T Cell Vaccines As An Immunotherapy For Type 1 Diabetes

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T CELL VACCINES AS AN IMMUNOTHERAPY FOR TYPE 1 DIABETES

Abstract

Type 1 Diabetes develops when CD4+ T cells aren’t taught to differentiate between self-antigens and foreign antigens during their maturation in the thymus. As a result, they attack and destroy the body’s own insulin-producing beta cells. If a vaccine can be created using CD4+ T cells attenuated with TCPTP, then, upon inoculation, the body will initiate apoptosis of circulating autoreactive CD4+ T cells. The CD4+ T cells will be isolated from diabetic peripheral mononuclear blood cells by first centrifuging the blood to fractionate it, then treating theuffy coat with a CD4+ T cell isolation kit. The isolated CD4+ T cells will be attenuated with TCPTP in order to inhibit autoreactivity and proliferation in vivo. The attenuated CD4+ T cell vaccine and placebo will each be administered to different groups of T1D patients in a double-blind clinical trial. Before and after inoculations, each participant will have their blood tested to determine the presence of circulating autoreactive CD4+ T cells. After all inoculations are complete, the participants who received the vaccine are expected to experience a reduction in circulating autoreactive CD4+ T cells, while participants who received the placebo are not expected to experience any significant change in circulating autoreactive CD4+ T cells. The reduction in circulating autoreactive CD4+ T cells in patients who received the vaccine will indicate an overall reduction of autoimmune activity within the body. The success of this vaccine could eliminate the need for an implantable device to protect beta cells in pancreatic islet transplantations, as well as benefit patients with recent-onset T1D by preserving their remaining functioning beta cells and allowing them to be less dependent on insulin.
Introduction

Type 1 Diabetes (T1D) is an autoimmune disease that usually develops in children and young adults. It is defined as the body’s inability to regulate glucose levels due to an absence in insulin production (Mayo Clinic Staff, 2014). Normally, beta cells located in the islets of Langerhans in the pancreas detect constantly changing glucose levels in the blood and produce insulin accordingly, ensuring that every cell in the body always has an energy supply and our blood glucose levels don’t get too high or too low. However, in patients with T1D, a molecular miscommunication causes the immune system to interpret the insulin-producing beta cells within the pancreas as foreign invaders. The body attacks and destroys the beta cells, thinking that it is merely eradicating an infection. As a result, the body becomes unable to produce its own insulin. This is why T1D patients must rely on daily insulin injections or insulin pumps in order to regulate their blood glucose levels.

T1D is the 7th leading cause of death in America (Hoyert & Xu, 2012). In 2012, 29.1 million Americans had diabetes, and that number has since grown exponentially (American Diabetes Association, 2014). Of these 29.1 million people, 8.1 million were undiagnosed, indicating that these individuals likely were not managing their disease well. If T1D is left untreated, it can cause many long-term complications such as cardiovascular disease, stroke, eye damage, coma, and death.

T1D is a prevalent and growing issue, not only in America, but worldwide. True, the disease may be manageable with daily insulin, but that doesn’t lessen the impact it can have on individuals’ lifestyles. The peak age for T1D diagnoses in America is 14 (Norman, 2014). It’s not uncommon for a teenager with T1D to be judged by his/her peers—having to wear an insulin pump or self-administer insulin injections, pricking fingers to test blood glucose levels, not being able to exercise for extended periods of time, or even just having to refuse a certain food with too many carbohydrates is enough to make anyone self-conscious.
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Background

In T1D, the insulin-producing beta cells within the pancreas are destroyed by T cells. T cells are a type of lymphocyte, along with B cells. Both types of lymphocytes are part of the immune system, and when the body becomes infected, they work together to identify and destroy the pathogen. This same process occurs in the development of T1D, except that something causes certain T cells to recognize the beta cells as a foreign pathogen and, accordingly, mount an autoimmune attack to destroy them. If the immune system of a T1D patient could learn to differentiate between normal T cells and autoimmune T cells, theoretically, it should induce apoptosis of all autoimmune T cells in the body and stop the self-destruction of beta cells. This could possibly be achieved with a T cell vaccine.

Attenuated vaccines work by administering a weakened form of a virus—not enough to induce illness, just enough to be noticed by the immune system. The body then produces specific T cells to destroy the virus and constantly “patrol” the lymphatic system for reinfection, thereby inducing lifelong immunity to that virus. If this same process could be used to weaken the autoimmune T cells responsible for mediating T1D and then administer those cells as a vaccine, the body should likely react in the same way, essentially making the body immune to T1D.

A vaccine to defend beta cells against autoimmune attacks could eventually assist in a treatment to cure T1D. Pancreatic islet transplantation is an experimental procedure that involves transplanting donor beta cell-containing islets of Langerhans into a patient with T1D. As a result, the patient becomes partially or completely independent from insulin injections. However, these results are only temporary, because the transplanted islets are eventually destroyed by the autoimmune mechanisms that caused the disease in the first place. A team of researchers at Harvard recently published a breakthrough in their goal to simplify this procedure. They managed to mass-produce beta cells from human embryonic stem cells, making the treatment much more readily available to patients. However, there’s still the matter of
the autoimmune mechanisms threatening the survivability of the transplanted beta cells. The team is currently developing an implantable device to protect the cells, but that means a surgical procedure will be necessary (Pagliuca, et al, 2014). It also opens a number of doors to the possibilities of implant rejections, infections, etc. Therefore, if the patients who receive the beta cell transplant could also receive a vaccine that prevents their autoimmunity from recurring, the need for a surgical procedure and an implantable device would be eradicated.

Assuming T1D patients seek a diagnosis immediately upon the onset of symptoms, they should experience a “Honeymoon Phase” in which the pancreas still contains a few functioning beta cells able to produce their own insulin. For many years, extensive research and funding has been dedicated to figuring out how to extend this phase and make T1D patients less dependent on insulin. Hypothetically, if a T cell vaccine were to be administered at the time of diagnosis, the autoimmune destruction of beta cells would stop, and the remaining beta cells would enable the patient to be less dependent or even independent from insulin.
Previous Work

The bone marrow is responsible for producing lymphocytes in the body. Once these lymphocytes are released into the bloodstream, some of them travel to the thymus to mature into T lymphocytes, or “T cells” for short. After a long process of differentiation within the thymus, the surviving T cells reach an area called the corticomedullary junction. Here, dendritic cells and macrophages present different self-antigens to the T cells. Self-antigens are molecular substances on cell’s surfaces that provoke antibody production in the case of infection. These self-antigens are presented to naïve T cells through little bridges created by Major Histocompatibility Complex (MHC) molecules on the surface of dendritic cells and macrophages. If the T cells bind tightly to the MHC molecules, this indicates potential autoreactivity against the organism that produces the given self-antigen. These cells are deleted by apoptosis, while the T cells tolerant to all self-antigens are released into the immune system. In the case of T1D, certain T cells mediate the destruction of the insulin-producing beta cells in the pancreas. Therefore, it can be assumed that the self-antigens indicative for beta cells (including insulin and certain peptides) are not presented properly by the dendritic cells and macrophages in the corticomedullary junction, resulting in autoreactive T cells surviving the maturation process through the thymus.

There are four basic categories of T cells. In simple terms, Regulatory T cells help keep the immune system running smoothly at all times, Memory T cells keep a long-term memory of different pathogens to allow immunity, Cytotoxic T cells kill foreign pathogens in the body, and Helper T cells detect the pathogens and signal for Cytotoxic T cells to come attack them. One common type of Helper T cell is CD4+. It is hypothesized that CD4+ T cells are responsible for mediating the autoimmune process in T1D. This hypothesis is supported by previous experiments with mice. In one study, the
CD4+ T cells of Non Obese Diabetic (NOD) mice were removed and purified, then transplanted into healthy mice. Within days, the healthy mice developed T1D (McDevitt & Unanue, 2008).

Attenuated viral vaccines are created using a weakened form of the target virus. The most common method for attenuating viruses is to grow them repeatedly either in cell cultures or in non-human embryos (typically chick embryos). Often, viruses are grown through cell cultures or embryos more than 200 times (History of Vaccines, 2014). While being grown through these cell cultures or embryos, the virus gets more used to replicating in these environments, and less used to replicating within a human body. After a certain number of growths, the virus will eventually become completely unable to replicate in a human. At this point, the viral cells are harvested and purified, then administered as a vaccine. Upon administration, the body will induce an immune response and attack the virus. When the immune system fights a viral infection, nonspecific B cells begin attacking the virus antigens. Meanwhile, helper T cells mediate the action of killer T cells, which attack and kill the infected body cell directly. After the virus is completely eradicated, some T cells will become memory T cells, meaning they’ll constantly “patrol” the lymphatic system for that specific virus in order to stop it in its tracks should it ever reenter the body.

Because T cells are not viral, traditional methods of attenuation are not possible. However, research has shown that an enzyme called T Cell Protein Tyrosine Phosphatase (TCPTP) can attenuate the activation and proliferation of T cells in vitro (Wiede, et al, 2011). In the study, naïve CD4+ and CD8+ T cells were isolated in clusters and stimulated with a TCPTP deficiency, then monitored for changes in lymphoblast formation and the expression of several cell-surface antigens involved in cellular interaction. After 48 hours, the T cells showed a significant increase in proliferation, as well as expression of cell-surface antigens. If a TCPTP deficiency caused an increase in the activity of CD4+ T cells, then, logically, an increase in TCPTP should attenuate their activity. Therefore, if the autoreactive
CD4+ T cells of a T1D patient were to be stimulated with TCPTP in vitro, they would decrease in autoreactive activity, and, when administered as a vaccine, would instruct the immune system to initiate apoptosis of all circulating autoreactive CD4+ T cells, leaving only healthy CD4+ T cells.

Currently, there are no published studies that specifically focus on creating a T cell vaccine to treat T1D; however, there have been extremely promising clinical trials of T cell vaccinations for Multiple Sclerosis (MS), another autoimmune disease that is similar to T1D in terms of pathogenesis. MS is a debilitating disease characterized by the deterioration of myelin sheaths, which protect nerve fibers. The most common complication of MS is trouble walking or simply moving in general; in severe cases, MS patients completely lose the ability to walk. MS is thought to be caused by the activation of a certain T cell that is autoreactive with Myelin Basic Protein (MBP), which is responsible for generating and maintaining myelin sheaths. In the pilot clinical trials, researchers isolated and cloned these MBP-reactive T cells from cerebrospinal fluid, then irradiated them to prevent proliferation in vivo. The vaccine recipients were each inoculated with 3 vaccinations at intervals of 2-4 months. There were no adverse side effects, and after 3 vaccinations, there was no detection of MPB-reactive T cells in the cerebrospinal fluid of all patients, indicating an overall reduction in the autoreactive T cells that cause MS (Zhang, 2004).
Problem Statement and Hypothesis

Type 1 Diabetes develops when CD4+ T cells aren’t taught to differentiate between self-antigens and foreign antigens during their maturation in the thymus. As a result, they mediate the destruction of the body’s own insulin-producing beta cells.

If a vaccine can be created using CD4+ T cells attenuated with TCPTP, then, upon inoculation, the body will initiate apoptosis of circulating autoreactive CD4+ T cells.
Experimental Design

The goal of this experiment is to create a vaccine that will benefit T1D patients by reducing or eliminating the circulating autoreactive CD4+ T cells that mediate T1D. This will help in the ultimate goal to find a cure for T1D in many ways, such as eliminating the need for an implantable device to protect beta cells in pancreatic islet transplantations, as well as protecting said beta cells from being destroyed by the immune system after transplantation. The vaccine will also allow recent-onset diabetes patients to lessen or even eliminate their future dependence on insulin.

The clinical trial phase of this experiment is to be double-blind with volunteer participants. All participants must be T1D patients (not recent-onset). All participants must be on no prescription medication and have no health complications aside from T1D. Additionally, all participants must not have suffered any recent illnesses prior to the trial, as an infection will have stimulated the immune system and yield inaccurate T cell measurements. Any participant who becomes ill during the trial will be dismissed.

Materials

- Type 1 Diabetic peripheral mononuclear blood cells
- CD4+ T cell isolation kit
- TCPTP enzyme
- Petri dishes
- Micropipettes and tips
- Feeder cells
- Incubator
- Centrifuge
- Syringes and needles
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Variables

- Independent: Attenuated CD4+ T cell vaccine
- Dependent: Amount of circulating autoreactive CD4+ T cells in vaccine recipients
- Control: Placebo vaccine and its recipients

Procedure
It may seem like this experiment would be much more efficiently conducted using laboratory mice rather than humans, but there is a big drawback that makes this impossible. When researchers order Non Obese Diabetic (NOD) mice to study T1D, the supplier induces the diabetes in otherwise healthy mice by injecting them with streptozotocin (STZ). STZ is a compound that has a preferential toxicity towards pancreatic beta cells (Graham, et al, 2011). Once injected, the STZ travels to the pancreas and selectively attacks beta cells, and the mice develop T1D. This is why the experiment would not work with NOD mice—they have T1D, but not the underlying autoimmunity that causes the disease in humans. Taking the CD4+ T cells from NOD mice would yield no results, as there would be no autoimmune factors.

Data

The most important data to collect will be the results of the inoculations. The number of circulating autoreactive CD4+ T cells will be measured for both the vaccine and placebo recipients by testing blood samples. These numbers will be tested and recorded for each participant before and after inoculations in order to detect changes in CD4+ T cell activity.

Anticipated Results

The participants who receive the CD4+ T cell vaccine are expected to experience a decline in circulating autoreactive CD4+ T cells. The participants who receive the placebo vaccine are not expected to experience any significant change in circulating autoreactive CD4+ T cells.
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