EDITORIAL

Trials and tribulations of overcoming xenograft rejection

Muhammad M Mohiuddin[1,2]

Throughout the world, there are millions of patients with terminal organ failure who are waiting for a human organ for transplantation. Over the last 20 years, due to improvement in our understanding of transplant organ rejection and progressively improved immunosuppression regimens, organ transplants have provided a significant extension of life. This success has saved many lives, but at the same time, due to increased public awareness and demand, it has created an acute shortage of organ donors.[1] Not every donor organ can be used for transplantation, and multiple factors contribute to the selection of a transplantable organ based on the stringent selection criteria of these donors and the organs. Therefore, a similar tough criterion is applied to the recipients based on the urgency of the organ requirement.

Patients with organ failure have limited options based on which organ is failing. For the kidney, there is an option of dialysis[2], which has saved many lives. Kidney failure patients on dialysis need this procedure performed on them from 2-5 days a week and after a period of time they lose their vascular access. At this time, they must find a donor organ to survive. For heart failure patients, there are very limited options. Mechanical assist devices[3] have improved over the years but still have the issues of size and blood clotting. Total artificial heart[4] has shown some promise but is still rarely used.

There has been a lot of effort in improving the quality of organs. This includes better preservation techniques[5,6] and reconditioning of unused organs like lungs to make them usable for organ transplantation. The organs are typically used when the donor is declared brain dead, but now significant success has been achieved by using organs after the circulatory death, which means after patient's heart has stopped functioning and circulation of blood has ceased. Using recently available machine perfusion mechanisms, the organs are made usable after even the circulatory death of the donor. This has allowed an increase in the pool of available organs.[6]

[1]Program in Cardiac Xenotransplantation University of Maryland School of Medicine, Baltimore, Maryland, [2]International Xenotransplantation Association (IXA).

Correspondence: Muhammad M Mohiuddin.
Email: MMOHIUDDIN@som.umaryland.edu
ORCID ID: 0000-0003-4654-783X

The newer methods to provide organs include 3 or 4 D printing of organs[7], decellularizing and then recellularizing organs[8] with recipients' own stem cells, growing human organs in non-humans[9] and finally, using non-human organs to overcome the organ shortage.[10] The transplanting non-humans' tissue, organs and cells into humans is called xenotransplantation. This procedure has been tried for many years and initially non-human primate's (Baboon[11] and chimpanzee[12]) organs were used with little success. But these transplants were done without any regulatory oversight and any preclinical research, so these organs were rejected acutely and finally when they demonstrated disease transmission from non-human primates (e.g., HIV) to humans, caused the abandoning of this procedure and also lead to a moratorium on xenotransplantation in certain countries.

Due to issues of transmission of disease with non-human primates, scientists started focussing on another donor for xenotransplantation and they ended up choosing pigs for the following reasons: a) The anatomic similarity of its organs with humans, b) their breeding potential, including the fast breeding cycle and large litter size, c) the fast growth rate (take 1 year for a pig's organs to grow to the size of a human, NHP organ takes about 20 years), d) The ability to manipulate their genetics to make them more humanized and avoid human immune response e) ethically less challenging as 90,000 pigs are slaughtered each day just in US for the dietary need.[13]

Besides the above similarities, there are several dissimilarities and concerns about pigs: a) Pigs are phylogenetically very distant from humans. There are about 10,000 genes that are different from humans, b) the physiological differences are not apparent, but pigs have a lower blood pressure and higher heart rate than humans and c) there are some religious restrictions in consuming pork and finally d) animal right and humane use concerns of pigs pose concerns.[14]

On top of all the above concerns, a significant concern arose when pig organs were transplanted in human surrogates (non-human primates). It was discovered that humans and old-world monkeys have pre-formed antibodies against specific carbohydrate antigens in pigs, and these antibodies induce hyperacute graft rejection within minutes.[15] A pig heart transplanted in a baboon is seen to turn black and stop functioning right in front of your eyes. This was a significant setback for the field, and progress halted for several years.

The technology of genetic modification revolutionized the field, and a significant breakthrough was achieved when a major carbohydrate epitope alpha 1-3 galactose was removed[16] from pig organs by knocking out a gene responsible for putting this terminal carbohydrate on the carbohydrate chain, eliminating a major culprit causing xenograft rejection. This advance eliminated the occurrence of hyperacute rejection but, unfortunately, could not completely avoid xenograft rejection.[17]

The research in the field kept progressing but at a snail's pace until the CRISPs -Cas 9 technology was introduced and allowed the scientists to make multiple changes to pig genes simultaneously.[18] By this time, more information about other immunogenic antigens in pigs and humans had been discovered, and many pathway incompatibilities between pigs and human complement regulatory and coagulation regulatory systems was discovered, which prevented the control of complement activation and coagulation. Further, this technology allowed the ability to introduce human genes into the pig genome, allowing the field to overcome the above incompatibilities.[19,20]

Overcoming the gene incompatibilities was not the only hurdle. The immunosuppression routinely used in allotransplantation with great success was unsuccessful in xenotransplantation. Tacrolimus, a CNI inhibitor that made a massive difference in the survival of allotransplants, failed to produce similar success in overcoming xenograft rejection.[21,22] As described above, the first insult to xenograft is from anti-pig antibodies, either preformed or elicited in response to exposure to xenoantigens. CNI inhibitors have no role in inhibiting antibody-mediated rejection. Removing B cells that produce antibodies provided some protection. However, the actual improvement in graft survival was observed when the CD40 -CD40 Ligand costimulation pathway was targetted along with B and T cell depletion.[23,24] This pathway prevented T cell help to B cells and also blocked the activation of B cells. With this regimen, along with some gene modifications, the graft survival was improved first for months and then for years.[24,25] This regimen provided real hope for xenotransplantation and was adopted by many groups who repeated a similar success.[26,27]

Though US Federal regulatory agency (FDA) was not convinced with these results, however, a provision that allows use of an experimental drug or procedure in patients where all traditional means of saving life are exhausted, enabled first ever heart transplantation from a genetically engineered pig into a severely ill heart failure patient who was at the brink of dying.[10] This first patient survived for 60 days and provided very useful information[28] to the clinicians and scientists to try another heart transplant into a patient who was also terminally ill but apparently in better shape than the first patient. However, the survival of the second patient was only 40 days, but in this patient, exogenous anti-pig antibodies that are present in human blood, and its products triggered an immune rejection similar to that seen in minimally genetically modified pig heart transplants into baboons.[29]

The learning from the above two transplants provided extraordinary knowledge that led to 5 kidneys xenotransplantations with three patients still living to date without apparent signs of rejection.[30-32] All these results and further studies in pig-to-baboon models convinced the FDA to grant the first permission for clinical trials for the kidney.

These events that transpired over almost 4 decades made it possible for the xenotransplantation field that was once considered fiction, transpired into an actual life-saving maneuver. The compassionate use of transplants provided a tremendous amount of information to accelerate the progress of xenotransplantation to the clinical arena. The planned clinical trials will provide an opportunity to improve the current genetic modifications and the immunosuppression regimen with an ideal goal to either induce xenograft immune tolerance or modify the pig genetics to an extent that no immunosuppression is required. With an unlimited supply of these pig organs, this life-saving procedure could be used at an early timepoint of organ failure or even in children where other mechanical options are not available.

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