

Toward a better understanding of immunosuppression and complete transplant tolerance

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Organ transplants save the lives of thousands of people every year, and continuing research is making that process increasingly safe. The main problem with organ transplantation is the organ rejection that occurs if the recipient immune system is not compatible with the donor cells. This causes organ failure, and long-term immunosuppression is often required. While this immunosuppression keeps the allograft healthy, it has serious side effects on both the organ and immune systems of the recipient. Further research into immunosuppression can lead to safer transplants methods and post-surgical therapies. The ultimate goal of this research is to create an environment of complete tolerance, allowing the immunosuppression to be stopped with no ill effects.

INTRODUCTION

The concept of taking an organ from one organism and putting it into another is relatively modern. The first recorded attempt at transplantation between a human donor and recipient was performed by a Russian doctor in 1936, who transplanted a kidney (Starzl, 1993). A study in the 1960s found that while kidney transplantation still did not extend lifespan by a significant amount, its acceptance as a treatment began to shift from that of a last ditch effort to a more viable option (Starzl et al., 1968). One of the earliest papers discussing the possibility of an immunological basis for transplant rejection was written by Richard Egdahl in 1957. This study, however, was carried out on dog transplants, and yielded no clear conclusions about humans and the immune reactions behind organ transplant rejection (Egdahl & Hume, 1957).

Since its inception, the field of organ transplantation has come a long way. We now know substantially more about the immune system and how rejection works. There are now sophisticated immunosuppressants which can keep a transplant from being rejected for longer periods of time. Patients with organ failure now have a chance at a new life, and numerous different organs are now able to be transplanted. Organ transplantation has come such a long way that, according to the Scientific Registry of Transplant Recipients, there were nearly 21,000 heart, lung, liver, and kidney transplants performed in 2009 (Arbor Research Collaborative for Health, 2011).

While the field of organ transplantation has vastly improved the lives of patients, allotransplants—a transplant between two genetically different members of the same species—often result in rejection of the graft. Genetic differences lead to important differences in the Major Histocompatibility Complex (MHC), also known as Human Leukocyte Antigen (HLA) in some sources (Fukami, 2009). The interaction between the MHC and the T-cell receptor is what drives the immune response against a foreign antigen. Normally, the body regulates immune cells so that they do not harm the body's cells, but in the case of an organ

transplant, a foreign MHC is introduced that the immune cells consider a threat and begin to attack.

There are two types of MHC: MHC class I and MHC class II. MHC class I is a cellular marker on all cells of the body that presents antigens to CD8+ T-cells. When a body cell is infected, it will present an antigen to a cytotoxic T-cell. This will cause the T-cell to kill the infected cell and those surrounding it by secreting cytotoxic molecules from secretory lysosomes (Topham & Hewitt, 2009). In the field of solid organ transplants, this is particularly important because all of the transplanted cells contain these MHC class I markers on their surface. MHC class II markers are found on dendritic cells and neutrophils, called antigen presenting cells (APCs), and present internalized antigen to CD4+ T-cells. B-cells are then stimulated to produce antibodies against the donated MHC and attack the allograft. Occasionally, these cells pick up pieces of actual cells as antigen and present it to the CD4+ T-cells. In organ transplantation, the self-antigen that is presented to the recipient T-cells is not recognizable as self, and causes a humoral rejection of the allograft.

This reaction of the recipient's immune system against the allograft is generally controlled with immunosuppressants over an extended period of time. Immunosuppressants prevent the immune system from attacking the organ, but can allow opportunistic infections to proliferate. There are also dangerous side effects from the drugs' interactions with the body's systems. With the risk of infection after surgery being so high (Vogel et al., 2007), the immunosuppression after an organ transplant is an even greater problem because it allows pathogens to get a foothold inside the body. A greater understanding of the human immune system could reduce the time a patient is on the immunosuppressant drugs and give the organ recipient a greater quality of life.

This paper has several purposes with regard to immunology and organ transplantation. First, the different types of rejection will be reviewed and explained, so that the necessity of immunosuppression can be understood. The second goal is to outline the different immunosuppressants that are available and most often used for post-transplant organ recipients. These drugs each have a unique interaction with the immune system that can make them better or worse for preventing rejection of the donated organ while still allowing the body's immune system to

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protect the body. This leads to the final, and most important, topic of this paper: transplant tolerance. Transplant tolerance is the ultimate goal of organ transplantation, even though it has proven to be elusive to researchers thus far. New research has included methods that attempt to manipulate the human immune system into regulating itself and also the discovery of more specific immunosuppressants.

REJECTION

Rejection of the allotransplant is the reason why immunosuppressants are so necessary after a solid organ transplant. There are three main types of rejection: hyperacute rejection, acute rejection, and chronic rejection. Due to the increased understanding of the human immune system, hyperacute rejection, in which the organ is rejected within a few days of the transplant, has become extremely rare, as therapies have been developed to treat it effectively (Ng, 2010) and therefore will not be addressed here.

Acute rejection

Acute rejection occurs within one to two years after the transplant and is caused mainly by HLA mismatching (Martinu et al., 2009). Since mismatched HLA indicates a foreign pathogen to the immune system, the closer the match between donor and recipient, the more likely the recipient immune system will accept the allograft as its own. This type of rejection can be caused by direct allorecognition, in which intact donor APCs present the donated organ's HLA molecules as antigen (Illigens et al., 2009), or indirect allorecognition, in which pieces of donor APCs present donor antigen to the recipients T-cells (Martinu et al., 2009). Once the donated HLA is presented to the T-cells as an antigen, cytotoxic T-cells begin to destroy the cells with that HLA (Seetharam et al., 2010). In the case of an organ transplant, the result can be catastrophic, as all of the donated cells would have the same HLA markers on their surface and would thus be marked for destruction.

With acute rejection, there is also a significant increase in inflammatory cytokines (Hirayama et al., 2006). These cytokines are the main reaction of the immune system to a perceived threat, such as a group of cells with foreign antigen. Of these cytokines, IL-17 is the most important in rejection because it increases the levels of other pro-inflammatory responses and also skews the body away from producing regulatory T-cells (T_{regs}) (Afzali et al., 2007). T_{regs} encourage the body to become tolerant of antigens (Fouchet & Regoes, 2008) and may therefore encourage the immune system of a transplant recipient to become tolerant of a new organ.

There continue to be disagreements concerning the diagnosis of acute rejection. Some researchers say that the only way to diagnose it is by identifying a decrease in general health (Afzali et al., 2007; Martinu et al., 2009; Smith et al., 2008). Others have outlined specific lists of symptoms, such as the presence of antibodies and swelling, associated with a strong immune response (Bickerstaff et al., 2008; Racusen et al., 1999). These specific symptoms were outlined in a table known as the Banff 97 diagnostic categories (Racusen et al., 1999).

The possibility of ending acute rejection is becoming more of a reality. LaRosa (2007) found that mice completely lacking in T-cells would accept mismatched allografts with no symptoms of rejection. This would result because the T-cells would not be present to recognize the foreign antigen in the donated organ (LaRosa et al., 2007). It was previously thought that presensitization would result in higher tolerance (Marks, 1983), though studies actually showed higher rates of rejection with presensitized mice when compared to those who were not presensitized (Bickerstaff et al., 2008; Weiss et al., 2010). Presensitization would give the immune system time to create an antibody response to the donated antigen and it would therefore respond faster when the actual organ transplant occurred.

Chronic rejection

Chronic rejection is now considered the biggest hurdle to successful transplantation (Ng, 2010). Years of research have resulted in making this type of rejection easier to identify than ever before. The exact symptoms vary according to the organ transplanted, but this type of rejection is always associated with increased fibrosis near or within the transplanted organ (Seetharam et al., 2010). A study by Smith et al. (2008) found that alloantibodies were the first evidence of chronic rejection, followed by C4d deposition, luminal occlusion, and then organ failure. The C4d deposition results from antibody attachment within the organ and the luminal occlusion occurs when the vessels within the organ swell up due to the influx of immune cells. Once the vessels are swollen, the organ would cease to work and ultimately fail.

The cause of chronic rejection is not well understood at this point, probably due to the complexity of chronic rejection and the variation from case to case. A significant problem that appears to affect chronic rejection is the immune system's memory B cells. These cells can retain antigens for decades and cause chronic rejection years after the transplant (Weiss et al., 2010). If a recipient has received a donation before, they will have antigens from that donor present in their memory B-cells, increasing the chance of rejecting subsequent donations from that donor.

There is the added complication of the T-cells that make up a large portion of the human immune system and the role they play in the rejection of transplanted organs. There is evidence that the more T-cells present in a transplant recipient, the higher the rejection rate is (Heeger et al., 2010). Because T-cells are such an important part of the adaptive immune response, an organ recipient struggles to maintain a balance between suppression of these cells and acceptance of their allograft. The effectiveness of T-cells in responding to the presence of antigen makes them the target of many lines of immunosuppression.

IMMUNOSUPPRESSIVE DRUGS

There are several types of immunosuppressants that are used after an organ transplantation to encourage acceptance of the allograft. The three main types of drugs used are calcineurin inhibitors, antimetabolites, and corticosteroids. These drugs work by reducing the responsiveness of the immune system or by killing off the immune cells that would attack the organ.

There are several complications and side effects that are associated with these drugs. Immunosuppression causes nephrotoxicity, neurotoxicity, hyperlipidemia, diabetes, and a host of other systemic problems (Pillai & Levitsky, 2009). Immunosuppression also results in higher rates of infection with bacterial, viral, and fungal infections (Rubin, 1993). This is because, in order to allow for the suppression of the immune system enough so that it does not attack the organ, immune system must also be disabled from attacking any other foreign substance within the body (Seyfert-Margolis & Turka, 2008).

Calcineurin inhibitors

Calcineurin inhibitors are another of the three main drugs taken by the majority of transplant patients. Their primary function within the field of organ transplantation immunosuppression is to prevent T-cell activation (Pillai & Levitsky, 2009). By preventing the T-cells from activating, they prevent the adaptive immune response against the organ and ensure that antibodies are not made against the donor HLA.

There are two main calcineurin inhibitors, Cyclosporin A and Tacrolimus. Their main function is to interfere with the transcription of the gene for cytokine IL-2 (Ng, 2010). IL-2 is necessary for the proper maturation of T-cells and for the recognition of self vs. non-self within the immune system.

Antimetabolites

Antimetabolites are yet another drug commonly given to transplant patients to prevent rejection. They work by inhibiting RNA and DNA replication (Duncan & Wilkes, 2005), therefore preventing the growth and expansion of cells and halting the body's production of immune cells that would attack the allograft. But by hindering DNA and RNA expression, these drugs also affect the growth and proliferation of other cell types within the body, causing problems when repairs need to be made in the other body systems.

Two antimetabolites, Azathioprine (AZA) and Mycophenolate Mofetil (MMF) are the most commonly prescribed to transplant recipients. AZA is effective because it interferes with the production of purines and therefore interrupts DNA synthesis. MMF also inhibits the synthesis of purines, however, it is more selective in targeting immune cells. This means that it does not interfere with DNA production in other body cells, though it becomes less effective after a few years of use (Ng, 2010).

Rapamycin inhibitors

Rapamycin inhibitors are slowly starting to take the place of the traditional antimetabolites as a second option when antimetabolites fail to work (Ng, 2010). The two currently being used clinically are Sirolimus and Everolimus. These drugs are unique in their ability to bind to a specific cell surface protein called the Mammalian Target of Rapamycin (mTOR) (Sabers et al., 1995), and this causes cell division to become arrested between phases so the cells cannot divide (Zimmerman, 2004). This affects proliferation of smooth muscle, fibroblast cells, and endothelial cells just as much as the immune cells (Duncan & Wilkes, 2005). The benefit of rapamycin inhibitors is that they

can be used to replace other immunosuppressants that no longer work or do more harm to the body over long term use.

Corticosteroids

Corticosteroids were the first immunosuppressive drugs used in transplant therapy (Duncan and Wilkes 2005). They work by redistributing the circulating immune cells into the lymph tissue (Ng 2010) and by lysing the immature T-cells (Duncan and Wilkes 2005). Another benefit to corticosteroids is that they reduce the inflammatory cytokines that would cause luminal occlusion and organ failure (Ng 2010).

Corticosteroids affect the entire body and their biggest side effects include weight gain and diabetes. This occurs because these drugs put the body into a long lasting state of readiness, the flight in the fight or flight response. They are critical to most transplant patients for the short term, but some patients have found that they can discontinue corticosteroid treatments with no problems.

Monoclonal antibodies

Monoclonal antibodies are a newer immunosuppressant and only one of them, OKT3, has been approved for use (Wilde & Goa, 1996). Monoclonal antibodies are made by fusing animal myeloma cells with human antibody producing B-cells (Ng, 2010). This creates antibodies designed to attach to the Fc epitope of the T-cell receptor, rendering it useless and causing apoptosis (Renders & Valerius, 2003). There are three different types of monoclonal antibodies: anticytokine receptor antibodies, inhibitors of cell migration, and antilymphocyte antibodies.

Anticytokine receptor antibodies include the drugs Daclizumab and Basiliximab. These medications are directed at the CD25 unit of the IL-2 receptor and can specifically target only that cytokine (Duncan & Wilkes, 2005). By preventing IL-2 from being used, these drugs prevent other pro-inflammatory cytokines from being produced and reduce rejection.

Inhibitors of cell migration are not well understood at this point in time. The only one currently known is FTY720. These drugs cause peripheral lymphopenia by holding lymphocytes in the lymph nodes rather than allowing them back into circulation (Duncan & Wilkes, 2005). This would prevent rejection by preventing the immune cells from encountering the foreign antigen on the allograft.

Antilymphocyte antibodies are the only monoclonal antibody currently prescribed to patients, in the form of OKT3. OKT3 works by inhibiting the effectiveness of CD3, a compliment component of the immune system. By reducing the effectiveness of CD3, this drug would halt the immune response against the allograft.

Polyclonal antilymphocyte antibodies

Polyclonal antibodies work much like monoclonal antibodies, except they are aimed at several epitopes rather than just one. They are more selective than most immunosuppressants, however not as specific as monoclonal antibodies. They directly affect lymphocytes by using the complement system to force apoptosis (Duncan & Wilkes, 2005). The benefit of using polyclonal antibodies is that they suppress all lymphocytes but

they do not appear to suppress regulatory T-cells (T_{regs}). This allows the body to utilize its natural immune system regulators and stops the lymphocytes from attacking the organ (Pillai & Levitsky, 2009). They are well tolerated, but they do cause slight increases in fibrosis and higher rates of infection than other treatments (Ng, 2010). This increase in fibrosis could become a problem if chronic rejection begins because chronic rejection is associated with fibrosis.

TRANSPLANT TOLERANCE

The ultimate goal of any organ transplantation is complete immune tolerance and the withdrawal of immunosuppressants, often referred to as the “holy grail” of organ transplantation (Starzl, 2007). The number of immunological determinants that work against this goal make complete tolerance seem impossible; but a study at the University of Pittsburgh showed that, in some cases, patients were able to withdraw all immunosuppression (Mazariegos et al., 1997).

The reasons why some patients seem to be able to tolerate their donated organs better than others is one of the least understood aspects of all transplant biology. Some studies have speculated that the complete removal of donor antibodies would result in higher rates of survival and longer functioning time for an allograft (Lynch & Platt, 2008). Other explanations include changing antibodies, changing antigen, changing complement system, and an innate resistance to injury (Lynch & Platt, 2008). The complete tolerance shown in monozygotic twins (Krishnan et al., 2008) is expected, however it has also been demonstrated in completely unrelated allografts.

Tolerance may be possible by affecting several factors of the immune system. The innate immune cells of the body, macrophages and dendritic cells, definitely contribute to the explosive inflammatory response of the body, even if they don't initiate it (LaRosa et al., 2007). By slowing or stopping the innate immune response, it may solve the problem of acute rejection and perhaps even give useful insights on solving chronic rejection. There is also the possibility that Natural Killer (NK) cells could actually assist with immune tolerance (Yu et al., 2006), though this is still being studied. T_{regs} could also prove to be extremely useful in controlling the rejection of a transplanted organ. By increasing the amount of T_{regs} compared to other immune cells, the body would perform as its own natural immunosuppression.

There is also the possibility that manipulation of the memory T-cells will induce transplantation tolerance. Memory T-cells are less controlled by T_{regs} (Ford et al., 2009) and they cause higher rates of rejection among organ transplant recipients (Heeger et al., 2010). They are affected by several factors, including previous exposure to donor antigen, previous infections, and number of times the cells have been exposed to a specific antigen (Ford et al., 2009). There is the hope that by getting rid of memory T-cells, there would be a drop in the rates of rejection but closer examination is needed.

CONCLUSION

By understanding the immune system and its interactions with the rest of the body, we have begun to understand the theories, methods, and technologies behind organ transplantation. We

have yet to discover the mechanisms behind complete transplant tolerance, and organ rejection is still treated mainly with immunosuppressants. These drugs have many side effects, from poisoning the body to preventing the immune system from fighting off pathogens that are actually harmful to the body. By reviewing the types of rejection that occur and the immune interactions that are unique to each, better immunosuppressants can be created which do not have as many side effects.

Though the topic of organ transplantation has been studied for many years, there is still controversy in some areas. For instance, researchers still cannot agree on specific symptoms of acute rejection. There are some who say there are very specific symptoms and others who say there are none at all. There is also disagreement on the symptoms and timeline of chronic rejections. These two disagreements could be solved if there was more in depth research done on the immune response for each type of rejection and if more studies were done on the timing of each type of rejection.

There is a desperate need for future research that is done on the cytokine response to organ rejection. There has been a host of research done thus far on the adaptive cellular response of both T-cells and B-cells. There has been very little research done with the innate immune system and the cytokines it produces when it responds to an organ transplant. By understanding this aspect of the immune response, we could increase our understanding of the adaptive immune response and maybe even become closer to finding what drives transplant tolerance.

Complete transplant tolerance is the ultimate goal of this field of study and yet, researchers are still focusing more on the cases when organ transplantation goes wrong. By focusing on these cases, we are only learning how to fix the problems when they arise and not how to prevent them from arising in the first place. More focus needs to be given toward the cases in which tolerance did occur.

The focus also needs to extend to immunological testing of the donor as well as the recipient. By changing our research to include the donor, a connection can be made between the donor and recipient immune systems. This connection, which we have not yet examined, could offer another explanation of transplant tolerance.

The future for organ transplantation looks bright. There are better immunosuppressants that are being created and the procedure for transferring an organ from one body to another is becoming less cumbersome and more exact. We are also getting closer to finding ways of preventing infection from occurring as a result of the immunosuppression that makes the organ transplant possible. The most important research being done is examining the forces behind complete transplant tolerance. We have not found it yet, but we will eventually find the reason why a select few people can completely accept a new allograft without the aid of immunosuppressants. Whether or not this leads to a method of inducing that tolerance, remains to be seen.

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