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ADVANCES IN OVERCOMING IMMUNOLOGICAL AND PHYSIOLOGICAL BARRIERS IN XENOTRANSPLANTATION

D.A. Velikiy[1], Yu.B. Basok[1], N.V. Grudinin[1], O.E. Gichkun[1, 2], A.P. Trashkov[3], A.V. Varlachev[3], O.P. Shevchenko[1, 2]

[1] Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation
[2] Sechenov University, Moscow, Russian Federation
[3] Kurchatov Institute, Moscow, Russian Federation

The growing number of patients with severe organ diseases, along with the increasing demand for retransplantations, has intensified the global shortage of donor organs – the primary limitation to expanding transplant programs. Advances in genetic engineering and cell therapy technologies are opening new opportunities for the use of animal organs in human transplantation. Enhancing the efficacy and safety of this approach requires overcoming significant immunological and physiological barriers inherent in xenotransplantation. This review summarizes recent progress in genetic modification of donor animals, use of cell-based therapy in xenotransplantation, and prospects for clinical application.

Keywords: xenotransplantation, hyperacute rejection, genetic modification, cell therapy.

INTRODUCTION

Organ transplantation remains the only definitive treatment for many patients with end-stage organ failure. Advances in surgical techniques, postoperative care, and immunosuppressive therapy have significantly improved transplant outcomes. However, the primary factor limiting the expansion of transplant programs is the persistent shortage of donor organs. In 2023, a total of 1,817 kidney transplants were performed in the Russian Federation [1], while 53,874 patients were on hemodialysis [2].

One possible solution to this problem is the use of animal organs. In the mid-20th century, the first attempts at transplanting primate organs into humans were conducted in the United States. However, these early experiments yielded poor outcomes, with most recipients dying within weeks due to graft rejection. The subsequent decline in interest in xenotransplantation was driven not only by immunological incompatibility but also by concerns regarding the transmission of zoonotic infections. The discovery of retroviruses and the potential emergence of recombinant strains pathogenic to humans led to an almost complete halt in animal organ transplantation research [3].

However, recent advances in genetic engineering and cell therapy have revived interest in xenotransplantation. Although apes share a closer anatomical, physiological, and immunological resemblance to humans, they are not considered an optimal source of donor organs. Compared to primates, pigs present several key advantages: rapid growth to adult size, early reproductive maturity, large litter sizes, and relatively low maintenance costs. It is also important to note that there is a certain amount of accumulated experience in the field of genetic engineering in cloning pigs [4].

The purpose of this review is to analyze the latest data on methods of genetic modification of animals and the use of cell therapy in xenotransplantation, as well as to assess the prospects for its clinical application.

RISKS OF XENOTRANSPLANTATION

Genetic disparities between species create immunological barriers to successful xenotransplantation. Early attempts to transplant pig organs into primates were largely unsuccessful: within hours of reperfusion, recipients developed hyperacute rejection [5, 6]. This reaction is driven by the recognition of xenoantigens expressed on the vascular endothelial cells of the pig graft by preexisting “anti-pig” antibodies in the recipient’s circulation. The ensuing cascade involved antibody-mediated activation of the complement system, leading to endothelial inflammation, formation of the membrane attack complex, and subsequent vascular injury. This triggered coagulation pathway activation, resulting in interstitial hemorrhage, thrombosis, and ischemia, ultimately destroying the transplant [7].

Membrane-associated regulatory complement proteins play a crucial role in the development of rejection during xenotransplantation. These molecules, expressed on the surface of most cell types, suppress excessive complement activation and thereby protect healthy cells.

Corresponding author: Dmitriy Velikiy. Address: 1, Shchukinskaya str., Moscow, 123182, Russian Federation. Phone: (499) 193-87-62. E-mail: dim_vel@mail.ru

Similarly, coagulation regulatory factors present in the vascular endothelium maintain an anticoagulant state under normal conditions.

However, in xenotransplantation, porcine complement and coagulation regulatory proteins fail to interact efficiently with their primate counterparts. As a result, the xenograft becomes susceptible to uncontrolled complement activation and coagulation cascade dysregulation [8].

Another major obstacle to successful xenotransplantation is the risk of transmission of zoonotic infections. Therefore, animals – particularly pigs – used as organ donors must be bred under sterile, pathogen-free conditions and rigorously screened for infectious agents hazardous to humans, such as gamma (γ) herpesvirus, swine influenza virus, porcine cytomegalovirus, hepatitis E virus, and porcine endogenous retrovirus (PERV) [9].

Unlike other pathogens, PERV is integrated into the pig genome and cannot be eliminated through pharmacological or vaccination measures [9]. Although no cases of PERV transmission to humans or nonhuman primates have been documented during experimental xenotransplantations, genetic inactivation of PERV loci is considered a promising strategy to mitigate the potential risk of zoonotic transmission [10].

GENETIC MODIFICATION OF DONOR ANIMALS

A significant body of research in genetic engineering and animal cloning is currently focused on enhancing the compatibility of pig organs for xenotransplantation into humans. The creation of genetically modified pigs with multiple gene deletions and human transgene insertions should be aimed at overcoming key immunological and physiological barriers to successful pig-to-human organ transplantation [11].

The advancement of these methods has been largely driven by the development of the CRISPR/Cas9 genome editing system, which is derived from a natural bacterial antiviral defense mechanism. This technology enables the induction of site-specific double-stranded DNA breaks within the genome, facilitating targeted insertion or deletion of genes followed by cellular DNA repair [12].

Another necessary condition for genetic modification is the ability to obtain a line of animals with a modified genome. This is achieved by transferring the nuclei of genetically modified somatic cells into an enucleated animal oocyte (cloning). The combination of gene targeting via homologous recombination in cultured somatic cells followed by nuclear transfer allows for the production of multiple heritable genetic modifications for xenotransplantation purposes [13].

In recent years, a series of experimental studies have been conducted to identify optimal strategies for genetic modification of the pig genome aimed at minimizing the risk of xenograft rejection [10, 14, 15]. One of the most effective approaches reported involves the modification of ten key genes – specifically, the deletion of four porcine antigens combined with the insertion of six human transgenes.

The inactivated porcine genes include the major carbohydrate antigen αGal (Galactose-α-1,3-galactose) and two additional carbohydrate antigens, CMAH and β4GalNT2, which participate in the synthesis of N-glycolylneuraminic acid and the sialyl dimeric antigen, respectively. The growth hormone receptor gene (GHr) is also deleted to limit donor pig growth, maintaining body weight below 150 kg.

At the same time, six human genes were introduced: CD46 (membrane cofactor protein) and CD55 (decay-accelerating factor) as complement inhibitors; THBD (thrombomodulin) and EPCR (endothelial protein C receptor) as coagulation inhibitors; CD47 (integrin-associated protein), which inhibits T-cell and macrophage activation; and HO1 (heme oxygenase-1), an anti-inflammatory enzyme [16].

Prior to organ harvesting for xenotransplantation, it is necessary to study the genotype and phenotype of donor animals for the presence of all intended modifications and to rule out any unplanned changes, such as unintended CRISPR/Cas9-induced breaks or the random insertion of extra copies of human transgenes [17].

CELL THERAPY

One of the major challenges in xenotransplantation is the lack of an effective immunosuppressive regimen. A promising direction for improving xenograft outcomes lies in the application of cell-based therapies designed to enhance the effectiveness of organ transplantation by mitigating the adverse consequences of prolonged immunosuppression [18].

Mesenchymal stromal stem (MSCs) are known to possess a set of unique properties, including immunosuppressive effects. The therapeutic potential of MSCs is mediated by the secretion of numerous regulatory and trophic factors, exosomes, microvesicles, lipoproteins, microRNAs, as well as apoptotic bodies, which significantly enhance regenerative processes in damaged organs, stimulate angiogenesis, and prevent cell apoptosis, inflammation, and fibrosis [19].

Further optimization of MSC-based therapy in transplantation may involve their pre-activation. One of the most promising strategies is MSC activation through autophagy induction [20]. The combined use of methods of genetic modification of animals and cell therapy can increase the effectiveness of xenogeneic transplantation and achieve long-term survival of the xenograft.

CLINICAL OBSERVATIONS

In recent years, an increasing number of studies have reported on the outcomes of xenotransplantation of genetically modified pig organs in both brain-dead human

models and living recipients [21]. The use of brain-dead patients as recipients in such experiments is scientifically justified, as it allows for minimizing the risks associated with early clinical trials [22].

Nevertheless, this model has several important limitations, including short observation periods and unstable hemodynamics in brain-dead recipients, which may lead to hypoperfusion, ischemic injury, and inflammatory responses within the xenograft [23].

A notable clinical case was described by Kawai et al., who reported the transplantation of a genetically modified pig kidney into a 62-year-old patient with end-stage chronic kidney disease. In the early postoperative period, the recipient developed an episode of T-cell–mediated rejection, which was successfully treated with antithymocyte globulin. No subsequent rejection episodes were observed. The xenograft remained functionally active for two months until the patient died of acute coronary pathology unrelated to the transplant [24].

In another notable report, a research group described the xenotransplantation of a genetically engineered pig heart – modified with ten specific gene edits – into a living human recipient. On the first postoperative day, the xenograft demonstrated satisfactory cardiac function. However, by day 13, endomyocardial biopsy revealed signs of acute antibody-mediated rejection. Despite intensive immunosuppressive therapy, hemodynamic decompensation occurred on day 30, necessitating extracorporeal membrane oxygenation (ECMO). The patient was declared dead 10 days later. The authors attributed the graft failure primarily to the recipient's critical preoperative condition and the extensive transfusion of blood components, both of which likely contributed to the rejection [25].

In a study conducted by Tao et al., heterotopic xenotransplantation of a genetically modified pig liver was performed in a brain-dead human recipient. The xenograft maintained stable hepatic perfusion and functioned effectively for 10 days, producing both bile and porcine albumin. Histological analysis of biopsy specimens revealed C3d and C4d complement deposition, along with IgM and IgG staining, consistent with early humoral immune activation. Despite the short observation period, the non-physiological nature of the transplant and the use of a brain-dead recipient, the authors suggested that such xenotransplants may provide temporary metabolic and synthetic support through xenotransplantation, serving as a bridge to to possible allotransplantation [26].

CONCLUSION

Advances in genetic engineering have made it possible to create genetically modified lines of animals (primarily pigs) whose organs do not trigger hyperacute rejection during xenotransplantation to primates in experimental settings or to humans in clinical practice. However, species incompatibility remains a major challenge, as long-term xenograft survival still requires recipients to take high doses of immunosuppressive drugs, which significantly increases the risk of malignant tumors and infectious complications. To mitigate the adverse effects of prolonged immunosuppression, several immune tolerance–induction strategies have been proposed, including hematopoietic stem cell transplantation to achieve mixed chimerism, combined transplantation of a solid organ and thymus, and infusion of regulatory T cells. Clinical studies have demonstrated that formation of mixed chimerism in kidney recipients following allotransplantation from HLA-incompatible related donors may allow for reduction or complete discontinuation of immunosuppressive therapy [27].

At present, a synergistic approach combining genetic modification of donor animals to reduce the immunogenicity of their organs with cell-based therapies aimed at inducing immune tolerance to the xenograft appears to be the most promising direction. This approach may not only enhance the efficacy and safety of xenogeneic transplantation and ensure long-term survival of both the xenograft and recipient, but also provide new insights into the fundamental regulatory mechanisms underlying the immune response in interspecies transplantation.

The authors declare no conflict of interest.

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