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Xenotransplantation-Progress and Problems: A Review

Joachim Denner*

Centre for HIV and Retrovirology, Robert Koch Institute, Berlin, Germany

***Corresponding author:** Joachim Denner, Centre for HIV and Retrovirology, Robert Koch Institute, Berlin, Germany, Tel: +4930187542800; E-mail: DennerJ@rki.de

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Abstract

Xenotransplantation using pig cells, tissues or organs is considered to be a solution to the shortage of human allotransplants. Pigs have been selected as optimal donor for several reasons, among them physiological and economical. Before xenotransplantation will be applied broadly in the clinic three hurdles need to be overcome: (i) rejection due to immune reactions and coagulation dysfunction, (ii) physiological incompatibility and (iii) microbiological risk. Although some clinical trials have been performed in the past and some are ongoing, most experience is gained from pig-to-non human primate experiments. To overcome immune rejection, numerous multitransgenic and knock-down animals were produced or are in preparation. The physiological compatibility is still badly studied, mainly due to the short survival time of the recipient animals. Last not least, xenotransplantation may be associated with the risk of transmission of porcine microorganisms. Most of them can be eliminated by designated pathogen free breeding of the animals; however, porcine endogenous retroviruses (PERVs) represent a special risk. PERVs are integrated as proviruses in the genome of all pigs, they can be released as viral particles and infect human cells. An extensive screening program and selection of donor animals with a low expression of PERV accompanied by the development of different strategies to prevent PERV transmission is therefore requested. Finally, a broad discussion within the scientific community and the society concerning ethical aspects of xenotransplantation had been taken place.

Keywords: Xenotransplantation; Diabetes; Organ transplantation; Porcine endogenous retroviruses

Introduction

Transplantation is a way to treat severe organ and tissue failures. Organ transplantation has been one of the preeminent successes in the field of medicine. An experimental procedure 40 years ago, organ allotransplantation is meanwhile so successful, that there is a shortage of human organs suitable for transplantation. In Germany for example, at present 11,000 persons are on the waiting list for a donor organ, among them 8000 for a kidney, approximately 25% of them will die being on this list [1]. With the increase in age of the human population, the need of different types of transplantations is increasing. For example, pancreas transplantations are applied to treat diabetes, the failure of the isle T-cells in the pancreas to produce insulin. Unfortunately, suitable human donor organs are very rare. For several reasons diabetes has become an epidemic, with the number of affected people in 2010 of 285 million (6.4% of the world population) and 382 Million in 2013 [2]. According to the International Diabetes Federation this number is expected to increase to 439 million by 2030 and 592 million by 2035 [2]. Moreover, because of the many long-term complications of diabetes, such as kidney and heart disease, impotence, limb amputations and blindness, treatment for diabetes accounts for 10% of health-care expenditure in Europe (€25 billion per annum) [3].

When discussing alternatives to allotransplantation, strategies to prevent the organ failure are usually neglected. However, prevention of organ failure is the best and least expensive way to prevent transplantation and should be enforced. In the case prevention failed, alternatives to allotransplantation are (i) mechanical devices, which are under development mainly for the treatment of heart failure, (ii) tissue engineering and (iii) the stem cell technology. Although mechanical heart devices have been improved with considerable good results, they are still expensive and cumbersome [4]. Tissue engineering and stem cell research are far from producing functioning differentiated tissues or even vascularized organs composed of different T-cell types [5]. Therefore, at present xenotransplantation is considered to be an optimal solution. Pigs have been selected as donors due to the physiological similarity, the low reproduction time, the high number of progeny, the possibility to produce cloned and transgenic pigs as well as the low costs (for details see [6]). Although non-human primates are immunologically closer to humans, the size of the organs from monkeys is too small and apes with a suitable organ size represent endangered species, they have very long gestation times and a small number of offspring [6]. The selection of the donor species is also influenced by the potential microbiological risk. It is a subject of discussion whether the transmission of microorganisms from a related species (non-human primates) using a closely related receptor is more effective than the transmission from an unrelated species (pigs) [7]. The transmission of the human T-cell lymphotropic viruses (HTLV) and the human immunodeficiency viruses (HIV) from non-human primates to humans was extremely successful for the viruses, partially due to the presence of the suitable receptors and a similar metabolism in the infected cells [8,9]. HTLV induces lymphomas and immunodeficiency, HIV the acquired immunodeficiency syndrome (AIDS) [10,11]. This does not exclude that transmission from an unrelated species may be also successful, especially when the receptor is highly ubiquitous. A well-studied example is the transmission of a gammaretrovirus closely related to PERV, the Koala retrovirus (KoRV), which most likely was transmitted from rodents [12,13] or from bats [14,15] to the koalas in Australia. The KoRV infection is spreading nation-wide, the infected animals suffer from lymphomas and chlamydia infection based on a severe immunodeficiency [16-18].

To summarize, three hurdles need to be overcome before xenotransplantation can be applied in the clinic: (i) rejection due to different immune reactions, (ii) physiological incompatibility of the cells, tissues and organs and (iii) the microbiological risk.

Figure 1: Genetically modified pigs for xenotransplantation.

Overview of the genetically modified pigs for xenotransplantation (hCD46-human membrane cofactor protein, MCP; hCD55-human decay-accelerating factor, DAF; hCD59-human protectin; H-transferase, competing for the substrates needed by the alpha-1,3-galactosyltranferase; hCTLA4-Ig-human cytotoxic T-murine lymphocyte antigen 4 fused with Ig heavy chains, as a surrogate ligand used to block CD28/CTLA4 T-cell costimulation; hTM-human thrombomodulin, anticoagulation, activates protein C; hA20-tumor necrosis factor-alpha-(TNF-alpha)-inducible gene, may control the AVR; HLA-E/beta-microglobulin-protection against human natural killer cell cytotoxicity, TRAIL-tumor necrosis factor related apoptosis inducing ligand, induces apoptosis; hHO-1, human heme oxygenase-1, anti-apoptotic, cell protective, Fas-L-Fas ligand, belongs to the tumor necrosis factor (TNF) family, its binding with its receptor induces apoptosis, GnT-III-β-1,4-N acetylglucosaminyltransferase III, catalyzes the formation of a bisecting GlcNAc structure in N-glycans, GalTKO-knock-out of the gene for the alpha-1,3-galactosyltranferase, shRNA PERV-PERV-specific short hairpin RNA, inhibits PERV expression by RNA interference (for details see [6] and the original references cited there).

Rejection

Various mechanisms of rejection are associated with xenotransplantation, the first and most destructive is the hyperacute rejection (HAR), which is absent in allotransplantation. It is characterized by an immediate loss of the function of the transplant and is associated with haemorrhage, thrombosis and infiltration of neutrophils [19]. HAR is the consequence of the binding of preformed antibodies to a sugar epitope, the Gal epitope (galactose-alpha-1,3-galactose), which is present on the cell surface of all bacteria and mammals with exception of humans, apes and Old world monkeys. The loss of the Gal epitope was certainly an enormous evolutionary advantage since it allowed effective protection from microorganisms and cells from other species. The pre-existing anti-Gal antibodies destroy together with the complement T-cells carrying the Gal epitope [20]. Since depletion of anti-Gal antibodies using affinity columns bearing Gal, depletion of complement by cobra venom factor, or inhibition of the complement cascade by soluble complement receptor 1 (CR1) or anti-C5 antibodies are not very effective [21,22]{Cascalho, 2001 #152}, genetically modified pigs have been created (Table 1).

Gene	Effect	References
1,3 Gal (alpha-1,3-galactosytransferase) knock out (GalTKO)	Reduced Galalpha-1,3-Gal (Gal) expression, reduced hyperacute rejection	[23,24]
hCD46 (hMCP, human membrane cofactor)	Human complement regulation	[24,25]
hCD46+GalTKO	Human complement regulation +Reduced Gal expression	[26]
hCD55 (hDAF, human decay accelerating factor)	Human complement regulation	[27,28]
hCD55+endo-beta-galactosidase C	Human complement regulation +Reduced Gal expression	[29]
hCD59	Human complement regulation	[30-32]
hCD55+hCD59	Human complement regulation	[33,34]
hCD46+hCD55+hCD59	Human complement regulation	[35-37]
H-transferase (alpha-1,2-fucosyltransferase)	Reduced Gal expression	[38]
hCD59+H-transferase (alpha-1,2-fucosyltransferase)	Human complement regulation +reduced Gal expression	[39]
hCTLA4-Ig (cytotoxic T lymphocyte-associated antigen)	Inhibits T-cell activity	[40]
hTM (human thrombomodulin)	Activate human anticoagulant protein C	[41]
hA20 (humanA20, tumor necrosis factor-alpha inducible gene)	Controls acute vascular rejection	[42]
HLA-E/ (human leukocyte antigen-E)+human beta2-microglobulin	Protection from NK cell-mediated cytotoxicity	[43]
TRAIL (tumor necrosis factor-alpha-related apoptosis-inducing ligand)	Reduced posthyperacute cellular rejection	[44]
GnT-III (beta-d-mannoside beta-1,4-N-acetylglucosaminyltransferase III)	Reduced antigenicity to human natural antibodies	[45]
hHO-1 (human heme oxygenase-1)	Antiapoptosis	[46]
Fas ligand (Fas L)	Antiapoptosis	[47]
GalTKO+CD55+CD59	Control of instant blood-mediated unflammatory reaction (IBMIR) when transplanting neonatal isle T-cell clusters	[48]
GalTKO+CD55+CD59+H-transferase	Reduced xenoantibody response to isle T-cells from transgenic animals	[49]
GalTKO+H-transferase	Reduced expression of alpha Gal antigen	[50]

Soluble TNFRI-Fc-hHO	Protection against oxidative and inflammatory injury	[51]
Optimized hTM	Overcome coagulation incompatibilities in pig-to-primate xenotransplantation.	[52]
GalTKO+CD46	Suppress in vitro human anti-pig cellular responses	[53]
HLA-E	Suppression of inflammatory macrophage-mediated cytotoxicity and proinflammatory cytokine production	[54]
CD55 CD59+H-transferase genes	Enhanced protective response to human serum-mediated cytolysis	[55]

Table 1: Strategies to overcome rejection and physiological incompatibility: Genetically modified pigs.

There are three strategies to overcome HAR by genetic modification of the pigs: (i) expression of the human complement regulatory proteins such as hCD59 (protectin), human decay-accelerating factor (DAF, hCD55) and membrane cofactor protein (MCP, hCD46), (ii) overexpression of enzymes such as H-transferase, competing for the substrates needed by the alpha-1,3-galactosyltranferase, which is responsible for the Gal epitope, and (iii) knock-out of the gene for the alpha-1,3-galactosyltranferase (GalTKO), these animals are unable to express the Gal epitope [23,56] (Figure 1). Using multitransgenic pigs and antibody depletion, HAR, once considered the most vexing problem for xenotransplantation, was thought to be overcome. However, meanwhile new carbohydrate non-Gal epitopes have been identified, which may play a role in HAR [57]. Nevertheless, when genetically modified pig hearts were transplanted heterotopically in baboons, a significantly reduced HAR and a survival of up to 8 months was observed. It is important to note that in contrast orthotopic life supporting heart transplantation has not extended beyond 57 days. Similar differences between ortho- and heterotopical-transplantations were observed when kidneys were transplanted (for review see [58]).

Concerning the next steps of rejection, there is still uncertainty, both in understanding the mechanisms as well as in naming the processes: acute vascular rejection (AVR) thought to be similar to AVR of allotransplants [59], delayed xenograft rejection (DXR) [60], or acute humoral xenograft rejection (AHXR) [61], a delayed form of antibody mediated rejection. Endothelial cells and antibodies are involved in this type of rejection. Finally, the classical cellular rejection has been described [58]. Rejection on this stage is based on the genetic differences between humans and pigs and may be controlled by immunosuppressive regimens more intense than the one used in allotransplantation. Transgenic pigs expressing CTLA4-Ig to provide a local immunosuppressive effect or a mutant MHC class II transactivator (CIITA) knocking down SLA expression in the endothelium, may be useful (for details see [58]).

Now it becomes clear that coagulation dysfunction between recipient and donor as well as inflammation contribute significantly to the loss of the transplant [62]. The induced thrombotic microangiopathy causes ischemic injury to the myocardium during heart transplantations and finally results in consumptive coagulopathy [63,64]. It is still unclear which part is contributed by the immune activation of the pig vascular endothelial cells or whether the presence of these cells is sufficient to activate primate/human platelets and PBMCs alone [65]. The expression of both the pig tissue factor and the primate tissue factor is increased [65,66]. In the regulation of the immune response, activating and inhibitory receptors and ligands are involved and it should be possible in future to knock out certain of these factors and to develop in vitro assays predicting whether the recipient is at high or low risk for rejection [67].

Liver and kidney transplantations showed a lower survival time compared with heart and kidney transplantations (see above). Cellular transplants, e.g, isle T-cells, function much better than organ transplants [68,69]. Transplanting pig isle T-cells, either an immunosuppressive regimen was applied, or the pig isle T-cells were encapsulated [70,71]. One report indicates that pig isle T-cells survived up to 9.5 year in the human recipient [72]. Other cell transplantations include pig neuronal cell xenotransplantation for the treatment of neurological diseases including Parkinson´s disease [73], and pig corneal transplantation (for review see [74]).

Physiological Compatibility

Assuming that the immune and physiological mechanisms of rejection are under control and that the tissues or organs were not rejected, additional physiological incompatibilities in the function and regulation can be expected [75]. These incompatibilities are not well studied and will become evident only when the xenotransplants survive for a longer time. The differences between pig and human organs include their size, their position and orientation in the body, and the mechanism of regulation of their function by hormones and other soluble factors. On the other hand there are common physiological processes, e.g., pig insulin has been used for the treatment of diabetes in humans for decades. Under discussion is the compatibility of pig erythropoietin produced in the kidney [76]. In the case it is not compatible, either transgenic pigs producing human erythropoietin have to be produced or human erythropoietin has to be injected after kidney transplantation. The liver produces more than 2000 factors including albumin and it seems unlikely that a pig liver will be functioning for a long period. In addition, ex vivo treatment of human blood with pig liver cells have resulted in unsatisfying results [77]. However, when livers from of genetically modified pigs (CD55 or GalTKO plus CD46) were transplanted into non-human primates, survival times up to 7 days were observed with near-normal function of the organ, except hypoalbuminemia, suggesting application as a bridge to allotransplantation [77].

Microbiological Safety

Xenotransplantation using pig cells, tissues or organs may be associated with the transmission of numerous porcine microorganisms and several strategies have been developed to increase the microbiological safety (Table 2). Long lists have been published with the names of pig parasites, fungi, bacteria, and viruses, representing a potential risk for the transplant recipient, especially when strong pharmaceutical immunosuppression is applied [78]. However, at present it is unknown which microorganism represent a real risk, inducing, when transmitted, a disease (zoonosis or xenozoonosis or xenosis). Breeding the animals under designated pathogen free (dpf) conditions will prevent at least the transmission of known zoonotic microorganisms such as circoviruses, herpesviruses and others [79]. Porcine cytomegalovirus (PCMV), porcine lymphotropic herpesvirus (PLHV), and porcine circovirus (PCV) are the main viruses screened for in the ongoing New Zealand clinical trial [80,81].

Strategy	Result	Reference
Designated pathogen free breeding	Defined microorganisms absent in the donor pigs	[82-86]
Selection of pigs with low expression of PERV-A and PERV-B and absence of PERV-C in the genome	Prevent PERV-A/C recombination, low expression, possibly no particle release	[87,88]
RNA interference: Expression of PERV-specific siRNA	Low expression, possibly no particle release	[89-92]
Zinc finger nucleases (ZFN)	Attempt to eliminate all PERV	[93]
Vaccine	Prevent transmission of PERV	[94-98]
Antiretroviral drugs	Prevention/therapy	[99,100]

Table 2: Strategies to increase microbiological safety.

In contrast to these microorganisms transmission of porcine endogenous retroviruses (PERVs) cannot be prevented by dpf breeding since they are integrated as DNA copy in the genome of all pigs [101]. PERVs can be released as infectious particles and infect human cells [102,103]. Retroviruses are able-using the enzyme reverse transcriptase which is unique for retroviruses-to transcribe their RNA genome into DNA and integrate the DNA copy into the genome of the infected host T-cell. In the case oocytes, sperms or their progenitors were infected, the integrated DNA copy, also called provirus, will be present in the fertilized oocyte and eventually in all cells of the developing organism and will be inherited like a cellular gene. Gamma- and betaretroviruses were found in the genome of all pigs [104,105]. The copy number ranges in dependence of the pig strain from 30 to 150 [105]. Two types of gamma retroviruses, PERV-A and PERV-B, are present in all pigs, a third, PERVs-C, is present in many, but not all pigs. PERV-A and -B infect human cells, PERVs-C infect only pig cells [102]. Retroviruses may induce in the infected host severe immunosuppression (example: HIV-1 and the feline leukaemia virus, FeLV), and/or leukemias, lymphomas or solid tumors (example: HTLV, FeLV) [8-10]. In rare cases retroviral infections are apathogenic (example: feline and primate foamy viruses) [106]. Transspecies transmissions of retroviruses are well known (for review see [18]) and the AIDS pandemic is the most disastrous example of a transmission of a zoonotic retrovirus to humans [10]. PERV is also the result of a transspecies transmission, probably from rodents [12,13]. PERV-A and PERV-B integrated 8 mio years ago, PERVs-C is younger and integrated 3 mio years ago [13,107]. Recombinant PERVs-A/C proviruses were detected in pigs in some tissues, but not in the germ line (for review see [108]). These viruses have a high replication rate, they infect human cells and can adapt to them with an increased titer [109,110].

To detect PERV and to prevent transmission, (i) sensitive and specific detection methods based on PCR or other molecular biological methods as well as indirect detection methods measuring PERV-specific antibody responses, and (ii) strategies how to screen donor animals and how to follow the recipient have been developed in the last years [80,81,87,88,99,101,102,105,107-114]. There are recommendations of the International Xenotransplantion Association (IXA), the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) how to screen the donor pigs and how the recipient of the xenotransplant [82-86,115]. In addition, strategies how to minimize PERV transmission are under development. These strategies include the selection of PERV-C free animals (in order to prevent the generation of high titer PERV-A/C) [88,111], the selection of pigs with low expression of PERV-A and -B [111,116], the treatment with antiretrovirals [99,100], the development of a vaccine [94-98] and the generation of transgenic pigs expressing a short hairpin RNA in order to suppress the expression of PERV by RNA interference [89-92] (Figure 1).

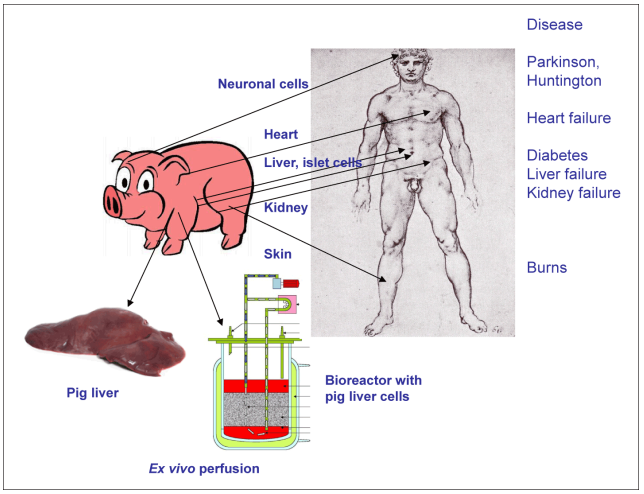

Figure 2: Examples of xenotransplantation.

Overview of clinically applied or planned xenotransplantations in order to treat different diseases. Cells or organs were directly transplanted into the human organism or pig cells or organs were used ex vivo to interact with human blood.

First Preclinical and Clinical Trials

Preclinical trials mainly using non-human primates have been performed in order to evaluate the efficacy and safety of xenotransplantation (Table 3).

In addition, in the past years more than 200 individuals had undergone xenotransplantation, mostly ex vivo perfusions with pig liver and spleen cells and some transplantations of pig islet and neuronal cells (for a detailed review see [6,83]) (Figure 2) (Table 4). Organs (heart, liver) were transplanted in two cases with a survival time of less than 34 hrs (for an overview of the history of xenotransplantation see [6]). PERVs or other porcine microorganisms have not been found transmitted until now.

Pig isle T-cells have been transplanted to diabetic individuals in several trials. In one trial performed in Russia, in two of eight persons a 100% insulin dose reduction was achieved after the first transplant [135]. A second trial was performed in New Zealand and in both trials no transmission of PERV was observed [81,114]. When pig cells or organs were transplanted into non-human primates (for review see [83]) or when high doses of infectious PERV were inoculated into non-human primates [85], no transmission of PERV was observed despite strong pharmaceutical immunosuppression in most cases.

Xenotransplantation: Strategy and treatment	Modification of the pigs	Recipients (number of animals)	Results after Tx	References
Cells and heart PAEC (1x107) iv one animal also pig heart, Cyp	None	15 baboons	No PERV transmission and no microchimerism 1 year after treatment, 12-24 months	[117]
Heart, skin, encapsulated islets, some CsA, MMF, Ster	None	23 (6 baboons, 6 bonnet macaques, 9 STZ rhesus, 2 STZ capuchins	No PERV transmission, 11-31 months	[118]
Kidney, Cyp, CsA, MMF, Ster	hCD59	6 cynomolgus monkey	No PERV transmission, 1-19 days	[119]
Kidney		12 cynomolgus monkey	No PERV transmission, transplant removed days 2-15, up to day 287	[120]
Encapsulated isleT-cells, no immunosuppression	None	12 STZ cynomolgus monkey	No transmission of PERV and other viruses, 3-8 months	[80]
Kidney (13) or heart (14) GAS914, Cyp, CsA, MPA	hDAF	27 baboons	No PERV transmission, 6-50 days	[121]
Liver perfusion 13-24 hours without immunosuppression	hDAF	6 baboons	No PERV transmission, 6-12 months	[122]
Pig neonatal isle T-cell clusters with immunosuppression	GalTKO +CD55+CD59+H-transferase	3 baboons	Reduced xenoantibody response to isle T-cells from transgenic animals	[49]
Pig neonatal isle T-cell clusters with immunosuppression	GalTKO +CD55+CD59+H-transferase	5 non-diabetic baboons	No coagulation incompatibilities, no thrombosis	[51]

Abbreviations: C1inh, complement C1 inhibitor; CsA, Cyclosporine A; CyP, cyclophosphamide; EC, endothelial cells; FTBI, fractionated total body irradiation; GAS914, a soluble, polymeric form of a Gal alpha-(1,3)Gal trisaccharide, hDAF, human decay-accelerating factor; MPA, mycophenolic acid; PAEC, primary aortic endothelial cells; Ster, steroids; STZ, streptocotozin (induced diabetes), Tx, transplantation.

Table 3: Summary of preclinical pig-to-non human primate xenotransplantations.

Strategy of xenotransplantation	Pig xenotransplant	Immunosuppression	Time after Tx	Number of patients	Result	References
Acute liver failure	Bioartificial liver device, 90-100 g wet weight	None	6 hrs pre/post	6	No PERV transmission	[123]
	Cryopreserved hepatocytes	N=2 for liver-tx after procedure	3 months – 5 yrs post treatment	28	No PERV transmission	[124]
	AMC-BAL		Before, 0 days to 2 yrs after	14	No PERV transmission	[125,126]
	Plasma perfusion though bioreactor Followed by liver Tx + immunosuppression	Exposure 8-46 hrs	Up to 2-5 yrs	8	No PERV transmission	[127]
Fulminant liver failure	Extracorporeal liver perfusion, tg-liver	Exposure 6-10hr		2	No PERV transmission	[128]
Chronic glomerulonephritis Renal dialysis	Extracorporeal kidney perfusion		6 hrs – 36 months	2	No PERV transmission	[129]
Neurological conditions (Parkinson, Huntington, focal epilepsy)	Fetal pig mesencephalon, lateral ganglionic eminence cells	CsA (n=11), graft pretreated with Abs	2-24 months post-Tx	11 PD 12 HD 1 FE	No PERV transmission	[130]

Diabetes	Encapsulated islets	No IS (n=1) CsA, AZA, pred (n=1) for kidney Tx	19 months	2	No PERV transmission	[131]
	Porcine fetal islets, 4x108 – 2x109 cells Porcine c-peptide up to 450d post-Tx (n=4)	CsA, pred, AZA maintenance	32-86 months post-Tx Mitochondrial DNA detectable 3d-1yr (n=1), d3 (n=6): all had Abs within 1wk post-Tx	10	No PERV transmission	[132]
	Beginning in 2009 treated with one of four different dosages of alginate-encapsulated porcine islets ranging from 5,000 - 20,000 IEQ/kg delivered in a single dose	No IS	1, 4, 12, 24, 52 weeks after Tx	14 with severe unaware hypoglycemia	No PERV transmission, reduction of hypoglycemia	[81]
Acute liver failure	Cryopreserved hepatocytes	Exposure 2 – 30 hours	No PERV transmission	28	No PERV transmission	[133,134]
	Extracorporeal liver perfusion	Exposure 4.25 hours, IS after subsequent liver Tx	No PERV transmission	28		
Burns	Skin	Exposure 10 days	36-150 months	15		
Chronic glomerulonephritis Renal dialysis	Extracorporeal kidney perfusion	Exposure 35, 65 min	32-36 months	2		
Various indications	Extracorporeal splenic perfusion	Exposure 50-60 min	0-102 months	100		
Diabetes	Islets		19-93 months	14		
Abbreviations: Abs, antibodies; AMC-BAL, Academic Center in Amsterdam, BAL, bio-artificial liver; CsA, cyclosporine; AZA, azathioprine; IS, immunosuppression; PBMC, peripheral blood mononuclear cells; pred, prednisolone; Ster, steroids; Tx, transplantation; PD, Parkinson´s disease; HD, Huntington´s disease; FE, focal epilepsy						

Table 4: Clinical xenotransplantations.

Ethical Aspects and Regulatory Requirements

Xenotransplantation is on the way from the lab to the clinic [135]. The ethical discussion came to the conclusion that this new technology should be applied if it is safe and effective [115]. The regulatory issues (informed consent, criteria for patient enrollment, the rights and obligations of third parties, the ethical management of safety measures, and the use of animals) remain central and the public health risk represents a major concern. After it was documented that PERVs infect human cells, it was perceived-mainly based on the knowledge that the infection of humans with HIV and the AIDS pandemic were the result of a trans-species transmission of this retrovirus-xenotransplantation might create a new epidemic infectious disease. Meanwhile the ethical concerns are minimal as (i) no transmission of PERVs was observed in the first clinical xenotransplantations, (ii) no transmission of PERV was observed in experimental settings and (iii) pigs are a major source of animal protein throughout the world. The International Xenotransplantation Association (IXA) [83,84,115], the European Medicines Agency (EMA) [85], and the US Food and Drug Administration (FDA) [86] developed appropriate guidelines how to perform clinical xenotransplantations.

Conclusion

Xenotransplantation may have several advantages compared with allotransplantation. It may overcome the shortage of organs, the transplantations can be planned, the organs are in a good status and well characterized, the microbiological risk may be low (note that HIV, rabies virus, Epstein-Barr virus, cytomegalovirus and other viruses had been transmitted repeatedly during allotransplantations) [136,137]. In the first clinical trials, isle T-cells from Auckland island pigs were used which were well defined concerning the microorganisms they carry [80,81,87]. Meanwhile the Göttingen minipigs are also well characterized [93], although additional tests for some relevant microorganisms have to be performed [138]. Due to their small size these animals are not suitable for organ transplantations, but certainly for isle T-cell transplantations for the treatment of diabetes.

On the other hand there is still a long way to go to solve the problems concerning rejection, physiological compatibility and microbiological safety. Looking back to the origins of allotransplantation 40 years ago, it would be interesting to see how far xenotransplantation will be advanced in 40 years from now.

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