XENOTRANSPLANTATION: AN UPDATE ON THE SAFETY OF USING PIGS AS SOURCE ANIMALS FOR TRANSPLANTATION

Xenotransplantatie: Een update van de veiligheid van het gebruik van varkens als brondieren voor transplantatie

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ABSTRACT

In this review we wish to update the progress which has been made in the art of xenotransplantation (the application of living animal-derived cells, tissues and organs for transplantation in humans), with a special emphasis on the barriers of its use as a clinical therapy. A brief overview of the history of xenotransplantation reveals the greatest barrier to clinical success: hyperacute rejection, a complement-mediated response to the source animal tissue that results in the destruction of xenografts within minutes. In the past decade, great progress has been made in countering this form of rejection, but further success is thwarted by the gradual awareness of subsequent processes of rejection and physiological incompatibilities. Nonetheless, reluctance to move forward to clinical application is predominantly related to the fear that xenotransplantation will unleash a new infectious disease in the prospective recipient and his or her surroundings. Animal breeders and caretakers play an important role in ensuring that the use of the source animals for this emerging therapy does not generate a xenozoonotic pandemic.

SAMENVATTING

In dit overzicht willen we een update geven van de vooruitgang die geboekt werd op het gebied van xenotransplantatie (het toepassen van cellen, weefsels en organen verkregen van levende dieren voor transplantatie bij mensen), met speciale aandacht voor de beperkingen ervan voor klinische toepassingen. Een kort overzicht van de geschiedenis van xenotransplantatie vestigt onmiddellijk de aandacht op de grootste barrière, namelijk hyperacute afstoting, een complementgemedieerd antwoord op het lichaamsvreemde dierlijk weefsel dat ervoor zorgt dat xenotransplanten binnen een tijdsbestek van enkele minuten volledig vernietigd zijn. In het vorige decennium werd grote vooruitgang gemaakt om dit soort van afstoting te verhinderen, maar verder succes wordt gedwarsboomd door het toenemende besef dat er nog andere processen van afstoting en fysiologische onverenigbaarheden een rol spelen. Toch houdt de voornaamste hinderpaal om de weg van klinishe toepassingen verder in te slaan verband met de vrees dat xenotransplantatie een nieuw soort van infectieuze ziekte zal veroorzaken bij de toekomstige ontvanger en zijn of haar omgeving. Dierenfokkers en dierenverzorgers spelen een belangrijke rol om ervoor te zorgen dat het gebruik van de donordieren voor deze ontwikkelende therapie niet zal leiden tot een xenozoötische pandemie.

STANDING THE TEST OF TIME

Xeno in xenotransplantation comes from the Greek word 'xenos' meaning "foreign". Xenotransplantation stands for different technologies that are aimed at substituting inadequate organs, tissues or cells of one species with a replacement taken from an individual of *another species*. In principle, xenotransplantation could involve

any cross-species transplants. However, current usage of the term primarily denotes the transfer of organs, tissues and cells from *animals* to *humans*. The use of such xenografts is being considered because of the growing shortage of human cells, tissues and organs available for clinical transplantation.

United States Public Health Service (2001) policy has defined xenotransplantation 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.

Note that this definition, which has been adopted by European authorities as well, does not include transplants of non-living animal products such as pig heart valves, porcine insulin and vaccinations from animal sources or animal sera used for the culture of human cells. This is not uncontested. It has been argued that the transplantation of such 'dead' animal material also evokes an immunological response in the host and that it is therefore arbitrary to distinguish between living and non-living grafts. However, the focus on living grafts is mainly because of regulatory issues. As we shall see, the transplantation of live animal material, in particular, involves a public health hazard and these products must therefore be more stringently controlled.

While xenotransplantation is generally regarded as an extraordinary field of contemporary medical research, the development of the technology started with transfusions of animal blood to humans as early as 1628 and has since featured many remarkable experiments. One famous example is the transfusion of lamb's blood conducted by Jean-Baptiste Davis, physician of King Louis XIV, and Paul Emmerez in 1667 (Deschamps *et al*., 2005). The recipient was a young man who was suffering severe fever. The physicians were convinced that the symptoms had vanished as a result of the transfusion and subsequently applied the procedure for various other conditions – including mental illness. Indeed, as people believed that the lamb's blood would transfer the animal's docile and calm character, such xenotransfusions were a particularly popular treatment for problems of temper in 19th-century Britain (Cooper and Lanza, 2000). The first documentation of tissue xenotransplantation also dates from the seventeenth century, with the report of successful engraftment of a piece of canine cranial bone to repair a soldier's injured skull in 1668 (Deschamps *et al.,* 2005). Remarkably, late nineteenth / early twentieth century attempts at endocrinology involved transplantations of slices of chimpanzee and baboon testicles in men for their alleged potential for human revitalization (Deschamps *et al.,* 2005). Xenotransplantation experiments also led to the earliest attempts at clinical kidney and heart transplantation. Whereas the first human kidney transplant was performed in 1933, kidney xenotransplants have been attempted since 1905 (Toledo-Pereyra and Lopez-Neblina, 2003). The first heart fully transplanted into a human was obtained from a chimpanzee in an operation conducted on January 23, 1964 (Hardy *et al*., 1964). The transplant surgeon, James Hardy, was almost four years ahead of the first human-to-human heart transplantation, which was conducted by Christiaan Barnard in December 1967 (Barnard, 1967).

The initial solid organ xenotransplantations were desperate measures to save terminal-staged patients in cases where no alternative treatment was available. The survival rates of the first animal organ recipients were extremely poor: the patients died in a matter of hours or days after the surgery. With evidence of better results with human grafts, allotransplantation (human-to-human transplantation) rapidly became the approach to which most interest and research was dedicated. Nevertheless, the appeal of using animals as sources of grafts resurfaced out of sheer necessity since human donors were hard to come by before the implementation and endorsement of clinical brain death (Toledo-Pereyra and Lopez-Neblina, 2003). Between 1963 and 1984, a total of 39 kidney, liver and heart xenotransplants into humans were reported. The organs were primarily obtained from baboons, rhesus monkeys and chimpanzees, which clearly provided better results than organs from other animal species. In 1964, Keith Reemtsma obtained 9 month survival in a recipient of a chimpanzee kidney showing no indication of organ rejection (Reemtsma *et al.,* 1964). This remains the longest survival ever recorded for the xenotransplantation of an organ. All subsequent xenotransplants of solid organs – which persisted sporadically until the mid 1990s – lasted no longer than 70 days.

More recent and successful clinical applications of xenotransplantation have consisted of cellular xenotransplants and *ex vivo* perfusions of diseased livers and kidneys. With perfusion, the major blood vessels of the organs are connected and cross-circulated to an animal kidney or liver placed outside the body. The first cross-circulation experiment dates from 1967, and involved

connecting the arm of a deep hepatic comatose woman to the leg of a baboon. The baboon's kidney excreted about 5 litres of the patient's fluid and allowed the patient to awake from coma (Deschamps *et al,* 2005). Alternatively, bioartificial liver devices, in which the patient's plasma is guided through primary porcine hepatocytes, have been applied to patients with acute liver failure (Chari *et al.,* 1994; Abouna, 1997; Levy *et al.,* 2000; Sheil, 2002). Currently, most hope and effort is devoted to direct implantations of animal cells (Bloom, 2001). Clinical trials have included injections of porcine pancreatic islet cells in insulin-dependent diabetic patients (Groth *et al.,* 1994; Groth *et al.,* 1996; Elliott *et al*., 2000; Elliott *et al.,* 2005; Wang *et al.,* 2005; Valdes-Gonzalez *et al.*, 2005), baboon HIV-resistant hematopoietic cells to treat a patient with AIDS (Thomson, 1995), encapsulated chromaffin cells from newborn calves to treat severe pain (Buchser *et al.,* 1996) and porcine neural cells for the treatment of patients with Parkinson's disease (Deacon *et al.,* 1997; Friedrich, 1999; Schumacher *et al.,* 2000), Huntington's disease (Fink *et al.,* 2000), epilepsy and stroke (Edge *et al.,* 1998; Savitz *et al.,* 2005).

BIOLOGICAL BARRIERS TO CLINICAL XENOTRANSPLANTATION

Xenotransplantation stands 'the test of time' in terms of the persistent pursuit of this approach to alleviate various human diseases for which human donor grafts are currently lacking. However, as is evident from the many dismal attempts in the past, whether xenogeneic organs may constitute a future replacement for human grafts is in the first place dependent on whether the tissue will be sufficiently tolerated by the human immune system. The incompatibility of cross-species grafts causes more intense and challenging immunological reactions than grafts transplanted between humans, and it continues to inhibit effective clinical use of most xenotransplantation applications (Cascalho and Platt, 2001).

Indeed, the immunological challenge has been heightened by choosing an evolutionarily disparate species as the source animal. Although the use of nonhuman primate grafts clearly provides the best results in terms of graft survival, pigs are currently the most preferred source animal for replacement of most cells and all organs due to both practical and ethical considerations. However, as has been reviewed in detail by Veraart *et al.* (2002) in an earlier issue of this journal, pig-to-human transplants evoke severe hyperacute rejection. This is a complement-mediated response to the source animal tissue that results in the destruction of xenografts within minutes. Hyperacute rejection occurs when a transplant between discordant (widely divergent) species elicits natural antibodies, known as 'xenoreactive natural antibodies', which target the species-specific donor tissue. In the early 1990s it was discovered that pig-to-human transplants evoked human xenoreactive antibodies against a sugar molecule called galactose-(α1-3)-galactose (Galα1-3Gal) that is present in the lining of porcine blood vessels (Cooper *et al.,* 1993). This carbohydrate is expressed by many animal species, but humans, apes and Old World monkeys have lost the capacity to produce it in the course of evolution (Lai *et al.,* 2002). As a result, our bodies produce natural antibodies to the antigen as a protection mechanism. Indeed, the Gal sugar molecules are also found on the surface of certain bacteria, viruses and parasites. It is known that at least 85% of human xenoreactive natural antibodies are targeted specifically to Galα1-3Gal (Cooper, 2003). The impact of xenoreactive natural antibody activation is profound. When the human immune mechanism comes into contact with a porcine vascularized xenograft, the organ turns into a black, swollen and mottled mass within several minutes or hours. The circulating natural antibodies quickly bind to the Galα1-3Gal and activate a destructive succession of nearly three dozen proteins, which is known as 'complement'. The process starts with damaging, and often destroying, the endothelial cells that compose the cell lining of the cavities of the heart and blood/lymph vessels. Consequently, the process of blood coagulation (for example, clot formation in the blood) is initiated, causing thrombosis, which in turn obstructs the xenograft and surrounding tissues from sufficient blood flow. Ultimately, the damage in the endothelium results in the exposure of the underlying matrix, the breakdown of metabolic and oxygen pathways, and the rapid death of the recipient. Hyperacute rejection develops in all organ xenotransplants between discordant species, but does not pose as much of a problem for cell or tissue xenotransplantation.

Hyperacute rejection cannot be overcome with conventional immunosuppressive regimens, so it requires that we circumvent the binding of the antibodies to the Gal sugar by either eliminating the xenoreactive natural antibodies or by inhibiting complement. The first successful approach to counter anti-Gal antibody mediated complement activation consisted of genetically engineering pigs to express human complement regulatory proteins (McCurry *et al*., 1995). The rationale underlying this approach is that the human complement-regulatory proteins will be able to protect better against human complement. Over the past decade, several strains of transge-

nic pigs that express one or more human complement regulatory genes have been bred and have increasingly provided better protection against human complement-mediated damage (Cowan *et al.,* 2000; Zhou *et al.,* 2005). In baboons, the transgenesis has effectively down-regulated activity of the complement cascade without any form of immunosuppression (Loveland *et al.,* 2004). The development of somatic cell nuclear transfer (cloning) technology in pigs (Polejaeva *et al.,* 2000) has facilitated the technique of multi-transgenesis. Through the cloning of pigs, alternative genetic modifications have also been generated which consist of 'knocking out' the gene for α1,3-galactosyltransferase so as to avoid synthesis of Galα1-3Gal. The production of cloned piglets that lack one allele of the gene – 'Gal-T knockout (GalT-KO) pigs'– was reported in 2002 by two independent research teams (Lai *et al.,* 2002; Dai *et al.,* 2002). Shortly after, in August, 2002, PPL Therapeutics announced the production of the first double knock-out piglets, which lack both copies of the α1,3-galacotsyltransferase gene (Phelps *et al.*, 2003). GalT-KO pigs have allowed for a further increase in survival rates in xenotransplantations of hearts and kidneys to primates in comparison with the use of hDAF pigs. Non-life supporting heart transplants (in which case the native heart is kept in place and the xenogeneic organ is transplanted in a different location within the body) in a pig-to-baboon model obtained maximum graft survival of 179 days, the longest survival of pig-to-primate organ transplantation recorded to date (Kuwaki *et al.,* 2005; Tseng *et al.,* 2005). Life-supporting xenotransplantation of a GalT-KO kidney resulted in survival of more than 80 days in nonhuman primates with no evidence of rejection (Yamada *et al.,* 2005).

These strategies are effective measures against hyperacute rejection. Further improvement of survival seems likely, provided that progress in site-specific genetic and transgenic modifications continues. Nonetheless, the progress in overcoming hyperacute rejection has revealed that this is only one of several rejection processes which inevitably develop within days after xenotransplantation. Due to the limited survival rates obtained so far, the impact of the mechanisms underlying subsequent processes of acute vascular rejection, acute cellular rejection and chronic rejection (for a discussion, see Veraart *et al.,* 2002) is not yet fully known.

Ideally, the xenograft would be tolerated by the human recipient as if it were part of his or her own body. With this purpose in mind, preliminary experiments have been conducted in which human stem cells are transferred into developing animals to generate animal grafts that express a significant amount of human cells (Airey *et al.*, 2004). Almeida-Porada and colleagues injected human stem cells in developing sheep and found that the human cells contributed to the sheep's blood, bone, liver, heart and nervous system (Almeida-Porada *et al.*, 2004). Ultimately, the hope exists that use can be made of a patient's own stem cells. If these cells were to be injected into developing animals, the animals may, once born, express a sufficient amount of that patient's cells in the grafts required. The genetic similarity between host grafts and recipient would thereby dismiss major immunological incompatibilities. An alternative, more remote approach could consist of using animals as growth environments of early-staged human embryonic organs (organ primordia). Although the field of regenerative medicine has made recent advances in the engineering of tissues, it remains an enormous challenge to reproduce the complex micro-anatomical structures and functions of multi-tissue organ structures. Theoretically, therapeutic cloning could be applied to create an early-staged embryo that is genetically identical to a particular patient. If successful, one could procure the early-staged organs (organ primordia) from the developing embryo, transplant them into an animal host, and allow them to mature. Once full-grown, the organs should be very compatible for transplantation into the recipient. The advantages of this approach include the fact that early staged organs, obtained at the proper time during embryogenesis, automatically differentiate into the desired tissue and facilitate vascularization. Furthermore, preclinical data suggests that kidney and pancreatic primordia can be effectively transplanted across both concordant (rat to mouse) and highly discordant (pig to rodent) xenogeneic barriers (Hammerman, 2003). Nonetheless, to date, there is still enormous controversy over the use of therapeutic cloning procedures. Also, the success of pre-clinical studies in large animal models has been constrained by the failure to identify the optimal gestation time for transplantation into the animal host environment (Eventov-Friedman *et al.,* 2005). Moreover, it will be essential that the embryonic organs do not come into contact with the host antigens. Such contact could cause the production of epitopes and thereby elicit an immune response.

Still another means to obtain long-term xenograft survival would consist of altering the recipient's immune system so that it completely, and permanently, tolerates the foreign graft. This state, known as 'immunological tolerance', is the destination of further allotransplantation research as well. Described as 'the immunological holy

grail'(Cooper and Lanza, 2000), tolerance is a condition in which the immune system tolerates specific donor cells, tissues and organs as if they were its own, but remains responsive to other invading microorganisms. It would completely alleviate the need for any additional immunosuppressive therapy. Apart from the medical utility associated with drug independence, tolerance may be the *only* chance for long-term effective discordant xenotransplantation. Various approaches to induce immunological tolerance exist - including xenoreactive antibody depletion, hematopoietic stem cell transplantation, transplantation of porcine thymus tissue (which generates T-cells), molecular chimerism using a gene therapy approach and temporary depletion of T-cells or induction of T-suppressor cells (U.S. SAXC, 2004). However, to date, none of these approaches have been able to overcome returning anti-pig antibody production.

While the feat of producing human tolerance to xenografts still lies ahead, long-term success of pig-to-human transplants is also left wanting by other potential incompatibilities between the species. These incompatibilities, which can manifest themselves in differences on physiological, homeostatic, metabolic and hormonal levels, remain largely unexplored (Bucher *et al.,* 2005). Initially, the major concerns regarding pig-to-human organ transplants related to the size of the organs and the horizontal posture of the animals. Nonetheless, postural changes do not appear to affect the function of the organ grafts and miniature swine breeds have been identified which provide organs that, in comparison to other species, best approximate adult human size. The major disparities between human and porcine physiology that have been identified so far include differences in hematology, enzymes, hormones and liver metabolism. The disturbances in hematology are of most concern. Many coagulatory disturbances have been indicated in various pig-to-primate xenotransplants and pose an increased risk of bleeding disorders and vascular thrombosis (Dobson and Dark, 2002; Bucher *et al.,* 2005). Adverse clotting may contribute to xenotransplant rejection, and problems related to circulation incompatibility may inhibit the delivery of oxygen and nutrients and the removal of waste substances. Differences between humans and pigs have also been found with regard to hematocrit, blood composition, blood viscosity, and red blood cell surface area and diameter – all of which may contribute to a weak integration of the xenograft in human microenvironments. The level of physiological compatibility between human and porcine lungs is currently the least understood, since porcine lung xenotransplants have not sustained survival of primates beyond a few hours.

CELLULAR XENOTRANSPLANTATION IS THE FUTURE

While the biological barriers to solid organ xenotransplantation remain substantial, various cellular replacements are proving to be more compatible with the functions of the native cells. The most imminent contribution of xenotransplantation to the clinic is likely to lie in the transplantation of porcine islets of Langerhans. This could provide an alternative to injections of human or porcine insulin, which are ineffective in fully restoring proper glucose homeostasis. Pig islet cells provide adequate blood levels of glucose in humans. Moreover, while xenogeneic islet transplantation is known to initiate an immediate inflammatory reaction in human blood, which causes coagulation, this effect appears to be manageable by appropriate drug regimens (Goto *et al*., 2004). For cellular xenotransplants of this sort, promising approaches exist in terms of eliminating the immune barrier (Opara and Kendall, 2002; Garkavenko *et al.,* 2005). One particular feasible procedure consists of encapsulating cells or small tissues in a semi-permeable membrane that cannot be penetrated by destructive factors but does facilitate two-way diffusion of nutrients from the host circulation and desired products from the xenograft. A recent report suggests that combining porcine islet cells with Sertoli cells (which have been found to consist of an immunomodulating factor) and encasing them in a semi-permeable encapsulation device allows for long-term survival of the grafts (Valdes-Gonzalez *et al.,* 2005). The follow-up data four years after the transplants indicate that 50 percent of the experimental human recipients (n=12) significantly reduced their demand for exogenous insulin. Two patients are reported to have achieved temporary insulin-independence. It must be noted, however, that the results of this trial are not left uncontested. Rood and Cooper (2006) reviewed this trial as well as three other recent reports involving clinical xenogeneic islet transplantation (Elliott *et al.,* 2000; Elliott *et al.,* 2005; Wang *et al.,* 2005) and indicate that each of the reports lack convincing results from pre-clinical primate models. The authors point out that without such preclinical data a decrease in patient insulin requirement could just as well have been obtained from the improved medical follow-up of the patients' condition.

Porcine neural cells are also a good match in terms of structure and function. Various animal studies have provided proof of principle that xenogeneic neural tissue can survive and function and that it can reduce the symptoms of neurodegenerative disease. The studies even suggest that the level of neural cell migration, innervation and in-

tegration is better compared to equivalent allografted tissue (Wictorin *et al.,* 1992; Hurelbrink *et al.,* 2002, Bucher *et al.,* 2005). The first neural xenograft survival in the human brain was documented in 1997 (Deacon *et al.,* 1997). Embryonic pig cells were implanted into a patient with Parkinson's disease and were found to have generated pig dopaminergic and other neural cells. The neurons had grown axon extensions into the host brain and evoked only low reactivity from human microglia and T-cells. Nonetheless, while some neurons survived over seven months, large numbers of dopaminergic neurons had only poor graft survival. A different study, three years later, reported follow-up results one year after the successful transplantation of embryonic porcine ventral mesencephalic tissue in twelve Parkinson's patients (Schumacher *et al.,* 2000). The results showed that the tissue was well tolerated without serious adverse effects. Overall rates of Unified Parkinson's Disease Rating Scale scores improved by 19 per cent and three patients, who had received particular immunosuppressive regimens including cyclosporine, improved over 30 per cent. These results are similar to the initial experience with unilateral human embryonic allograft transplantation, although in the latter case much less cells are transplanted (in this study, 12 million embryonic pig neurons were transplanted!). Apparently, although the immune rejection in the brain is thought to be rather weak, most of the xenografts nevertheless undergo rejection when there is a lack of proper protection measures.

RESTRICTING THE EMERGENCE OF XENOGENEIC INFECTIOUS DISEASE

It is clear that many challenges remain to be addressed before xenotransplantation of organs, especially, will be a viable routine therapy for waiting list patients. Precisely at what point pre-clinical efficacy is sufficient to warrant clinical applications in humans is unclear. The Spanish Xenotransplantation Commission suggested survival and proper function of the grafts for at least six months in primates (Council of Europe Committee of Ministers, 2003). In comparison to the start of allotransplantation, this is a demanding requisite. Norman Shumway, for instance, felt that survival for 4 to 21 days among 85 percent of dog recipients of cardiac transplantation was sufficient to warrant the move to clinical trials (Fox and Swazey, 2004). An argument could be made that, in a situation where a patient is sure to die soon without a transplant, even the most extreme operative mortality rate is acceptable. It has also been noted that the preclinical survival rate requirements should distinguish between the various types of xenografts. In this sense, perhaps, short survival rates of xenogenic cellular xenotransplants may be acceptable to warrant further progress in clinical research, provided that the graft malfunction is not dangerous for the patient. Nevertheless, currently, authorized xenotransplantation trials of whichever type of xenograft are extremely rare. The major brake on further progress is clearly related to the possibility that xenotransplantation will transfer infectious agents from the animal to the xenotransplant recipient and endanger public health.

Hypothetically, the source animals could bear particular viruses, bacteria, or prions that can be transmitted to humans and elicit an infectious disease (zoonosis). Conceivably, the infectious agents could be transmitted to animal caretakers or to the prospective recipients of the xenografts. Contamination could then extend beyond these individuals to their close surroundings and, eventually perhaps, to the public at large. Notwithstanding the long history of attempts at xenotransplantation, serious concern regarding this public health hazard materialized particularly in the second half of the 1990s. Arguably, this is due to heightened understanding of and sensitivity towards zoonotic disease, a looming threat of biological terrorism and the potential for rapid, global spread of infectious disease through mass air travel. The need to provide tough regulations to protect the public from xenogeneic infection was particularly felt in the United Kingdom, which had just experienced the crisis over BSE (Anonymous, 1997). The real dangers specific to primate-to-human xenotransplantation have since been affirmed: a post-mortem blood analysis of a baboon liver recipient indicated infection of simian cytomegalovirus infection (Michaels *et al.,* 2001). Pigs have lived in domestication with humans for thousands of years and therefore initially elicited less theoretical concerns regarding transmission of novel pathogens. Nonetheless, the 1997 discoveries that a family of porcine endogenous retroviruses (PERVs) is able to infect human primary cells and cell lines *in vitro* and adapt to these cells by serial transmission on uninfected cells (Patience *et al.,* 1997; Le Tissier *et al.,* 1997) evoked a new wave of concern.

To this day, the risk of creating a xenotransplant related epidemic, or at worse pandemic, remains unquantifiable. No PERV-related disease has been shown to occur in humans and there is no way of estimating how low or high the risk of infection is. Nevertheless, a cautious approach is supported by the fact that it is precisely viruses which persist asymptomatically in quiescent or latent phases that constitute the greatest hazard to public health. These

viruses could easily spread without being noticed. Moreover, the severity of the danger of PERVs was emphasized in light of known homology to other retroviruses, such as feline leukemia virus (FeLV) or murine leukemia virus (MuLV), which induce tumors or immunodeficiency in the infected host (Specke *et al.,* 2002). Evidence of PERV infections *in vitro* and fears that there may be other, undiscovered transmissible agents compelled several pleas for a moratorium on clinical xenotransplantation (Bach and Fineberg, 1998; Butler, 1998; Council of Europe Committee of Ministers, 1999). Currently, in most nations, a temporary *de facto* moratorium between 1997 and 1999 has been replaced by strengthened national oversight. Various regulatory and advisory authorities world- wide have published detailed safety protocols for xenotransplantation research and clinical trials (the latest and most influential documents are: US FDA, 1999; US PHS, 2001; UKXIRA, 1998/1999a/1999b; COECM, 2003; Steering Committee on Bioethics, 2003; EMEA, 2003; OECD, 1999; Working Party on Biotechnology, 2001; WHO 2001/2004). The protocol reviews pertain to the procurement and screening of source animals, the clinical and preclinical testing of xenotransplantation products and the post-xenotransplant monitoring/surveillance of recipients.

The safety protocols attribute an important role to animal breeders and caretakers. The conditions for breeding and housing the animals, as set out in the guidelines mentioned above, are extremely stringent. As such, the animals must be derived from closed herds with documented health screening programs only. The prospective source animals should be bred and housed in barrier facilities that are free of designated pathogens. The source animal's health status should not be jeopardized by the use of natural, non-sterile feeds. The herd should be continuously screened for a list of infectious agents as generated by experts on infectious diseases of the species involved. Particularly those infectious agents known to infect the source animal, to cause zoonoses, or to occur in latent state should be granted special attention. Biological specimens should be routinely procured from the herd and tested for infectious agents by appropriate assays. These samples must be archived for future purposes to facilitate identification of infections after the grafts have been retrieved and/or transplanted (the recommended durations of storage range from 20 to 50 years). In addition, all details concerning the health status of the source animals – including all illnesses, treatments, drugs and medical care involved – should be documented consistently. Several weeks prior to harvest of the grafts, the individual source animals should be quarantined and screened extensively. Importantly, humans who are in regular contact with the animals must also be screened. Minimally, this will involve the procurement of baseline samples, but periodic sampling and storage of serum or plasma is also possible. This level of surveillance is followed by very strict and far-reaching safety measures during preclinical research and clinical trials. The regulation of xenotransplantation can be seen as an important step in effectively approaching the emergence of new zoonoses.

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REFERENCES

An extended literature list can be obtained from the author.