endogenous retroviral genes – at least part of the whole genes – can still be expressed. It is hypothesised that viral proteins synthesised within cells (i.e. endogenous virus) can bind to the cell surface molecules that are recognised by equivalent but exogenous virus, thereby blocking viral binding. It may be assumed, therefore, that certain semi-endogenous retroviruses, in which retroviral proteins are expressed and viral particles formed, can prevent the host from becoming infected by exogenous retroviruses. In this way, endogenous retroviruses provide a survival advantage to their host by promoting resistance to superinfection by their exogenous counterparts. This may explain why none of the known human infectious retroviruses have endogenous counterparts in the human genome [16, 36, 84].

Evolutionary engines for their hosts

When endogenous retroviral genes are expressed and give rise to viral particle formation, even though their infection titres are generally much lower than those of exogenous retroviruses, endogenous retroviruses can reintegrate into a new site in the host genome. If this occurs in a strategic location in the genome, it would have a significant effect in changing the

host genome type; thus endogenous retroviruses could serve as engines of host evolution [16, 36, 84].

Protecting the human embryo against maternal immunological rejection

Generally, large amounts of CpG dinucleotides reside in the proviral genome of endogenous retroviruses. These regions are the target of mammalian DNA methylation and thus in the mammalian genome about 70 per cent of all CpG dinucleotides are methylated [33], which means that expression of endogenous retroviruses is strongly suppressed and their spread through the host genome is limited. However, when DNA methylation is decreased – during embryo development, for example – some endogenous retroviruses could be reactivated [66]. In man, endogenous retroviral expression is highest in the placenta and in a recent study placental expression of a HERV-W derived Env protein was linked with cell fusion of the human trophoblast. By binding to the specific receptor on an adjacent cell surface, HERV-W Env protein induces trophoblast cell fusion and assists in the formation of the syncytiotrophoblast, a one-cell-deep continuous structure on the outside of the placenta at the feto-maternal interface that facilitates exchange of nutrients and waste products between mother and fetus. It is speculated that the immunosuppressive domain present in the TM domain of the HERV-W Env protein may help prevent maternal immunological rejection of the semi-allogeneic fetus [46, 66].

Antigen in autoimmune responses

In contrast to the benefits a host may gain from endogenous retroviruses, their expression can also contribute to the pathogenesis of autoimmune disease. If endogenous retroviral expression occurs in the lymphoid generative organ during T- and B- cell development, then the immune system may destroy any developing T- or B- cells that react with self-antigens, including endogenous retroviral proteins expressed in other tissues. Thus, the immune system is tolerant to endogenous retroviral expression. However, if expression occurs only in a specific tissue and not in lymphoid tissue, or if expression is delayed in lymphoid tissue, or there is cross-reaction with a protein in an infectious agent, the self-tolerance of the immune system may become defective. Thus, an autoimmune response to an endogenous retroviral protein could occur, which could lead to immunological rejection of the tissue expressing the viral protein. Antibodies against human

endogenous retroviral proteins have been observed in many patients with systemic lupus erythematosus [32, 74].

Endogenous retroviruses and xenotransplantation

Endogenous retroviruses present a serious threat to the application of certain medical biotechnologies. For example, both retrovirus-based gene therapy and retroviral vaccines that use attenuated or inactivated virus, provide the chance for recombination between endogenous retroviruses in the host genome and the retroviral elements introduced by the treatment. Recently, xenotransplantation – the transfer of organs, tissues, or cells from one species into another (usually from animals into humans) – has become one of the most exciting and controversial fields in medical biotechnology [7, 9, 64].

History of xenotransplantation

The transplantation of organs such as hearts or kidneys from one individual to another has become a highly successful mode of therapy, so much so that the demand for human donor organs far exceeds their supply. According to reports of the FDA (US Food and Drug Administration) and CBER (Center for Biologics Evaluation and Research), ten patients die every day in the United States because organs needed by these patients are not available. The United Network for Organ Sharing (UNOS) claim that the supply of organs remained static throughout the 1990s, at around 5,000 donors per year, while demand rose from about 20,000 patients in 1990 to more than 60,000 waiting for organs in 1998 [2, 3]. This huge demand for organs has driven transplantation researchers to focus on the use of xenografts as donor organs.

Xenografts are divided into two categories: concordant, e.g. between non-human primate and human, and discordant, e.g. from pig to human [9, 64]. In cases of discordant xenotransplantation, an untreated recipient rejects the immediately vascularised organ in one to two hours [56]. This hyperacute rejection (HAR) is uncommon in cases of concordant xenotransplantation, but delayed xenograft rejection (DXR) occurs instead [12, 15].

The desire to create a large pool of donors for patients requiring transplantation has caused enormous interest in clinical xenotransplantation and scientific research in this field in recent years. At the same time, xenotransplantation-related topics have raised concerns in both investigators and society as a whole. These include: (a) the physiological functional compatibility

of xenografts; (b) xenotransplantation associated HAR and DXR; and (c) xenozoonoses, infections associated with xenotransplantation that are caused by organisms transmitted from the organ donor [47].

Baboons and xenotransplantation

Due to the high risk of HAR occurring in patients receiving discordant xenografts and the absence of efficient immunosuppression drugs to suppress the rejection, most xenotransplantations performed between the early 1960s and 1990s used concordant xenografts (Table 2). During this period, about 30 organ transfers were performed, including kidney, heart and liver from chimpanzee and baboon donors to human recipients [60]. The most successful of these was a chimpanzee-to-human kidney transplantation, performed in 1964, in which the patient survived for nine months with normal renal function before dying from the side effects of immunosuppression. In addition, a baboon heart kept an infant alive for four weeks [13, 64]. These achievements gave the first indication that long-term survival and function of a concordant xenograft could be achieved in humans, despite the relatively inefficient immunosuppression therapy available at that time. Since the chimpanzee is unlikely to be accepted as a donor because of the emotional ties associated with using an animal phylogenetically so close to humans – especially one whose numbers are extremely limited – the baboon was the most preferable and popular donor species. However, there are two major drawbacks to using baboon organs as xenografts have driven scientists to look for alternative species as donors, for example the pig, since 1990s.

Experience in clinical xenotransplantation				
Donor	Organ	Outcome	Number of Cases	Year
Chimpanzee	Kidney	< 9 months	12	1964
Monkey	Kidney	10 days	1	1964
Baboon	Kidney	4.5 days	1	1964
Baboon	Kidney	< 2 months	6	1964
Chimpanzee	Heart	< 1 day	1	1964
Chimpanzee	Liver	< 14 days	3	1969-1974
Baboon	Heart	< 1 day	1	1977
Chimpanzee	Heart	< 4 days	1	1977
Baboon	Heart	4 weeks	1	1985
Baboon	Liver	70 days, 26 days	2	1993

Table 2. Experience in clinical xenotransplantation 1964-1993 [64]

Barriers against baboons as xenotransplantation donors The first major barrier to using baboons as xenotransplantation donors is the physiological incompatibility between baboon and human organs. In general, baboon organs are too small for human adults [13]. But besides the incompatibility of organ size, studies have indicated that some physiological functions of transplanted baboon organs will remain intact while others may not. For example, recipients who have received baboon livers have insufficient levels of serum cholesterol (but consistent with normal levels for baboons) and remarkably low levels of serum uric acid, because the baboon liver does not produce uric acid as the human liver does [63].

The second barrier to the use of baboons for xenotransplantation is xenozoonoses, infections transferred from animal donors to human recipients by xenotransplantation. There are three classes of highly risky organisms that can potentially cause xenozoonoses: bacteria, parasites and viruses, especially retroviruses. Today, modern biological techniques can be employed to screen and breed potential animal donors within specific pathogen-free (SPF) environments to eliminate the risk of most bacteria, parasites and viruses. However, it is almost impossible to avoid transmission of retroviruses, especially endogenous retroviruses that already exist in the host germ line with multiple copies [7, 18].

Retroviruses in the baboon genome

Cross-species infection by retroviruses is considered possible. In the baboon, simian immunodeficiency virus (SIV), which is similar to human immunodeficiency virus type 2 (HIV-2), is probably non-pathogenic, but it can infect human peripheral blood lymphocytes *in vitro* [58]. Results from virus monitoring programmes for patients who received baboon livers showed that baboon cells could be detected in several of their organs, including lymph nodes, suggesting that cells from the xenograft were circulating systemically. One of these baboon liver recipients, whose survival was shorter than 70 days, showed antibody response to simian foamy virus three weeks after transplantation [5, 79].

In addition to the many exogenous retroviruses able to infect baboons, there is one semi-endogenous retrovirus present in the baboon genome. This is BaEV, one of the best-characterised endogenous retroviruses in Old World monkeys. In 1974, BaEV was isolated from baboon placenta by co-cultivation with permissive cell lines, including a human rhabdomyosarcoma cell line. Electron micrographs showed that the BaEV particle belonged to the C-type retroviruses. By using DNA hybridisation, researchers found that BaEV viral genes were present in the baboon genome and could be continually expressed in baboon placenta but not in liver [14, 72]. In 1987, the complete nucleotide sequence of BaEV was obtained [20, 21, 30]. Gene analysis results showed that the BaEV proviral genome is chimeric, containing type-C *gag* and *pol* genes and a type-D *env* gene, suggesting that BaEV is the result of a recombination event between two retroviruses in the past. A similar chimeric genome construction is also present in RD114, a feline semi-endogenous retrovirus [61]. Recently, the cellular receptor for BaEV virus entry, the sodium-dependent neutral amino acid transporter type 1 (ASCT1), was identified. This receptor is also present on the surface of human cells, giving BaEV the potential to infect human cells during baboon-to-human xenotransplantation [39, 59, 68]. Results of viral distribution studies showing that BaEV is present only in the genomes of the *Papionini* tribe and in African green monkeys *(Cercopithecus aethiops*; Table 3) [22, 31, 40], indicated that BaEV was not inherited from a common ancestor of all African monkeys [75]. The baboon genome contains 10 to 30 copies of BaEV, with very little sequence variation, implying that BaEV

has a relatively short evolutionary history [76, 77]. Two ancestors of BaEV, simian endogenous retrovirus (SERV), a type-D virus, and papio cynocephalus endogenous retrovirus (PcEV), which belongs to type-C viruses, have been identified in the baboon genome [37, 38, 81]. Results from phylogenetic analyses based upon nucleotide sequences have indicated that SERV is at least 9 million years old. It is the eldest of the three related retroviruses and contributed its *env* gene to BaEV; PcEV is younger than SERV and its type-C *gag* and *pol* genes were obtained by BaEV.

Classification of Old World monkey, ape, and human							
Order	PRIMATES						
Sub-order	ANTHROPOIDEA (anthropoids)						
Infra-order	CATARRHINI						
Super-family	Hominoidea (Apes and humans)			Cercopithecoidea (Old World monkeys)			
Family	Hominidae	Pongidae	Hylobatidae	Cercopithecoidea (Old World monkeys)			
Sub-family				Cercopithecinae		Colobinae	
Tribe				Cercopithecini	Papionini		
Genus	Homo (human)	Pan (chimpanzees) Gorilla (gorilla) Pongo (orangutan)	Hylobates (gibbons)	Cercopithecus Erythrocebus Miopithecus	Macaca (macaques) Cercocebus Lophocebus Papio (baboons) Theropithecus (gelada) Mandrillus	Prebytis (langurs) Rhinopithecus Nasalis Colobus Procolobus	

Table 3. Classification of Old World monkey, ape and human [22, 78, 80]

Pigs and xenotransplantation

Given the limitations of using non-human primates as donors, discordant donors such as the pig may have greater potential. Natural antibodies in man can, of course, cause HAR, the greatest immunological barrier to using discordant xenografts, but a number of recently developed strategies in genetic engineering may help to overcome HAR.

Pigs have been selected as the best of the potential xenotransplantation donors because they are available in large numbers, share elements of anatomy and physiology with man and can be housed in SPF environments. Furthermore they have a relatively short breeding cycle, thereby enabling the introduction of genetic modifications that might make them more suitable donors [23]. Several transplantations have already been performed using pig cells, tissues and organs, including fetal pig-to-human islet transplants for diabetic patients [28] and fetal pig-to-human neural cell transplants for patients with Parkinson's or Huntington's disease [25]. Nevertheless, numerous steps still have to be taken to overcome the problems of pig-to-human transplantations caused by physiological and immunological incompatibilities as well as potential xenozoonoses.

Molecular mechanism of HAR HAR is the key topic associated with immunological incompatibility between pigs and man. At least two elements make HAR of pig organs by human patients an explosive and nearly universal event. During the 1990s, experimental studies of HAR revealed that a single dominant epitope expressed by pig endothelium – $\alpha(1,3)$ gal determinant – was responsible for binding a large proportion of preformed human natural antibodies. This α-gal determinant is formed by the action of an α-galactosyl transferase enzyme, which glycosylates N-acetyllactosamine. Man and Old World monkeys do not have a functional gene for this transferase.

The second element that can cause the HAR response in man is related to human complement and its regulatory proteins. Thus, the full manifestation of HAR requires formation of the membrane-attached complex at the end of the complement cascade, which is usually regulated by CD55 (decay accelerating factor, or DAF), CD46 (membrane co-factor protein, or MCP) and CD59, which act as inhibitors at various points along the cascade. Each of these regulatory proteins typically shows strict homologous restriction, functioning effectively only with the complement proteins of their own species [24, 26, 27, 44].

Techniques to overcome HAR Some advances have been made to prevent HAR after the xenotransplantation of pig organs: 1) Several techniques have been developed to reduce the level of preformed antibodies in recipients to bring it below the threshold level required to trigger HAR [11]; 2)

Since the main target of preformed natural antibodies is the α(1,3) gal determinant expressed by pig cells, HAR could also be avoided if the pig galactosyl transferase gene were to be eliminated. Recently, a pig cell line lacking the α-galactosyl transferase gene has been isolated that could be used to clone genetically engineered pigs [2]; 3) Genetic engineering to inhibit human complement activation has been accomplished by creating transgenic pigs that express human or other primate complement regulatory proteins, including CD55, CD46, and CD59. Studies of non-human primates have shown that this achievement can significantly postpone HAR, extending the interval before pig organ rejection from minutes to days. With strong immunosuppressive therapy, a transgenic pig heart has survived in a non-human primate for up to 60 days without evidence of rejection [45, 83].

Further progress in pig-to-man xenotransplantation In 2000, two different research groups simultaneously developed technologies for cloning pigs which were based on a similar strategy [51, 57]. They first fused porcine granulosa-derived donor cells with enucleated mature oocytes to form pronuclei. After 18 hours, the donor nuclei were removed from the oocytes and transferred to the cytoplasm of fertilised eggs (Figure 3). By using this so-called double-nuclear transfer strategy, 72 reconstructed embryos were prepared and transferred to the surrogate sow, resulting in a litter of five live piglets [57]. This success overcomes one of the major problems for pig cloning: how to produce at least 4 good quality embryos that are required to induce and maintain a pregnancy. It provides a great opportunity to generate a specific transgenic pig 'breed', for example, a pig free of the α-galactosyl transferase enzyme with organs that might be tolerated by the immune system of human recipients.

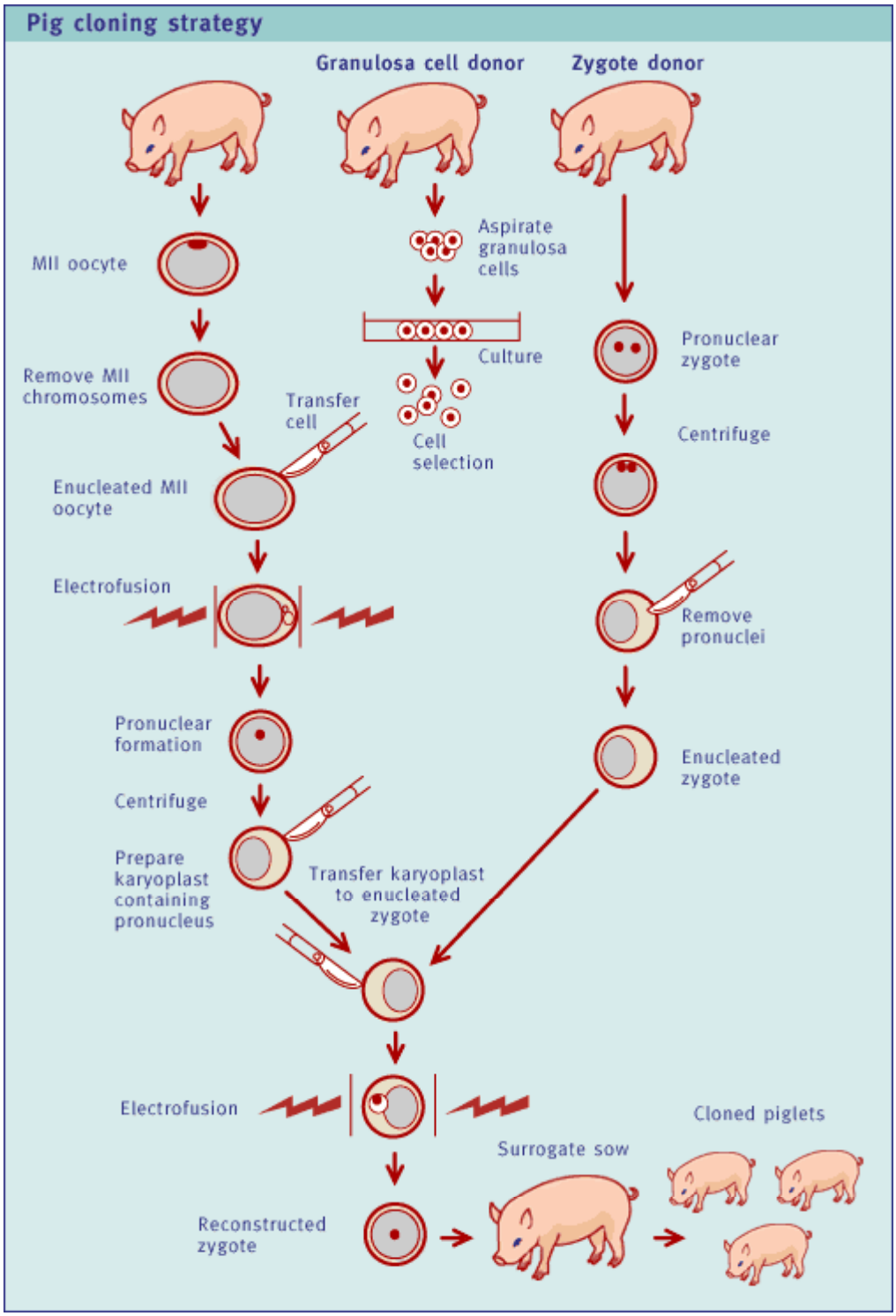

Figure 3. Pig cloning strategy [57].

Progress has also been made in the field of xenotransplantation-related immunology. To inhibit human complement activation, transgenic pigs that express human DAF and MCP have been developed by Imutran (Cambridge, UK). Researchers are trying to establish whether the combination of two inhibitors confers an advantage over

the expression of DAF or MCP alone. PPL Therapeutics plc (Roslin, UK) has constructed a pig cell line lacking the α-gal gene, which could be used to engineer HAR-free pigs. BioTransplant Inc. (Charlestown, Massachusetts, USA) has bred herds of miniature swine that grow to only 90–130 Kg and thus have organs a suitable size for transfer into man. Researchers from this company have also developed several ways of inducing the recipient's immune system to recognise pig organs as less foreign, one of involves the transplantation of pig thymic tissue into the recipient followed by treatment to create temporary immuno-suppression until the thymic tissue induces tolerance for future pig tissue implants [2, 3].

Endogenous retroviruses of pigs

Gains are always combined with risks and xenotransplantation is no exception to this rule [8, 9, 10, 11]. Public dread of using pigs as xenograft donors is mainly caused by the existence of potential pathogens in pigs, especially endogenous retroviruses, which cannot yet be screened out by traditional breeding methods or by modern gene knock-out technology.

In the 1970s, several groups reported that two porcine kidney cell lines, PK15 and MPK, could continuously produce C-type retroviral particles, but at the time nobody thought to check if they could infect human cells [6, 35, 48, 67, 70].

In 1996, part of the *pol* gene sequence of a porcine endogenous retrovirus named PoEV (porcine endogenous virus) was identified in a domestic pig genome. Phylogenetic analysis showed this virus to be C-type and closely related to gibbon leukemia virus (GaLV) [73]. One year later, a research report showed that viral particles released from PK15 cell could infect some cells from a range of hosts *in vitro*, including cells from the human 293 kidney cell line [54]. The virus was consequently named PERV-PK. In addition to the infection of specific human cell lines, human peripheral blood mononuclear cells (PBMC) also became non-productively infected by PERV-PK [85]; although no virus replication could be detected, viral DNA was amplified from these human PBMC. Sequence analysis showed that the RT gene of PERV-PK is closely related to the RT gene of GaLV, suggesting that PERV-PK was a different isolate from PoEV, but it is likely that both belong to the same viral species. Further investigation demonstrated that the PK15 cell line could produce two classes of PERV: PERV-A and PERV-B. The *gag* and *pol* genes from these two classes are

almost identical, but there is 92 per cent amino-acid identity to one another in the SU domain of ENV protein, suggesting that they probably use different cellular receptors to enter the host cells. Multiple copies of PERV-A and -B have been observed in the pig genome [34].

Besides PERV-A and -B, a third PERV class, PERV-C, has been identified from a cDNA library of a miniature swine [4]. Sequence analyses indicate that all three PERVs are members of the mammalian type-C retrovirus genus, showing closest homology to GaLV. They are closely related to one another in their *gag* and *pol* genes, with maximum nucleic acid divergence of around eight per cent (Figure 4). However, PERVs have three distinct classes of SU-encoding region, implying that they may have different host range specificities. *In vitro* experiments for the host range of the PERVs demonstrated that PERV-A and PERV-B have wider host ranges, including several human cell lines, than PERV-C, which can infect only two pig cell lines and one human cell line [41, 69]. Unfortunately, none of these PERVs could infect non-human primate cell lines *in vitro*, raising doubts about using a non-human primate model to study PERV zoonosis [42, 43, 69].

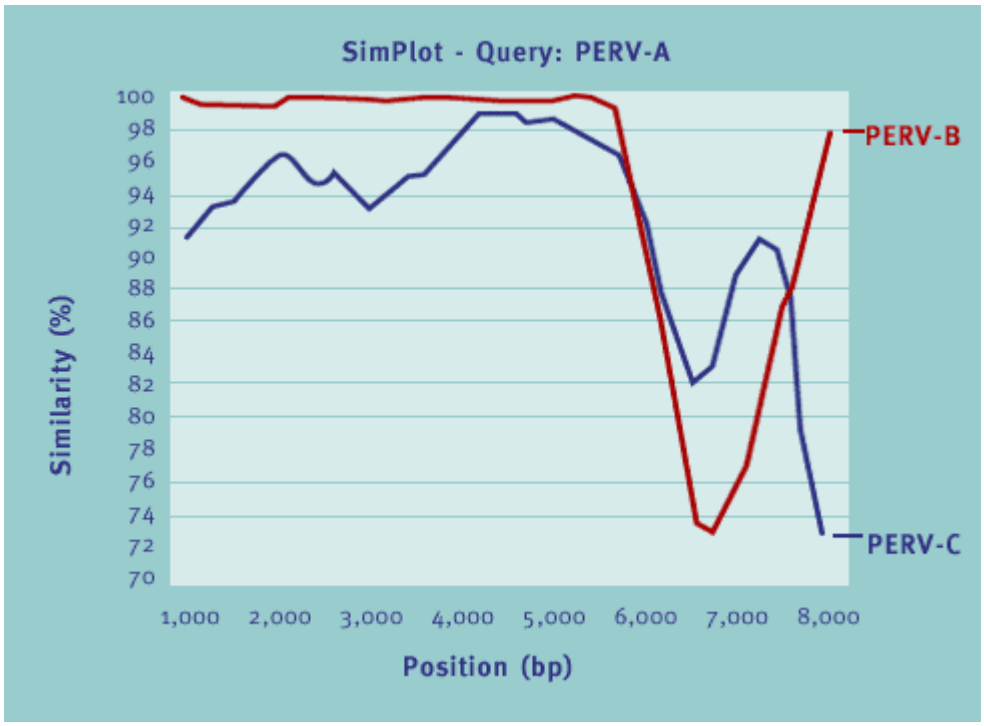

Figure 4. Nucleotide sequence similarity of PERVs. Analysis was performed by using SIMPLOT. The full-length sequences of PERV-A, -B and –C were included in the analysis, and the sequence of PERV-A was used as the query.

All the above conclusions, which address the concern that PERVs could be transmitted across species from pig to human when using pig organs as xenografts, were made on the basis of results from *in vitro* experiments. A number of studies to establish whether the recipients of pig organs or tissues were already infected by PERVs gave no indication that PERVs can infect human cells after xenotransplantation [29, 52, 53, 86]. It appeared that people were over-threatened by the existence of PERVs until, most recently, one group reported that after transplanting pig pancreatic islets into NOD/SCID (non-obese diabetic, severe combined immunodeficiency) mice, ongoing PERV expression could be detected and several tissue compartments had become infected [55, 82]. Since cross-species infections have only been found in mice compartments chimeric for pig cells, this finding suggests that cell-cell contact might be one important mechanism for spreading infection. It was the first evidence that PERV is transcriptionally active and infectious across species *in vivo* after transplantation of pig tissues and the

results showed that a concern for PERV xenozoonoses in immunosuppressed human patients should be taken very seriously.

In August of 2000, BioTransplant Inc. announced its creation of an inbred miniature swine containing no replication-competent PERVs, which could supply 'PERV-free' organs and tissues for xenotransplantation [1]. However, viral expression of PERVs in these swine might be only temporarily inactivated with complete proviral sequences still present in the genome, and the question remains whether PERVs will be reactivated and then jump to a new host when the xenotransplantation is performed.

Not only can retroviruses cross species to infect a new host, as PERV did with the mouse, but endogenous retrovirus already existing in the host genome could be reactivated by recombination with exogenous virus. For example, infectious BaEV arose through the recombination of SERV and PcEV [37, 38] and infectious FeLV-B (feline leukemia virus B type) was generated through recombination of FeLV-A and endogenous FeLV in the cat genome [65]. Enormous amounts of retroviral sequences are present in the human genome. Apart from three classes of PERVs, a porcine endogenous retrovirus designated PERV-E has been identified from a pig genomic DNA library. Sequence analyses showed that HERV 4-1 – a member of the HERV E family – is a retrovirus most similar to PERV-E, especially in the *env* gene. Multiple copies of PERV-E-like and HERV 4-1-like proviral sequences exist in the pig and human genome respectively, and at least part of the HERV 4-1 proviral genes is still expressed. Given the gene sequence similarity between PERV-E and HERV 4-1, the possibility of viral recombination in pig organ recipients cannot be ruled out.

In general, multiple copies of endogenous retrovirus are present in the genome of their natural host, and copy numbers have increased in the time since they first integrated into the germline [16]. Proviral copy numbers of different viruses range from several copies to several hundred copies. The pig genome has multiple copies of PERVs [4, 17, 54], which complicates the generation of PERV-free pigs by gene knock-out technologies or selective breeding.

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