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Xenotransplantation: history, problems and development prospects

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The paper reviews the milestones and prerequisites in the history of the emergence and development of xenotransplantation. The currently existing barriers (immunological, infectious, genetic, ethical, and regulatory) to the development of this organ and tissue transplantation type have been studied. Available data on theoretical research and experimental studies have been reviewed. The prospects for performing xenotransplantation in various combinations of species have been assessed. The forms and variants of the xenograft rejection reaction have been described. Genetic engineering approaches to overcoming xenoimmunological incompatibility are described. An assessment is made of ways to overcome existing barriers and prospects for the further development of xenotransplantation as a scientific section of transplantology.

Keywords: xenotransplantation, history of medicine, genetic engineering

C1-INH, C1 inhibitor

EMCV, encephalomyocarditis virus

GTKO, galactose-α -1,3-galactosyltransferase

IL-2, interleukin 2

PCMV, porcine cytomegalovirus

PCV, porcine circovirus type 2

PERV, porcine endogenous retrovirus

PLHV, porcine lymphotropic herpes virus

vWF, porcine von Willebrand factor

XNAs, xenoreactive natural antibodies

Introduction

Organ transplantation has achieved significant success in the treatment of various diseases at decompensation stage, reaching a high percentage of survival. From 1988 to 2014, more than 617,000 transplants of solid organs were performed in the United States, and about the same in the rest of the world [1]. The actual problem is a growing shortage of organs available for transplantation compared to the number of patients waiting for them [1]. In the USA, 30 deaths are recorded every day among the patients on the waiting list who never received a saving surgery [2]. Changes in the legislation have only partially resolved this problem. The US National Organ Transplant Act was signed in October 1984, which led to an increase in the donor organ pool by 85%, while the number of recipients on the waiting list had increased by 243% [3].

In the first decade of the 21st century, an increase in the number of transplants was associated with an extension of the criteria for using "high-risk" donors, but the number of patients on the waiting list had increased even more [2–4]. It is worth emphasizing that the current system of organ donation, including the use of extended criteria donors, does not provide a sufficient number of solid organs. In this regard, the development of xenotransplantation, i.e. the transplantation from one biological species to another biological species, has been considered one of the solutions to the organ shortage problem. For example, the use of porcine organs could have provided humanity with donor organs to the full. Pigs are considered by many authors to be an acceptable choice as an alternative source of organs for humans [5]. In the recent 25 years, a significant progress has been made in understanding the immunobiology of organ xenotransplantation, which allows for a better understanding of other barriers, such as molecular incompatibility, coagulopathy hindering its successful results [6]. These advances have returned hopes that xenotransplantation problems can be clinically resolved. At the same time, there are still considerable doubts remain that even after overcoming immunological and biological barriers, animal organs after transplantation to humans will function properly and the risk of this transplantation will be acceptable.

The purpose of this review has been to highlight the history of xenotransplantation, the progress in using organs of genetically modified animals as a source for transplantation to primates and humans, the prospects for the clinical use of organs and the cells that generate lost functions, as well as existing problems in this branch of science.

Definition

According to the definition of the US Food and Drug Administration, xenotransplantation refers to any procedure that involves transplantation, implantation, or infusion into the human recipient's body, either (1) live cells, tissues, or organs derived from a different species, or (2) fluids, cells, tissues or organs from the organism of the same species as that of the recipient, but who had ex vivo contact with live animal cells, tissues or organs of another species [7]. Fetal neurons (stem cells), porcine pancreatic cells, encapsulated bovine chromaffin adrenal cells, primate bone marrow, extracorporeal devices using a whole organ or its cells also belong to this category. Biological preparations or materials obtained from animals, but not containing live cells, such as porcine heart valves or porcine insulin, are not considered to be xenotransplantation products and run out of this definition.

Classification

In his classification R. Calne proposed to distinguish between two types of xenotransplantation according to the phylogenetic proximity degree and the severity of rejection reaction [4].

- **Concordant** xenotransplantation of organs i.e. the transplantation performed between phylogenetically close or related species, such as a mouse and a rat, a monkey and a Javanese macaque; or, presumably, between primates and humans. The organ rejection reaction in these cases develops in a few days.
- **Discordant** xenotransplantation i.e. the transplantation performed between different species (for example, a pig and a monkey, or a pig and a human). With discordant xenotransplantation, a hyperacute

rejection develops that lasts from several minutes to several hours. Clinical experience in humans with discordant xenotransplantation has been very limited. In discordant xenotransplantations performed to humans, the recipients developed humoral rejection, despite any treatment, and the organ functioning did not exceed 34 hours [8, 9].

It is easier to overcome the immunological barrier and obtain relative tolerance in concordant organ transplantation rather than in discordant one. A low titer of circulating antibodies in a concordant combination makes it nearly possible to avoid an immediate organ rejection in case of a hyperacute rejection, compared to a discordant combination. The cellular immunity is the main obstacle to the success of concordant xenotransplantation [10, 11].

The history of development

In the 1960s, Keith Reemtsma at Tulane University in Louisiana (Fig. 1) suggested that primate kidneys could be used in the treatment of renal failure in humans. At that time, neither chronic hemodialysis, nor human kidney transplantation were performed. Kidney xenotransplantation was an actual alternative to death. K.Reemtsma chose chimpanzees as the source of organs because of their close evolutionary relation to humans. He performed 13 double kidney transplants from chimpanzee to humans [12].

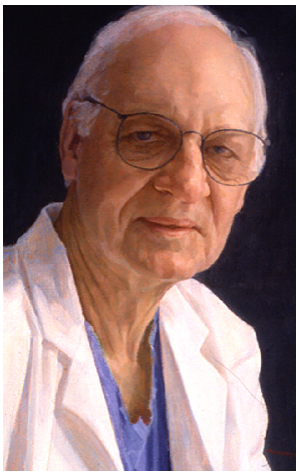

Fig. 1. Keith Reemtsma (1925-2000). Fragment of the portrait, 1996, College of Physicians & Surgeons of Columbia University, painted by Sarah Belchetz-Swenson [http://belchetz-swenson.com/work]

Most of the transplants he performed had unsuccessful outcomes within 4 to 8 weeks either as a result of an acute rejection or infectious complications. Nevertheless, one of Reemtsma's patients survived for 9 months, returned to work as a school teacher. The concept of using primates as kidney donors was supported by several surgeons, in particular by Thomas Starzl, the father of current transplantation (Fig. 2) in Colorado, who used monkeys as donors [13]. His results were similar to those of Reemtsma, except that Starzl did not achieve any long-term survival. James Hardy (Fig. 3), a pioneer of lung transplantation, visited Reemtsma and was impressed with the condition of some patients with kidney transplants from chimpanzees. In 1964, Hardy planned to perform the first heart transplant and intended to use chimpanzees as potential donors in case a posthumous donor would be unsuitable. A severely ill patient with advanced atherosclerosis after limb amputation was considered as a recipient. Due to a sudden death of the donor, Hardy was forced to perform a heart transplant

from a chimpanzee [14]. The heart turned out to be insufficient in size to maintain adequate hemodynamics even for several hours.

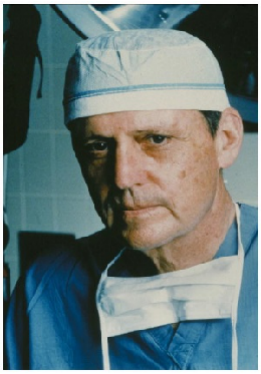

Fig. 2. Thomas Starzl (1926−2017)

[[https://www.gettyimages.com/photos/thomas-starzl]

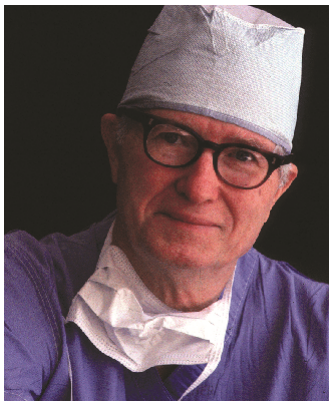

Fig. 3. James Hardy (1918−2003)

[https://www.findagrave.com/memorial/7364547/james-d-hardy]

Inspired by Hardy's attempt, Leonard Bailey (Fig. 4) performed a xenotransplantation to a little girl in 1983. The case is known as the "Baby Fay case". At that time it was almost impossible to obtain human organs from babies. Technically, the operation was performed successfully, but the

girl died of rejection on the 20th day. A possible cause in that case was AB0-incompatibility, since blood group 0(I) is actually not found in monkeys [15].

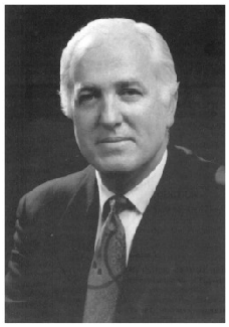

Fig. 4. Leonard Bailey (born 1942)
[https://www.sciencedirect.com/science/article/pii/S1743919115003337?via%3Dihub#fig4]

Thomas Starzl, one of the pioneers in kidney and liver transplantation, performed several liver transplants from primates to children in Colorado in the 1960s without significant success [15, 16]. After the tacrolimus implementation had crucially changed the results of immunosuppressive therapy, he and his team in Pittsburgh performed two liver transplants from monkeys to adult patients in the 1990s, with one patient surviving and living for 70 days [17]. The results, however, were not successful enough to justify the continuation of that study.

A Swedish group headed by Karl Groth made the first attempt to transplant porcine pancreatic islet cells to diabetic patients in 1993 [18]. Although porcine C-peptide was detected in blood of some patients, indicating that some islets survived, the clinical outcome remained unsatisfactory.

Choosing a potential animal donor for xenotransplantation to humans

The availability of primates for medical purposes in the 1960s quickly declined when they were listed as endangered species. Moreover, baboons, for example, do not breed well in captivity, have a long gestation period and produce little offspring. The negative attitude to xenotransplantation has increased in the 1990s, after the detection of retroviruses and a possible xenogeneic infection transmission to a recipient. Due to ethical problems and, especially, due to the risk of transmitting dangerous viruses, organ xenotransplantation from chimpanzees and other monkeys to humans was prohibited [19]. Theoretically, xenogenic viruses can pose a risk to the human genome due to the risk of integrating the genome of an animal to a human when transferred by retroviruses. Fears of cross-infection transmission led to setting a moratorium on clinical trials in xenotransplantation from monkeys to humans [20].

Another likely organ donation source for xenotransplantation to humans is a pig. Considering the immunological characteristics, pig is a less suitable source of organs than primate. Meanwhile, breeding of transgenic pigs with the galactose-α-1,3-galactosyltransferase gene locus (GTKO), comparable sizes of human and pig organs, ease of breeding mean that the pig is a likely potential donor of organs, tissues, and cells for xenotransplantation [21]. The significant phylogenetic distance between pigs and humans reduces the risk of viral infection transmissions, and a careful examination and skilled breeding of animals contribute to minimizing the risk of transmitting zoonotic infections. Progress in understanding the barriers of xenoimmunobiology of pigs and primates and overcoming these

barriers by means of genetic engineering give hopes for obtaining an unlimited number of organs and cells necessary for transplantation needs.

Physiological barriers in xenotransplantation

When using pig organs for xenotransplantation to a human, two fundamental questions arise: the size of the organ and its lifespan. The heart or kidney of a young pig can grow, but how long it will be growing and to what extent is unknown. Moreover, the pigs have a natural lifespan of about 15 years, and there is no data on aging in the organs subjected to xenotransplantation. There are doubts about the efficacy of hormonal factors. It is known that porcine insulin can impact human glycemia, but not all porcine hormones effectively overcome the species barrier. So, in humans, porcine renin and porcine erythropoietin are ineffective [22]. The liver produces more than 2000 proteins, and it is clear that many of them will be incompatible or will not be able to perform the necessary functions across the species barrier. This makes liver xenotransplantation less promising than that of other organs. Xenotransplantation of pig kidneys to primates was able to achieve an effect on plasma-electrolyte metabolism, although not all functions of the human kidney were reproduced. Another problem is a body temperature. The body temperature in pigs is about 39° C, while the human body temperature is about 37° C. The functional consequences of this fact for the activity of porcine enzymes at a lower temperature remain unclear [23].

Hyperacute xenograft rejection and the ways to resist it

The main problem of discordant xenotransplantation is the phenomenon of hyperacute rejection. It is characterized by a rapid onset of edema, bleeding and vascular thromboses, resulting from the presence of preformed antibodies, and occurs within a few minutes after xenograft reperfusion [24, 25].

The hyperacute rejection reaction is mainly mediated by the activity of xenoreactive natural antibodies. Xenoreactive natural antibodies (XNAs) are similar to those naturally produced against the blood group antigens. The epitope, which is the main target of these antibodies, is the trisaccharide group of galactosyl (α-(1,3)-galactosyl-β-1,4-N-acetyl-glucosaminyl), also called gal. There is no such epitope in humans due to the lack of the enzyme synthesizing it. Therefore, higher primates recognize this epitope as alien and produce an immune response against it. Due to the gal epitope expression by microbes in the intestine, humans are immunized to it and have pre-formed antibodies. XNAs produce the effect of a hyperacute rejection, mainly through the activation of the complement system involving NK cells, and the damage to the endothelium. A number of therapeutic strategies have been focused on fighting the XNA synthesis by plasma sorption, plasmapheresis. Unfortunately, anti-gal returned to normal levels within several days. Attempts have been made to prevent the anti-gal synthesis by using cytotoxins against plasma cells capable of producing these antibodies [26].

The main path of inflicting the xenograft damage is the complement system activation with the release of various cytokines and the platelet activating factor. One of the proposed approaches to circumvent this

mechanism suggests the complement system depletion by using the cobra venom. An alternative strategy involves the use of a C1 inhibitor (C1-INH), the only physiological inhibitor of the first step of complement activation. Unfortunately, both these methods impair the physiological functioning of the complement system. Genetically engineered pig clones have been created that express human complement regulators CD55 (decay accelerating factor), CD46 (monocyte chemotactic protein) and CD49. An in vitro experiment showed that such expression protects cells from complement-induced lysis [27].

The ultimate cause of xenograft loss in hyperacute rejection is the vascular thrombosis. The thrombin inhibition has been used to prolong the graft survival. Currently, promising seem the studies aimed at the expression of anticoagulant molecules by xenograft cells [28].

Another concept is the breeding of genetically engineered pigs with a complete blocking of the gal epitope expression. Such clones of various kinds, called knockouts, are currently bred under laboratory conditions. The first encouraging results were obtained from xenotransplantation of organs from knockout mice and pigs [29].

Acute humoral rejection

The next barrier to be overcome in xenotransplantation is an acute humoral rejection. Its main histopathological manifestations include edema, vascular thrombosis, hemorrhage, and intercellular edema. These phenomena usually occur within 24 hours after transplantation and progress to an irreversible damage inflicted to xenografts over the coming days. The initial response is mediated by the formation of IgM antibodies,

predominantly against the gal epitope, followed by an increase in IgG levels. Their damaging effect is ultimately mediated by intravascular coagulation. The approaches to prevent the acute humoral rejection of xenografts include depletion of anti-gal antibodies through the use of immunoaffinity columns for plasma immunoadsorption. Robson et al. have shown that the use of a synthetic low molecular weight thrombin inhibitor can prolong survival, improving the xenograft function [30].

Cellular rejection

Another means of xenograft damage is the cellular rejection reaction. The xenogenic antigen-producing cell represents the receptor for recipient's CD4+ T cells. This leads to the interleukin 2 (IL-2) production by CD4+ T cells. IL-2 acts on the CD8+ T cell that itself recognizes the xenogenic protein [31]. This mechanism of a direct immune response is similar to that one in allogenous transplantation, and, therefore, will be amenable to standard immunosuppressive therapy. However, it is known that besides the direct immune recognition in allotransplantation, there is also an indirect recognition mediated through phagocytosis. This also occurs in xenotransplantation; however, there are many more peptide differences between different species than between different organisms of the same species. Thus, the potential of indirect xenorecognition is much higher than that with the indirect allorecognition [32]. A possible solution to this problem may be the strategies aimed at achieving the immunological tolerance. These include the creation of donor chimerism, that is, the coexistence of the donor and recipient hematopoiesis in one and the same organism. There is a long-standing task to create a protocol enabling to

achieve the donor chimerism without toxic myeloablative strategies. The initial protocols included the elimination of non-specific pre-formed mature donor-reactive T cells and NK cells. In recently developed models, it is expected that only donor-specific T-cells can be deactivated and eliminated, while other T-cells be kept essentially intact, using co-stimulation. The induction of donor chimerism leads to a stable tolerance in concordant xenotransplantation. Early evidence suggests that this may also be possible in discordant pairs [33].

Kosimi et al. have the experience of induced tolerance to allotransplanted kidneys in monkeys by using mixed hematopoietic chimerism, even when the immunosuppressive therapy is discontinued. However, a known risk of this strategy is the development of a graft versus host reaction, i.e. the attack of donor immune cells on the recipient's tissues. In case of xenotransplantation, the severity of this reaction is likely to be more prominent [34].

Infection risk

The risk of transmitting infectious agents across the species barrier is a serious threat to humanity [35, 36].

The term xenosis (xenozoonosis) was proposed to describe the infection transmission by transplanting xenogenic tissues or organs. Xenosis potentially creates previously non-existent epidemiological hazards due to the transmission of pathogenic microorganisms, specifically viruses, with viable cellular transplants. When an infectious agent gains access to a new host species, its pathogenetic abilities become unpredictable [37].

For example, herpesvirus Cerkopiteca 1 (B virus) in its natural host, the macaque monkey, has a clinical profile that is very similar to the infection of the herpes simplex virus in humans. However, the infection virus B in humans or other primates (not macaques) results in rapidly progressive myeloencephalitis with a mortality rate of approximately 70% [38].

Microorganisms that cause a serious concern in terms of their potential danger in xenotransplantation include herpesviruses and retroviruses, toxoplasma gondii, mycobacterium tuberculosis, encephalomyocarditis virus, filoviruses (Marburg and Ebola), monkeypox virus and hemorrhagic viruses, monkeys, and hemorrhagic viruses, filoviruses (Marburg and Ebola), monkeypox virus, hemorrhagic fever virus [39].

Retroviruses, due to the presence of the reverse transcriptase enzyme, integrate their genome into the DNA of the host cell chromosome. Convincing arguments suggest that the HIV pandemic was caused by the adaptation of monkey retroviruses introduced into the human body in various ways. The latent infection of people before 1970 led to more than a decade of an active HIV transmission from person to person before AIDS was first recognized as a public health problem in the 1980s [40].

Endogenous retroviruses exist as part of the genomic material of most, if not all mammalian species, including humans. Endogenous retroviruses raise an equal concern and a greater uncertainty than external retroviruses. Endogenous retroviruses, supposedly, descended from external viruses that have become permanently integrated into the genome, vertically passing down through the inheritance. In original species, this process is benign, whereas in close-related species, the same viruses can be pathogenic [23].

Increasing the phylogenetic distance between pigs and humans presumably makes pigs safer donors than primates. However, this presumption has not been completely studied. The detection of porcine endogenous retroviruses (PERVs) capable of infecting human cells in vitro has raised serious questions regarding the safe clinical use of xenotransplantation from pigs to humans [24].

Phylogenetic analysis has shown that PERVs are closely related to the gibbon leukemia virus, the endogenous koala retrovirus, and the inducible mouse endogenous retrovirus [25]. PERV RNA has been detected in several types of pig tissue (for example, kidneys, lungs, liver, heart, pancreatic islet cells). However, the isolation of virus mRNA does not necessarily correlate with the release of infectious particles. Many human cells clearly express receptors that are specific to PERV A and B, while PERV C-specific receptors cannot be detected in most cases [36].

Besides PERVs, the porcine cytomegalovirus (PCMV), porcine lymphotropic herpes virus (PLHV) and porcine circovirus type 2 (PCV) can have pathogenic potential in humans. In New Zealand, the pigs prepared for xenotransplantation turned out to be carriers of encephalomyocarditis (EMCV) and hepatitis E [38]. It is important that pigs do not have an exogenous antiretroviral equivalent of the HTLV virus or HIV. Two strains of the PERV virus (strains A and B) are present only in a subset of pigs and have the potential to infect human cells in vitro. It is important to note that PCMV strains can be isolated from a pool of potential xenografts by early weaning piglets from breast milk.

Experimental xenotransplantation of organs from pigs or nonhuman primates demonstrated no PERV transmission. One study suggested that

reducing the risk of endogenous retroviruses transfer from pigs to monkeys correlates with a decrease in the number of circulating anti-α-gal antibodies [26]. Other studies showed no PERV infection in monkeys after transplantation of a transgenic liver. Japanese researchers are trying to create genetically engineered transgenic pigs that would be genetically incapable of supporting the infectious process caused by endogenous retroviruses. This breed of pig will express the so-called "silence genes" of RNA [37].

Transmissible spongiform encephalopathies are equally fatal both for humans and animals. Referred to them Creutzfeldt–Jakob disease, a chronic wasting disease, and bovine spongiform encephalopathy are caused by prions. Various reports have documented the ability of prions to overcome the species barrier from cattle, squirrels, and rabbits to humans. There are proven cases of transmissible spongiform encephalopathy transmitted during transplantation [24, 26]. Another risk factor is the fact that a human xenogenic transplant recipient can be infectious in terms of xenosis and will transmit this disease non-typical to human population, to people from his/her environment. The potential clinical application of xenotransplantation to people might require special anti-epidemic measures in relation to recipients and their close environment.

Current state of organ xenotransplantation

Heart transplantation. Anatomically, the pig heart, although not identical to the human, has a significant similarity with it, forms a stroke volume comparable to that of the human heart. Mean arterial pressure, heart contraction rates are also comparable. Currently, the incidence of hyperacute

rejection in heart transplantation from genetically modified pigs to primates is minimized. In 1998, the longest survival of a heterotopically transplanted xenogenic heart was 31 days, whereas at the end of 2013, such organ could function for more than 12 months [27]. The heterotopic xenogenic heart from GTKO/hCD46 pigs can survive average for 236 days [38].

Transplantation of circulation-assisted devices has been practiced for over 50 years. With gaining a comparable experience in cardiac xenotransplantation, the advantages of a natural, complete implantation of a pig's heart will outweigh the advantages of a mechanical heart. Ezzelarab et al. [22] studied the coagulation profile of healthy baboons and recipient baboons who received hearts from GTKO-modified pigs. Some of them developed severe coagulopathy. In porcine heart transplants to a primate, the autoimmune injury takes the form of thrombotic microangiopathy. For example, ischemic myocardial damage occurs at the early stage of consumption coagulopathy [18]. In that situation, the blood plasma fibrinogen and platelet concentrations in the recipient decrease, and the D-dimer level and INR increase [28].

After the removal of the transplanted organ, the coagulation parameters return to normal. Coagulopathy in heart xenotransplantation may be associated with the expression of different gene profiles of the vascular endothelium. Hearts obtained from CD39 transgenic mice were resistant to thrombosis during xenotransplantation on the "mouse-to-rat" model. Ricci et al. [18] investigated the significance of right ventricular endomyocardial function, and concluded that the histological evaluation of right ventricular endomyocardial biopsy samples is an effective method of monitoring a hyperacute rejection after heart xenotransplantation. Generating transgenic

pigs (hCRP) having one or more regulatory proteins of the human coagulation system, for example, thrombomodulin (CD39), the endothelial cellular protein C-receptors, is being studied.

Kidney transplantation. The kidney plays an important role in maintaining homeostasis, excretion of metabolites, in the regulation of electrolyte, fluid balance and osmolarity of blood plasma. The anatomy of a pig kidney is surprisingly similar to human. The maximum concentrating capacity (1080 mOsm/L) and the glomerular filtration rate (126-175 ml/h) of pork kidneys are similar to human ones. Consumptive coagulopathy during kidney transplantation develops less frequently than during heart transplantation, but other complications develop faster. For these reasons, the model of a kidney transplant from a pig to a primate was more complicated than with a heart transplant. The longest survival rate reached was up to 90 days. According to Shimizu et al. thrombotic microangiopathy and glomerulonephritis were the main causes of hDAF-transgenic kidney graft loss [15]. The deposition of IgM antibodies with subsequent activation of the complement play an important role in the mechanism of glomerular damage to the endothelium and in the formation of multiple blood clots [28].

Yamada et al. [22] achieved an 80-day kidney survival in 2 baboons. But when transplanting a combination of a pig kidney and the thymus tissue as an immunomodulator, the maximum graft survival was 83 days [27]. Kidney histological structure was kept safe, but the continuing intensive immunosuppressive therapy quite per se caused complications. Kelishadi et al. [39] showed that the addition of the hDAF (CD55) gene to GTKO pigs improved the survival and function of the transplanted pig kidney in baboons.

Clinical trials of kidney xenotransplantation to patients with chronic renal disease are unlikely, since severe coagulopathy is life threatening and cannot become an alternative to a safe dialysis. Additionally, proteinuria has been identified, requiring a massive infusion of donor albumin, which also complicates the clinical practice. Whether this is caused by the activation of antibodies against the graft endothelial cells or by the physiological incompatibility between pigs and primates is still unknown. Pig kidneys adequately support homeostasis, electrolyte balance, and osmolarity in primates [18, 27, 39].

Thus, the potential factors contributing to the development of thrombotic microangiopathy include the presence of preexisting antibodies, the activity of natural killer cells or macrophages, the preexisting physiological discrepancy between hemostasis of pigs and primates.

Liver transplantation. Despite the anatomical differences (the pig's liver has 3 lobes, the vena cava passes intraparenchymally in the caudate lobe), technically the pig's liver can be transplanted to primates. The main problem remained unsolved is associated with the development of thrombocytopenia in recipient-primates after pig liver transplantation. The liver has various functions, ranging from the synthesis of many vital proteins, blood coagulation factors, biochemical molecules to detoxify harmful substances. The graft survival could be increased from <3 days in 1998 to 9 days in 2014 [18]. The graft failure primarily results from a hyperacute rejection with congestion, hemorrhage, and necrosis caused by severe coagulopathy.

Orthotopically transplanted liver obtained from genetically modified pigs functions adequately; the porcine circulating fibrinogen is noted on the

first day after transplantation being involved in the homeostasis system, but in all cases, an acute renal failure develops. A tubular damage was confirmed by a histological evaluation, and a serum electrophoresis showed a range of low molecular weight proteins eliminated with urine, the recipient survival reaching approximately 1 week. Light microscopy demonstrated focal infarctions, intercellular hemorrhages, the coagulopathy signs widespread in the microvascular bed. The process is characterized by cytokine expression, progressive penetration of monocytes and natural killer cells into the tissue. Only a small amount of T-cells (~ 5%) was noted. The role of macrophages and natural killers in acute rejection is still to be determined; however, neither xenoreactive natural antibodies, nor T cells were significant in rats with a reduced complement concentration [14, 16, 28].

The presence of porcine liver causes immediate platelet aggregation and phagocytosis, or both, on hepatic sinusoidal endothelial cells and hepatocytes. Platelets are lost from the circulating blood almost immediately, causing spontaneous intraorganic bleeding in several sites. It is believed that the activation of endothelial cells plays a key role, but the factors causing it have not yet clearly defined. Studies by Ekser et al. [12] indicate that pig liver can function adequately in a primate, producing the coagulation factors that maintain its normal profile. Hara et al. [13] note that after transplantation, the pig liver will produce complement proteins.

The pig that has acquired the CRP genes is likely to be protected from the pig complement even in the presence of human anti-pig antibodies. The hDAF transgenic liver of pigs will express both human and porcine antigens. There is evidence that human CRPs can effectively neutralize the activated pig complement. But Tai et al. [34] proved that the combination of pig anti-human antibodies and pig complement could damage human tissue.

Inflammatory immune complexes can be formed when the proteins secreted by a transplanted organ bind. Other protein interactions, for example porcine von Willebrand factor (vWF) with human platelet receptor 1b, can lead to thrombocytopenia. But the ability of the liver to clear itself of soluble immune complexes may decrease the liver damage as well. Kupffer cells in the xenograft liver phagocytize the erythrocytes of primate recipients, and frequent hemotransfusions are required to maintain a normal hematocrit level. But organs from GTKO pigs, in combination with additional CRP transgenes, probably provide a protection against a hyperacute rejection, which might prolong survival in order to use xenograft as a bridge to allotransplantation [29].

Lung transplantation. The first experience in lung xenotransplantation was gained in 1968 by Bryant et al. [31], who, by using the perfusion machine ex vivo, showed that, unlike monkey lungs, the pig lungs quickly lose their function due to a high pulmonary vascular resistance and massive edema. The complex of pathological phenomena occurring in the meantime was defined by the authors as “hyperacute pulmonary xenograft dysfunction”. The fragile structure of a pig lung makes it more susceptible to immune-mediated damage in primates. Despite considerable efforts made so far, the porcine lung graft survival after transplantation to baboons had been prolonged only by 9 hours by 1998 and up to 5 days by to date. A pig lung xenograft is most susceptible to a rapid dysfunction due to coagulopathy. Neither the use of various immunosuppressive protocols and the schemes to remove antibodies or complement, nor the introduction of new genetically modified pigs, such as hDAF and CD46, could provide a longer survival. Unlike other organs (heart and kidneys), lungs release large

amounts of vWF. Under normal conditions, the platelet activation and adhesion occur when vWF binds to GPIb receptors on platelets, but only if the platelets have been exposed to a sheer stress. But the porcine von Willebrand factor binds to a human (or monkey) GPIb without a sheer stress, which leads to a platelet aggregation even in the absence of the activation. The vWF–xenoantibody complexes remain able to aggregate primate platelets. With the removal of von Willebrand factor from the donor and the depletion of pulmonary macrophages, the survival is prolonged [18]. These observations suggest that porcine vWF plays an important role in the pathogenesis of pulmonary xenograft dysfunction [19]. Gonzalez-Stawinski et al. [17], when using CD46 transgenic pigs, demonstrated the importance of anti-gal antibodies in lung xenotransplantation.

The hyperacute dysfunction of a lung xenograft seems to be related to factors other than anti-Gal antibodies, since the survival and pulmonary vascular resistance of the lungs obtained from normal pigs were better than in genetically modified animals. Though having modest results, porcine lung transplantation can help selecting genetic mutations or exogenous drugs to improve the survival of kidney or heart xenograft. Experimental experience of pig lung xenotransplantation is limited, partly due to a severe vasoconstriction that occurs in newly transplanted lungs; although it has been demonstrated that a porcine lung provides an adequate oxygenation and carbon dioxide exchange in primates [37]. Perhaps future studies with genetically modified pigs expressing TFPI or CD39 will make further progress.

Transplantation of pancreatic islets. Porcine insulin differs from human by a single amino acid only and is functionally comparable to the

human one. Pig insulin has been used for decades to treat diabetic patients [37]. Therefore, there is reliable evidence that islet cells that are well functioning in pigs will be able to maintain normoglycemia in diabetic patients. Experimental studies on the transplantation of porcine islets to primates are more encouraging than xenotransplantation of pig organs, so these studies can expand significantly over the coming years [35]. Without immunosuppression, the pancreatic islets obtained from conventional pigs survive 250 days, and those from genetically modified pigs do for > 1 year. The data indicate the presence of antibodies, complement, which get involved in the process of graft damage. Fetal and neonatal pigs express the galactose-α-1,3-galactosidase, the islets in adult pigs do not express this antigen. Pancreatic islets obtained from hCD46 pig, survive for 396 days. And with moderate immunosuppression, normoglycemia persists for more than 1 year in monkeys with experimental diabetes mellitus [19].

Neonatal islets are more resistant to damage than adult ones [21]. Genetically modified pig islets can be used for several days after pig's birth and will not need to be grown for several months or years to obtain enough quantity. Casu et al. [11] investigated the aspects of glucose metabolism in pig islet xenografts transplanted to monkeys. There were differences in glucose metabolism between monkeys and pigs, which increased the difficulty in obtaining normoglycemia in animals after transplantation of pig islets. Probably, it will be easier to get normoglycemia in humans after transplanting pig islets.

Neuronal cell transplantation. The potential of pigs as a source of cells capable of correcting various neurodegenerative abnormalities is being studied [32]. The graft function in monkeys with extrapyramidal disorders

kept safe for over 1 year. For example, there is considerable potential for dopamine-producing pig cells in conditions such as Parkinson's disease. Preliminary reports from Cozzi et al. indicated a significant improvement in motor function in the monkeys in whom a condition similar to Parkinson's disease had been induced [26].

Hepatocyte transplantation. Pig hepatocyte xenotransplantation has several advantages over liver xenotransplantation. Very few studies have been conducted, but the use of arterialized xenografts has proven its worth when using various immunosuppressive regimens. Hepatocytes from a conventional pig survive for 80 days without reimplantation [30].

Skin transplantation Wiener et al [15] reported a long-term survival of the pigskin obtained from GTKO-modified pigs and grafted to baboons. Skin grafts from GTKO survived for 14 days, while those obtained from unmodified pigs were rejected on the 4th day.

Cornea xenotransplantation. Xenotransplantation can provide an unlimited corneal resource for patients with corneal blindness. Although the number of deceased human donors in most of the Western world countries is sufficient for this purpose, in many parts of the world there is a very significant shortage of human donor cornea [37]. In vivo studies on primates show that even corneas of wild-type pigs (unmodified) remain functional for several months with local treatment with corticosteroids. The current experimental data in vitro have shown that the corneas of GTKO/CD46 pigs exhibit significant resistance to the human immune response [19]. With new genetic modifications, it is likely that, from an immune point, the pig's cornea will soon be comparable to the human cornea. From a biomechanical point of view, they are also comparable [22].

Ethical issues

The very idea of xenotransplantation raises many ethical questions concerning both humans and animals [1, 19, 26].

Animals used for xenotransplantation should be raised in isolation in order to exclude known potential pathogens for humans from the colony. The extensive experience of human contact with pig tissue, including the patients receiving pig insulin, clotting factors, and skin grafts, is encouraging. However, none of these situations was associated with the long-term presence of a large number of porcine cells or organs in an immunocompromised human body [27].

Potential risks of xenotransplantation for society create a unique situation with obtaining the informed consent from the potential recipient of the xenoorgan. His/her consent to xenotransplantation must be accompanied by an undeniable, non-revocable agreement, according to which the recipient agrees to lifelong monitoring, stops blood donation, and informs all people who are in close contact with him about the xenotransplantation and its potential risk of spreading infections. The recipient of the xenogenic organ waives the right to refuse from the treatment and investigations at any time, which denies the fundamental individual rights, as defined in the Helsinki Declaration and the US Federal Code [9]. Perhaps the consent to xenotransplantation creates a precedent for the imposition on the recipient of a non-revocable obligation (the so-called Odyssey Pact, referring us to the story of Odyssey who tied himself to the mast of the ship and forbade unleashing him, despite his requests) [24]. The Odyssey Pact for xenotransplantation will allow the transplant team to perform certain actions necessary to ensure the fulfillment of obligations given by the recipient

before the operation. If the subject changes his/her opinion in the future regarding his/her previous agreements, such as the right to refuse court proceedings or to inform relatives about contacts, about the potential risks of xenotransplantation, the Odyssey Pact will be binding in the form of quarantine or even detention, thus protecting society as a whole [21].

From a public health perspective, notifying the family and caregivers of potential infectious risks associated with a xenotransplantation recipient may violate confidentiality principles. In this regard, questions arise concerning the need for obtaining an informed consent from third parties when selecting a patient for such transplantation [22].

Another problem is that people who are in close contact with xenograft recipients should also refrain from donating blood and agree to monitoring if this becomes necessary. The application of such rules may turn impossible, given that intimate contacts can vary many times over the course of a person's life [18].

Given these difficult issues, public involvement and government oversight are needed to decide whether a country will be allowed to conduct xenotransplantation research [18]. Raising public awareness about xenotransplantation problems is the only adequate mechanism to ensure public alertness regarding the potential dangers of xenosis to human health [34].

Potential risks of xenotransplantation cannot be limited to the geographic boundaries of the country in which the surgery is performed. In the absence of international rules and control procedures, the most aggressive security measures of any state are likely to be unsuccessful. This issue is relevant because of the constant migration of the population and the

widespread use of intercontinental air travel, which can quickly spread the infectious agent to geographically distant areas. The ethical principle of equity requires all states to be responsible for controlling the risks of infectious diseases. This problem is extremely complex and requires a globally recognized international treaty with a unified system of immigration surveillance to check the penetration of potentially dangerous infectious pathogens [37].

Xenotransplantation can cause various psychosocial problems associated with the emotional and personal identity of the recipients. These issues should also be thoroughly discussed with the potential recipient in advance [27].

The notion of animal rights as an organ donor is controversial. Higher primates have complex social patterns of behavior, so there are various ethical problems regarding their use; as far as pigs are concerned, the issue is much less controversial. However, regarding the use of animals to obtain xenogenic biological meshes for the treatment of soft tissue defects, representatives of the *People for the Ethical Treatment of Animals* stated that they were “against using animals and animal tissues for experiments, medical training, and clinical treatment ..., including the use of biological meshes" [19]. Many animal rights activists oppose xenotransplantation because they assert that humans do not have the right to use the lives of animals for these needs.

Religion plays a significant role in the daily life of many people and thus affects the lifestyle, eating habits, and attitudes towards treatment. Three main monotheistic religions put humanity on a unique place in the hierarchy of living creatures [8]. Christian Churches believe that humanity

has the mandate to govern the life of creation to its common good. On the other hand, both Judaism and Islam prohibit the breeding and consumption of pigs for food. However, the use of pig organs for xenotransplantation is not considered pork ingestion. In addition, both Judaism and Islam allow exceptions to nutrition laws, especially in situations where human life can only be saved in this way [22].

Buddhism views organ donation as a matter that must be left to every person's conscience. The Hindu principle is that the body must remain intact for the transition to eternal life, so the transplantation is not performed. However, Hindu legislation does not prohibit a person from his/her organ donation or receiving a donor organ. With the exception of cows that are sacred in Hinduism, there are no prohibitions on using animal parts to alleviate human suffering [29].

Conclusion

Thus, xenotransplantation provides a large field for research and contains a number of complex questions that have not been answered yet. Finding the ways to ensure the safe and efficient functioning of animal organs in a human body allows a deeper understanding of the organ rejection mechanisms after allotransplantation, the development of strategies to improve patient outcomes via organ transplantation. The issues raised include new fundamental aspects in molecular biology, cytology, immunology, ethics, and therefore, serve as points for further growth and development of clinical and experimental transplantology.

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