Growing Human Organs in Animals: Interspecies Blastocyst Complementation as a Potential Solution for Organ Transplant Limitations

by

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ABSTRACT

Prior to the first successful allogeneic organ transplantation in 1954, virtually every attempt at transplanting organs in humans had resulted in death, and understanding the role of the immune mechanisms that induced graft rejection served as one of the biggest obstacles impeding its success. While the eventual achievement of organ transplantation is touted as one of the most important success stories in modern medicine, there still remains a physiological need for immunosuppression in order to make organ transplantation work. One such solution in the field of experimental regenerative medicine is interspecies blastocyst complementation, a means of growing patient-specific human organs within animals. To address the progression of immune-related constraints on organ transplantation, the first part of this thesis contains a historical analysis tracing early transplant motivations and the events that led to the discoveries broadly related to tolerance, rejection, and compatibility. Despite the advancement of those concepts over time, this early history shows that immunosuppression was one of the earliest limiting barriers to successful organ transplantation, and remains one of the most significant technical challenges. Then, the second part of this thesis determines the extent at which interspecies blastocyst complementation could satisfy modern technical limitations of organ transplantation. Demonstrated in 2010, this process involves using human progenitor cells derived from induced pluripotent stem cells (iPSCs) to manipulate an animal blastocyst genetically modified to lack one or more functional genes responsible for the development of the intended organ. Instead of directly modulating the immune response, the use of iPSCs with interspecies blastocyst complementation could theoretically eliminate the need for immunosuppression entirely based on the

establishment of tolerance and elimination of rejection, while also satisfying the logistical demands imposed by the national organ shortage. Although the technology will require some further refinement, it remains a promising solution to eliminate the requirement of immunosuppression after an organ transplant.

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CHAPTER 1

INTRODUCTION

Organ transplantation has been touted by many as a discovery that can add years to the lives of those with otherwise disabling and fatal conditions. (Sharp, 1995) Since the first successful human organ transplant in 1954, researchers have expanded the ability to treat previously fatal chronic conditions, enabling them to provide patients with over 700,000 organ transplants (American Transplant Foundation, 2020) which have provided an estimated extension of 2.3 million "life years" to organ recipients.

However, these numbers do not tell the stories of those sentenced to lifelong treatments such as dialysis because they are not sick enough to warrant an immediate intervention, or those figuratively sentenced to death after their own organs have left them too sick to qualify for surgery. Conversely, the numbers also ignore the lifelong responsibility and disease susceptibility placed on those who do qualify for organ transplants, whose transplanted organ is delicately held in a balance of diligent medication schedules and infection avoidance, in addition to the long-term risks associated with immunosuppressive medications. They also disregard the lives of those who die waiting for an organ.

Before society reached the point at which physicians were capable of transplanting organs from donor to patient in 1954, it first had to arrive at the consensus that organ replacement was even a worthwhile prospect. (Schlich, 2011) Between 1900 and 1954, researchers contended with the physiological barriers that impeded any successful attempts at transplanting tissues and organs. They learned over time that the root of many of these barriers was the immune system and its response to foreign tissues.

Within the span of about fifty years, researchers went from a concept loosely supported by a handful of animal-based models to actual live human transplants capable of saving and extending lives. Physicians executed the first successful human-to-human kidney transplant in 1954, which was celebrated as one of the most significant surgical contributions of the century. (Starzl, 2000) (Howard, Cornell, Cochran, 2012)

Given the fact that organ transplantation has been successfully performed for nearly seventy years, it would be reasonable to expect that scientific progress would have resolved some of the initial challenges and limitations. However, the current statistics and their associated historical narratives reveal an apparently implicit acknowledgement that there are some fundamental shortcomings that scientists have yet to resolve. While short-term survival rates have increased, likely due to the advent of immunosuppressive therapies, long-term survival rates following an organ transplant have remained relatively stagnant in the last thirty years. (Lodhi, Lamb, Meier-Kriesche, 2011) There has also been little headway made into how to manipulate the human immune system to accommodate alternative strategies for organ transplantation, immunological acceptance, and to prevent the rejection of organs in a safer manner than lifelong immunosuppressant medications. (Orlando, Soker, and Wood, 2009)

In 2012, immunologist Peter Fairchild claimed that "the immunology of transplant rejection has [only recently] begun to take center stage in the field of regenerative medicine and is now recognized to be one of the most intransigent obstacles to the success of stem cell therapies in the future." (Fairchild, 2012) He further claimed that the general disregard for the immune system and its influence over the emerging areas of stem cell biology and regenerative medicine may have unwittingly enabled

scientists to underestimate its role as one of the key sources of organ transplant limitations as a whole in the last 120 years. There is plenty of evidence to support his assertions.

Initially, between 1900 and 1954, scientists were just learning the immunological principles of transplantation that would serve to restrict and limit it. After the 1950s and 60s, scientists then progressed toward determining who could serve as the best source of organs for transplantation. That determination was made more precise as the relevance of both rejection and compatibility became clear. Technology continued to advance, and organ transplantation methods became relatively "standardized," in the sense that physicians generally accepted immunosuppression to some degree as inescapable and that humans were the only reasonable origin of transplantable organs.

Presently, the defining barrier of modern organ transplantation is often attributed to the organ shortage. (De Los Angeles, Pho, Redmond, 2018) However, while the organ shortage directly limits the number of people who can receive organ transplants each year, if a technology existed that could eliminate the need for organs to originate in humans as the source, then that limitation would be resolved. If the way by which that limitation was resolved also could address the necessity of immunosuppression, then that alternative would reasonably be expected to garner attention and resources, as it would be a potentially better transplantation strategy.

One technology that may serve as a reasonable solution for modern organ transplant restrictions is interspecies blastocyst complementation, which provides a means of growing a patient-specific organ within an animal; this organ would in theory eliminate the need for immunosuppression because it would be grown with the use of the

patient's own cells. However, this technology, while somewhat simple conceptually, has some technical shortcomings and ethical implications that researchers must resolve before it can become viable as an alternative source of organs for transplant.

Therefore, this thesis presents the argument that the immune system has been the underlying theme to the discoveries, failures, successes, and restrictions of organ transplantation over time. It also argues that the need for immunosuppression is one of the most important restrictions to modern-day organ transplantation, and that any alternative or solution would need to address the necessity for lifelong immunosuppressants.

This thesis seeks to describe three general points. First, it aims to show that the underlying current throughout the history of organ transplantation has been the unseen and sometimes ignored impacts of the immune system. Also, it seeks to describe that the current limitations of organ transplantation have minimally improved beyond the limitations that were established shortly after the first successful organ transplants in the 1960s. That is, the primary limitation is the requirement of immunosuppression, which connects proximally with the unrelenting search for a perfect source for organs. This comparison will be further delineated by the examination of short and long-term survival statistics over time in addition to the development of new strategies and techniques that have progressed organ transplantation forward in terms of its accessibility and overall application. Finally, it strives to assess the use of interspecies blastocyst complementation as a solution to many limitations associated with modern organ transplantation. Specifically, the potential for this technology to overcome the need for immunosuppression will be analyzed. This assessment is made with regards to its

potential to resolve existing limitations of organ transplantation and its uniqueness compared to other approaches, with respect to the technical challenges that remain.

Overall, using historical methods, this thesis describes some of the hurdles leading to the development of organ transplantation, the limitations that have persisted and diminished its advancement, and the plausibility of an alternative solution, interspecies blastocyst complementation, to overcome the continuing limitations of organ transplantation. These goals culminate together to answer the research question, which is: What impact did early immunological discoveries impose upon the long-term technical development of organ transplantation, and how might a modern solution, interspecies blastocyst complementation, address any persistent challenges?

CHAPTER 2

ORGAN TRANSPLANTATION

The era of early organ transplantation covered within this thesis stretches from the pre-1900 era, to 1900 through 1954, with a final section assessing how the discoveries made within these formative years of organ transplantation have been resolved. While this history is incomplete, this section attempts to uncover the sequence of events that ultimately led to the discoveries associated with the immune system and its effects on transplantation. This chronology explains why a solid understanding of the immune system was crucial before organ transplantation could be successfully performed. Additionally, in comparison with modern limitations of organ transplantation related to the immune system, this section assesses the extent at which the immune system continues to restrict the success and application of organ transplantation.

Before 1900

Across myth and reality, humans have been curious about the origins and limits of the human body for millennia. Acknowledging these earliest hurdles of organ transplantation is critical, not just for the lessons learned or the achievements won, but also to highlight the momentum at which these discoveries amassed amidst constant skepticism and failure. Ultimately, this history shows that there were break-through discoveries and actions taking place nearly every other decade, making any present reluctance at the thought of growing human organs in animals as subversive as the thought of transplanting an organ may have been two centuries ago. And while much of this early curiosity was fueled by nonscientific influences, the notion that one could

replace a faulty part of the body with an alternative was not groundbreaking once it reached scientific feasibility in the 1950s. The idea of hybridized bodies had arisen much earlier.

Lending to the eponymous name associated with many branches of modern interspecies research, the Chimera was first a creature of Greek mythological origins, with a lion's head, a goat's body, and a snake's tail. Although the Chimera does not return to the timeline of organ transplantation development until much later, many researchers claim its invention to be one of the earliest conceptions of transplantation. The Chimera was not alone in this generation of fantastical creatures, sharing the mythical prestige with the Centaur, Minotaur, and Sphinx. This generation of Greek mythology showed that the absence of a reproductive barrier between species was something that deserved to be memorialized and shared. (Bazopoulou-Kyrkanidou, 2001) Greek poet Homer (762 B.C.) wrote of the treachery of the Chimera in *The Iliad*: "When he had received the wicked written signs, he first commanded Bellerophon to kill that savage monster, the Chimera, who was not a human being, but a goddess, for she had the head of a lion and the tail of a serpent, while her body was that of a goat, and she breathed forth flames of fire; but Bellerophon slew her, for he was guided by signs from heaven." And whether it stemmed from fear, marvel, or disgust, that intrigue has transcended beyond Homer's (762 B.C.) first description of a man slaying the chimera; that such a creature could be weaponized and envied was surely as fascinating then as it remains today.

In many other early examples across cultures and religions, the mere vision of transplantation could only be justified as the outcome of miraculous intervention.

(Howard, Cornell, and Cochran, 2012) As such, any early example of transplantation can only be substantiated through spiritual texts. The *New Testament* detailed autogenous transplantation examples such as the divine restoration of severed ears or hands. Additionally, within *Legenda sanctorum,* Jacopoda Varagine described the miracle of Saints Cosmas and Damian transplanting a leg from a dead man onto a man whose leg was wounded while defending a church. That instance was estimated to have occurred sometime around the third century CE. (Howard, Cornell, and Cochran, 2012) Anything related to surgery was limited by the dogma of the Catholic Church, which emphasized that attempting to fix God's work was a sin. (Ang, 2005) While not excluding the possibility of other primitive types of transplants around the time, some of the only historic examples substantiated through archaeological research involved tooth transplants, many of which were pulled from corpses, therefore making deceased donors one of the first transplantation tissue sources. (Howard, Cornell, and Cochran, 2012)

More scientifically minded efforts for animal-to-human transplants began around the seventeenth century. Until the latter half of the nineteenth century, it was generally accepted that ailments were caused by some sort of internal disruption with the external environment. Therefore, most treatments focused on balancing the body's fluids, such as bloodletting, which prompted early transfusion interests. (Schlich, 2011) While acknowledging that there is an extensive list of organ and tissue transplant accomplishments throughout the modern era, early experimental blood transfusions and skin grafts between humans and animals were some of the most recognizable instances where humans began grappling with the biological constraints of the immune system. The persistent curiosity resulting from these early seventeenth century discoveries in

transplantation provided the foundation for early tissue transplant attempts among both humans and animals.

In 1658, French monk Dom Roberts des Gabets at Habert de Montmort (later, the French Academy of Sciences) was one of the first to raise the possibility of transfusing blood from animals to humans. Around the same time, French physician Jean-Baptiste Denis began experimenting on how to transplant animal blood into humans, which he successfully carried out in 1667. After initially experimenting with dogs and cows as his test subjects, Denis successfully transfused lamb's blood into a febrile teenaged patient whose doctors had bled over twenty times. Not only was this the first xenotransfusion (transfusion across species), it was also the first known blood transfusion altogether. (Cooper, 2012) (Roux, Sai, Deschamps, 2007) Denis justified his use of animal blood to transfuse into humans because he claimed that "the blood of animals is less full of impurities than that of men because debauchery and irregularity in eating and drinking are not so common in them as in us." (Roux, Sai, Deschamps, 2007)

While Denis is largely credited for the first documented case of successful animal-to-human transfusion of blood, other sources point to a controversial claim from English physician Richard Lower, who argued he first successfully transfused blood between dogs and humans in the 1660s. (Roux, Sai, Deschamps, 2007) Regardless of its origin, this initial research laid the groundwork for future discoveries involving histocompatibility, immunological responses to transplants, and interspecies chimera research.

After his initial success, Denis continued his xenotransfusion experiments, initially noting only one death, and claimed that his first series of transfusions had

successfully ‘cured’ patients from paralysis and psychosis. Denis’ success is largely attributed to the cessation of bleeding, rather than the introduction of the animal blood itself. After the death of a second patient, whose psychosis Denis had previously cured, Denis faced a court trial instigated by the patient’s wife. While the verdict was ultimately not guilty (after establishing the patient died via arsenic poisoning from his wife), the French Parliament prohibited future blood transfusions on 10 January 1670 for an unspecified amount of time. (Roux, Sai, Deschamps, 2007) That prohibition carried through until the end of the nineteenth century in France. Many other countries’ medical societies had followed suit in declaring a prohibition, but researchers began venturing back into animal-human organ and tissue transplantation experiments in the 1800s as restrictions loosened. (Roux, Sai, Deschamps, 2007) As an interesting parallel with the present day, there exists a similar prohibition on the funding of types of human-animal chimera research in the US, thus preventing a majority of research to continue on interspecies blastocyst complementation.[1]

After declining in most countries by the end of the seventeenth century, blood transfusion experiments resumed throughout the nineteenth century. Because coagulants were not used until after 1914, physicians renewed their interest in blood transfusions, as transfusions could provide a means of relatively efficient revival of blood volume following any sort of significant loss of blood, particularly that experienced by people at

[1] There were three notices passed by the NIH between 2015 and 2016 on the topic of research involving the experimentation on animal embryos with human pluripotent stem cells. The original notice passed on 23 September 2015, NOT-OD-15-158, detailed that there would be no funding granted for research until a new policy revision was passed. Then, on 4 August 2016, NOT-OD-16-128 called for public comments on a new policy revision to amend part of the NIH Guidelines for Human Stem Cell Research. Since an extension was granted to the public commenting period with the release of NOT-OD-132 on 9 August 2016, the NIH has remained in a holding pattern for funding such research until further notice.

war. In the early 1800s, physicians in England and Scotland continued transfusing blood among animals of differing species and discovered that blood transfusions were more effective if the blood donor and host were of the same species. They speculated the same would apply in humans, and in 1829, British obstetrician James Blundell performed the first human-to-human blood transfusion on a woman who had hemorrhaged shortly after childbirth. (Roux, Sai, Deschamps, 2007)

This 1829 experiment marked a significant point when physicians acknowledged the potential for humans to replace non-human animals as the predominant source for tissue and organ transplantation. However, other researchers stated that the benefits of animal blood included its unlimited supply and availability, in addition to the fact that a human did not have to risk their own life to donate blood to another. (Roux, Sai, Deschamps, 2007) Nonetheless, this early work on the science behind blood transfusions was essential for researchers to understand the immune response to foreign DNA, although at the time, they were unaware it was an immune response directing the failures. (Starzl, 2000)

Also important in establishing early understandings of immune response were animal-based pedicle skin grafts and free skin grafts. With a pedicle graft, a physician would strap an immobilized animal on to a patient, affixing its skin (and oftentimes, fur) onto the patient's wound. That reportedly enabled the donor skin to unite with the patient's skin, and after the donor skin allegedly became vascularized with the patient, the physician would then cut the animal off of the patient, leaving the skin graft. However, modern researchers assume that very few, if any, of these pedicle grafts were successful. (Cooper, 2015) Free skin grafts were often produced with the skin from frogs,

which were cheap, easily accessible, and free of fur or feathers. These involved the fresh removal and application of a layer of skin from the animal to the recipient. Physicians also noted that the green pigment in the frog skin often faded after only a few days. During the process of grafting, a surgical assistant would hold the frog - often alive - above the patient's wound. The physician would then cut pieces of skin off of the live frog to fit over the wound, which was typically a burn or ulcer. Then, they would lay the skin on top of the wound without any stitches or fasteners. Again, modern researchers claim that these grafts likely did nothing more than provide a cover for the skin to heal on its own. (Cooper, Lanza, 2000)

Disagreements surrounding blood transfusions continued as new issues surrounding compatibility became apparent. While some physicians were moving away from using animals as tissue donors based on recent experiments demonstrating unfavorable safety and efficacy results (Crawford, Patel, McGhee, 2013), others continued to uphold the therapeutic value of animal blood for human transfusion. Around this same time, Louis Pasteur and Joseph Lister were publishing their contributions to germ theory and asepsis, which may have aided in increasing patient survival rates by decreasing infection rates and increasing surgical survival outcomes. In 1874, multiple physicians began reporting noticeable adverse effects after transfusing animal blood into humans. Their published results revealed high rates of death and adverse effects such as jaundice, blood in the urine, fever, and the reported emission of animal odors. Despite these results, some physicians such as Oscar Hasse in Germany continued to defend the value of xenotransfusion. (Roux, Sai, Deschamps, 2007)

However, toward the end of the 1870s, physicians began understanding the physiological mechanisms related to the species barrier that caused the adverse effects. In 1875, Leonard Landois showed that human blood was capable of lysing the red blood cells from the animal donors, which provided evidence for species-specific antibodies. This was early evidence that foreign cells could potentially cause a serious bodily response and was further supported after researcher Jules Bordet observed that the same antibodies became elevated in the blood after vaccinations. (Roux, Sai, Deschamps, 2007) These findings were especially useful as physicians began to focus on how to navigate the immune response during attempts at full organ transplantation in the twentieth century.

By the end of the nineteenth century, as surgical success increased, physicians began to physically cut disease out of the body. By 1883, surgeon Theodor Kocher had surgically transplanted healthy human thyroid tissue back into a patient who had exhibited symptoms we now associate with hypothyroidism after undergoing a radical thyroidectomy. This was one of the first examples of a successful autograft and resulted in an increased curiosity about the notion of "organ deficiency" (not to be confused with organ inferiority) as a root of disease. (Starzl, 2000, 759) (Barker, Markmann, 2013)

In 1894, surgeon Otto Lanz cautioned his fellow physicians not to dismiss the possibility that a patient may return to a normal, healthy quality of life after the removal and replacement of a whole organ. (Schlich, 2011) And despite initial denial, surgeons began working toward the possibility of transplanting organs, beginning first among animals. After Kocher had established the relative safety and efficacy of his version of a thyroid transplant, physicians began surgically experimenting on animals, often involving

the removal of tissue or organs from one part of an animal's body and moving it to another to see what would happen. (Schlich, 2011) Surgeons' use of animals resulted in some of the first non-human models of surgical impacts on organ function, which eventually led to one of the first interspecies organ transplants in different species of dogs. (Bezinover, Saner, 2019) Eventually, the experience gained during these early non-human transplants gave physicians the confidence to delve into similar surgeries on humans, resulting in some of the first attempts at whole organ xenotransplants. (Schlich, 2011) These massively unsuccessful first attempts at xenotransplantation of organs very likely contributed to the collective move away from animal organs and toward human organs for the purposes of transplantation.

1900 to 1954

At the turn of the twentieth century, scientists and researchers rapidly began uncovering the physiological reasons why early transplant attempts had failed. Around this time, many scientists became focused on achieving the first successful transplant of an organ, while others continued focusing on blood transfusions. Because many researchers around the world were working on similar projects simultaneously, scientists had limited means of sharing their findings with one another quickly, which often resulted in miscommunications then, and timeline and attribution confusions now. Also, many of these discoveries were made through trial and error, sometimes resulting in seemingly unnecessary loss of life (at least by modern standards). However, the groundwork built in the first half of the twentieth century enabled scientists to make

critical discoveries on the immune system response to transplants, making the first successful human organ transplant possible in 1954.

It was during this time that scientists were interested in determining who could qualify for an organ transplant and what factors limited who could serve as the organ donor. Scientists began to reconsider the use of animals as direct donors for organ transplants. Although some researchers continued to use animals as the donors of direct organs via xenotransplantation throughout the entirety of the twentieth century, the majority focus swiftly shifted in favor of allogeneic human-to-human transplants after a series of highly unsuccessful xenotransplantation attempts. The outcomes of these experiments ultimately served to damage the reputation of xenotransplantation long-term. The first attempts at organ transplantation in general, whether animal-to-human or human-to-human, established how the immune system imposed functional limits on what types of DNA it would and would not accept. Essentially, the discoveries made between 1900 and 1954 indicated that the immune system was one of the most critical barriers in determining the scarcity of suitable organ donors.

Alexis Carrel's Early Impact on Transplantation

Although Kocher's initial transplant experiments in the 1880s encouraged an early focus on transplanting the thyroid and other endocrine organs such as the pancreas, many physicians changed their focus to the kidney, presumably due to the prevalence of animal models for kidney transplants before 1910. (Schlich, 2011) Generally, surgeons had accepted that the exchange of organs was feasible, but their early experiments

revealed the need for a technical expertise on how to fit seemingly mismatched organs together to make the transplants functional.

As a student, Alexis Carrel, who would later be known as the "Father of Transplant Surgery," had suggested that it would be possible to one day replace diseased organs with healthy organs to treat diseases. (Cooper, Lanza, 2000) He also claimed physicians would be able to save more patients if they knew how to repair blood vessels as well as they could repair tissues. Although other scientists had begun successfully repairing large arteries within the body, the same techniques could not be applied to smaller vessels. This concept evolved into some of his earliest research on vascular anastomoses, in which he used small needles and thread to connect small blood vessels with each other. He published his initial research on these methods in 1902. (Sade, 2005)

After his initial findings on vascular anastomoses, he began applying this research to early experimental attempts at tissue and organ transplantation. In 1902, Carrel transplanted a dog's kidney into its neck. He attached its blood supply to the dog's carotid artery and attached the ureter to the esophagus. According to Carrel, the kidney began producing urine immediately, though the dog died a few days later due to infection. In collaboration with Charles Guthrie, he published these findings in 1905, at which point he also published research on his successful transplantation of a thyroid gland in a dog. (Carrel, Guthrie, 1905) (Sade, 2005)

Thus, Carrel used dogs as early models for organ transplantation in the early 1900s. He and Guthrie developed strategies for transplanting kidneys and ovaries from one dog to another. He also experimented by transplanting a dog's heart into a larger dog's neck. Carrel and Guthrie found that the transplanted heart began functioning within

an hour of transplant. He then continued more detailed research into the functional importance of blood vessels during organ transplantation. He used this research to transplant several organs among dogs, including the intestines, heart, adrenal glands, and even a full limb reattachment in 1906. This work culminated with his execution of the first coronary artery bypass graft on a dog in 1910. (Sade, 2005) These discoveries earned Carrel a Nobel Prize in 1912 and his designation as one of the "fathers of transplant surgery." (Cooper, 2012) (Vernon, 2019)

Furthermore, Carrel's research in tissue culture was amongst the first to establish that bodily tissues could live, grow, and survive outside the body. His research on organ and tissue preservation was based on his interpretations on what it means for a tissue to remain alive. In 1912, he described a dichotomy of what he called the "secret of life," between active and latent living. He further divided the concept of latent life into a form of un-manifested actual life, or the typical progress toward death, and potential life, or the temporary suspension of vital functions. These concepts were the foundation for determining that organs and cells do not instantly become unviable when a human dies, which benefited later justifications for cadaver organ donations. (Dutkowski, Rougemont, Clavien, 2008) Using these foundations, he developed techniques for preserving organs and tissues on ice, invented the first perfusion pump to keep organs alive during transplant surgery, and created some of the fundamental techniques for tissue engineering with his cultivation of immortal chicken heart tissue. (Jiang, 2010)

Carrel ultimately had concluded that there was a type of "biological force" that acted to innately prevent most successful transplantation between individuals. His hypothesis was later shown to be associated with the immune system's response and

rejection of the foreign tissues. (Nobel Prize, 1990) While Carrel's discoveries were vital for organ transplantation to progress and become successful, neither allogeneic nor xenograft organ transplantation methods were possible without further understanding of the extent of the immune response.

First Attempts at Transplantation

Carrel's theories about the immune system as a "biological force" foreshadowed the disastrous results of some of the earliest attempts at transplanting organs, both from animals to humans, and humans to humans. The earliest efforts at transplantation in humans were xenotransplants, which occurred in the midst of Carrel's discoveries. And while there was also simultaneous research being done by scientists outside of transplant experimentation on the fundamentals of the antibodies and the immune system that will not be included here, these early catastrophes were vital in prompting scientists to understand what led to these surgical failures.

Between the earliest transplant attempts through the early 1910s and the first successful human organ transplant in 1954, scientists had begun forming the framework behind the concepts of tolerance, rejection, and compatibility. Together, these principles later defined who could receive an organ from whom and provided a framework for the many methods of pharmacologic immunosuppression. In the midst of these early efforts at xenotransplantation, Carrel's key discoveries on tissue culturing, organ transplant animal models, and his theory on the "biological force" were occurring simultaneously and shortly thereafter.

The earliest attempts at organ transplantation were xenotransplants, all of which were blatantly unsuccessful. While some sources disagree, the first attempt at a xenograft was likely performed by Emerich Ullman, who among many other transplant attempts, had surgically attached a pig kidney to the blood vessels in the arm of a woman, though little else is known about the outcome or reason for the experiment. In 1905, French surgeon Princeteau unsuccessfully implanted slices of a rabbit kidney into a child's failing kidneys, noting the child died sixteen days after the surgery. In 1906, Mathieu Jaboulay used a pig and a goat kidney in two different kidney transplant attempts. As this was prior to Carrel's research, Mathieu attached the kidneys' largest blood vessels to the veins in the patients' arms, allowing the organ to remain outside of the body. (Cooper, Lanza, 2000) Then, in 1910, Ernst Unger published two attempts at xenotransplanting kidneys between humans and monkeys. For one experiment, he transplanted a monkey kidney into a human, and for the other, he transplanted a stillborn infant's kidney into a baboon. (Cooper, Lanza 2000) Other experiments involved transplanting slices of testicles from monkeys into men to renew their "zest for life." (Cooper, Eksor, Tector, 2015) None of these patients survived these early procedures, and interest in xenografts temporarily subsided as a result. (Barker, Markmann, 2013) No further xenografts were performed until 1963, after immunosuppression therapies were made available. (Starzl, 2000,)

Between the early attempts at xenotransplantation and the first successful organ transplant in 1954, there were relatively few attempts at transplanting organs, arguably due to the previous failures. Other historians state that the lapse in experiments was due in part to World War I. (Schlich, 2011) Regardless, in 1933, Soviet surgeon, Yu Yu (also

documented as Yurii) Voronoy performed one of the first attempts of an allogeneic kidney transplant. The recipient was a woman in acute renal failure who had attempted suicide by ingesting large amounts of mercury. However, Voronoy made two decisions that turned out to be recognized as mistakes when performing the surgery. Not only was the organ donor's blood type B+ while the recipient's was O+, but Voronoy also had chosen to remove the organ from the donor over six hours past the time of death. He implanted the kidney into the patient's thigh (which was the standard location in the animal models) and observed – based on the absence of urine production – that the kidney never gained function. (Starzl, 2000) (Barker, Markmann, 2013) And although the patient died two days after surgery, the failure of the transplant was likely due to ischemia and organ warming from the expansive time between the death of the donor and the transplant, in addition to the incompatibility of the patients' blood types. (Matevossian et al, 2009) It later became a standard that a surgeon should avoid transplanting an organ between patients with ABO-incompatible blood type based on the destruction induced by antibodies. (Brent, 2014)

The process Voronoy unintentionally highlighted with his failed first kidney transplant attempt was one of the first suggestions of hyperacute rejection. However, before scientists could fully reach those conclusions, they first had to establish the principles of what would later be known as immunological tolerance, which ultimately led to the discoveries of rejection and compatibility, catalyzing the success of allogeneic organ transplantation. (Matevossian et al, 2009)

Tolerance and Rejection

In 1945, Ray Owen published initial results that established the potential mechanisms of both tolerance and natural chimerism. At the time, Owen worked as an immunogeneticist at the University of Wisconsin and was studying the inheritance of red blood cell antigens in cattle. He discovered that non-identical twin calves were capable of carrying different blood phenotypes. After noting that embryologist Frank Lillie had discovered that fetal calves share a blood supply in 1916 (Barker, Markmann, 2013), Owen theorized that foreign red cell precursors would have had to have spread via that shared blood supply, enabling the precursor cells to implant in an early fetal stage and then adapt and proliferate at a smaller capacity within the growing fetus. Not only was this one of the first verifications of chimerism, but it also showed that an organism could survive with an undetermined number of foreign cells in the body and that it was possible for the body to adapt to foreign tissues. Owen published these results in *Science* in 1945 but received minimal praise until 1949. (Owen, 1945) (Brent, 2014)

In 1949, Australian virologists Macfarlane Burnet and Frank Fenner found Owen's research while publishing theories related to antibodies on what would later be dubbed 'immunological tolerance.' They had theorized that immature fetuses had a type of protective mechanism that would induce immune unresponsiveness if foreign cells were present or introduced to the fetus at an early age of gestation. They called those cells 'cellular self-replicating antigens.' If the cells were present before the age at which the fetus began developing its immune system, they theorized that the cells would be accepted as "self." This distinction, between self and non-self, was another key early concept for organ transplantation.

Then, in 1951, Medawar and Billingham demonstrated that Burnet and Fenner's theories were accurate and established the existence of immune 'tolerance.' Previously, they had found that humans would reject skin grafts from other humans, which they theorized was due to immunological interference. However, years following that observation, they then found that dizygotic cattle twins could accept skin grafts from one another. Because they had not yet heard of Burnet and Fenner's findings, they could not explain why the twin cows could accept skin grafts from one another while their earlier research had shown them that humans could not accept skin grafts from other humans. This solidified the presence of an innate tolerance among closely related individuals. They applied these findings to skin grafts among rabbits. Assuming it was an immunological phenomenon, they weakened the immune response by giving the rabbits prednisone or total body radiation to protect the graft. And, although those therapies only preserved the grafts for a few days longer, this was the basis of immunosuppression therapies for transplants of all kinds. (Starzl, 1995) (Medawar, Billingham, 1951) (Brent, 2014)

Then, after accounting for the conclusions made by Burnet and Fenner, Medawar and Billingham published one of the first papers on actively acquired tolerance in 1953. In their experiment, they inoculated fetal mice with donor spleen cells to determine whether the mice would later accept a skin graft with the same cells as the donor strain. Unintentionally, they had used a pair of mouse strains that were unable to induce a rejection reaction on the basis of an antibody reaction rather than a cellular reaction. They concluded that the mice would accept skin grafts that carried cells from the splenic donor strain, and that the exchange of precursor cells in early fetal life could induce an inability

to respond to foreign cells in the skin, much like Owen's findings on calf twins years before. They did note that this tolerance only remained for a few days following birth before the mice started to reject the grafts, stating it was not an all-or-nothing phenomenon. This experiment also demonstrably made the mice chimeras, which they estimated as having a low level between 1 and 10 percent chimeric cells. (Medawar, Billingham, 1951) (Brent, 2014) (Barker, Markman, 2014) For this discovery of acquired immunological tolerance, Medawar and Burnet earned the Nobel Prize in Medicine and Physiology in 1960.

While their findings showed that scientists could experimentally channel how the body reacts to foreign cells, only a few years later, the same team found that sometimes the donor cells could divert an attack on the host's body if there was not a decent tissue match. This was based on the concept of graft-versus-host disease (GVHD), which is the opposite of rejection, because the white blood cells that are present within the graft itself end up rejecting the surrounding host tissues. And while much of acute organ transplant rejection is based on a mismatch of human leukocyte antigens (HLA) within the donor tissues, GVHD can occur even in HLA-identical matches. (Billingham, Brent, Owen 1959) (Brent, 2014)

Although these critical discoveries were transpiring around the same time as transplantation efforts reignited, the transfer of information around the world was not instantaneous, thus restricting scientists' ability to apply the concepts of tolerance and rejection to the next round of allogeneic organ transplant attempts. In the early 1950s, because there was not yet any form of dialysis, a cluster of physicians in France resumed attempting kidney transplants for those in renal failure, using kidneys from what was later

loosely defined as a “living donor.” (Starzl, 2000) In 1952, Rene Kuss and Charles DuBost attempted a series of kidney transplants that were unique in that the origin of the donated kidneys were convicts who had been guillotined immediately before they collected the kidneys. Because they were freshly deceased, Kuss and DuBost considered the kidneys to be living. Shortly after those experiments, French surgeons Jean Hamburger and Louis Michon attempted one of the first living donor kidney transplants of closely related individuals, from a mother to her son in 1953. Rather than allowing the urine to drain out of the body, they attached the kidney ureter to drain back into the patient’s bladder. And although the kidney functioned for three weeks, the son died shortly thereafter due to rejection. However, the procedure established the benefits of manipulating the surgical placement, of living donors, and of familial matching. (Howard, Cornell, Cochran, 2012) (Barker, Markmann, 2014)

After the French surgeons’ results were relayed around the world, more surgeons began experimenting on kidney transplants between humans, although many physicians rejected the thought of continuing to perform allogeneic transplants because they found it unethical due to the high mortality rates. These surgeries occurred between 1951 and 1953 out of Peter Bent Brigham Hospital in Boston, led by David Hume, who documented at least nine transplants, but likely performed many more but did not report them due to mortality rates. (Barker, Markmann, 2014) Of these kidney transplants, some were treated with immunosuppression medications such as cortisone. Only four kidney transplants showed any function, and all but one ceased functioning within a few days. However, the last attempt stayed functional for over five months before rejecting, though it is not clear why it stayed functional for so long. According to Clyde Barker and James

Markmann, this gave some physicians hope that allogeneic grafts would be more successful than xenografts. (Barker, Markmann, 2014)

Ultimately, the first successful allogeneic organ transplant is credited to Joseph E. Murray, who transplanted a kidney from one identical twin to another. This surgery, occurring in 1954, sparked an immediate enthusiasm around the world for allogeneic grafts over xenografts, despite the fact that there was minimal technical significance with Murray's approach, since it was known that identical twins accepted skin grafts from one another and the surgical technique mirrored previous attempts as well. This ignition in the field of organ transplantation immediately shifted, especially as new methods of immunosuppression, radiation, and bone marrow transplantation technologies began to emerge. (Barker, Markmann, 2014)

Limitations

In the span of just over half of a century, scientists progressed from implanting autografted thyroid and kidney tissue in dogs, to the first successful allogeneic organ transplant. While unapparent to those conducting some of the earliest human-involved transplant efforts, the realization that there was something physiological in nature that was impeding a successful transplant was likely inferred once human patients began surviving for a few days following surgery before succumbing to what was likely organ failure or sepsis. Furthermore, the fact that the first successful allogeneic organ transplant was a result of an identical twin donation likely conferred further suspicion that there were biological mechanisms at play. Through much trial and error, scientists uncovered

the major components of the human immune system that were implicated as the underlying reasons for most of the early failures.

Altogether, the discoveries of the mechanisms involved in tolerance and rejection made it so that organ transplantation could not occur without some measure of immunosuppression, which is often lifelong and can cause some serious consequences. Therefore, a goal of organ transplantation science would be to create a transplant that would require no suppression of the immune system. (Fuchs, 2014)

Tolerance

Despite the fact that these innovations made organ transplantation possible at all, they also served to limit the capacity of organ transplantation based on physiological limitations of the human body and the extent to which it could be manipulated. First, early studies on tolerance informed scientists that natural tolerance was really only achievable between twins. If researchers wanted to channel natural tolerance around the time of the late twentieth century, they would have had to manipulate human fetuses.

In clinical induction of tolerance, the production of a transplanted organ that functioned and had no histological evidence of rejection would be successful. Experiments involving clinical tolerance induction have varied, but it appears one of the only promising methods involves the use of hematopoietic stem cell transplants at a level surpassing the typical use of these transplants, such as certain bone marrow or blood cancers. This invasive procedure involves the simultaneous transfer of donor bone marrow in conjunction with the organ transplant but maintains the goal of establishing systemic chimerism that enables graft acceptance and tolerance. (Otsuka, Wada, et al,

2020) Not only does this require systemic eradication of the host's immune system through chemotherapy and radiation, but many studies demonstrating any positive effects have been found within small samples and with inconsistent results. (Sachs et al, 2014) (Leventhal et al, 2016)

While it does not appear that researchers will ever be able to induce clinical tolerance without significant intervention, there have been some reports of patients developing what resembles tolerance to their organ transplant after discontinuing their immunosuppressant drugs, typically due to noncompliance and adverse side effects. (Orlando, Soker, and Wood, 2009) However, such appearances tend to be documented only with liver transplants, and that tolerance is questionable when patients' immune systems are eventually challenged by infections like influenza. (Watson, Dark, 2012) Additionally, this finding appears to only persist for a limited time, is non-representative of a majority of the population, and may compromise the longevity and survival of the transplanted organ. (Orlando, Soker, and Wood, 2009)

Rejection and Immunosuppression

Despite modernized approaches to transplantation surgery, organ and recipient selection criteria, and post-surgical care, transplant rejection remains an imminent risk for all transplant recipients. One of the most influential ways to reduce the risk of rejection is through diligent and scrupulous immunosuppressant therapies. These medications are designed to impede organ rejection for as long as possible and are commonly also known as anti-rejection medications for that very reason.

Methods of immunosuppression varied throughout the years, although the two general categories are pharmacological therapies and radiation. Prior to scientists' attaining a full understanding of the immune system and its effects on transplantation, much of the early hypotheses about the extent of immunosuppressant efficacy was built on other disease models (such as the early use of cortisone to treat rheumatoid arthritis in the late 1940s) or based on an assumption that many of the negative effects seen after transplantation had something to do with an overactive immune response. (Wiseman, 2016) The advent of Cyclosporine therapies in the 1970s enabled the ability to control rejection rather than just suppress the immune response. (Colombo, Amirrati, 2011) Other scientists have suggested that immunosuppressant research should focus on generating reliable evidence on the use of monoclonal antibodies for immunosuppressants post-transplant. This therapy could be potentially advantageous in improving long-term transplant survival, specifically for patients with greater HLA mismatching, a history of nonadherence to pharmacologic therapy, or a history of prior transplants and/or acute rejection. For patients with those risk factors, they are at a greater risk of graft failure with more traditional means of immunosuppression therapies, and often require more intensive levels of immunosuppressants. (O'Leary, Samaniego, et al, 2015)

In order to produce immunosuppression, physicians administer powerful induction drugs at the time of surgery and then maintenance drugs that are used long-term. Much like an antibiotic reduces preventable risks associated with surgical infection, induction drugs are necessary to prevent acute rejection immediately following the surgery. There are four general classes of immunosuppressants, including calcineurin

inhibitors, antiproliferative inhibitors, mTOR inhibitors, and steroids. (UNOS, 2020) (Barker, Markmann, 2013) The type and strength of drug may change based on individual risk factors, and the types and dosages may change over time. Barriers involved with immunosuppressants include medication costs, regimen complexity, loss of medical coverage, and lack of education or awareness of the outcomes of nonadherence. (Field, Lawrence, Zwanziger, 2000)

If for whatever reason, organ recipients become noncompliant with their immunosuppressant regimen, they immediately risk the rejection and loss of their transplant. Medication nonadherence is the second most common cause of graft failure in kidney transplant recipients. (Mellon, Doyle, et al, 2017) Researchers have described effective patient adherence to medication when their behaviors and actions surrounding taking their medications match the prescriber's guidelines. Factors that contribute to patterns of nonadherence include young age, lack of social support, decreased cognitive aptitude, high transplant-related stress, minority race or ethnicity, and the perception of poor health. (Nevins, Nickerson, Dew, 2017) (Mellon, Doyle, et al, 2017) Unintentional nonadherence can occur when a patient is outside of their normal routine or is sick. Intentional nonadherence may occur due to stress, anxiety, depression, paranoia, and external events that cause heightened distress levels. However, even when taken diligently, immunosuppressants only forestall rejection. Because the tissue DNA will always be "not-self," rejection is imminent rather than fully preventable.

Additionally, rejection can limit a transplant patient's quality of life. As organ transplantation stands today, there is always some degree of rejection that occurs, regardless of the methods of transplantation or immunosuppression, and at least one

episode of acute rejection is common within the first year following transplant surgery. (UNOS, 2020) Acute rejection can vary in severity, from reduction of the organ's function to flu like symptoms and organ-specific symptoms like heart failure or renal failure. That can also lead to chronic rejection involves a gradual reduction in organ function, and typically will occur in any transplant eventually. This requires long-term immunosuppression via the pharmacological therapies discussed above, sometimes requiring multiple different types of medications per day for the rest of a patient's life.

Rejection typically occurs due to an activation of the immune system as a result of a variety of factors, but historically this was often due to overtly mismatched Major Histocompatibility Complex (MHC). Although locating an exact MHC match is very rare even today, unless the recipient has an identical twin willing to serve as a live donor, researchers have uncovered MHC partial matches that are less likely to result in severe rejection reactions. Although human leukocyte antigen (HLA) mismatches can be less detrimental to the rejection potential of the organ than MHC mismatches, antibody-mediated rejection can trigger an immune response that becomes difficult to manage and treat given current methods of immunosuppression. (Otsuka, Wada, et al, 2020) While certain immunosuppressants can broaden the range of HLA tissue types, there is still ongoing research occurring into the limits of the MHC and its effect on transplant rejection with tissue-matched donors and recipients as well. (Nakamura et al, 2018) Thus, despite the gaps in overall understanding over time, researchers were able to conclude that the immune system imposes some of the most stringent restrictions on the scope and application of organ transplantation moving forward.

State Of Modern Organ Transplantation

A patient's long-term quality of life after an organ transplantation in the year 2020 differs to an extent from that which was common in the mid twentieth century. However, although immunosuppression methods have become somewhat more sophisticated, patients are still subjected to lifelong pharmacological therapy to avoid rejection of their organ graft and researchers have uncovered very few practical alternatives. Many of the challenges associated with modern organ transplantation are generally a result of the need for immunosuppression, the shortage of organs, or a mixture of both variables. The current state of organ transplantation is marked by the ways scientists and physicians attempt to grapple with the perpetual dependency on immunosuppressants and the ongoing organ shortage.

Organ Allocation and Shortage

While learning and adapting to new information about the limitations of the immune system on transplant rejection, physicians were able to rapidly expand the types of organs transplanted as a result of better immunosuppressive therapies and enhanced technical surgery approaches. In 1967, physicians performed the first successful liver transplant. In 1968, the first successful heart and pancreas transplants were performed. Between the 1970s when cyclosporine was first used and today, surgeons achieved variations of living and deceased donations for heart and lung transplants. Living donors for organs like the liver, kidneys, and even lungs became possible as well. As the science and technique behind organ transplantation grew, physicians were able to treat more people with more complex diseases. However, that further emphasized the growing organ

shortage. Because the organ shortage meant that multiple people could qualify for any single organ, the United Network for Organ Sharing system was formed in 1977 and became one of the first means of assigning who gets which organ. It was made a non-profit after the 1984 passing of the National Organ Transplant Act. (UNOS, 2020)

Despite researchers' efforts at finding alternative sources, the organ shortage remains one of the most impactful limitations of modern organ transplantation in addition to the requirement for immunosuppression. In 1967, it was estimated that 8,000 of the estimated 50,000 people who died each year from kidney failure could have been candidates for kidney transplants, noting that only about 300 patients were treated with kidney transplantation that year. (Dukeminier, 1970) As of March 2020, over 112,000 people were on the organ waiting list in the US alone. Only 39,718 transplants were performed in 2019 and over 20 people die each day waiting for an organ. (HRSA, 2020) As organs are at such a shortage, matching them with donors who are in good enough condition to survive the surgery, but who often also hold an urgent enough need for it to be a nearly life-or-death requirement causes significant challenges in who does and does not get approved for organ transplantation.

According to the Organ Procurement and Transplantation Network working in affiliation with the US Department of Health and Human Services, because of the organ shortage, the process of allocating an organ requires consideration for patients' medical needs and overall circumstances, in addition to medical utility. They define medical utility as the balance between increasing the number of successful surgeries, considering the length of time a patient is expected to survive, and estimating the length of time the transplanted organ will survive before failing. When a donor organ is identified, typically

by a procuring organization working with UNOS, a list of potential recipients is generated and separated based on certain variables that may raise or decrease their candidacy for the organ. Some of these variables include age, blood type, medical urgency, survival benefit, waiting time, and distance between the donor and recipient. Once identifying a patient who fits the criteria, an organ procurement specialist reaches out to that patient's surgeon, who makes the final decision as to whether that patient's current health status makes them a suitable candidate for the organ. (OPTN, 2020)

After finding a suitable recipient, organ procurement teams fully recover and transport the organ in a cold-organ preservation solution to the recipient's location. The viability of organs once procured from the donor varies. The current standard for preservation allows hearts and lungs to survive for four to six hours outside of the body, for the liver to survive up to twelve hours outside of the body, for a pancreas to survive twelve to eighteen hours outside of the body, and for kidneys to survive twenty-four to thirty-six hours outside of the body. These time restraints may limit who is determined to be a potential recipient based on their geographical proximity to the donor. (OPTN, 2020) Additionally, only three in 1,000 individuals die in such a way that allows for their organs to be donated. Factoring in the fact that only about 60 percent of adults in the US are registered as organ donors, these serve as additional limitations of organ transplantation. However, with the option of living organ donation, whether from a family member or a stranger, a patient's odds of receiving an organ may improve. In 2019, out of a total of 19,267 organ donors, living donation accounted for 7,397 transplants and deceased donation accounted for 11,870 transplants. (HRSA, 2020)

Data Review

While one could examine a surplus of statistics to fully investigate the differences of organ transplantation efficacy over time, there are some general overarching statistics that represent a more comprehensive perspective of the current state of organ transplantation. For example, each day, about eighty people receive an organ transplant. Overall improvement of organ donation and selection techniques have enabled the ability for one suitable donor to save up to eight lives by donating their organs. (OPTN, 2020) It appears that researchers generally agree that modern organ transplantation has improved the short-term period following transplantation but disagree on the extent at which organ transplantation has improved long-term variables, such as overall patient survival, graft survival, and quality of life. (Lodhi, Lamb, Meier-Kriesche, 2011) (Rana, Godfrey, 2019)

Additionally, more than 270,000 kidney transplants have been performed since 1987. Receiving a deceased kidney graft can double a recipient's chance of survival, whereas a living kidney donation can quadruple a recipient's chance of survival. Recipients of kidney transplants have a median survival time of about 12.4 years past transplantation, whereas those on the waiting list have a median survival time of about 5.4 years. (Rana, Godfrey, 2019)

Up to fifty percent of those who receive liver transplants survive for seven years, which is nearly identical to the survival rate seen with heart transplant recipients. Adults who receive a liver transplant have a median survival time of 11.1 years, whereas pediatric liver transplant recipients may survive longer than twenty-five years. Adults who receive a heart transplant have a median survival time of 9.4 years, whereas pediatric heart transplant recipients have a median survival time of 12.8 years. However, some

researchers concede that an over-reliance on reporting median survival numbers may negatively inform overall interpretations of data, stating that average survival times may indicate problematic statistics. (Lodhi, Lamb, Meier-Kriesche, 2011) Overall, as of 2017, the average survival estimate after a liver donation was 4.3 years per recipient, and the average survival estimate after a heart transplant was 4.9 years per recipient. (Rana, Godfrey, 2019)

Although short-term survival rates have increased, many researchers claim that there appears to be a trend of stagnation for long-term health and survival outcomes in transplant recipients. In the 1980s, the one-year survival rate after a liver transplant was only about sixty seven percent. In 2014, that number increased to over ninety percent. However, long-term survival rates have not significantly improved since the 1980s. Long-term stagnation can be seen when analyzing the long-term attrition rates associated with kidney, liver, heart, lung, and intestinal transplants. (Lodhi, Lamb, Meier-Kriesche, 2011) Researchers have also claimed that the increase in one-year survival rates in contrast with the stagnant long-term survival rates may be due to the fact that immunosuppressive medications only require a one-year clinical trial period before being approved. This reliance on premature trials and reporting of early data may not accurately reflect the long-term attrition of certain therapies, thereby possibly explaining why patients experience only marginally improved long-term survival rates. (Lodhi, Lamb, Meier-Kriesche, 2011)

After factoring in the long-term sacrifice and responsibility associated with lifelong immunosuppression, some researchers disagree on the extent at which transplantation improves recipients' quality of life under its current restraints of

immunosuppression and imminent potential for rejection. For example, some researchers have found that the data suggests any estimated extension of life span after a heart or liver transplant specifically may only be slightly longer than that without a transplant. They claim that patients must be fully aware of how their quality of life may change with and without an organ transplant before deciding to proceed with the decision. Without a transplant, a patient may risk a progressive increase of suffering as the organ dysfunction and/or failure progresses, whereas with a transplant, a patient may be given a slightly longer lifespan estimation but must factor that with the lifelong constraints and need for immunosuppressive medications. (Rana, Godfrey, 2019)

Since the 1980s, there have been moderate improvements made to the ways organs are procured, preserved, oxygenated, and implanted. However, when comparing these innovations with the absence of any increase to long-term survival rates, it is evident that current strategies of immunosuppression remain a significant factor leading to graft failure and recipient mortality. While an overall improvement in acute rejection rates is likely due to the use of more potent immunosuppressive drugs, the effects of those drugs may also factor into the relatively static long-term survival rates and an increase in malignancy rates. (Rana, Godfrey, 2019) While drug choices have diversified, and the efficacy has improved over time, deciding the type and strength of immunosuppressant medications is largely dependent on the type of transplant received and the overall risk of rejection.

For example, a group of researchers constructed a hypothetical scenario to communicate the limitations involved in raising the overall strength of immunosuppressive drugs given to a population in an effort to reduce rejection rates.

They state that a raise in drug strength would not actually result in more significant increases in short or long-term survival rates. “For example, one may consider a hypothetical recipient population with an initial rejection rate of 40%, and immunosuppressive therapy is then intensified to decrease rejection rates to 20%. This move will benefit possibly those 20% of patients who now will not experience rejection, yet the 60% of patients who would not have had rejection on the previous regimen are now by definition over immunosuppressed.” (Lodhi, Lamb, Meier-Kriesche, 2011) Because immunosuppressants have a narrow overall therapeutic index, potent and unnecessary immunosuppression can lead to higher rates of cancer, infection, and death. However, many physicians advocate for individualized approaches to immunosuppression, factoring in the overall risks of rejection and drug toxicity. (Abboudi, MacPhee, 2012)

Specifically, the use of calcineurin drugs contributes to an overall increased risk of hypertension, hyperlipidemia, and diabetes which may sacrifice the integrity of the organ graft, especially heart, pancreas, and liver transplants. Furthermore, that same class of drugs is highly toxic to certain cells within the kidney, which often leads to Chronic Kidney Disease across all transplant types. Up to ninety percent of liver transplant recipients will go on to develop some form of Chronic Kidney Disease and can widely vary from ten to ninety percent risks among other forms of organ transplants. This can lead to a significantly elevated risk of renal failure and need for kidney transplant among those who have already received a non-kidney transplant. Among those who have already received a previous non-renal transplant, mortality risks from dialysis and kidney transplant increase substantially, and survival after a kidney transplant is further

complicated by post-surgical infection risks in an already immunocompromised patient. (Bloom, Reese, 2007) As these are some of the most commonly prescribed immunosuppressants, some researchers have called calcineurin immunosuppressants the "Achilles' heel" of transplantation, as they can have negative effects on both short- and long-term survival and quality of life. (Lodhi, Lamb, Meier-Kriesche, 2011)

With uncertain long-term survival rate increases and the outcomes associated with immunosuppressant medications, the current state of modern organ transplantation appears relatively bleak. As it stands, patients with organ dysfunction, injury, or failure are not given many options than to either, a) live with their condition and allow themselves to decline naturally without the need for advanced therapeutic interventions (such as dialysis) or immunosuppression, b) partake only in advanced therapeutic interventions without the need for immunosuppression, or c) wait on an organ transplant waiting list, potentially needing short-term therapeutic interventions before an organ match is made, at which point they begin lifelong immunosuppressant medications. Thus, a solution is needed that addresses and overcomes these limitations.

CHAPTER 3

INTERSPECIES BLASTOCYST COMPLEMENTATION

Although allogeneic organ transplantation has persisted as the only treatment option for end-stage organ failure, there remains a large gap in the accessibility of organ transplantation. In this regard, several researchers have made it a priority to discover an alternative that would allow for unlimited access to viable organs, while also dismissing the need for immunosuppression. Thus, scientific attention was turned toward the regenerative medicine field upon its rise in popularity through the late 1990s and early 2000s. (University of Nebraska Medical Center, 2020)

Organogenesis is a highly complex process that results in an intricate organ consisting of billions of specialized cells. While many scientists have touted that a key goal of regenerative medicine is to one day be able to grow functional organs, those dreams have been quashed – perhaps temporarily – by the inability to determine how to vascularize an engineered organ and at what extent engineered immunological cells are also needed when that organ is transplanted. (Zhou, 2017) Thus, any innovation would have to accommodate these inherent processes without disrupting it to the point of deformation or mortality to the organ or recipient. It is in this stage that the concept of the chimera returns, and the modern approach at engineering human and animal cells to work together to benefit humans rather than terrorize them as was portrayed by Homer in *The Iliad*.

Among many alternative solutions for traditional organ transplantation that have been posited in the last twenty years, one of the most promising appears to be interspecies blastocyst complementation. This technology presents the potential to generate human-

specific organs within animals with the use of precise and targeted chimerism which, through genetic engineering, reduces the likelihood for the migration of human cells throughout the host. Not only would this resolve the organ shortage, but importantly, it could address and eliminate the need for immunosuppression. (Otsuka, Wada, et al, 2020) Once researchers demonstrated that interspecies blastocyst complementation was possible between mice and rats in 2010 (Kobayashi, Yamaguchi et al, 2010), their findings suggested that this technology may become a plausible therapy, establishing it as a potential solution for many of the current limitations surrounding modern organ transplantation.

If successful, interspecies blastocyst complementation would provide a means of creating patient-specific organs that would not require lifelong immunosuppression. As a brief summary, this process would begin at a very early gestational age for the embryonic host, which will have been genetically modified to lack a specific functional gene for the organ of interest. That modification would make the developmental niche technically empty, although none of the extrinsic organogenesis factors would be affected. That means that a subsequent microinjection of human progenitor cells derived from the patient's induced pluripotent stem cells (iPS) cells would then be able to fill the organ niche left vacant by the gene knockout, enabling the growth of a patient-specific organ. (Rashid, Kobayashi, Nakauchi, 2014) If the technology functions as expected and the organs reach a degree of chimerism that eliminates the possibility for the immune system to react to the organ as "not-self" (Matsunari, Watunabe, et al, 2020), it would require no immunosuppression based on the establishment of central and peripheral tolerance, due to its use at an early embryonic stage. (Suchy, Yamaguchi, Nakauchi, 2018)

First described in 1993, early blastocyst complementation models involved the addition of mice embryonic stem cells into mouse embryos. Modern interspecies blastocyst complementation builds upon that early technology by using interspecies stem cells rather than stem cells from the same species. (Chen, Lansford, Stewart, Young, Alt, 2010) However, researchers suspect based on early results that this crossing of the interspecies barrier will require some significant and time-intensive investigation before interspecies blastocyst complementation can be fully plausible, especially in humans. (Suchy, Yamaguchi, Nakauchi, 2018) Thus, although researchers claim that their technology will one day be successful at creating organs in animals (Rashid, Kobayashi, Nakauchi, 2014), the human applications of this technology remain largely theoretical, with most of the assumptions being made on the animal models that have been developed to date.

Biological Proof Of Concept

In the first portion of this thesis, the focus was on the timeline of initial transplant development, and how scientists attempted to reconcile early failures and successes with the observed effects of the immune system before fully understanding its effects. While looking for a suitable source for organs, researchers felt that the most logical location for obtaining transplantable organs was from another human. After being reinforced by the early failures of animal xenograft experiments, researchers upheld the premise that organs could only be recycled and reused from other humans throughout a majority of the transplantation biology-focused research in the late twentieth century.

According to Clyde F. Barker and James F. Markmann, following the initial short burst of successful transplants for kidneys and the discovery of early immunosuppression therapies, there was a "period of consolidation," lasting between 1964 to 1980, during which there were no critical advances made in transplant biology. (Barker, Markmann, 2013) And although organ transplantation was simultaneously becoming more sophisticated, as was shown in the previous section, those improvements which came after 1980 did not yield many improvements due to the constraints imposed by the immune system.

Before researchers eventually established a framework for interspecies blastocyst complementation, scientists had utilized a variety of stem cells and tissues of various origins to attempt to grow organ-like structures. Researchers have attempted to transplant human tissue directly into animals. (Dekel, Burakova, Ben-Hur et al, 1997) (Hammerman, 2002) Researchers have also tried to use mesenchymal stem cells to grow hepatocytes in sheep in order to produce a functioning human liver. (Almeida-Porada, Porada, Chamberlain et al, 2004) Scientists have also attempted to use genetically modified pigs to streamline xenotransplants to omit the antigens that initiate acute rejection and reduce certain risks for zoonotic transmission. (Cooper, Lanza, 2000) In some of these preliminary attempts at developing models for organ or tissue transplantation, scientists relied on hematopoietic and mesenchymal stem cells. Hematopoietic stem cells are responsible for generating all of the necessary components of peripheral blood. (Jackson, 2019) Mesenchymal stem cells are multipotent stem cells that are responsible for repairing bone and cartilage. Both of these types of stem cells can

be isolated in adults, and both exist within the bone marrow, although mesenchymal stem cells make up a very small fraction of the stem cell content. (Ullah, Subbarao, Rho, 2015)

While hematopoietic stem cells are capable of differentiating into any of the cells within the peripheral blood and some other kinds of cells, mesenchymal stem cells were originally thought to only be capable of differentiating into tissues that could repair bone and cartilage. Later research established that other parts of the body also contain mesenchymal stem cells (and mesenchymal-like stem cells), such as the liver, brain, placenta, cord blood, dental pulp, and certain types of fat tissue. (Thirumala, Goebel, Woods, 2009) (Cordeiro-Spinetti, de Mello, et al, 2014) Essentially, they had the capacity to produce a variety of mesodermal phenotypes in an adult organism. (Caplan, 2017) However, these stem cells presented a variety of disadvantages that make them ultimately incapable of generating or transmitting any biological matter other than stem cell transplants with hematopoietic stem cells and bioactive therapeutic techniques with mesenchymal stem cells. (Caplan, 2017) Therefore, attempts at generating or regenerating organs could not proceed without the use of a different type of stem cell.

Evidence and Experimentation

Interspecies blastocyst complementation was made possible by many technological breakthroughs. One of the most crucial technological components of interspecies blastocyst complementation is its ability to inactivate genes that code for the development of certain organs. While the historical development of this technology is extensive, gene knockout technology primarily began with the creation of the knockout mouse, which was first introduced in 1989 by Mario Capecchi, Martin Evans, and Oliver

Smithies. (Nobel Prize, 2007) This discovery provided scientists with the ability to introduce and target specific gene modifications in mice using different types of stem cells.

In the midst of early research on gene knockouts, in 1993, Chen and colleagues first demonstrated the success of intraspecies blastocyst complementation, generating chimeric mice. The researchers used mouse blastocysts that were deficient in a gene that eliminates their ability to produce B and T cells, and injected them with normal mouse embryonic stem cells, which resulted in the generation of mice capable of producing a full immune response. (Chen et al, 1993) Despite the fact that this first example was not as targeted as later technology, nor was it specifically interspecies, it established the foundations for interspecies blastocyst complementation that eventually enabled its success. (Yamaguchi, 2019)

Also essential to the currently endorsed approach to interspecies blastocyst complementation is the use of induced pluripotent stem cells (iPSCs or iPS cells). iPSCs may serve as a reasonable substitute for hematopoietic stem cells, mesenchymal stem cells, and embryonic stem cells in regenerative medicine. Embryonic stem cells are pluripotent, which means that they give rise to all types of cells within an organism, are capable of self-renewal, and are derived from the inner cell mass of the blastocyst. These cells are most operative during a time that is short, yet crucial for early embryogenesis, setting up the developmental framework, while also carrying characteristics of indefinite self. (Wu, Izpisua Belmonte, 2016) This stage also signals the start of organ development, progressing through cycles of cell proliferation, migration, differentiation, and apoptosis. (Suchy, Yamaguchi, Nakauchi, 2018) Thus, because iPS cells could

emulate these effects, they became more favorable for regenerative medicine efforts. The first experimental production of human embryonic stem cells was performed in 1998, and only nine years later, researchers were able to reprogram somatic cells into an embryonic stem cell-like pluripotent cell – the induced pluripotent stem cell. (Wu, Izpisua Belmonte, 2016) (Takahashi et al, 2007)

In 2006, Takahashi and Yamanaka published their experiments in "Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors." In short, they demonstrated the capability to reprogram somatic cells to revert back to a pluripotent state. Blastocyst injections were also used in these experiments to prove the ability for the pluripotent cells to give rise to differentiated cells, as seen with their capacity to produce embryonic teratomas upon transplantation. (Hockemeyer, Jaenisch, 2016)

Some of the noted benefits of iPSCs revolve primarily around their capacity to replace the need for embryonic stem cells in research, based on the similarities between the cell types. Both iPSCs and embryonic stem cells are capable of expressing core pluripotency factors, of maintaining genomic stability in long-term cultures, and of producing tissue types derived from each of the three germ lineages. (Wu, Izpisua Belmonte, 2016) And although technically relevant to some of the early research surrounding interspecies blastocyst complementation, some of the functional differences between human embryonic stem cells and mouse embryonic stem cells, in addition to the differing potential applications of primed and naive state stem cells, will not be discussed at length in this thesis. (Robinton, Daley, 2012) Briefly, there is still not enough known about the immunogenicity of these different types of cells and how they may help or

hinder interspecies blastocyst complementation. (Fairchild et al, 2016) (Zhao et al, 2011) Additionally, some researchers claim that iPS cells may hold a lower differentiation capacity than embryonic stem cells, whereas others claim that the differentiation capacity of iPS cells may depend on the origin source of the reprogrammed cell. (Robinton, Daley, 2012)

Once gene knockout technology evolved in the early 2000s, scientists obtained better control over the ability to select the gene of interest for an experiment. (Capecchi, 2005) For the majority of interspecies blastocyst complementation studies, scientists use CRISPR-targeted knockouts, which has given researchers better control over how many of the mice are complete knockouts opposed to mosaics. These strategies for gene editing have been used in many of the animal-based models beginning in 2010.

In the 2010 article, "Generation of Rat Pancreas in Mouse by Interspecific Blastocyst Injection of Pluripotent Stem Cells," researchers were able to demonstrate the capacity of the technology in one of the first successful experiments using interspecies blastocyst complementation. (Kobayashi et al, 2010) This experiment enabled the creation of a mouse with a rat-based pancreas. The genetic modification in this case involved the knockout of the *Pdx1* gene in mice, after which researchers added rat pluripotent stem cells to the *Pdx1*-deficient mice, resulting in a viable mouse-rat chimera. (Wu, Luengo et al, 2016) Researchers chose this particular gene because previous models had shown that mammalian embryos could develop normally in-vitro without a pancreas. (Suchy, Nakauchi, 2017) The pancreases were fully functional as indicated by the measurement of glucose levels once the chimeras reached adulthood. However, despite the fact the pancreas was composed nearly entirely of the donated rat PSCs, the vessels,

nerves, and interstitial cells all had varying levels of chimerism belonging both to the host and donor. Regardless, the effects of mixed chimeric tissue structures outside of the targeted organ did not appear to affect the success of the embryo, but it is unclear whether similar conclusions could be applied to humans. (Kobayashi et al, 2010) (Rashid, Kobayashi, Nakauchi, 2014)

The 2010 study established interspecies blastocyst complementation as a viable option for organogenesis, and subsequent research showed that the technology is capable of targeting multiple organs and multiple genes at once. Using gene knockout, researchers generally suspect that interspecies blastocyst complementation will be able to generate human organs such as the pancreas, liver, heart, kidney, and lung. Additionally, researchers have also experimented with the technology to create progenitor cells to treat human neurological conditions and immune cells to treat immunodeficiencies. Moreover, researchers have also spent time considering how differences in the vascular endothelial cells between the organ and the host may impact the rejection potential of the organ. These specific organs are targeted through the functional genes responsible for the development of these organs in vitro.

In 2017, researchers published a study that attempted to generate rats with mouse-based pancreases, effectively serving as an inverse of the original 2010 study. Similar to the successful findings in the 2010 study, researchers were able to produce rats with fully functional mouse-based pancreases using targeted gene knockout and interspecies blastocyst complementation. (Yamaguchi, Sato, et al, 2017) This experiment was interesting for a few reasons. First, almost every organ within the rat had some mixture of both rat and mouse cells. The main cellular composition of the pancreas was rat-based,

and the pancreas was fully functional, as measured through its production of insulin and ability to maintain blood glucose levels.

In the same experiment, the authors then proceeded to remove those interspecies pancreases from the rats, and harvested isolated clusters of pancreas beta islet cells, which they then transplanted into type I diabetic mice. Those mice shortly thereafter began producing insulin and attained the ability to manage their blood glucose. To avoid any rejection of the rat-based vasculature and immune cells inherent within the grafted islet cells, Yamaguchi and his team treated the mice with a low dose of immunosuppressant for only five days. Those cells maintained function within the mice for over 370 days following the transplant, at which point it is assumed that the researchers sacrificed the mice as the experiment had demonstrated a subjective proof of concept that PSCs could result in functional interspecies organs. (Yamaguchi, Sato, et al, 2017) Other researchers have speculated that it is possible that the mouse was able to absorb any small amount of rat DNA, which would shorten the immune response enough that the five-day immunosuppressant course was enough to instill tolerance. Because islet clusters tend to be quite small, this would potentially make it more feasible than a fully developed organ within the same experimental parameters. (Zhao, 2017)

In 2018, researchers led by Sanae Hamanaka, Ayumi Umino, et al., in collaboration with Hiromitsu Nakauchi, published the article, “Generation of Vascular Endothelial Cells and Hematopoietic Cells by Blastocyst Complementation.” Acknowledging that there is reasonable suspicion that mismatched endothelial cells could potentially be rejected by a host’s immune system in experiments separate from rodents, the researchers attempted to generate PSC-derived vascular endothelial cells. By

targeting an additional gene knockout, the researchers were able to demonstrate that the same pluripotent stem cells were capable of producing vascular endothelial cells with no structural abnormalities after one year in mouse studies. (Hamanaka, Umino et al, 2018) However, they did note a few limitations that involved an inability to grow certain vessel wall cells. In theory, this would mean that the vasculature and endothelial cells would match the PSC origin of the organ itself, reducing risks of rejection.

Research has progressed to show interspecies blastocyst complementation models to provide early evidence for the production of functional lungs by targeting a plausible gene for the organ niche (Kitahara, Ran et al, 2020). Additionally, there have been publications demonstrating its potential for growing pancreatic islet cells (Yamaguchi, 2019) and the potential for utilizing the principles of the technology to grow additional organs such as the thymus (Isotani, Hatayama, et al, 2011) and forebrain (Chang, Liang, et al, 2018).

For practical applications outside of rodent studies, researchers have used pig blastocysts to determine at what extent this technology transfers to other animals. Initially, in 2013, researchers had established that it was possible to use the same gene knockout approach on pigs as was done on rodents through intraspecies blastocyst complementation. Despite some shortcomings, the authors stated that their study proved it was possible to fill a developmental organ niche in a pig, specifically vacating the pancreatic niche. (Matsunari et al, 2013) Later, in 2020, researchers demonstrated it was possible for the same technology to successfully vacate the porcine organ niches responsible for liver, kidney, and vascular development. (Matsunari et al, 2020)

Since 2010, researchers have published a number of studies covering interspecies blastocyst complementation. However, it should be noted that experiments such as these are highly technical and leave minimal room for error, especially when manipulating embryos to operate outside of their typical functions. (Freedman, 2018) Additionally, it should also be emphasized that many of the early mouse and rat experiments exhibited low numbers of successful chimeras, and later studies investigating the roles of non-rodents resulted in even fewer successful chimeras and low rates of measurable chimerism within the targeted organ. While researchers theorize this may be due to a difference in the cellular status of human and rodent pluripotent stem cells in vitro, whether these same results may be better or worse in larger animals with the use of human induced pluripotent stem cells is an area that requires more time and resources than it is currently capable of receiving. (Yamaguchi, 2019) This may be in partial consequence of ongoing funding restrictions. (Freedman, 2018) In the US, the National Institutes of Health remains in a holding pattern after enacting a funding moratorium in 2015. This funding moratorium withholds federal funding from researchers manipulating early non-human embryos with human DNA. (NIH, 2015)

Discussion

When compared to traditional allogeneic organ transplantation, interspecies blastocyst complementation addresses many of the limitations that have existed in traditional organ transplantation since its inception in 1954. As a reminder, those main limitations included a common requirement of lifelong immunosuppression, an imminent risk of organ rejection over time, and tissue compatibility challenges making it more

difficult to locate a suitable organ donor. Additionally, the technology behind interspecies blastocyst complementation is arguably much more complex than traditional organ transplantation, and therefore, there is more room for errors.

Any comprehensive conclusions made about interspecies blastocyst complementation should be addressed in two ways. The first method of drawing conclusions would be made under the assumption that this technology is possible and will satisfy all of the requirements that researchers have suggested throughout their publications. (Rashid, Kobayashi, Nakauchi 2014) However, as it stands today in 2020, interspecies blastocyst complementation is still in an early modeling phase and thus faces a variety of limitations. Thus, because there appear to be many areas needing attention before this technology becomes fully feasible, a less speculative approach would focus on determining how to solve these challenges. While this thesis acknowledges considerations for some of the technical concerns, the final conclusions assume that interspecies blastocyst complementation will work as intended.

Many hesitations surrounding this technology involve its potential to disrupt ethical boundaries of humanization and personhood. The overall notion of chimeras has been subjected to a myriad of ethical interpretations and hesitations in the last twenty years. While it would be impossible to present each idea and the development of those postulations within the constraints of this thesis, there is a need to include a broad interpretation of what researchers consider to be the underlying ethical hesitation: the potential for the humanization of the animal hosts. (Wu et al, 2016)

Scientifically, researchers have acknowledged the importance of preventing human cells from crossing over unintentionally into gametes or neural tissues, which

could raise serious implications about the possible humanization of non-human animals, as that may mean they confer an ethically ambiguous status of personhood in the case of neural cells, or of reproductive mayhem in the case of gametes. Therefore, it is critical that any genetic modification involving human DNA exclusively affects the organ or organs of interest within the animal host. In theory, if the technology only affected the organs of interest, it would not have any capability to cross over into other organs because the cells would not function. While certain scientists have expressed opinions that imply the possibility for interspecies blastocyst complementation to contribute to neural or gametic tissues is minute (Wu et al, 2016), if it does happen, it could have morally significant implications: the engraftment of neural progenitor cells has improved loose definitions of learning and cognition in mice. (Han et al, 2013)

Researchers have suggested that there are two main approaches that interspecies blastocyst complementation can avoid the systemic engraftment of human iPSCs into animal embryos. They suggest that the deletion of transcriptional factors that give rise to neural or gamete development within iPSCs would likely prevent the transmission of human DNA away from the designated organ niche. Another option would be to substitute specific progenitor cells rather than iPSCs. However, this process would require further investigation to determine how researchers could navigate the propensity for more differentiated cells to stimulate apoptosis when transplanted into blastocysts. While these processes need some further examination, they provide evidence that researchers are taking these ethical matters seriously and are moving forward with innovating new ideas to resolve these challenges. (Suchy, Yamaguchi, Nakauchi, 2018) (Wu et al, 2016)

Logistically, interspecies blastocyst complementation addresses some of the community-based challenges associated with traditional organ transplantation. Whereas traditional allogeneic organ transplantation is specifically recommended only for end-stage organ failure, there remains a gap for those whose quality of life becomes impacted based on health deficits caused by chronic, but not imminently life-threatening diseases. If interspecies blastocyst complementation is one day able to produce patient-specific organs, eliminating both the need for immunosuppression and the organ shortage, then organ transplantation or tissue grafting using the technology could be justified to treat chronic conditions. (Fairchild, 2012)

Based on early studies, researchers have determined that a similar technology could be used on a variety of organs. Using gene editing technologies such as CRISPR and projecting early rodent findings (Yamaguchi, Sato, et al, 2017) to the human potential, the injection of donor iPSCs into an embryonic host engineered without genes for a pancreas may be able to solve chronic conditions such as diabetes and pancreatitis. (Crane, Aravilli, Asakura, 2019) Additionally, this same technology can be applied to liver generation using the *HHEX* gene to solve hepatic issues such as cirrhosis and hepatitis. Heart challenges such as dilated cardiomyopathy and coronary artery disease could be supplemented with cardiac progenitor cells using this technology. Health challenges related to the lungs and kidneys could also be candidates for interspecies blastocyst complementation technologies. (Crane, Aravilli, Asakura, 2019)

While interspecies blastocyst complementation could potentially make organ transplantation available for more people (setting aside considerations of cost), it also solves many of the social and policy-based initiatives designed to improve the number

and quality of transplantable organs. Initially, there was a requirement that donors had to be deceased before an organ transplant could occur. However, the development of concepts such as “brain death,” technologies like coronary bypass or respirators, and facilities such as intensive care units enabled higher numbers of people who could be considered ‘live donors’ around the 1960s. (Howard, Cornell, Cochran, 2012) Later solutions included donor education programs, presumed consent programs, rewarded organ gifting, and paired organ donation. (Abouna, 2008) Despite these attempts at raising the threshold and any resulting positive outcomes, the shortage continued to increase. Eventually, the organ shortage justified the use of experimental techniques for organ transplantation. Physicians had previously argued that a patient who was going to die from organ failure anyway may as well be given the best shot at survival, even if that meant from questionable organ sources. (Cooper, 2012)

Organs suitable for transplantation into a grown human adult would need to come from a source that shares similar stature to a fully grown human. Although preliminary studies have shown that the technology is successful to an extent in some smaller mammals such as rodents, the technology would need to utilize larger animals such as cows, sheep, or pigs in order to produce a viable organ of the appropriate size for human use. Some researchers suggest that using pigs may be less morally questionable than non-human primates, are relatively tame, have large litters and short gestational intervals, and can be genetically modified to reduce the likelihood of interspecies zoonoses. (Casal, Williams, 2019) However, the use of a livestock mammal as the host may further reduce the application of the early rodent findings because of the interspecies barrier. Whereas mice and rats diverged sometime in the last 30 million years, humans and livestock such

as pigs or sheep diverged more than 60 million years ago. (Cooper, Ekser, Tector, 2015) (Suchy, Yamaguchi, Nakauchi, 2018)

This interspecies barrier between humans and livestock is important for two major reasons in the context of using human iPSCs for interspecies blastocyst complementation. First, chimera formation using blastocyst complementation among organisms of the same species results in significantly higher rates of both the frequency of intraspecies chimera formation and the resulting degree of chimerism, whereas interspecies chimeras do not. Not only do interspecies chimeras appear less frequently and exhibit a lesser extent of total chimerism within the targeted organ, they also have a higher rate of malformations and death while in vitro. While noting that there is clearly something provoking this incompatibility, researchers have only been able to theorize about the cause and have suggested that there is some sort of discontinuity in progenitor signaling or overall fetal development in which the transplanted cells are lethal. (Suchy, Yamaguchi, Nakauchi, 2018)

Some researchers have suggested using monkeys, such as baboons (Cooper, Ekser, Tector, 2015) or rhesus macaques (De Los Angeles, Pho, Redmond, 2018), due to their shared genetic characteristics and stature with humans. However, the ethical impact of using a monkey more closely related to humans, that reduces the species barrier more significantly, such as a chimpanzee, would be more difficult. First, chimpanzees receive greater protections in research based on their status as endangered animals. (Casal, Williams, 2019) Also, some argue that chimpanzees satisfy the standard requirements for personhood, which raises questions about the use of non-human persons and non-person

humans, such as individuals who are brain dead without any form of consciousness, as a means of producing organs for human use. (Casal, Williams, 2019)

Additionally, antigens in both primate- and livestock-based xenografts are capable of producing an uncontrollable innate immune reaction. (Starzl, 2000) (Platt, Saadi, 1999) In primates, this is often due to the complement cascade, similar to ABH blood antigen reactions. In pigs, this antigen is Galα1-3Gal, which differs from the expression of traditional ABH blood antigens from the human vascular endothelium. Because the Gal antigen is present in pigs and not in humans, the human immune system mounts a hyper-acute reaction that can occur within minutes of attempts at xenotransplantation. (Cooper, 2017) However, in the 2000s, researchers began genetically modifying pigs to prevent the synthesis of Galα1-3Gal (Cascalho, Platt, 2005), in addition to genetically modifying pigs to hold increased resistance to the human complement-mediated injuries. (Cooper, 2017) In one experiment, upon transplanting a genetically modified pig organ into a primate, the researchers found that the typical hyper-acute rejection did not occur, although the graft survival was no longer than Gal-positive pigs with human complement regulatory protein transgenes. (Zhong, 2007) With human-animal chimeras, there is also the potential for zoonotic transmission of certain viruses, such as porcine endogenous retroviruses. Although there is no firm resolution, researchers have generally moved forward with experiments on livestock animals. However, this decision could change based on the engraftment efficacy of human iPSCs into livestock blastocysts to create chimeric embryos, a hesitation which has been expressed by some researchers. (Wu et al, 2016)

Future Implications

Despite the fact that it appears interspecies blastocyst complementation could potentially solve many of the most critical limitations associated with modern organ transplantation, its future applications remain uncertain. Not only does this technology remain relatively theoretical in its application to humans, but it also will likely require a high demand for funding and resources that are not currently available within the United States. Research teams working on this technology are generally working in California where the restrictions are less stringent, or outsourcing overseas, particularly to Japan.

While interspecies blastocyst complementation may address the need for immunosuppression, there are still a variety of technical and biological limitations that will need addressed. Briefly, researchers would need to address how to improve the rates and efficiency of chimerism within the organs produced in these foundational models, to consider the immunogenic potential existing within epithelial cells, immune cells, vascular cells, and any other surrounding tissue that may need transplanted in tandem with the organ, and to consider any new research that further weakens the potential for iPSCs to maintain a low immunogenic burden.

As noted above, most of the animal models show a low chimeric efficiency and many of the attempts to overcome those inefficiencies have resulted in embryonic deformations and deaths thought to be caused by unknown mechanisms. (Zhou, 2017) In some of the earliest studies, donor cells populated between 0.01 to 10 percent of the generated organ, but often less than 1 percent. Additionally, some researchers find fault with the exclusion of specific data for chimeric content among individual animals. (Freedman, 2018) Much of this assessment stems from the debate between whether

iPSCs must be naive or stable state in order to produce chimeras. Additionally, this same assessment likely governs the debate surrounding the synchronization of the developmental timelines of iPSCs and host development. (Suchy, Yamaguchi, Nakauchi, 2018) This is pertinent to much of the discussion involved in the unknown immunogenic potential of iPSCs. However, because this mechanism is still speculative, it will not be investigated further at this time.

However, reflecting back to chapter two, researchers began the twentieth century without any practical conception as to how they were going to successfully perform an organ transplant, or whether it was even scientifically feasible. Within nearly five decades, that progressed to the first successful allogeneic organ transplant. In 1910, scientists had generally established some of the rudimentary understandings on the interaction of organs when transplanted among animals. In 2010, scientists demonstrated the first model for interspecies blastocyst complementation. In the ten years since, researchers have made some significant headway into further outlining the parameters and areas for growth associated with the technology, especially given the constraints imposed upon the funding and resources for this research. These are early days for interspecies blastocyst complementation research, and its future prospects rest on more than a century of scientific attention to the immune system at the heart of the organ transplant enterprise.

The impact of early immunological discoveries established not only the principles that ultimately made organ transplantation possible, but also defined a pattern that would repeat throughout history and into the modern era – that the immune system plays a key role in determining the extent at which future research may be possible. If resources and

time are spent resolving the immunological issues presented earlier within this chapter, the feasibility of interspecies blastocyst complementation may progress quicker – if not on a purely technical level, at the very least from a regulatory perspective when considering if this technology is actually capable of resolving the harms associated with the current system, or if it will impose new, previously implausible harms upon the human population.

The development of these components of the technology will also serve to define the extent to which interspecies blastocyst complementation can address the persistent challenges and limitations associated with modern organ transplantation. In theory, if the limitations are resolved with this technology, interspecies blastocyst complementation would likely enable the extension of survival rates, improve the access to curative strategies for a variety of chronic and acute conditions, and decrease or eliminate the need for immunosuppression and the many side effects that come with it.

One idea that will require long-term consideration is the monetary aspect of this technology. Based on the resource demands associated with making it plausible, it is reasonable to expect that this will be an expensive process. With the US government's current funding restrictions, financial support may reduce who is able to use this technology and therefore, lessen its impact, especially within disenfranchised populations. Considering who will pay for these services or the establishment of the necessary education to make these services possible in general also indicates a need to determine under whose discretion will the care and ownership for the animal hosts fall and to what extent will these animals be regulated? This implies a need for government and regulatory participation that does not currently exist.

For future research, it would be interesting to assess how ethical justifications for certain solutions for organ transplantation have evolved over time. Additionally, it would also be worthwhile to establish how this technology may be facilitated through existing regulatory networks if trying to determine at what extent the government will be forced to become involved. All future research is dependent on how the technology progresses, and how the technology progresses should depend, at least in part, on good governance.

In conclusion, the overarching barrier to modern organ transplantation is the need to accommodate the immune system through tissue matching and immunosuppression. The earliest discoveries divulged that a physiological mechanism was at work, and even after researchers realized the exact extent to which the immune system was limiting the success of organ transplants, there was still little progress made outside of immunosuppression techniques alone. Interspecies blastocyst complementation may address and eliminate the need for immunosuppression, although it will require more research before such a claim can be fully substantiated. However, for now, it remains a promising solution to eliminate the need for immunosuppression, thereby improving the access to and outcomes of organ transplants for patients with organ failure.

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