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Xenotransplantation and Its Adverse Effects On the Immunology of the Human Body and Solutions

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ABSTRACT

The current demand in the U.S. for a transplant far exceeds of what is available. In fact, 22 people die each day because they do not get the necessary organ in time. With the human population growing, this problem will surely continue to get worse, but innovative solutions exist. Xenotransplantation, a method where a non-human organ is transplanted into a human, offers exciting potential like none seen before. An unlimited number of cells, tissues, and organs could be "manufactured" through genetic engineering, and this would truly be able to solve the high demand of organs in the United States. Obviously, tons of hurdles exist. Based on multiple sources, specific effects because of hidden diseases inside the graft, or organ of the animal, can cause damage to the patient which is alarming. Organ rejection is also deeply covered: the several types and what it entails in the body. Analyzing from various sources, the purpose of this research paper is to explain the adverse effects that transplantation can cause and to promote further research and investigation on combating the adversities presented.

Introduction

Various diseases and chronic conditions require patients around the world to make a difficult choice. An example of one would be Stage 4, chronic kidney disease, and the choice they would have to make is whether to proceed with dialysis, a blood filtering system that requires numerous hours, or transplantation. The issue resides in the fact that there are not enough organs to supply the demand of the growing human population. Xenotransplantation, on the other hand, would provide an unlimited number of cells, tissues, and organs. Currently what is known is that genetically modified pigs are raised by knocking out pig genes that can have the expression of antigens that can bind with primate (human) antibodies to complement-mediated destruction. For this paper, the major issue I will be focusing on is the immunological differences existing between a pig and a primate since the physiological differences are overall similar. The complications of these methods are extreme though. A lot of fixing is required since the organs of animals such as pigs are not always compatible inside a human's body. Hence, my discussion will proceed to be about the adverse effects on the immunology caused by Xenotransplantation, and techniques of how genetic engineering and other methods can combat these hardships.

Transplant demand by organ

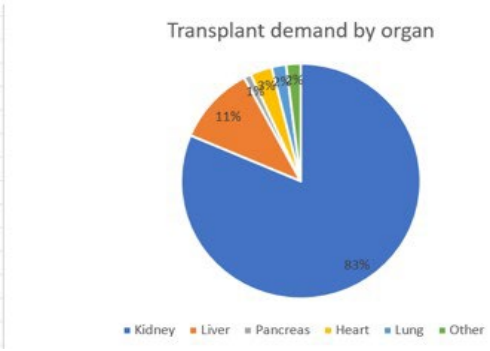

Figure 1. As of February 2022, the kidney organ is in an overwhelming demand compared to other organs, coming in at an astounding 83%

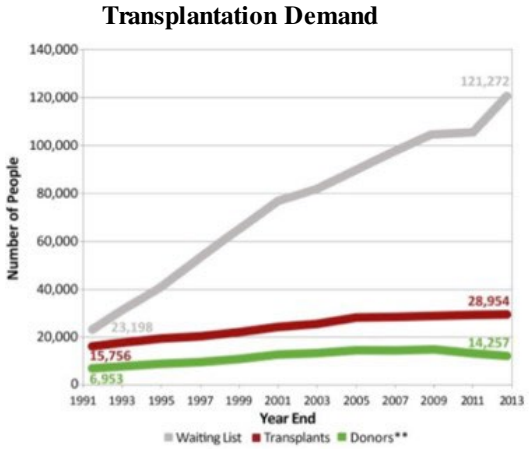

Figure 2. The number of people that require a transplantation far exceeds availability.

Origin of Xenotransplantation

The idea of transfusing an animal's organ into a human was first proposed back in 1667. Non-human primates (NHPs) had similar characteristics to humans, so it was said to be suitable. It was first believed that the chimpanzees would be most suitable to be animal organ donors, because they had a similar genetic composition since we all originated from one common ancestor. The first two chimpanzee kidneys ever were transplanted into humans in 1963 and 1964, where one patient managed to survive nine months.

Soon, the field of xenotransplantation began to take a turn. Experimentation with pigs began because of their large litter size and its physiological similarities to humans. Also being easier to genetically modify, certain life-threatening aspects such as viruses can be avoided through gene-editing techniques. Ethical concerns and the risk of cross contamination were addressed, and it became clear that the best animal for transgenic processes was the pig. Currently, underworks are using modified pig tissues and marrow to solve complex diseases such as Huntingdon's and Parkinson's Disease.

Table 1. The pros and cons of each animal with respect to being transplanted in a human body

Animal	Organ size	Immune system	Risk of infection	Offspring	Cost of maintenance
Pig	Satisfactory	Distant	Low	5-12	Low
Baboon	Unsatisfactory	Close	High	1-2	High

Several Types of Organ Rejections During Xenotransplantation

Hyperacute Rejection

Imagine an organ from an animal's body is put inside a human's body, but the graft (section of tissue in the organ) gets destroyed. Happening in a matter of minutes to a few hours, this type of rejection is known as Hyperacute rejection. For this to occur, the patient usually has pre-existing anti-bodies, and the binding of these antibodies to xenoantigenic epitopes triggers the forming of proteins. The activation of the protein causes more issues and complications in the endothelial cells (inside heart and blood vessels) which then leads to destruction and then organ failure.

The characterization of this rejection is by the graft developing a beefy red, or even blue-like appearance and the formation of platelet thrombi in small blood vessels. One reason for the extreme prominence of Hyperacute rejection in regulatory proteins in the graft is not at all compatible with the complement system of the recipient. One counter example of a regulatory protein would be CD59, but being slow and inefficient, this membrane co-factor protein does not inhibit the complement of the primate. The lack of function and communication between the pig and primate protein contribute to the rejection since the activated complement proceeds rapidly and unchecked.

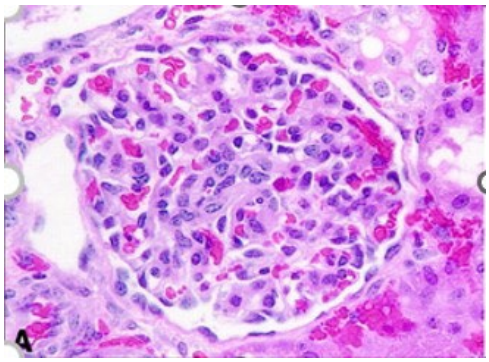

Figure 3. Hyperacute rejection in pig-primate renal xenotransplantation

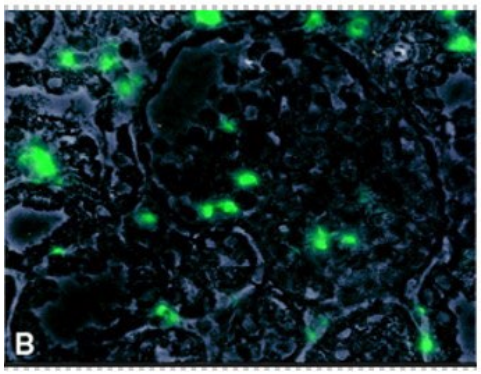

Figure 4. Staining for CD41 for kidney undergoing hyperacute rejection; reveals platelet aggregation

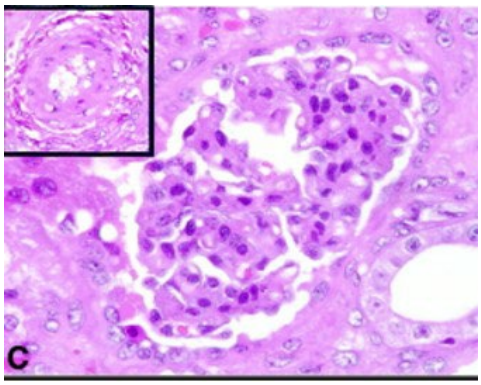

Figure 5. Acute Vascular Rejection; signs of vasculitis in the biopsy

Acute Vascular Rejection

This condition is also known as "delayed" xenograft rejection, but acts different in many ways, this rejection is the main obstacle to transplanting animal organs. This type of rejection is usually identified with characteristics such as focal ischemia (when a blood clot blocks a cerebral vessel), thrombosis consisting of fibrin (blood clots block veins or arteries), and sometimes infiltrating cells (discussed more in cellular rejection).

One of the main reasons for the rejection to occur is the xenoreactive antibodies. Observations show that after the exposure of porcine tissues or the graft to the human body, many antibodies are synthesized. Another reason would be endothelial cells. There are different ways by which these endothelial cells get activated. The first are the xenoreactive antibodies which react with the surface antigens which can create signals which then lead to pathways. The next way is the activation of the complement, which then activates the endothelial cells. The incompatibilities with the porcine proteins, such as protein C and thrombomodulin, are also a huge cause in the rejection process.

Cellular Xenograft Rejection

The graft for this specific type results in rejection days or weeks after the transplantation happens. Cellular rejection, such as by that of T cells, Natural Killer (NK) cells, and macrophages, are all propelled by certain immune responses and these rejections are all cell mediated.

Macrophages (cells found as a white blood cell at site of infection) are present in the immune system and respond to any kind of tissue invasion, such as rejected xenografts. The result of the macrophage activity can be due to T cells. These T cells can cause infiltration with the microphages and then the destruction of the xenografts. Macrophages can also activate simply by the interaction between donor epithelial antigens and the receptors on the surface of macrophages. They have toxic effects because the macrophages release tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and IL-6. Toxic effects such as those can cause apoptosis, or cell death, and is catastrophic for the xenograft.

Being part of a group known as lymphocytes, NK cells can show signs of cytotoxicity inside human blood by mediating xenograft rejection. NK cells release something known as lytic granules, which contain a series of proteins that mediate cell destruction after secretion. This leads to lysis of the donor cells, specifically the epithelial cells. The pig molecule NKp44 can trigger lytic granule release, and the NK receptors NKG2D and pULBP-1 bind to NKp44 to trigger the release of the lytic granules. Once again, the chain of reactions, or the activation on the endothelial cells causes cell apoptosis.

Chronic Rejection

In the final type of organ rejection, Chronic Rejection, this would typically take months to years. It is also the major cause of graft (organ)- loss, and the main sign or feature of this specific rejection is thinning of the graft vessels. This is caused by both cells which mediate other activation channels or antibodies that reject the xenograft. Basically, what happens is fibrosis on the endothelium leads to a blockage, and this then causes nephrosis because of the increase in permeability. Eventually, the fibrosis leads to failure of the organ by the localized inflammation, scarring of the tissue, and buildup. By this stage, there is no treatment.

One final type of rejection is the Graft vs Host Disease. Organs that have T-cells can cross react with the host and attack the "foreign-looking home", also known as the host. The rapid attacks will eventually cause failure of the xenograft.

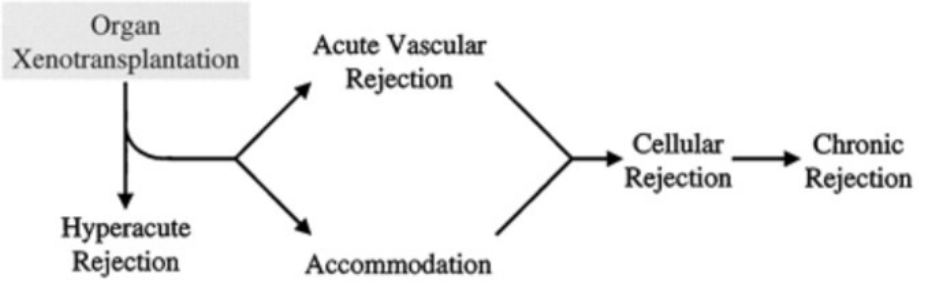

Figure 6. The pathway of rejection for a Xenotransplantation;

Infection Diseases/Viruses

Infectious diseases passed from an animal to a human by unnatural means are called xenozoonoses. Xenotransplantation carries the risk of transmission of infections with the cells or tissues of the graft. The degree of risk is usually unknown because of the absence of clinical trials, but some common principles exist: The risk of infection is related the quantity of the organism transmitted, and any experiment or scientific document of people that implement a transmission of an animal part must be documented in that scientific journal.

First, infectious diseases are such a risk is because of immunosuppression. Patients are on this when the transplant occurs, which also means that their ability to counteract a normal immune response to an infection is lost. Increasing the dangers by bringing an animal's organ with their already suppressed immune system possess many risks, so when experimentation occurs, doctors must be extremely careful and test the animal and the animal's organs to make sure it in not an agent for a particular virus. The same concept occurs if you are meeting someone in an ER room: either mask up or be sure you are not sick so you do not spread them even the common cold because that itself could damage their immune system leading to horrible and even life-threating complications.

Second, the infectious microbe being inside the host (animal) may not show any signs or symptoms which makes it highly unpredictable when it enters a human body. Considering that the effects that may be present are masked, it is important to figure out a way to identify any dangerous microbes before transmitting an organ into the human body. An example would be cercopithecine herpesvirus, or B virus. Clinically known to act normally and harmless in its host the macaque monkey, things change drastically if a xenotransplantation were to occur. It would initiate a chance of 70% mortality rate by causing a neurological disease in humans. Another virus would be pseudorabies virus (pig herpes), which does not show any signs of being a virus inside

of its host which is pigs but can cause neurological disease inside human which quite literally fatal. This is the case for many adult pigs and baboons, which are positive for herpesviruses unless raised in specific conditions (and genetically engineered).

What Are Some of the Non-Immunological Hurdles?

The physiological issue begins with complex cascades of proteins, and the porcine organs may not know how to deal with this complexity. For example, consider human protein C. Porcine thrombomodulin does not interact properly with this protein, so it leads to a trigger that will eventually cause rejection of the xenograft. The only way to solve issues like these would be with genetic engineering. Expressing the human proteins would make sure that coagulation, or the clotting of blood to become a solid state, does not occur due to genetic manipulation of the processes.

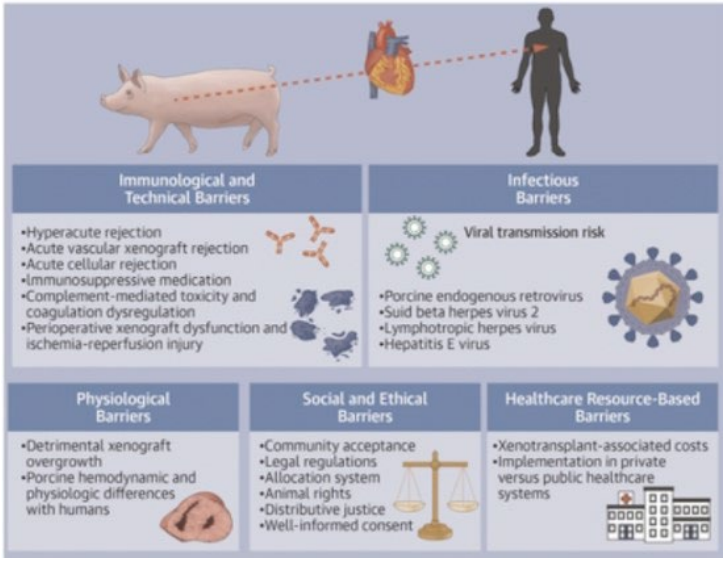

Figure 7. The various barriers involved in the transgenic process.

Combating Organ Rejection

Prior to new technology and innovative strategies, organ rejection was simply that. There was no way or device to combat it, but times have changed. In fact, there are biotechnology companies that have transplanted pig organs into humans with only around a 2% rejection rate (hyperacute). One way that has been clinically proven by reducing or completely eradicating the patient's xenoreactive antibodies. This is difficult though, and the method to do it is known as immunoadsorption, involving the removal of plasma into a particular device that removes immunoglobulins. The plasma is then re-filtered back into the human body and does not bind with the surface antigens as no antibodies are present. Specifically, the columns that possess Gala1, 3Gal are the ones that prevent hyperacute rejection.

Another factor besides the antibodies that can be inhibited is the complement. Two ways are with the use of cobra venom factor and CR1, both disrupting the cascade but stimulating the chance of other infections because the immune response is weakened.

The final strategy that has emerged is transgenic pigs, or genetically modifying pigs in order to produce results that are compatible with the human body. The pigs are modified such that they contain human complementary proteins so that they do not reject once placed inside the body. One of the genes, α-galactosyl transferase, is deleted inside pigs since it is rejected inside a human's body. It is present in mammals such as pigs but

must be taken out in order to prevent rejection by any means. Humans have a loss of the GGTA1 gene, so they cannot synthesize galactose, leading to the development of antibodies and eventually rejection.

For acute vascular rejection, a practice known as "tolerance" can be used to solve the rejection. Because it leads to a decreased amount of xenoreactive antibodies produced, it is useful to prevent this rejection. One of the ways to execute this is to "engraft bone marrow cells by expressing xenogeneic antigens" (Samstein). A study that expressed α1, 3galactosyltransferase in bone cells in mice produced the anti-Galα1,3 Gala antibodies. Inside humans, the transplantation into the bone marrow directly impacted the antibody count which was overall decreased amount.

The prevention of cellular xenograft rejection proceeds after hyperacute and acute rejections are solved. The first, and most obvious way, to combat this rejection is by immunosuppressive drugs. An example would be Rapamycin or Deoxyspergaulin that affect the T cell functioning which then leads to less xenoreactive antibodies being naturally produced (Samstein). Other medications and drugs do the same: by inhibiting cell function, it allows a greater chance for the transgenic graft not to fail. Additionally, blocking of the CD28 and CD40 pathways prolong survival is mice experiments, so it is likely that the same occurrence happens inside a human's body. The only problem is that the blocking of these pathways to not guarantee survival forever, it is only a temporary fix for a much more complex and difficult issue which will take time to resolve.

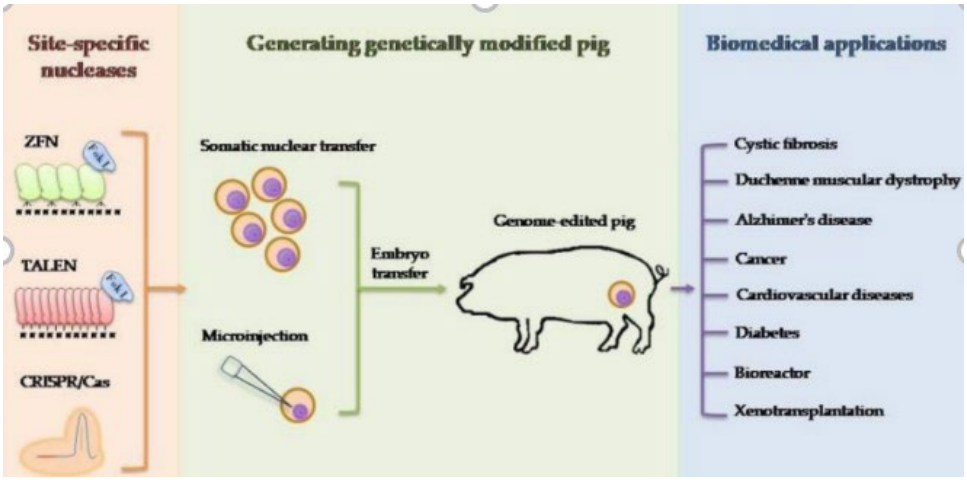

Figure 8. Gene-editing technique for pigs and their applications (specifically xenotransplantation in this paper)

Future Outcomes

Being one of the most promising ways to solve the organ shortage issue amongst patients, xenotransplantation holds power like no other. Recently enough, at NYU Langone Health, the first ever investigative transplantation took place with a genetically engineered, non-human kidney being transplanted into a human body. After getting consent from the family, the doctors studied for 54 hours (about 2 and a half days), waiting for any signs of rejection or other breakdowns that would cause the body to have unexpected outcomes. The genetically engineered pig had a specific gene knocked out which was mentioned in this research paper, alpha-gal, so that the anti-bodies that would be created by the body when pig organ is inside. The alpha-gal molecule specifically initiates the hyperacute rejection response, so it was crucial to delete that gene from the genetically engineered pig. All the kidney levels were monitored closely, and everything mimicked that of a normal, human kidney.

Studies like these show promises. We are still hopeful for the first transgenic pig organ to be placed inside of a living human, not a brain-dead one. Ethically and for all the effects that a porcine (pig) graft could

serve to the human body, it is still considered a risk to transplant a non-human organ into a living patient, but with more hopeful transplantations like the one at Langone Health Center, the future is extremely close.

Conclusion

Throughout this paper, the main discussion has been around xenotransplantation: an introduction, the adverse effects it poses, solutions, and the great future it has. Although transgenic animals are an innovative solution to an organ shortage, they pose many risks to the human body. As per my research, the first risk it poses are hidden infections and diseases. Pigs and baboons may have diseases that do not affect themselves but do affect the human pathological system in dangerous ways, causing severe neurological damage. A huge effect is the rejection of the organ itself, and there are multiple types depending on how long it takes and whether it is reversible. Types like hyperacute rejection are being solved right now. Countering the “antibody effect”, which is when the xenoreactive antibodies reject the antigens of the organ, it is possible to use tactics such as immunoadsorption to help the body not go into complete rejection. It is crucial to understand that this idea has always been around: goat testicles were used to cure impotence back in the 1920s. And xenotransplantation has steadily been improving, so we are extremely hopeful for what the future holds.

Acknowledgments

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