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(Medically Speaking) The Good, the Bad, and the Ugly in Xenotransplantation

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Introduction

A large percentage of the deaths in Canada, Europe, and the U.S are caused by chronic illnesses such as cardiovascular disease, diabetes, cancer and liver disease[1]. Many of these individuals would potentially benefit from an organ transplant but unfortunately the demand for organs greatly supersedes the supply[1]. It is estimated that there are more than 400 individuals awaiting organ transplant in B.C. alone and many of the individuals waiting for an organ die while on the waiting list[19].

The supply of organs is from healthy donors who have died in a trauma, which means that the donor supply is small[2]. Another problem is that the families must consent to the donation (which occurs only approximately 20% of cases)[2]. Since the supply of organs is so small, other solutions to the organ shortage problem are being investigated[3].

One solution is to xenotransplant which is defined as the transplantation of organs from non-human animals to humans[4]. From an immunological perspective, primates would be the preferred source of organs for transplant but almost all of the species are endangered or too small to provide organs capable of sustaining a human[5]. There are also concerns about the ability of primates to transmit infectious agents to the human population as many primates are caught from the wild or have been housed in colonies for many generations[5]. Also, primates are difficult and time consuming to keep and breed and there is a lack of experience genetically engineering them in the scientific community[3]. The pig is believed by many investigators to be the preferred donor as the organs are of similar size and physiology[1]. Another benefit to using pigs is that they proliferate quickly[1]. Pigs reach sexual maturity at nine months, take three to five months to gestate, have up to 16 piglets in a litter, and have been extensively farmed so the supply of potential organs for donation is unlimited[1]. Pigs can be genetically engineered to render them more compatible for organ donation and the histocompatibility is well established[1]. Finally, there is much less social opposition to killing a pig than killing a primate[1]. The reasons are that they have been used for food for many years and lack the human-like intelligence and social skills of primates[1]. Zoonotic infection from pigs to humans is thought to be limited[1]. The reason is that pigs have been captively bred for food for many years with reasonable quality control[1]. One problem is that pigs are distant from humans both in terms of their immune and coagulation systems and potentially require substantial genetic manipulation in order to provide useful donors [4].

So why is xenotransplant not a current option? The answer is that there are many technical problems with xenotransplant including rejection, the possibility of a transfer of disease, and physical compatibility issues.

History

In general, transplantation has been around for a long time. In ancient Egypt, 8000BC, surgeons were making cosmetic operations on the nose, face and ears[2]. In ancient India, doctors were transplanting skin[2]. In 1682 a Russian physician reportedly repaired the skull of a wounded nobleman using a bone from a dog[2]. The church however frowned upon the surgery and the bone was subsequently removed[2]. The first documented animal to human transplant occurred in 1906 when a physician named Jaboulay transplanted a pig's kidney into a woman and a goat's liver into another[3]. Both transplants however, were rejected in one hour[3]. In 1963 one patient survived 9 months after receiving a chimpanzee kidney[3]. The kidney was excised post mortem and showed no sign of rejection[3]. One of the more

publicized cases of xenotransplant was in the case of Baby Fae[6]. At birth, Baby Fae was diagnosed with hypoplastic left heart syndrome[6]. She received a baboon heart and three weeks later died of organ rejection[6]. Another interesting case was that of Tom Getty[6]. Tom was an AIDS patient who was given a baboon bone marrow transplantation in hope that it would combat the AIDS virus[6]. Baboons normally are not susceptible to infection by AIDS and it was hypothesized as being due to the inability of the AIDS virus to infect the immune cells[6]. In terms of Tom Getty, the transplant ended up being unsuccessful as the transplanted cells failed to engraft however, mysteriously his white cell count and condition seemed to improve[6]. The results were inconclusive, the improvement may have been due to the transplantation or due to the irradiation used to deplete his current immune system[6].

Rejection

There are four recognized phases of xenotransplant rejection:

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

Each phase is discussed in detail below.

Hyperacute rejection

One of the major hurdles in xenotransplantation is hyperacute rejection. Hyperacute rejection occurs immediately and is characterized by interstitial hemorrhage, congestion, disruption of the vascular endothelium, edema within the graft and the formation of platelet thrombi resulting in the rapid loss of graft function[7]. Leakage of fluid and blood through the capillary walls is closely followed by necrosis of the graft endothelial cells[5]. It is caused by the interaction of xenoreactive antibodies with the graft endothelium[4]. Ninety-percent of the xenoreactive IgM antibodies are specific for the sugar Gala-3Gal and make up 1-4% of the circulating immunoglobulins in humans[8]. Gala-3Gal sugars are normally present on the porcine endothelium[9]. The binding of the antibodies causes the activation of the complement regulatory proteins of the recipient through the classical pathway, which results in the destruction of the foreign tissues [10]. The presence of xenoreactive antibodies is not the only reason for rapid activation of the complement system[4]. Rapid activation also reflects the lack of complement regulatory proteins expressed on the donor epithelium[11].

One solution to the problem of xenoreactive antibodies is to deplete them from the serum[4]. Since most of the antibodies are involved are specific for Gala-3Gal it is reasonably easy to isolate the xenoreactive antibodies using columns bearing Gala-3Gal however there may be less common antibodies to other epitopes which could still function in hyperacute rejection[12]. Alternatively, soluble synthetic Gala-3Gal can be continuously infused into the blood in order to bind anti-Gala-3Gal antibodies[5]. The problem with this approach is that at high levels the sugars can be toxic and the results of experiments done have not shown prolonged xenograft survival[5]. Another possible solution is to use complement inhibitors[4]. These inhibitors are designed to inhibit the cleavage of the pro-enzymes involved[4]. The problem with inhibitors is that complement normally acts as a first line defense against bacterial infection so the recipient becomes more susceptible to bacterial infection[4]. Now, with the availability of transgenics, it is possible to engineer animals that present complement regulatory proteins like human decay accelerating factor or membrane cofactor protein on their cell surfaces[4]. These proteins regulate the activation of complement by dissociating and degrading the complement enzymes involved in the formation of the membrane attack complex[13]. When these procedures are combined with immunosuppressive therapy, xenografts can continue to function for several weeks experimentally[3].

Acute vascular rejection

Once hyperacute rejection has been successfully prevented, the xenograft can become subject to acute vascular rejection[4]. Acute rejection begins after 24 hours of reperfusion and results in the failure of the graft within days to weeks [4]. It is characterized by endothelial swelling, ischemia and vascular thrombosis with blood extravasation [3]. Acute rejection is generally thought to be due to an antibody (both Gala-3Gal and non-Gala-3Gal) mediated activation of the graft endothelium[5]. Endothelial activation induces the expression of many inflammatory and prothrombotic molecules that lead to the changes in the tissues of the graft[4]. For example, endothelial activation by antibody deposits on the vascular endothelium results in the transcription of paracrine factors like interleukin-1a, which activates other endothelial cells[4]. Also, the binding of antibodies to the endothelial cell epitopes may direct killer cells through interactions with the Fc receptors of the antibody to release their cytotoxic compounds on the endothelial cell surface[5]. Therapeutic strategies for combating acute rejection are similar for those of hyperacute rejection including the depletion of xenoreactive antibodies along with the expression of complement regulatory proteins and immuno-suppressant drugs[4]. Recent studies have shown that co-stimulatory blockade with anti-CD154 monoclonal antibodies inhibits the induction of an antibody response although it does not suppress the production of anti-Gal antibodies[14]. Another approach is to genetically engineer the donors

to inhibit donor endothelium activation[4].

Cellular rejection

Cellular rejection occurs after 7 days due to the infiltration of tissue specific T cells, B cells and antibodies[15]. Natural killer (NK) cells may also be a potential problem in terms of cellular rejection[15]. There is evidence that NK cells may fail to receive inhibitory signals from the xenograft tissue[15] . Not much is known about cellular rejection in xenografts as the hurdles of hyperacute and acute rejection still stand in the way[4]. Cellular rejection is a major problem in the tolerance of allografts (same species transplants) and so the same approaches to solutions may be applicable[2]. One approach is the formation of mixed chimeras where the bone marrow of the donor is simultaneously injected into the irradiated host resulting in the deletion of xenoreactive cells[16]. Another strategy is to transplant the donors thymus in order to ensure that the T cell repertoire is not reactive to the donor[17]. Both approaches are being experimentally investigated with good results however the current treatments are composed of cocktails of immunosuppressant drugs[4].

Chronic rejection

As for cellular rejection, not much is known about chronic rejection in xenografts; however, as in all forms of rejection, it probably occurs in an accelerated form[5]. In allografts, chronic rejection affects long term graft survival[2]. The average half life for kidney grafts is approximately 8 years[2]. Chronic rejection is characterized by concentric arteriosclerosis of the graft blood vessels, along with fibrosis and atrophy of the tissue[5]. Chronic rejection is due to ischemia-reperfusion injury which occurs at the time of grafting along with chronic inflammation and associated scarring[5]. It can also be caused by infection and cyclosporin toxicity[5]. The only solution thus far to chronic rejection is the transplantation of a new tissue or organ[2]. As the availability of organs from animals would greatly exceed the available organs from humans, chronic rejection may not be such a problem in terms of xenotransplantation.

Transfer of disease

One of the more recent concerns of xenotransplantation is xenozoonosis[1]. Zoonosis is defined as a disease that can be transmitted from animals to humans[1]. Well known zoonosis include HIV, hauntevirus, Ebola, and Bovine spongiform encephalopathy (mad cow disease)[1]. Xenozoonosis is defined as the transmission of pathogens from animal organs and blood products to human recipients of animal organ transplants [1]. It may be possible to avoid transfer of microorganisms with xenotransplantation by the use of gnotobiotic techniques in the delivery, weaning, housing and handling of the piglets, which would theoretically make them safer organ donors than other humans[18]. The reason, however, that xenozoonosis has become such a major concern is due to the discovery of endogenous retroviruses like porcine endogenous retrovirus (PERVs)[3]. PERVs make up 1% of the genome in pig cells and are similar to human endogenous retroviruses[3]. Although there is no evidence showing that PERVs can lead to health problems in pigs or humans, the fear is that these retroviruses could mutate and become harmful to the human recipient and potentially spread through the population[3].

Physical compatibility

If the problems with rejection and zoonosis can become overcome, there is still the question of the physical compatibility of the xenograft with the human system. Is the donor's organ going to be sufficient to fulfill the requirements of a human organ? Since humans and pigs are anatomically and physiologically different, the transplanted organs may not function adequately in order to sustain the human recipient[5]. For example, pigs normal body temperature is 103^{o}, while the average human body temperature is 98.6^{o} indicating that the pigs cell metabolism may not be optimal at human body temperatures[5]. Also, it is unclear whether porcine organs can generate the factors that would be necessary to sustain a human and whether human cytokines and hormones are able to support the organs[5]. Another potential problem is the physiological function of many of the organs for example human hearts must perfuse a high blood flow organ (brain) against gravity where as a pig's brain is a low blood flow organ[5].

Pig insulin has been used for years to treat diabetes and pig aortic valves that have been rendered inactive have been used successfully in transplantation so there is optimism that the organs will function adequately in humans[5].

Conclusion

Xenotransplantation may provide an unlimited supply of organs to patients suffering from chronic diseases, however, there are still many barriers to overcome before xenotransplantation can become common practice. As rejection is still a problem in the transplantation of allografts, it will probably be a while before xenotransplantation becomes a realistic alternative. With the advent of genetic engineering and new drug regimens, however, it seems as though the problems associated with the initial forms of rejection in xenotransplantation are being overcome. Once a major barrier, hyperacute rejection has become less of a determinant in the success of a transplanted organ. This is important in terms of using animal organs tem-

porarily as bridge organs while the patient is waiting for an allotransplant. Likewise, our understanding of the mechanisms of acute rejection has increased greatly in recent years extending the amount of time a foreign organ can survive in a host. In terms of cellular and acute rejection, not much is known. There have been many advances in the field of allotransplantation but our ability to conquer acute and chronic rejection is still beyond our reach. In time there is no doubt that the problems will eventually get worked out and with luck, the solutions will apply to xenotransplantation as well. In terms of the problem of xenozoonosis, hopefully with time, research and genetic engineering we can eradicate the threats of endogenous viruses in the porcine genome and with better technology, gnotobiotic conditions for the housing and care of the donor animals should also reduce xenozoonosis. As we get closer to unlocking the secrets of the immune system, xenotransplantation will undoubtedly become a major achievement in the field of science; unfortunately, we still have a long way to go before xenotransplantation becomes a reality.

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