The Role of T Regulatory Cells in Allergy and Autoimmune Disease and Potential Therapeutic Options

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Abstract

T regulatory cells are part of the immune system and act to control and suppress immune responses. A small population or a population of T regulatory cells with impaired function has been associated with allergic and autoimmune diseases. Without suppression from these cells, immune dysfunction can become prevalent and lead to disease. Previous studies have shown that exposure to microbes and parasites such as Helminths can boost the T regulatory cell population. In developed countries, where microbial and parasitic exposure is diminished, allergies and autoimmune disorders are on the rise. In this review, scholarly articles and recent clinical trials were examined to see what therapies are currently being tested using T regulatory cells. One of the most favorable therapies is adoptive transfer of T regulatory cells. This therapy has had promising results in the early stages. Patients who received adoptive transfer therapy have had more alleviation of their disease than those in the control groups who received traditional treatment. Future studies need to be done to come to clear conclusions about the effectiveness of adoptive transfer, which would include a larger sample size of patients and longer follow up periods after therapy. By improving our understanding of adoptive transfer as a treatment, patients with these disorders can hopefully have an improved quality of life in the future.

Table of Contents

Introduction

In Western countries the rates of allergic diseases, including asthma, food allergies, and hay fever, have been on the rise for the past several decades (Versini et al. 2015). Hay fever was first described in 1819 by a man named John Bostock. Bostock had symptoms for years but could not find a physician who could diagnosis him. Being a doctor himself, he found other people who had similar symptoms and were undiagnosed (Platts-Mills 2015). It wasn't until the 1870's when Dr. Blackley, after using a skin test with grass pollen, concluded that hay fever was a reaction to pollen. Unlike hay fever, asthma had been around for years, however, it was not common until the 1960's. Similarly, the rates of food allergies, specifically peanut allergy, did not rise until the 1990's (Platts-Mills 2015).

Why have allergic diseases increased so suddenly and rapidly? In 1989, Dr. David Strachan noticed an inverse relation between hay fever and number of older siblings. Doctor Strachan suggested that this disease was prevented in younger siblings through "infection in early childhood, transmitted by unhygienic contact with older siblings."(Brookes and Cheng 2015). He called this idea the "hygiene hypothesis" and it has expanded greatly since then. Now, the general belief is that through the increase of hygiene in developed countries, exposure to microbes has decreased. The decrease in exposure has led to an unregulated immune system, resulting in not only allergic disorders, but autoimmune diseases too. Microbes, or microorganisms, are things too small to see with the naked eye, such as bacteria, viruses, or a fungus. Factors that have led to the decrease in microbial exposure include decontamination of water, vaccination, widespread use of antibiotics, and lifestyles changes (Okada et al. 2010).

Several population studies have been done that support the hygiene hypothesis. One study was conducted by Dr. Mark Holbreich and colleagues. They studied the prevalence of

allergic diseases among Amish children, versus Swiss farm and non-farm children. The Amish immigrated to the United States about two hundred years ago from Switzerland (Holbreich et al. 2012). Amish families typically live and work on farms. Their lifestyle includes no electricity, large families, drinking raw milk, and daily exposure to livestock. Prevalence of asthma was 5.2%, 6.8%, and 11.3% among Amish, Swiss farm, and Swiss non-farm children. Overall allergic sensitization was 7.2%, 25.2%, and 44.2% respectively. Reaction to dust mite and grass pollen was lower among Amish children as well (Holbreich et al. 2012). This study gives insight that living in a pre-hygienic community, such as the Amish, provides protection against allergic disorders. Another study comes out of Finland. The region of Karelia is characterized by a Finnish section, with high standards of hygiene, and a Russian section, with lower standards of hygiene. Finnish Karelians have much higher rates of allergic and autoimmune disorders than Russian Karelians, despite sharing the same genetic background. One example is that the incidence of the autoimmune disorder Type 1 Diabetes (T1D) is six times higher among Finnish than Russian Karelians (Kondrashova et al. 2013).

While genetics does play a role in the development of these disorders, it is not the sole factor. The case above is a prime example, as the Karelian population shares similar genetic backgrounds with one another. Migration studies also show that descendants of immigrants who came from a country of low disease incidences, acquire the same incidence as the host country (Versini et al. 2015). This is seen in African Americans, who have a much higher rate of the autoimmune disorder systemic lupus erythematosus than West Africans do (Symmons 1995). To understand why this is occurring, it helps to understand how the immune system responds to microbes.

Overview of the Immune System

The immune system of an individual has two immune responses, the innate immune response and the adaptive immune response. Though different, these two responses work together to defend the host from invading pathogens. Pathogens are disease causing microbes or parasites, but not all microbes cause disease. The innate immune response and the skin barrier are considered the first line of defense when fighting off a pathogen and are known to be nonspecific because it doesn't recognize specific pathogens, but a spectrum of microbes that don't belong to the host. It consists of natural barriers such as the skin, as well as white blood cells known as leukocytes. The main types of innate leukocytes are neutrophils, macrophages, eosinophils, basophils, and mast cells, each with a specific role. Eosinophils and basophils primarily defend against parasites. Mast cells are found in tissue such as the skin and can release histamine to instigate an inflammatory response (Stranford et al. 2013). Neutrophils and macrophages are known as phagocytes because they consume dead cell debris and microbes such as bacteria. This process is called phagocytosis. Macrophages can also be classified as antigen presenting cells (APCs) along with dendritic cells. APCs will phagocytize a pathogen and then present an antigen from the pathogen on their surface. Antigens are molecules that initiate an immune response. In the case of APCs, antigens come from a pathogen and are used to activate an adaptive immune response.

APCs take the antigen and present it to T cells of the adaptive immune response. Cells of the adaptive immune response are highly specific and will only recognize one antigen each. These cells are called lymphocytes which include B cells and T cells. APC's will present an antigen to T cells. When they are presented with an antigen which they recognize, they will become activated. B cells have antibodies on their surface, which can recognize antigens and

activate the cells. When activated, B cells will produce more antibodies to that antigen. The antibodies can bind to the antigen, which is part of the pathogen, and neutralize it or attract other immune cells to kill the pathogen. T cells serve many different functions. T helper cells serve as a helper to the adaptive immune response. When activated by an APC, they will go and find other B or T cells that recognize the same antigen and activate them using chemical signals called cytokines. Cytotoxic T cells, once they are activated, will kill cells infected with that specific pathogen. They do this by inducing cell death in infected cells by a process called apoptosis (Stranford et al. 2013).

Since lymphocytes are capable of recognizing specific antigens, it is necessary that they only recognize foreign antigens, and not one's own antigens, known as self-antigens. To exclude any self-reactive lymphocytes, they undergo a selection process. This occurs in the bone marrow for B cells and in the thymus for T cells. The thymus is an immune organ near the front of the heart that T cells get their name from. Any cells that are self-reactive are not allowed to survive and die by apoptosis. This is called self-tolerance, as the surviving cells will tolerate the self-antigen and not respond to it.

Mechanism of Tolerance

The population studies that have supported the hygiene hypothesis have led researchers to wonder what is happening inside the body that promotes either tolerance or dysfunction. Studies have concluded that it comes down to different subsets of T cells. In one subset, T helper cells, researchers have been able to identify connections to allergic and autoimmune disease. Two specific types of T helper cells (T_H) are T helper Type 1 cells (T_H1) and T helper Type 2 cells (T_H2). In autoimmune patients, T_H1 is the dominant T helper cell, while in allergic patients, T_H2

is the dominant type (Versini et al. 2015). In normal, healthy individuals, the two types are in a reasonable balance, but with patients that have immune dysfunction, there is an imbalance.

There is also another subset of T cells that is critical; the T regulatory cell (T_{reg}). The T_{regs} role in the immune response is to suppress it. The idea of T_{regs} was first developed in the 1970's when researchers noticed that tolerance to foreign cells could be transferred among different mice using T cells (Gershon and Kondo 1971). Due to technology at the time however, it was impossible to identify the specific subset of T cells that caused tolerance. Since there was no way to identify these cells, the idea of transferred tolerance was not well favored. It wasn't until many years later that the subset would be identified as T_{regs} (Kuhn et al. 2016). As stated, these cells induce immune suppression and prevent the immune system from becoming overactive and harmful to the individual. There are many types of regulatory T cells that have been discovered, but the main two are native T regulatory cells (nT_{regs}) and induced T regulatory cells (iT_{regs}) (Braga et al. 2012). Native T regulatory cells are selected for in the thymus and are then used to prevent autoimmune disorders. Normally, any self-reactive T cells would not be allowed to survive in the thymus, but occasionally a couple may slip through the selection process. The purpose of the nT_{regs} would be to suppress the activity of these self-reactive T cells. Induced T regulatory cells were once T_H cells that encountered a foreign antigen. In the presence of the signal interleukin 10 (IL-10) and transforming growth factor beta (TGF-β), a small percentage of T_H cells will become iT_{regs} (Braga et al. 2012). Since T_{regs} show the capability of suppressing the immune system, which is overactive in allergies and autoimmune disorders, they have become a central focus in research to prevent the development and alleviate the symptoms of such diseases.

T_{regs} get their suppression function from the transcription factor known as forkhead box P3 (FOXP3). FOXP3 is considered a master regulator and is what gives T_{regs} their regulatory function. This factor is used to identify T_{regs}, thus they are said to be FOXP3+ T cells (Ng et al. 2013). It is worth noting however, that other forms of T_{regs}, lacking FOXP3, have been identified (Braga et al. 2012). Loss of function mutations in the FOXP3 gene have resulted in T_{reg} deficiency and severe immune disorders (Ng et al. 2015). One genetic disorder is IPEX syndrome where there is a complete loss of function from FOXP3. Individuals with IPEX syndrome will demonstrate multiple organ autoimmunity (Aricha et al. 2016). Studies have shown that in patients with overactive immune systems, the population of T_{regs} is either diminished or does not function properly.

T_{regs} use a couple of different mechanisms to achieve immune system suppression. One of the most well documented ways is through the secretion of the cytokine IL-10. Cytokines are substances secreted by one immune cell, which can have an effect on another immune cell. It is a way for immune cells to communicate with each other. Most cytokines are referred to as interleukins, such as interleukin 10, which is considered to be an anti-inflammatory cytokine. Animals that had a deficiency in IL-10 or are treated with anti-IL-10 antibodies died rapidly when infected with parasites such as *Toxoplasma gondii*. Death did not occur from the parasite itself, but rather from overproduction of inflammatory cytokines (Gazzinelli et al. 1996). *T. gondii* is a common parasite that can infect any warm blooded animal. Infection may produce mild flu like symptoms, but most infections are asymptomatic. The parasite is harmless in many cases so death occurred because in the absence of IL-10, the immune response was not able to be suppressed and hyperactivation occurred.

In healthy individuals, T_{regs} will function to suppress the immune system by suppressing antigen-presenting cells and T_H1 and T_H2 cells, using IL-10 and TGF-β. If given antibodies to block these cytokines, suppression is also blocked and levels of these cells will return to what they were prior to suppression (Braga et al. 2012). Similarly, T_{regs} can also suppress mast cells, basophils, and eosinophils. T_{regs} also perform regulatory functions on B cells. Secretion of IL-10 can downregulate the production of IgE and enhance the production of IgG4 and IgA (Braga et al. 2012). Ig stands for immunoglobulin, also known as an antibody. There are five classes of antibodies shown in the table below.

Table 1: Antibody Classes (Rahman 2016)

Antibody Name	**Role**	**Subclasses**
IgM	- Responses to first exposure of antigen	None
IgD	- Located on the surface of B cells with IgM	None
IgG	- Expressed on the surface of B cells - Involved in secondary immune response	4
IgE	- Triggers histamine release - Protects against parasitic worms	None
IgA	- Found in saliva, tears, and mucus	2

There is also another type of regulatory cell known as the B regulatory cell (B_{reg}). B cells secrete antibodies which fight off invading pathogens, but in allergies and autoimmune disorder, antibodies can cause an array of problems. In autoimmunity, these antibodies can be self-reactive, while in allergies, IgE activates the release of histamine from mast cells, eosinophils, and basophils which causes an allergic reaction (Braza et al. 2014). However, similarly to T_{regs},

B_{regs} exist to suppress any unwanted activity from the B cell population and they achieve this by the secretion of IL-10. B_{regs} are able to inhibit cytokine secretion from T_H1 and T_H2 cells, particularly proinflammatory cytokines. B_{regs} are also able to recruit or induce a population of FOXP3+ T_{regs}. Lastly, B_{regs} are also capable of producing apoptosis in activated T cells (Smits 2012). Unlike T_{regs}, which are mainly characterized as being FOXP3+, there is no specific marker to identify B_{regs}. Therefore, they are hard to identify and understand where they originate from.

In the Case of Helminths

Helminths are parasitic worms that often reside in the intestine of humans. Years ago, infection with Helminths was very common. Almost everybody at some point in their lifetime would come into contact with a worm and become infected (Maizels 2016). Today, about 37% of the world's population is infected with Helminths, mainly in developed countries (Versini et al. 2015). The decrease in infections is associated with sanitation of water, as well as access to antihelmintic treatments. Certain types of Helminths can be highly pathogenic, but most infections are often asymptomatic (Maizels 2016).

Helminths have a long evolutionary history and it is believed that humans co-evolved with them. The reason for this is that the parasite does not want to kill their host, but rather live inside of them. To achieve this the parasite must modulate the immune system to keep the host from attacking and killing it. The parasite is capable of living peacefully in the human body because an attack on the parasite from the immune system would be very burdensome, and the body is likely to become infected again shortly after. The immune system is able to overlook the infection and focus on more harmful pathogens (Weinersmith and Earley 2016). The parasite is

also capable of upregulating T_H2 while inhibiting the T_H1 response. Normally, a high T_H2 response would be a characteristic of allergic disorders, however, the Helminth worm also upregulates a population of T_{regs}, which will prevent the T_H2 cells from having abnormal activity (Versini et al. 2015). The T_{reg} population with its immune suppressive capabilities not only allows the parasite to survive, but can aide in the prevention of allergic and autoimmune disorders in the host.

Since Helminths have been able to aid humans, they have been give the name "old friends". The human population has lived in contact with "old friends" for millennia, up until a couple of decades ago (Versini et al. 2015). As society began to change, people moved away from farms and more hygienic ways were developed, we successfully decreased exposure to harmful microbes, but also decreased the exposure to "old friends". It has been theorized that since we evolved for so many years with the parasites, that many of us now do not possess the capabilities of creating a substantial T_{reg} population on our own (Weinersmith and Earley 2016).

Knowing that undeveloped countries have lower rates of allergic and autoimmune disorders while having higher rates of infections from parasites, has prompted numerous studies to look at the role infections play in the development and the treatment of these disorders. There have been both positive and negative results from these studies. One study out of Argentina followed twelve patients with the autoimmune disorder multiple sclerosis. The patients who had active helminth infection all went into remission for four years, while uninfected individuals had numerous relapses (Correale and Farez 2011). The patients in remission showed increased levels of IL-10, TGF-β, B_{regs}, and T_{regs}. Another study looked at inflammatory disorders of the gut such as Crohn's disease and ulcerative colitis. Patients were infected with the eggs from the parasite *Trichuris suis.* About 70% of the patients showed improvement in their symptoms (Summers et

al. 2005). While these results are promising, a problem with both studies is that the study size is too small to consider the results significant.

Other studies have had mixed results. There are a couple reasons for this. One is that there are many different types of Helminth species being investigated for a wide range of disorders. The different species and different diseases increase the variables, which makes it difficult to come up with uniform conclusions about whether a specific parasite can benefit a specific disease or not. Another reason for mixed results is that these disorders are not caused solely by the absence of parasites, but also by genetic and environmental factors. Infection by a Helminth parasite may not be able to help individuals who are genetically susceptible to a disorder as parasitic infection is not the cause. In patients whose disease is not well controlled, it is worth the investigation to see if Helminths can alleviate their symptoms.

Some researchers have looked at Helminths not as a treatment, but as a preventative measure. They have studied the effect of parasitic infections on infants and children. These studies have had more consistent results. One study concluded that infection by the worm *Trichuris trichiura* in early childhood is associated with reduced odds of allergic reactivity in adulthood (Amoah et al. 2013). During pregnancy, infection with Helminths of the mother is associated with lower odds of the newborn developing allergic eczema. When given antihelminthic treatment, children had an increased reaction to allergens (Amoah et al. 2013). This occurred because allergens promote B cells to produce the antibody IgE. During infection, the Helminth worms can upregulate T_{regs}, which reduce the responsiveness of the IgE. After treatment, with no infection, there is no T_{reg} upregulation, and the IgE antibodies are able to induce an allergic reaction (Amoah et al. 2013).

Altogether, worm infections early in life have a preventive effect on the development of allergic and autoimmune diseases. In parents with one of these diseases that may increase the chance of the child developing it, Helminth treatment as a possible preventive measure may be possible. More research needs to be done on the safety of Helminth treatment in children beforehand, however.

T_{regs} in Immunotherapy

Immunotherapy is the prevention or treatment of disease with a substance that will stimulate an immune response. In allergies, allergen-specific immunotherapy (SIT) has been used for many years. With SIT, the patient is given gradually increasing doses of an allergen, usually through a shot in the skin, over a period of time with the hopes that the patient will become tolerant to that allergen. The aim of this treatment is to restore balance of the immune system in allergic individuals. Though SIT has been around for 100 years and has been known to work, the mechanism of how SIT worked was unknown for quite some time (Eiwegger et al. 2012).

As researchers discovered more information about T_{regs}, they began to realize the important role these cells play in the development and alleviation of allergic diseases. In children who outgrow allergies to cow's milk, they have a higher frequency of circulating T_{regs} in their blood when compared to children who continue to have the allergy (Karlsson et al. 2004). The T_{regs} suppress the inflammatory response and contribute to the generation of IgG4 antibodies, rather than IgE antibodies that incite an inflammatory response. The cytokine IL-10 is found to be a key molecule secreted from T_{regs} that inhibits T cell activation and ultimately restores balance in the immune system to a state of tolerance. Another effect of SIT is that the small dosage of allergen does generate some release of histamine from mast cells. This release is below

the threshold to cause an allergic reaction. By doing this, the contents of the cells are diminished and the level of allergen exposure needed to cause a reaction is increased (Eiwegger et al. 2012).

During SIT, the gradually increasing exposure to an antigen that the patient is allergic to allows for the immune system to produce IgG4. This antibody exhibits a blocking effect as it will bind to the antigen before IgE is able to. Thus, IgE antibodies are unable to bind and cause histamine release of neighboring mast cells (Eiwegger et al. 2012). The increase in IgG4 levels can serve as a marker for T_{reg} cell activity, as T_{regs} are able to promote the production of IgG4 instead of IgE. Overall, SIT actually increases the T_{reg} population and increased levels of IgG4 antibodies are a product of that. As well as restoring T_{reg} levels, SIT can shift away from the pro-allergic state of dominant T_H2, to a more tolerant state of T_H1 cells (Eiwegger et al. 2012).

A study on mice demonstrated the principle of acquired tolerance through the use of SIT. Professor Yamashita and colleagues studied allergic reaction in mice while treating them with SIT (Yamashita et al. 2011). Pretreating mice with a gradually increasing doses of the allergen prevented allergic reaction from occurring. The researchers also studied adoptive transfer in the mice. Here, they took T_{reg} cells from mice that had a tolerance to the allergen and injected them into mice that had the allergy. After treatment, the mice did not demonstrate an allergic reaction to the allergen as they previously did (Yamashita et al. 2011). One aspect that was different however was that levels of IgE were reported to have increased in the mice, though no allergic symptoms were seen to be occurring in the mice. They found that the antibodies were able to bind to the allergen, but it was believed that the T_{regs} prevented the bound antibodies from starting a reaction (Yamashita et al. 2011).

T_{regs} in Type 1 Diabetes

Type 1 Diabetes (T1D) is an autoimmune disorder that affects the pancreas. Causes of T1D include genetic and environmental factors, as well as infection with certain viruses (Creusot et al. 2016). Cells in the pancreas that are affected are called beta islet cells. These cells produce insulin that allow other cells in the body to take up glucose, a form of sugar. In T1D, T cells are self-reactive and they recognize the beta islet cells, attacking them and eventually destroying them (ElEssawy and Li 2015). The destroyed cells can no longer produce insulin, causing the rest of the body's cells to be affected where they cannot take in glucose, leading them to starve. To avoid this, patients take insulin shots to allow the cells to take in glucose from the blood. T1D affects about 1.25 million people in the United States and rates are increasing every year (Gitelman and Bluestone 2016). Projections suggest that the number of affected individuals will triple by the year 2050 (Gitelman and Bluestone 2016). Insulin replacement has improved the life of individuals affected but blood sugar levels are still hard to control and patients develop different complications over the years (Perakakis and Mantozoros 2016). Unsatisfactory treatment has led researchers to continually investigate not only new treatments, but preventative measures for high risk individuals.

Numerous studies have shown that T_{regs} play a large role in T1D. In patients with T1D, T_{reg} numbers are deficient, the cells have impaired function, or other immune cells are unresponsive to the T_{regs} (ElEssawy and Li 2015). Secondly, though self-reactive T cells are the main effector of T1D, they are not the first to cause damage. Recent studies have shown that cells of the innate immune system, such as macrophages and dendritic cells, are the first to enter the pancreas and cause increased inflammation followed by the infiltration of the self-reactive T cells. It is believed that this is caused by infection from certain viruses. The viruses may kill the

beta islet cells, than macrophages present islet cell antigens to T cells, where self-reactive ones will become activated (Perakakis and Mantozoros 2016). In mice studies, non-obese mice showed an increase in the rates of T1D when they had a decreased population of T_{regs} (Perakakis and Mantozoros 2016). Relating back to the hygiene hypothesis, mice that were raised in a germ free environment were also more likely to develop T1D (Wen et al. 2008). This type of T1D was not actually caused by self-reactive T cells, but from cells of the innate immune system. The lack of microbes in the mice led to a poor development of T_{regs} causing the overall immune system to become dysregulated (Wen et al. 2008).

T_{reg} cells have become the focus of potential treatment because restoration of the T_{reg} population could potentially suppress the self-reactive T cells that are causing damage to the pancreas. They are also being studied for possible preventative measures because T_{regs} can suppress the initial inflammation caused by the innate immune cells. Preventative treatment for high risk individuals is very significant because by the time an individual is diagnosed with T1D, on average about 40% of their beta islets cells have already been destroyed (Perakakis and Mantozoros 2016).

T1D is just one example of many autoimmune disorders which seem to be following the same trend, that one of the major underlying problems is the T_{reg} population. The population of T_{regs} in affected individuals is decreased and if not decreased, the T_{regs} have impaired function and are ineffective at suppressing other immune cells. Though this is not the case for every individual, it is a general trend that has been noticed over the years. This has led to the focus on T_{regs} as potential targets for immunotherapy.

Adoptive Transfer of T_{regs}

The therapy of adoptive transfer consists of taking cells and transferring them into a patient. The cells can come from either the patient or another individual. Since T_{regs} have been shown to be an important immune cell in allergies and autoimmune disorders, they have been implemented in this type of therapy. Typically, samples are taken from the patient and the sample undergoes a purification process to isolate T cells. These cells undergo further isolation so that only T_{regs} remain. The sample of T_{regs} is then cultured so that a substantial population exist. This is done by introduction of cytokines such as IL-10 and IL-2 which stimulate the production of T_{regs} (Trzonkowski et al. 2015). The T_{regs} must then be tested to make sure that they function properly. In the case of autoimmune disorders, the cells must also be tested to be sure that none of them are able to recognize and attack host cells. The original self-reactive T cells must not be in the new T_{reg} population. From here, the cells are transferred back into the patient, either through the blood or into a site effected by the disorder, like the pancreas in T1D.

Testing adoptive transfer therapy was first done using mice as subjects. It has been shown that T_{regs} from a non-allergic mouse that are transferred to an allergic mouse will induce tolerance in the recipient mouse (Yamashita et al. 2011). Likewise, mice with T1D who undergo adoptive transfer therapy of T_{regs} have been able to either alleviate symptoms of the disease or stop further damage to the pancreas and restore insulin production (Gitelman and Bluestone 2016). T_{regs} have also been tested in transplants, to prevent graft-versus-host disease (GVHD) where cells from the donated blood or bone marrow recognize the recipient cells as foreign and attack the recipient. T_{regs} have also been evaluated to see if they can prevent or improve the risk of rejection of the transplanted organ. Transfer of other immune cells, such as T_C and T_H cells are also being investigated to determine if these immune cells can attack cancer cells in the body.

The first human studies done with adoptive transfer were done only to determine if it was safe to transfer T_{regs} into a person, not to determine if there was any alleviation of disease (Gitelman and Bluestone 2016). So far, transfer of T_{regs} has been seen to be safe with minimal side effects. Some studies however, have reported an increase in the rate of cancer and infections, but in the control group there was also increased rates of cancer and infection (Todo et al. 2016). Once safety was established, researchers needed to know how many T_{regs} need to be transferred into a patient to achieve suppression and what is the maximum limit of T_{regs} that should be given to the patient. In mice, large numbers of T_{regs} are needed to reverse T1D, around $5x10^6$ cells infused. However, mice are much smaller creatures than humans, therefore a much larger sample is needed, around $3x10^8$ cells per dose (Tang and Lee 2012). This is just a general estimate, as every disease and person is unique and will require different amounts of cells to achieve suppression. The upper limit of how many cells that can safely be transferred is near the limit of the number of cells that can be produced in culture with current methods (Gitelman and Bluestone 2016). Once safety and numbers had been established trials went on to test the effectiveness of adoptive transfer in treating autoimmune and allergic disorders, as well as preventing GVHD and organ transplant rejections. Results of some trials are explained below, while many trials are still being currently conducted.

Autoimmune Disorders

Type 1 diabetes is possibly one the most popular autoimmune disorders that is being used in research of the effectiveness of T_{reg} cell transfer. One group out of the University of California recently published results from their trials this year, 2016 (Gitelman and Bluestone 2016). They first established that there was no increased risk of infection among patients. They also

established that it is feasible to obtain blood from a patient, isolate the T_{regs}, allow them to grow in culture, and infuse them back into the patient. The T_{reg} population that was infused in the patient declined over the course of nine months but a prolonged presence was seen till the end of the study, a year out from initial infusion. Twelve patients were used and one year after the study, eight of those patients achieved clinical remission, while in the control group, only two out of ten patients had achieved remission (Gitelman and Bluestone 2016). The patients were also sorted into different groups and each group was given a different dose of T_{regs}. Results suggested that a higher dose of T_{regs} is better as the patients that received the higher dose were completely off of supplemental insulin (Gitelman and Bluestone 2016). One significant problem with this study is that the sample size is very small, only twelve patients being studied. The small size prevents any results from being considered significant.

Another study done by Dr. Marek-Trzonkowski and colleagues had similar results to the previous study. At the end of the study, one year after infusion, patients that received T_{reg} infusion on average required half the amount of insulin injections than those in the control group, while two individuals did not require insulin injections at all (Trzonkowski et al. 2008). These patients had all received T_{reg} infusion around two months after initial diagnosis of T1D. Transfer early on in diagnosis is important because throughout the course of the disease, beta islet cells are slowly destroyed. The longer an individual has had T1D, the more cells in the pancreas have been destroyed. To preserve optimal function of the pancreas, it is necessary to try treatment very early.

Since large numbers of cells in the pancreas are destroyed by the time of diagnosis, adoptive transfer has been tested as a preventative measure for high risk individuals in order to prevent pancreatic damage. Individuals are deemed high risk if they have a family history of

T1D or they have genetic risks factors (Creusot et al. 2016). Several animal studies have shown that adoptive transfer of T_{regs} amongst animals at risk for T1D can prevent the development of the disease. Future trials need to be conducted to see if results from animal models will correlate to human subjects. One potential problem is that children are typically the patients that are considered to be high risk as the majority of T1D cases are diagnosed at age fourteen. The problem here is that adoptive transfer requires a large initial volume of blood to be taken from the patient, a volume that children may not be able to safely give (Gitelman and Bluestone 2016). A way around this would be to take a smaller amount of cells from the patient, but increase the growth rate of the T_{regs} in culture.

Another autoimmune disorder being investigated is myasthenia gravis (MG). MG is a disease characterized by muscle weakness and fatigue from immune cells attacking skeletal muscle receptors (Aricha et al. 2016). Overtime, the communication between muscles and nerves is broken down. Some individuals can live normal lives with medications, while others still develop serious complications. Researchers at The Institute of Science in Israel looked at T_{regs} as a possible treatment for MG. They used rats as their model and induced them with experimental MG, which is very similar to normal MG (Aricha et al. 2016). First, they took T_{regs} from healthy rat donors, and gave them to the rats with MG. The recipient rats achieved suppression of MG from the transfer T_{regs}. When the researchers took T_{regs} from the sick rats, cultured them, and transferred them back, MG was not suppressed (Aricha et al. 2016). This was because the T_{regs}, though normal in number, had functional impairments and could not suppress immune activity. They tried this step again, but improved the function of the rat's T_{regs} by stimulating them with dendritic cells to improve function. When the functionally improved T_{regs} were administered back into the rats, MG was successfully suppressed. (Aricha et al. 2016).

Type 1 diabetes is currently one of the most studied autoimmune diseases for the therapy of adoptive transfer using T_{regs}. Early clinical trials, as the one described above, have had hopeful and beneficial results. The next step is to increase the number of trials and increase the number of patients in each trial. Many trials are currently underway and we will have to wait for results. For other autoimmune disorders, like MG, trials are still being done on animal models, but as successful experiments show positive results, human trials will begin. Early clinical trials on other autoimmune diseases are currently begin done or are in the process of recruiting patients. Again, we will have to wait for the results of these trials to see if what we learn from animal models can be transferred over to humans. It is imperative that researchers share results with one another, so data on timing of transfer, method of transfer, characteristics of patients, and other factors can be looked at and concise conclusions can be made about the effectiveness of adoptive transfer of T_{regs}.

Allergic Disorders

When looking at allergic disorders, adoptive transfer therapy has only been tested in mice so far, but with promising results. It has been shown that mice with food allergies can overcome them by receiving transferred T_{regs} from non-allergic mice (Yamashita et al. 2011). The mice no longer showed any signs of allergic reaction when exposed to the allergen. In another study, normal T cells were taken from mice with asthma and then converted to iT_{regs} in culture. When reintroduced into the mice, the iT_{regs} were capable of controlling airway inflammation (Agua-Doce and Graca 2012). These researchers used the cytokine TGF-β to convert the T cells into functional iT_{regs}. IL-10 and IL-2 are also known cytokines that drive the development of T_{regs}. The environment of a T cell is important because what the T cell is exposed to will dictate what

it develops into. Mice deficient in IL-10 show signs of airway inflammation (Agua-Doce and Graca 2012). This may be because the environment, lacking IL-10, is not favorable for T_{reg} development. T_{regs} secrete both IL-10 and TGF-β, so the presence of them will induce more to be converted.

Aside from the few mice studies looking into food allergy and asthma, there are not many other studies that have been conducted. A reason for this may be that for most people, allergies are burdensome, but do not affect quality of life that greatly. While other health issues, such as autoimmune disorders or transplant rejection, significantly affect the health of the individual. These diseases have taken front stage in researching adoptive transfer of T_{regs} because those affected would have the greatest benefit. Another possible reason is that the process of adoptive transfer is laborious and costly, so the focus is on the more taxing diseases. In the future, if more data comes out showing the significant benefit of T_{regs}, the therapy may open up to allergic diseases, especially for those individuals who are greatly affected.

Transplants

When an individual requires a transplant, it is important that they receive an organ that is a "match". With blood, a recipient must receive blood from a person whose blood type is compatible with theirs. For an organ, this process is similar, but more complicated. Not only must blood type match, but human leukocyte antigen (HLA) must also be matched. HLA is a type of antigen on cells that allow for the body to recognize these cells as belonging to itself, thus they will not be attacked by the immune system. There are one hundred different antigens, but of these, six are the most important when matching organs (UC Davis Transplant Center 2016). Except in the case of twins, it is rare for two people to have the same identical six.

Doctors try and match as many HLAs as possible to ensure a match. Without matching, the immune system of the recipient patient would recognize the donor organ as foreign and attack it, leading to organ rejection. Still, even with matching, there still is a chance that organs may be rejected by the patient (UC Davis Transplant Center 2016). For this reason, patients receive antirejection medication to prevent rejection from occurring. However, long term satisfaction is still low, as patients can be on medication for the rest of their lives and can suffer complications due to long term immune suppression (Todo et al. 2016). These complications include infection, malignancies, kidney disorders from the toxicity of the medication, and still the chance of rejection. T_{regs} with their immune suppression capabilities are being looked at as a potential new therapy to prevent rejection.

Dr. Todo and colleagues from the Research Institute of St. Mary's Hospital in Japan, studied the effect of adoptive transfer of T_{reg} cells on ten patients who received a liver transplant. At the end of the study, all ten patient were maintaining good transplant function and none of these patients had any adverse effects of treatment (Todo et al. 2016). Seven of these patients no longer needed immunosuppressive (IS) drugs. IS drugs were discontinued within eighteen months of the transplant and the patients were drug free until the end of the study. Some patients have been drug free for twelve months, while others have been drug free for thirty-three months at the end of the study. These patients are considered to have achieved operational tolerance (OT), which means to have maintained transplant function without the need of IS drugs (Todo et al. 2016). This is considered a rare event. The other three patients, who originally had an autoimmune disorder affecting the liver, developed mild rejection after cessation of IS drugs and needed to be placed back on them.

The researchers concluded a few significant results. One is that the process they used to extract, develop, and test T_{regs} was practical and less expensive than other methods (Todo et al. 2016). The amount the researchers used is lower than other reported values in previous studies. Secondly, this study, published in 2016, is the first to report OT achievement using T_{reg} therapy. They demonstrated that patients could be weaned off of IS drugs as soon as six months after transplantation occurred and completely terminate the use of IS drugs by eighteen months (Todo et al. 2016). OT rates of patients in this study were higher than that of patients who received only traditional IS drugs (Todo et al. 2016). Of the three patients that developed mild rejection, Dr. Todo had originally believed that the transfer of T_{regs} would aid the patients with autoimmune disorders, but this was not the case. As to why this happened, Dr. Todo theorized that it has something to do with the genetics of the patients, but this was not looked into as it was not included in the realm of the study (Todo et al. 2016).

Several limitations of this study exist. First, to achieve statistically significant results, Dr. Todo needed at least forty patients to participate, but was only able to get ten patients in the time frame of the study (Todo et al. 2016). Secondly, patients had received transplants from living donors, rather than deceased donors. Living donors typically have a higher HLA match then deceased ones, thus the chance of achieving OT is possibly higher among these patients (Todo et al. 2016). Another limitation was the limited follow up period. Long term follow up, five years or even ten years after transplantation, was not possible so we cannot be sure of the long term outcome of these patients. Still, this study displays the potential that T_{reg} therapies have on the success of transplants. As a result, further study is being conducted on a large sample size with a longer follow up period (Todo et al. 2016).

Other Therapies using T_{regs}

Other types of treatment using T_{regs} other than adoptive transfer is possible. One therapy is to treat patients with low dosages of IL-2. In patients who have a normal population of T_{regs}, but the cells show diminished function, exposing them to IL-2 can improve their function. This is a possible therapy for autoimmune and allergic disorders, as well as transplant patients. A study out of Harvard Medical School looked at this type of therapy used in corneal transplants on mice. The cornea is a transparent part of the eye that covers the pupil. Transplant survival is low in patients whose eye shows signs of chronic inflammation, chronic infection, or have received trauma (Tahvildari et al. 2016). This is because there is already prior inflammation at the site of the transplant. Even with IS treatment, long term survival of the transplanted cornea is not guaranteed and complications can arise. Similar to adoptive transfer in liver transplants, promoting T_{reg} function with IL-2 will hopefully increase good outcomes of transplants.

The mice in this study received low doses of IL-2 starting three days before transplantation in order to begin boosting T_{reg} function. During the first week, dosage was continued as this is the peak time for rejection (Tahvildari et al. 2016). From week two to week six, IL-2 was given twice a week, by this time, most corneal transplants are rejected (Tahvildari et al. 2016). At the end of the study, the mice showed significantly improved rates of transplant survival. This was also the first study that used IL-2 alone, with no other IS drugs (Tahvildari et al. 2016). Enhancing T_{reg} numbers and their function was enough to overcome the prior inflammation at the site of the transplant. Low dose IL-2 has also been used on autoimmune disorders such as T1D and has improved disease prognosis as well.

Obstacles in Therapy with T_{regs}

Many obstacles exist in T_{reg} therapy which can impair results. One problem is that there is no clear way of identifying all T_{regs}. We know that most T_{regs} can be identified by the FOXP3 transcription factor, but not all T_{regs} have this marker. Without a clear marker, it can be difficult to isolate a population of T_{regs} from a patient (Trzonkowski et al. 2015). When isolating T_{regs}, there is not just one method of doing so, but many. Different experiments use different methods, which can make it difficult to come to a conclusion on which method is the best one. Another conclusion that needs to be made is which method is the most practical for researchers to use. Ways to create a functional T_{reg} population are also disputed. Both IL-10, IL-2, and TFG-β can stimulate a functional T_{reg} population. There is also debate on whether low doses or high doses of these cytokines is the right exposure (Tahvildari et al. 2016; Trzonkowski et al. 2015). Creating a substantial population size can also be difficult. Some patients already have a diminished T_{reg} population so getting enough viable cells from them can difficult. Also previously mentioned is that smaller individuals, such as children, cannot give enough blood for researchers to obtain a substantial amount. Consequently, the samples have to be cultured even more, possibly beyond what current equipment can do (Trzonkowski et al. 2015).

Regulations placed on facilities and trials can severely limit what researchers are trying to accomplish. While these are meant to benefit patients for their safety, it hinders researchers. Adoptive transfer is considered a cell-based therapy and thus has harsher restrictions than pharmaceuticals (Trzonkowski et al. 2015). For academic institutions and small companies with limited resources, it can be a great challenge for them to comply with regulations. It is possible for certain cell-based therapies to be given orphan drug status. Orphan drug status is given to drugs that are designed to treat a specific rare disease. The profit of these drugs is far less than a

generic drug. By giving it orphan drug status some restrictions are lifted, making testing easier (Trzonkowski et al. 2015).

The clinical trials themselves possess a few problems. Researchers as of right now are not sure if T_{regs} work best at preventing the development of disease, or suppressing the disease after the development. Each case comes with its own set of obstacles. In terms of preventing, high risk patients must be identified. Genetics can help identify high risk patients. There is a vast array of autoimmune diseases, most with some genetic component. Family history can help identify those who might be genetically predisposed to certain disease. However, if no family history is available, genetic testing must be done. Genetic testing will only work if certain genes have been linked to disease, but for many disease, it is still unknown what genes can cause them. If therapy is given after the development of a particular disease, the question raised is when is the right timing? Is it most beneficial for treatment to begin right after diagnosis? Would patients who have had the disease for ten or twenty years benefit as well? All these questions means that extensive experimentation needs to be done. During the trials, it is also important that there is a long term follow up period. The reason why most of the time there isn't, is because grants and funding only last a few years. When funding stops, research stops. As a consequence, long term results are not thoroughly studied. Since T_{regs} suppress the immune system, there remains the possibility of increased risk of infection and the development of malignancies. These risk cannot be known unless there is long term follow up of patients.

Lastly, the cost of the therapy is currently very expensive. The cost of a single transfer can range anywhere from sixteen to forty thousand dollars (Trzonkowski et al. 2015). Most of this cost comes from the equipment needed to isolated and increase the T_{reg} population. Some of this equipment can only be used once and the facilities they are located at can be expensive. On

top of that, personnel must be paid and the products need to be tested to ensure functional quality. If the process can be made less labor intensive over the upcoming years, the price should fall (Trzonkowski et al. 2015). There is also the risk that insurance companies may not cover this type of therapy. One main reason why is that, at the moment, T_{reg} therapy has not been proven to be effective. To have insurance companies cover the cost, therapy needs to be proven to benefit a specific set of individuals.

Conclusion

Over the years, researchers have learned more and more about the complexity of the immune system. Much of this started with Dr. Strachan suggesting that being born with older siblings would help protect against allergic disease. Since then, his idea called the hygiene hypothesis has grown and now includes autoimmune disease. The general belief is that less exposure to microbes causes the immune system to be underdeveloped. Whereas exposure to these microbes can actually train the immune system. This is also seen with parasitic worms. Countries with low incidence of worm infections have a higher incidence of allergic and autoimmune disorders. As technology has improved, researchers have been able to see why this is. The key player ends up being the T regulatory cell. Parasitic worms will promote a population of T_{regs} in order to increase their survival because the T_{regs} can suppress immune activity. T_{regs} were studied and it was shown that many individuals who have allergic or autoimmune disorders possess either a substantially lower population of T_{regs} or a population with compromised function. These general studies further cemented the role of microbes and parasitic worms in allergic and autoimmune diseases.

With connections becoming too apparent to ignore, the big question of what to do with this information came about. One specific idea was the therapy of adoptive transfer, where T_{regs} would be taken from an individual, would be grown in culture, tested for appropriate function, and then transferred back into the patient. It is hoped that this new and improved population of T_{regs} would benefit the patient and alleviate the disease. Potential recipients would include those with autoimmune or allergic disorders, as well as transplant patients to reduce the risk of rejection. Early clinical trials have shown promising results. Patients with T1D have been able to get off insulin. Transplant patients have been able to stop using immunosuppressive drugs. Experiments using mice have shown that allergies can be alleviated and other autoimmune disorders can be treated as well. Future trials need to clearly assess any side effects of the treatment, including the possibility of increased risk of infection and malignancies. Long term follow up needs to be planned as well to know how this therapy will work for patient many years out. It is imperative that institutions researching adoptive transfer share results with one another. This way, a clear conclusion on whether therapy works or not can be more easily made. Communication will also improve future trials and lower some barriers that adoptive transfer therapy has. Learning about the advantage of T_{regs} in therapy can advance the field of immunology and hopefully bring about alleviating the suffering of those burdened by allergic and autoimmune diseases.

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