The immune response and its role in renal transplant rejection

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Abstract
Renal transplant rejection is a common issue among transplant recipients. During transplant rejection, the immune system attacks the transplanted organ, causing it to fail and possibly leading to the death of the patient. The administration of immunosuppressive medications throughout the life of the patient reduces the chances of transplant rejection. However, the medication is not always effective and rejection can still occur. Pre-transplantation screening can provide better donor-recipient matches that reduce the chances of developing renal transplant rejection. Decreases in immunosuppressive medication doses can also have a positive effect on transplants. High doses can lead to the over suppression of the patient’s bone marrow, allowing for other infections to manifest themselves. Infections have the possibility of triggering the rejection of a transplanted organ. Understanding the various factors that contribute to renal transplant rejection can provide insight into new medications and treatments. Better treatments can lead to longer organ survival rate as well as an increase in the patient’s quality of life. One possible treatment targets co-stimulatory factors that activate T cells. By inhibiting these factors, T cells will recognize transplanted organs but will not mount an immune response. Solid organ transplant allows for patients who would otherwise die to have an increased life span. Given that transplant rejection is a common occurrence, research needs to be done regarding causes and possible treatments.

Introduction
The immune system is a combination of cells, tissues, and organs that work together to protect the body from infection. The source of infection can come from foreign microbes, such as viruses, or diseased cells within the host’s body, such as tumor cells. The immune system is able to distinguish between healthy cells and unhealthy cells that pose a threat to the body (National Institute of Allergy and Infectious Diseases, 2016). The immune response in vertebrates involves two components, the innate immune response and the adaptive immune response. The innate immune response acts as a general defense and involves almost any cell type in the body. Vertebrates rely on the innate immune system to defend against potential infections during the first critical hours or days of exposure to a new pathogen. The adaptive immune response is a more specialized defense that involves more sophisticated but slow reacting mechanisms. Specialized white blood cells known as lymphocytes are responsible for the adaptive immune response. These cells are classified as either B cells, which produce pathogen specific antibodies, or T cells, which can either directly kill infected cells or signal to other cells for aid (Alberts et al. 2015). Together, the innate immune response and the adaptive immune response work to protect the body from foreign pathogens and diseased cells.

The transplantation of solid organs is commonly done when an organ has failed due to illness or injury. Organs that can be transplanted include the heart, intestine, kidneys, liver, lungs, and pancreas (Martin, 2015). Before a transplant occurs, a donor must be matched to a recipient to reduce the risk of transplant rejection. When transplant rejection occurs, the immune system of the recipient attacks the donor organ. Three types of rejection are known: hyperacute, acute, and chronic. Hyperacute rejection occurs within minutes of the transplant and can lead to death if the transplanted tissue isn’t removed immediately. Acute rejection occurs within the first 3 months after the transplant. Chronic rejection occurs years after the transplant. Regardless of rejection type, the organ’s function begins to decrease and can eventually fail (Martin, 2015). Solid organ transplantation is inevitably associated with the activation of the recipient’s immune system.

In order to reduce the possibility of transplant rejection, recipients must use immunosuppressive drugs for the rest of their lives. Treatments are rather broad but advances in research and technology are providing opportunities for immunosuppressive therapies to be tailored to the recipient. Biomarkers related to the activation of the immune response have been identified and can be monitored over time to determine the type of immunosuppressive treatment required for the recipients (Shipkova, 2016). Transplant rejection remains a serious issue today despite the advancements in technology. Understanding the various aspects of transplant rejection can provide recipients with better post-operation treatments that reduce the risk of rejection later in life. The goal of this paper is to examine different aspects of transplant rejection through a review of current literature and to develop an experiment related to transplant rejection using available techniques.

Current Research
Pre-transplant observations can decrease the frequency of transplant rejection in renal transplant patients

Despite advances in technology and transplantation methods, acute rejection of transplanted organs remains a serious problem. The development of chronic allograft dysfunction (CAD) remains a limiting factor for long-term kidney graft survival. By determining individuals’ risk of acute rejection prior to transplantation and adapting an immunosuppressive treatment post transplantation decreases in the number of acute rejections in transplant patients will occur. The risk of acute rejection can be determined by analyzing the reactivity between peripheral blood mononuclear cells from both the donor and the recipient. Furthermore, the concentrations of sCD25, sCD30, and sCD44 in recipients prior to transplantation can predict allograft rejection (Vondran et al. 2014). There are a variety of steps that must occur before transplants occur in order to reduce the possibility of transplant rejection.

The reactivity of recipient and donor peripheral blood mononuclear cells can be observed through a mixed leukocyte culture. The peripheral blood mononuclear cells from the kidney of the recipient were used a responder cells. The recipient cell reactivity to irradiated peripheral blood mononuclear cells from the donor kidneys was observed. In order for reactivity to be observed, 1μCi of 3H- Thymidine was added to each well. Reactivity was detected using a scintillation counter to obtain the counts per

Figure 1. Mixed lymphocyte culture results. Figure (a) shows the ideal results of the inhibition of co-stimulatory factors. Low reactivity between the peripheral blood mononuclear cells of the recipient and the donor is related to low counts per minute and low immune response. Figure (b) shows the results of the mixed lymphocyte culture if the co-stimulatory factors are not inhibited by the given medication. High reactivity is related to high counts per minute and a strong immune response.
High counts per minute are related to increased reactivity. Patients who experienced no transplant rejection within one year of transplantation showed significantly lower counts per minute than patients with borderline transplant rejection and acute transplant rejection before and after transplantation (Vondran et al. 2014). High counts per minute are related to a high reactivity between donor and recipient cells because 3H-Thymidine is incorporated into the DNA of proliferating cells. When the peripheral blood mononuclear cells of the recipient come into contact with the donor cells an immune response will occur. This response will cause lymphocyte production, causing proliferating cells to absorb the 3H-Thymidine. An increased uptake and incorporation of 3H-Thymidine in recipient cells is related to a strong immune response.

Other biomarkers that can predict allograft rejection are sCD25, sCD30, and sCD44. sCD25 is a soluble form of the interleukin 2 receptor. These receptors can be found in serum after the activation of T cells. sCD30 is the soluble form of a transmembrane protein that is related to the tumor necrosis factor receptor family. Similar to sCD25, sCD30 is shed from T cells after their activation. sCD44 is a soluble transmembrane glycoprotein that is released from lymphocytes, endothelial cells, and macrophages following their stimulation by pro-inflammatory cytokines (Vondran et al. 2014). The concentrations of these biomarkers can be determined through enzyme-linked immunosorbent assay (ELISA). Low levels of each of these biomarkers was related to the patient being rejection free while high levels were related to patients with acute rejection. Patients with borderline transplant rejection showed similar levels to the rejection free patients but their levels were not statistically different from the acute rejection patients. Pre transplant levels of these three biomarkers in serum are related to the level of rejection in patients after transplantation (Vondran et al. 2014). The frequency of transplant rejection can be lowered by observing a variety of factors in the recipient’s body as well as interactions between the donor and recipient blood cells.

Immune profiling can be used to determine patients with a high risk for transplant rejection

Sensitization, the formation of human leukocyte antigen antibodies, is a major barrier to successful kidney transplantation. Human leukocyte antigen antibodies are acquired through exposure to foreign human leukocyte antigens usually via pregnancy, previous transplantation, and transfusions. Recipients who have become sensitized have a high chance of transplant rejection and often have difficulty finding a suitable donor. Desensitization techniques use medications to prevent transplant rejections by targeting both the human leukocyte antigens and the immune system. However, many recipients do not respond to the desensitization therapy and are unable to receive a donor kidney that will lead to a successful transplant (Yabu et al. 2016). Immune desensitization increases the chances of a successful transplantation but does not affect a wide range of candidates.

Desensitization started before transplantation and was continued afterward. The protocol used required the administration of immunosuppressive medications. Peripheral blood samples were taken following transplantation and further analyzed. Immunophenotyping was conducted using single-cell mass cytometry time-of-flight phenotyping. A total of 33 antibodies were

Figure 2. Cytometric Bead Array results. The 7 cytokines tested for are shown in these graphs. The healthy control levels are compared to the pre-transplant and post-transplant levels of cytokines in the serum. Inhibition of the co-stimulatory factors will prevent the activation of T cells, preventing the secretion of the various cytokines.
used to determine the differences between patients who responded to the desensitization and patients who did not. There were not significant differences between responders and non-responders. However, there were two cell subsets which were lower in responders versus non-responders. Further research concluded that patients who responded to desensitization had high numbers of transitional B cells and low levels of regulatory T cells. High levels of transitional B cells are commonly seen in patients who have successful transplantations. In addition, regulatory T cells may cause inflammation or other immune events which would lead to transplant rejection (Yabu et al. 2016). Desensitization therapy is used to decrease the immune response in transplant patients. There are few transplant patients who respond to the desensitization and experience no rejection, while there are many patients who experience some level of rejection.

**Low doses of immunosuppressive drugs administered before and after transplantation can reduce the frequency of transplant related complications**

Kidney transplantation is characterized by better growth, development, quality of life, and longer survival time when compared to dialysis. However, given the risk of organ rejection, it remains a dangerous operation in some areas. In order to reduce the immune response, Rabbit antithymocyte globulin (ATG-F) is used to induce an immune response during and after transplantation. These antibodies attack human T cells, acting as an immunosuppressant. The use of tacrolimus and mycophenolate mofetil also provides immunosuppression to transplant patients (Shang et al. 2014). Although these drugs offer some protection against transplant rejection, they do not always work effectively. It is possible that their overuse can lead to the rejection of the donor kidney.

Children undergoing renal transplantation were given 1.5 mg/kg/day of ATG-F for 4-6 hours during surgery and received an equal dose daily for three days. Patients also received a declining dosage of tacrolimus and mycophenolate mofetil over the course of the following year. Post-operation complications were reduced when compared to patients taking a higher dosage of ATG-F. Furthermore, acute rejection rates were lower as was the suppression of bone marrow. Severe bone marrow suppression was common in patients who had received a higher dose of mycophenolate mofetil in previous studies (Shang et al. 2014). Lower doses of immunosuppressive drugs have the potential to reduce complications associated with transplants such as infection.

**Mesenchymal stromal cells may provide a novel way to prevent transplant rejection**

As previously stated, transplant patients must undergo immunosuppressive therapy for the rest of their life in order to lower their chances of transplant rejection. This makes research into alternative treatments that decrease the need for immunosuppressive drugs, increase long-term survival, and induce tolerance very important. Mesenchymal stromal cells have gained an interest to researchers due to their capacity to enhance tissue repair. These cells secrete bioactive molecules which inhibit apoptosis and limit the extent of damage, inhibit fibrosis and scarring, protect the microvasculature and stimulate angiogenesis, and promote mitogenesis of tissue-intrinsic progenitor cells. Furthermore, mesenchymal stromal cells influence various parts of the immune system. Extracellular vesicles have been shown to play an important role in intercellular communications. Vesicles generated by mesenchymal stromal cells may have potent immunomodulatory properties (Koch et al. 2015). Due to their wide range of capabilities, mesenchymal stromal cells have been studied to determine their effects on organ transplantation.

Lewis rats received kidney grafts from other weight and aged matched Lewis rats. The rats shared the same genetic background, causing them to have different MHC haplotypes. These different haplotypes led to MHC class I and MHC class II incompatibilities. After the transplantation, the rats received either medium or microvesicles (extracellular vesicles) from mesenchymal stromal cells (Koch et al. 2015). Kidney function in both rat groups was impaired with no group showing significantly more or less impairment. In addition, there was no significant difference between the compositions of the peripheral blood mononuclear cells of both groups. A significant increase in T cells and B cells in the transplanted kidney was observed for the EV group while a significant decrease in natural killer cells was observed (Koch et al. 2015). The increase in B cells and T cells in the transplanted kidneys of the group that received the EV suggests that the immune response was activated in that region. Although the immune response was activated, the EV rats did not show significantly worse signs of kidney function impairment or rejection (Koch et al. 2015). Treatments for the prevention of transplant rejection primarily use immunosuppressive drugs. Novel treatments such as the use of mesenchymal stromal cell extracellular vesicles are being researched but provide little protection so far.

Complement regulatory protein levels can affect the survival rate of renal transplants.

The complement system is part of the innate immune system. It is composed of numerous proteins with antimicrobial activity, some of which are produced in response to infection, while others are produced constantly. The proteins of the complement system enhance the innate immune response by marking attaching to pathogens and cleaving themselves. The cleavage of the proteins attracts a variety of cells to the pathogen. These cells can cause an inflammatory response, phagocytize the pathogen, or make antibodies against the pathogen (Alberts et al. 2015). Due to its involvement in the immune response, the role that the complement system plays in the development of acute T cell mediated rejection is important to examine.

Patients of renal allograft biopsies were obtained at the Osaka University Medical Hospital between 1989 and 2012. All 67 patients were diagnosed with acute T cell mediated rejection type I and type II. The expression of the membrane cofactor protein and decay accelerating factor, complement receptor 1-related gene/protein y (Cryy), during acute T cell mediated rejection was examined to determine how the complement system affected the rejection of the transplanted kidney (Yamanaka et al. 2016). Cryy acts as a complement regulatory protein which is responsible for turning off the complement system. Inhibition or removal of Cryy and other complement regulatory proteins from the body can cause acute T cell mediated rejection to worsen. Two groups of patients were formed, a high membrane cofactor protein group and a low membrane cofactor protein group. Both groups had no difference in the function of the grafted kidneys at the time of diagnosis. After the anti-rejection treatment, patients with the high levels of membrane cofactor protein maintained significantly lower serum creatinine levels in their kidneys than the low membrane cofactor protein group (Yamanaka et al. 2016). Serum creatinine levels are maintained by the kidneys. Normal functioning kidneys maintain a concentration of 0.7-1.3 mg/dL in men and 0.6-1.1 mg/dL in women (Martin, 2015). Over the course of 5 years, the survival rate for renal transplants was significantly higher in patients who had had high levels of membrane cofactor protein.

**Figure 3. Renal Functional Assay results.** Creatinine levels in the serum of healthy control mice is compared to the creatinine levels in the serum of mice 7 days post-transplant. Normal levels are between 21.9 μmol/L and 26.7 μmol/L. As shown in the graph, the both the healthy control and post-transplant mice show normal levels of creatinine in their serum.
than in patients with low levels of membrane cofactor protein (Yamanaka et al. 2016). The complement system plays a role in the rejection of transplants. By increasing the expression of complement regulatory proteins, it is possible to increase the life span of a transplanted organ. Elevated levels of inflammatory cytokines are related to renal transplantation rejection.

Previous research has shown that there is an imbalance of T cells and T cell activity in the body of patients experiencing transplant rejection. A cytometric bead array was used to determine the role that cytokines play in transplant rejection. A cytometric bead array was used to determine the role that cytokines play in transplant rejection. A cytometric bead array was used to determine the role that cytokines play in transplant rejection. A cytometric bead array was used to determine the role that cytokines play in transplant rejection. A cytometric bead array was used to determine the role that cytokines play in transplant rejection.

The immune response to transplanted organs is mediated by antibodies, T cells, and innate immune cells. Specifically, a disturbed T cell homeostasis plays a role in the acute rejection of transplanted organs. T-helper cells and regulatory T cells are the primary components of the T cell balance in the body. Inflammatory cytokines have also been associated with the development of rejection. However, their role is not yet clarified (Ma et al. 2015). Understanding the role that various cells and proteins in the immune system play in transplant rejection is important to reducing the frequency of transplant rejections.

End-stage renal failure patients had their serum levels from before and after transplantation to determine the role that cytokines play in transplant rejection. A cytometric bead array was used to determine the role that cytokines play in transplant rejection. A cytometric bead array was used to determine the role that cytokines play in transplant rejection. A cytometric bead array was used to determine the role that cytokines play in transplant rejection. A cytometric bead array was used to determine the role that cytokines play in transplant rejection. A cytometric bead array was used to determine the role that cytokines play in transplant rejection.

Figure 4. T cell activation. The above graphic shows the activation of the T cell through contact with a foreign antigen and co-stimulatory factor. Figure (A) shows the normal pathway in which both the antigen and co-stimulatory factor attach to receptors on the T cell. Figure (B) shows the inhibited pathway in which only the antigen attaches to the T cell receptor.

Research Proposal

I propose that acute transplant rejection can be prevented by the administration of co-stimulatory factor inhibitors. I will perform experiments to determine whether or not the administration of co-stimulatory factor inhibitors provides protection from transplant rejection. I will use three different experimental techniques. A mixed lymphocyte culture will be done prior to transplantation to determine if inhibition of co-stimulatory factor decreases the reactivity of recipient cells to donor cells. A cytometric bead array will be used to observe the amount of cytokines released by T cells before and after the transplantation. A renal function assay will be conducted to determine if there is any impairment in kidney function. Based on previous research, Lewis rats will be used as the model organism in this experiment (Koch et al. 2015). Inhibition of co-stimulatory factors will prevent the development of renal transplant rejection in the mice models.

Mixed lymphocyte culture

In order to determine an ideal concentration of co-stimulatory factor inhibitor, a wide range of dose concentrations will have to be administered to different rats prior to the mixed lymphocyte culture. Using the mixed lymphocyte culture protocol described in Vondran et al. 2014, the reactivity levels between the peripheral blood mononuclear cells of recipients and donors can be measured. A scintillation counter will be used to record the counts per minute of each of the tests. If the inhibitors work as hypothesized, there will be a low level of reactivity between the donor and recipient serum resulting in low counts per minute (Figure 1a). If the inhibitors do not prevent T cells in the serum from being signaled by co-stimulatory factors then an elevated level of reactivity will be observed as a high counts per minute (Figure 1b). The mixed lymphocyte culture will be run multiple times to ensure that the data is accurate.

Cytometric Bead Array

Prior to transplantation, a serum sample will be taken from each rat. Serum samples will be taken for 7 days following transplantation to monitor any changes in cytokine expression. Using the cytometric bead array protocol described in Ma et al. 2015, the expression of cytokines in patients with low levels of membrane cofactor protein (Yamanaka et al. 2016). The complement system plays a role in the rejection of transplants. By increasing the expression of complement regulatory proteins, it is possible to increase the life span of a transplanted organ. Elevated levels of inflammatory cytokines are related to renal transplantation rejection.
tient serum will be observed and compared. Elevated levels of IL-2, IFN-γ, TNF-α, IL-10, and IL-17 have been previously seen in patients diagnosed with transplant rejection while IL-4 and IL-6 levels remained constant (Ma et al. 2015). The co-stimulatory factors should prevent the cytokine levels from being elevated in the rats (Figure 2). Cytokine levels are directly proportional to the levels of T cells in the body. Low levels of cytokines in the serum will indicate low levels of T cells and that no immune response has been initiated.

**Renal function assay**

In order to determine if the kidney is functioning properly, a renal function assay will be conducted 7 days after transplantation. The protocol used by Koch et al. 2015 will be used in this experiment. The renal function assay examines serum creatinine levels in transplant patients several days after surgery. High creatinine levels in the serum are related to renal impairment which is a symptom of renal transplant rejection. If the co-stimulatory factor inhibitors successfully block the activation of T cells in the transplant patient, there will be normal levels of creatinine in the rats (Figure 3). For rats, normal creatinine levels are between 21.9 μmol/L and 26.7 μmol/L (Palm and Lundblad, 2005). Normal kidney function should be observed in rats that have been given co-stimulatory factor inhibitors.

**Conclusion**

Renal transplant rejection is a serious issue in transplant patients. By testing for specific biomarkers prior to transplantation the rate of rejection can be decreased. Biomarkers such as sCD25, sCD33, and sCD40 have higher levels of expression prior to transplant in patients who are more likely to experience transplant rejection. In addition to pre testing, a lower dose of immunosuppressive drugs during and after transplantation may increase the life span of the transplanted organ, thus extending the life of the recipient. Lower doses of immunosuppressive drugs will prevent bone marrow from becoming over suppressed. If bone marrow becomes suppressed, the immune system will fail and the recipient will be more likely to contract an infection elsewhere. The prevention of infection is important in transplant recipients because infections can trigger transplant rejection (Sternfield et al. 2009). Due to transplant recipient dependency on immunosuppressive drugs, new treatments are being researched that do not require recipients to constantly use immunosuppressive medications for the rest of their lives. Studies on the complement system have provided more insights into how the body reacts to transplants. By understanding the different ways in which transplant rejection occurs, new treatments can be developed. T cells play a large role in the rejection of transplanted organs. Based on their significant role in transplant rejection, they are critical targets for further research.

Activation of T cells requires two steps: contact with a foreign antigen and contact with a co-stimulatory factor (Figure 4). The body develops a self-tolerance by preventing the co-stimulatory factors from coming into contact with the T cells. It may be possible to extend this self-tolerance to transplanted organs by inhibiting the co-stimulatory factors with certain medications. Inhibition would only be required for a short time, allowing T cells to recognize the foreign body but not activate an immune response. This method could reduce the need for immunosuppressive drugs and increase the life span of the transplanted organ and the transplant recipient. New methods of treatment need to be developed in order to increase both the life span and quality of life of transplant recipients.

Solid organ transplantation often results in the rejection of the transplanted organ. Immunosuppressive medications are used to lower the risk of rejection. However, they are not always effective. Transplant rejection can lead to the failure of the organ and the death of the recipient if not immediately treated. While treatments have become more advanced since transplants were first started, they remain problematic. Through the research of new treatments, transplants can become significantly more effective and longer lasting.

**Note:** Eukaryon is published by students at Lake Forest College, who are solely responsible for its content. The views expressed in Eukaryon do not necessarily reflect those of the College.

**References**


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