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# XENOTRANSPLANTATION – A REVIEW

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## ABSTRACT

The transplantation of living cells, tissues or organs from one species to another is called Xenotransplantation and such organs are called xenografts or xenotransplants. Human xenotransplantation offers a potential treatment for end stage organ failure. A continuing concern is that many animals, such as pigs, have a shorter life span than humans meaning that their tissue age at a quicker rate. Xenozoonosis and permanent alteration to the genetic code of animals are also cause for concern. It is not uncommon for patients and physicians to use the term "allograft" imprecisely to refer to either allograft (human to human) or xenograft (animal to human). The first xenotransplantation appeared in scientific literature in the year 1905, when slices of rabbit kidney were transplanted into a child with renal insuffiency. Human organs have been transplanted into animal as a powerful research technique for studying human biology without harming human patients. This technique has also been proposed as an alternative source of human organs for future transplantation into human patients.

**KEYWORDS:** Xenotransplantation, Xenografts, Xenotransplant, Xenozoonosis, Allograft.

## INTRODUCTION

Xenotransplantation (xenos- from the Greek meaning "foreign"), is the transplantation of living cells, tissues or organs from one species to another. Such cells, tissues or organs are called xenografts or xenotransplants. It is contrasted with allotransplantation (from other individual of same species), syngeneic transplantation or isotransplantation (grafts transplanted between two genetically identical individuals of the same species) and autotransplantation (from one part of the body to another in the same person).

Xenotransplantation of human tumor cells into immunocompromised mice is a research technique frequently used in pre-clinical oncology research.

Human xenotransplantation offers a potential treatment for end-stage organ failure, a significant health problem in parts of the industrialized world. It also raises many novel medical, legal and ethical issues.[1] A continuing concern is that many animals, such as pigs, have a shorter lifespan than humans, meaning that their tissues age at a quicker rate. Disease transmission (xenozoonosis) and permanent alteration to the genetic code of animals are also causes for concern. A few successful cases of xenotransplantation are published.[2]

It is not uncommon for patients and physicians to use the term "allograft" imprecisely to refer to either allograft (human-to-human) or xenograft (animal-to-human), but it is helpful scientifically (for those searching or reading the scientific literature) to maintain the more precise distinction in usage.

## HISTORY

The first serious attempts at xenotransplantation (then called heterotransplantation) appeared in the scientific literature in 1905, when slices of rabbit kidney were transplanted into a child with renal insufficiency. In the first two decades of the 20th century, several subsequent efforts attempts to use organs from lambs, pigs and primates were published.[3]

Scientific interest in xenotransplantation declined when the immunological basis of the organ rejection process was described. The next waves of studies on the topic came with the discovery of immunosuppressive drugs. Even more studies followed Dr. Joseph Murray's first successful kidney transplantation in 1954 and scientists, facing the ethical questions of organ donation for the first time, accelerated their effort in looking for alternatives to human organs.[3]

Human organs have been transplanted into animals as a powerful research technique for studying human biology without harming human patients. This technique has also been proposed as an alternative source of human organs for future transplantation into human patients.

## POTENTIAL USES

A worldwide shortage of organs for clinical implantation causes about 20–35% of patients who need replacement organs to die on the waiting list.[8] Certain procedures, some of which

are being investigated in early clinical trials, aim to use cells or tissues from other species to treat life-threatening and debilitating illnesses such as cancer, diabetes, liver failure and Parkinson's disease. If vitrification can be perfected, it could allow for long-term storage of xenogenic cells, tissues and organs so that they would be more readily available for transplant.

Xenotransplants could save thousands of patients waiting for donated organs. The animal organ, probably from a pig or baboon could be genetically altered with human genes to trick a patient's immune system into accepting it as a part of its own body. They have re-emerged because of the lack of organs available and the constant battle to keep immune systems from rejecting allotransplants. Xenotransplants are thus potentially a more effective alternative.[9,10,11]

Xenotransplantation also is and has been a valuable tool used in research laboratories to study developmental biology.[12] It seeks to be a mutable architecture that, like open source software, remains available for perpetual modification and enhancement following the navigational impulse of militant ethical reasoning.

Patient derived tumor xenografts in animals can be used to test treatments.[13]

**POTENIAL ANIMALS ORGAN DONORS**

Since they are the closest relatives to humans, non-human primates were first considered as a potential organ source for xenotransplantation to humans. Chimpanzees were originally considered the best option since their organs are of similar size, and they have good blood type compatibility with humans, which makes them potential candidates for xenotransfusions. However, since chimpanzees are listed as an endangered species, other potential donors were sought. Baboons are more readily available, but impractical as potential donors. Problems include their smaller body size, the infrequency of blood group O (the universal donor), their long gestation period, and their typically small number of offspring. In addition, a major problem with the use of nonhuman primates is the increased risk of disease transmission, since they are so closely related to humans.[11]

Pigs are currently thought to be the best candidates for organ donation. The risk of cross-species disease transmission is decreased because of their increased phylogenetic distance from humans.[15] They are readily available, their organs are anatomically comparable in size,

and new infectious agents are less likely since they have been in close contact with humans through domestication for many generations. Current experiments in xenotransplantation most often use pigs as the donor, and baboons as human models.

In the field of regenerative medicine, pancreatogenesis- or nephrogenesis-disabled pig embryos, unable to form a specific organ, allow experimentation toward the *in vivo* generation of functional organs from xenogenic pluripotent stem cells in large animals via compensation for an empty developmental niche (blastocyst complementation).[16] Such experiments provide the basis for potential future application of blastocyst complementation to generate transplantable human organs from the patient's own cells, using livestock animals, to increase quality of life for those with end-stage organ failure.

## BARRIERS AND ISSUES

### Immunological Barriers

To date no xenotransplantation trials have been entirely successful due to the many obstacles arising from the response of the recipient's immune system. This response, which is generally more extreme than in allotransplantations, ultimately results in rejection of the xenograft, and can in some cases result in the immediate death of the recipient. There are several types of rejection organ xenografts are faced with, these include

- Hyperacute rejection
- Acute vascular rejection
- Cellular rejection
- Chronic rejection

A rapid, violent, and hyperacute response comes as a result of antibodies present in the host organism. These antibodies are known as xenoreactive natural antibodies (XNAs).[15]

## HYPER ACUTE REJECTION

This rapid and violent type of rejection occurs within minutes to hours from the time of the transplant. It is mediated by the binding of XNAs (xenoreactive natural antibodies) to the donor endothelium, causing activation of the human complement system, which results in endothelial damage, inflammation, thrombosis and necrosis of the transplant. XNAs are first produced and begin circulating in the blood in neonates, after colonization of the bowel by bacteria with galactose moieties on their cell walls. Most of these antibodies are the IgMclass, but also include IgG, and IgA.

The epitope XNAs target is an α-linked galactose moiety, Gal-α-1,3Gal (also called the α-Gal epitope), produced by the enzyme α-galactosyl transferase. Most non-primates contain this enzyme thus, this epitope is present on the organ epithelium and is perceived as a foreign antigen by primates, which lack the galactosyl transferase enzyme. In pig to primate xenotransplantation, XNAs recognize porcine glycoproteins of the integrin family.

The binding of XNAs initiate complement activation through the classical complement pathway. Complement activation causes a cascade of events leading to: destruction of endothelial cells, platelet degranulation, inflammation, coagulation, fibrin deposition, and hemorrhage. The end result is thrombosis and necrosis of the xenograft.

Also known as delayed xenoactive rejection, this type of rejection occurs in discordant xenografts within 2 to 3 days, if hyperacute rejection is prevented. The process is much more complex than hyperacute rejection and is currently not completely understood. Acute vascular rejection requires de novo protein synthesis and is driven by interactions between the graft endothelial cells and host antibodies, macrophages, and platelets. The response is characterized by an inflammatory infiltrate of mostly macrophages and natural killer cells (with small numbers of T cells), intravascular thrombosis, and fibrinoid necrosis of vessel walls.

Binding of the previously mentioned XNAs to the donor endothelium leads to the activation of host macrophages as well as the endothelium itself. The endothelium activation is considered type II since gene induction and protein synthesis are involved. The binding of XNAs ultimately leads to the development of a procoagulant state, the secretion of inflammatory cytokines and chemokines, as well as expression of leukocyte adhesion molecules such as E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1).

This response is further perpetuated as normally binding between regulatory proteins and their ligands aid in the control of coagulation and inflammatory responses. However, due to molecular incompatibilities between the molecules of the donor species and recipient (such as porcine major histocompatibility complex molecules and human natural killer cells), this may not occur.

**Overcoming acute vascular rejection**

Due to its complexity, the use of immunosuppressive drugs along with a wide array of approaches are necessary to prevent acute vascular rejection, and include:

- Administering a synthetic thrombin inhibitor to modulate thrombogenesis
- Depletion of anti-galactose antibodies (XNAs) by techniques such as immunoadsorption, to prevent endothelial cell activation
- Inhibiting activation of macrophages (stimulated by $CD4^+$ T cells) and NK cells (stimulated by the release of Il-2). Thus, the role of MHC molecules and T cell responses in activation would have to be reassessed for each species combo.

**Accommodation**

If hyperacute and acute vascular rejection are avoided accommodation is possible, which is the survival of the xenograft despite the presence of circulating XNAs. The graft is given a break from humoral rejection when the complement cascade is interrupted, circulating antibodies are removed, or their function is changed, or there is a change in the expression of surface antigens on the graft. This allows the xenograft to up-regulate and express protective genes, which aid in resistance to injury, such as heme oxygenase-1 (an enzyme that catalyzes the degradation of heme).

**Cellular rejection**

Rejection of the xenograft in hyperactute and acute vascular rejection is due to the response of the humoral immune system, since the response is elicited by the XNAs. Cellular rejection is based on cellular immunity, and is mediated by:

- Natural killer cells, which accumulate in and damage the xenograft; and
- T-lymphocytes – which are activated by MHC molecules through both direct and indirect xenorecognition.

In direct xenorecognition, antigen presenting cells from the xenograft present peptides to recipient $CD4^+$ T cells via xenogeneic MHC class II molecules, resulting in the production of interleukin 2 (IL-2). Indirect xenorecognition involves the presentation of antigens from the xenograft by recipient antigen presenting cells to $CD4^+$ T cells. Antigens of phagocytosed graft cells can also be presented by the host's class I MHC molecules to $CD8^+$ T cells.[15]

The strength of cellular rejection in xenografts remains uncertain, however it is expected to be stronger than in allografts due to differences in peptides among different animals. This

leads to more antigens potentially recognized as foreign, thus eliciting a greater indirect xenogenic response.[15]

**Overcoming cellular rejection**

A proposed strategy to avoid cellular rejection is to induce donor non-responsiveness using hematopoietic chimerism. Donor stem cells are introduced into the bone marrow of the recipient, where they coexist with the recipient's stem cells. The bone marrow stem cells give rise to cells of all hematopoietic lineages, through the process of hematopoiesis. Lymphoid progenitor cells are created by this process and move to the thymus where negative selection eliminates T cells found to be reactive to self. The existence of donor stem cells in the recipient's bone marrow causes donor reactive T cells to be considered self and undergo apoptosis.[15]

**Chronic rejection**

Chronic rejection is slow and progressive, and usually occurs in transplants that survive the initial rejection phases. Scientists are still unclear how chronic rejection exactly works, research in this area is difficult since xenografts rarely survive past the initial acute rejection phases. Nonetheless, it is known that XNAs and the complement system are not primarily involved. Fibrosis in the xenograft occurs as a result of immune reactions, cytokines (which stimulate fibroblasts), or healing (following cellular necrosis in acute rejection). Perhaps the major cause of chronic rejection is arteriosclerosis. Lymphocytes, which were previously activated by antigens in the vessel wall of the graft, activate macrophages to secrete smooth muscle growth factors. This results in a build up of smooth muscle cells on the vessel walls, causing the hardening and narrowing of vessels within the graft. Chronic rejection leads to pathologic changes of the organ, and is why transplants must be replaced after so many years. It is also anticipated that chronic rejection will be more aggressive in xenotransplants as opposed to allotransplants.

**Dysregulated coagulation**

Successful efforts have been made to create knockout mice without α1, 3GT; the resulting reduction in the highly immunogenic αGal epitope has resulted in the reduction of the occurrence of hyperacute rejection, but has not eliminated other barriers to xenotransplantation such as dysregulated coagulation, also known as coagulopathy.

Different organ xenotransplants result in different responses in clotting. For example, kidney transplants result in a higher degree of coagulopathy, or impaired clotting, than cardiac transplants, whereas liver xenografts result in severe thrombocytopenia, causing recipient death within a few days due to bleeding. An alternate clotting disorder, thrombosis, may be initiated by preexisting antibodies that affect the protein C anticoagulant system. Due to this effect, porcine donors must be extensively screened before transplantation. Studies have also shown that some porcine transplant cells are able to induce human tissue factor expression, thus stimulating platelet and monocyte aggregation around the xenotransplanted organ, causing severe clotting. Additionally, spontaneous platelet accumulation may be caused by contact with pig von Willebrand factor.

Just as the α1, 3G epitope is a major problem in xenotransplantation, so too is dysregulated coagulation a cause of concern. Transgenic pigs that can control for variable coagulant activity based on the specific organ transplanted would make xenotransplantation a more readily available solution for the 70,000 patients per year who do not receive a human donation of the organ or tissue they need.

**Physiology**

Extensive research is required to determine whether animal organs can replace the physiological functions of human organs. Many issues include:

- **Size** – Differences in organ size limit the range of potential recipients of xenotransplants.
- **Longevity** – The lifespan of most pigs is roughly 15 years, currently it is unknown whether or not a xenograft may be able to last longer than that.
- **Hormone and protein differences** – Some proteins will be molecularly incompatible, which could cause malfunction of important regulatory processes. These differences also make the prospect of hepatic xenotransplantation less promising, since the liver plays an important role in the production of so many proteins.[15]
- **Environment** – For example, pig hearts work in a different anatomical site and under different hydrostatic pressure than in humans.
- **Temperature** – The body temperature of pigs is 39 °C (2 °C above the average human body temperature). Implications of this difference, if any, on the activity of important enzymes are currently unknown.[15]

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