Using Zinc Finger Nucleases and Homologous Recombination to Treat HIV/AIDS

Section 1: Idea

Introduction:

HIV/AIDS is a sexually transmitted infection that is transmitted by coming into contact with infected bodily fluids. According to the World Health Organization (WHO), it is the number 6 cause of death in the world, killing about 1.5 million people each year, and the current lifetime cost of treating HIV is about $379,000. In industrialized countries this maybe a possible option, but in third world countries, where the disease is most prevalent, if the treatment is even available, most people are not able to afford it. Some people are born with immunity to HIV/AIDS due to a genetic mutation, which causes misshapen proteins to form on the surface of their white blood cells. The deformed proteins prevent the HIV/AIDS virus from binding to the cells and ultimately infecting them. Using gene therapy, we can manually insert this mutation into cells and prevent them from getting infected by the HIV/AIDs virus.

Background:

The HIV/AIDS virus primarily binds to and replicates in CD4 T-cells, a type of white blood cell that is key in fighting off infections. Once it enters the cell the virus begins to replicate, and once the replication process is complete, the virus particles lyse the host cell and spread to replicate in other cells. The constant lysing of cells leads to a drastic drop in the number of CD4 T-cells in the body. In healthy uninfected individuals, a normal CD4 count, which measures the number of healthy and functioning CD4 T-cells in the blood, ranges from 500 cells per cubic millimeter to 1,200 cells per cubic millimeter. Once a person's CD4 count drops below 200 cells per cubic millimeter, they are considered to have AIDS, the final stage of the HIV infection (5). As the immune cells are being destroyed the body looses its ability to fight off infection, and the patient usually dies from an opportunistic illness.

In order to enter the cell and begin replication, the virus must first bind to receptor proteins on the surface of the T-cell. The main binding protein for the virus is known as the CCR5 protein, which is coded for by the CCR5 gene. In some people, there is a mutation in the CCR5 gene resulting in a new gene called CCR5-delta 32. The mutation is the result of a 32-base pair deletion in the CCR5 allele (10), and the mutated version of the gene codes for a misshapen version of the CCR5 protein that the virus cannot bind to. Figure 1 shows, how the lack of the correct protein expression prevents most forms of the virus from binding to T-cells, essentially making these individuals immune to

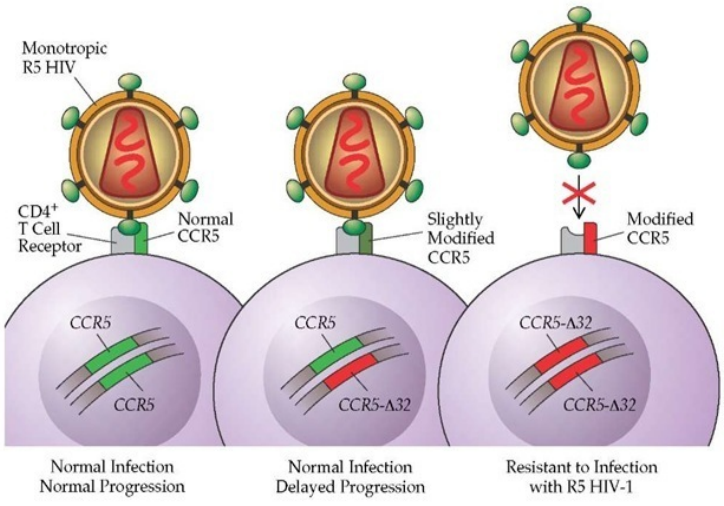

Figure 1. In individuals who have one copy of the allele that codes for the delta 32 mutation, the CCR5 receptor is only slightly misshapen, and the HIV/AIDS virus is still able to bind to it, but the progression of the disease is slow. When both copies of the allele code for the delta 32 mutation, the CCR5 protein is completely dysfunctional, and virus cannot bind to it (9).

HIV/AIDS virus. If we are able to genetically modify the CCR5 gene to inhibit the correct production of the CCR5 surface protein, then we are able to prevent the virus from replicating in the body. Decreasing the replication of the virus in people who are already infected with HIV will greatly decrease the viral load and possibly cure those who are infected.

Zinc Finger Nucleases (ZFNs) are small proteins that act as artificial restriction enzymes. To create ZFN's Zinc Finger Proteins are combined with Fok-1 restriction enzymes (11). Zinc Finger Proteins (ZFPs) are the binding domains of the complex. Each ZFP can recognize three to four base pairs in a DNA sequence, and it binds to the DNA by inserting its alpha-helix structure into the grooves of the double helix of the DNA (4). ZFPs can be created to target different domains on the DNA, and multiple ZFPs can be paired together to generate a recognition site of nine to eighteen base pairs (4). Fok-I endonucleases are able to recognize specific DNA sequences and cleave the DNA at the target region (4). Figure 2., shows how these complexes are paired together so the ZFP functions as the binding domain, which identifies and binds to the target sequences in the DNA, and the Fok-1 endonucleases make the cuts in the DNA. The combination of these two complexes creates a novel protein, which is highly specific and has very accurate genome-editing capabilities. With this technology we can target the specific DNA sequence, which codes for the CCR5 surface protein. We can then cleave or cut the DNA at this specific region, and insert the CCR5 delta 32-gene to produce the misshapen surface protein. As the number of CCR5 negative CD4 T-cells in the body increases, the number of replicating viruses will decrease. This decrease in replication will help the immune system fight off the infection and ideally destroy the all of the virus particles within the body.

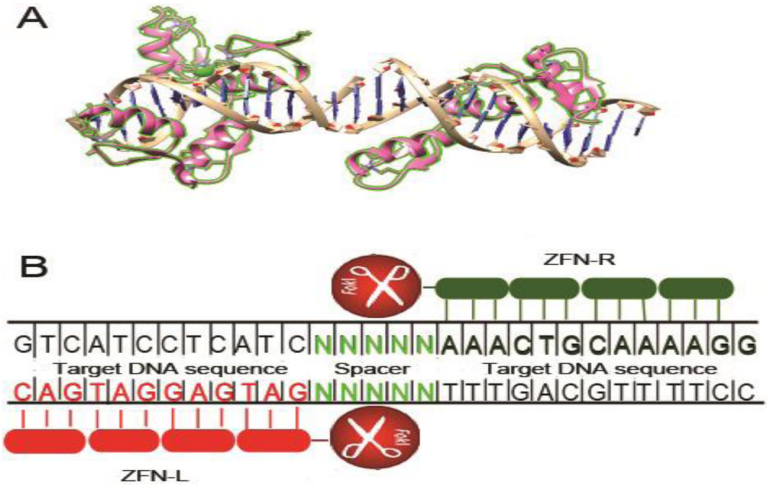

Figure 2. A. ZFN bind to DNA by using its alpha-helix structure to wedge itself into the grooves of the double-helix structure of DNA. B. Zinc Finger proteins act as the binding domains in ZFN; they identify the target nucleotide sequence in the DNA. Fok-1 restriction enzymes cleave the DNA at the targeted sequence. There are essentially two parts to the ZFN a left half and right half, which are both imperative to form the double stranded breaks in the DNA (11).

After ZFNs cause the double stranded break in the DNA, the gene is edited by using either homologous recombination (HR) or non-homologous end joining (NHEJ). In HR, a corrected donor gene is inserted into the genome to replace the gene that was cut out (4). When using NHEJ, the DNA is left to repair and rejoin itself. This process can result in the random insertion and or deletion of nucleotides at the target site, which in turn, can result in defunct or "knocked out" genes (12). Figure 3. shows that in HR a donor gene is inserted into the genome, rejoining the ends in the target sequence that were cut by the ZFNs. In NHEJ, no gene is used to rejoin the ends, so the random insertion and or deletion of nucleotides results in a knocked out gene. Both of these processes can be applied to mutating the CCR5 gene. By using HR we can insert a constructed gene that contains the same sequence as the CCR5-delta 32 gene. The insertion of this donor gene would result in a modified version of the CCR5 surface protein, one

that the HIV/AIDS virus could not bind to. We can also prevent the production of the protein through NHEJ. If the CCR5 gene is cut out of the genome, then the CCR5 protein cannot be coded for; and in turn, it would not appear on the surface of the T-cells. Theoretically either method of causing the CCR5 mutation should prevent the virus from infecting the T-cells; however, the CCR5 protein is the main receptor that the virus binds to, but it is not the only receptor. The virus also binds to a CD4 co-receptor and by knocking out the gene completely the virus may still be able to enter the cells (11).

Figure 3. After ZFN makes double stranded breaks in the DNA, the DNA is pieced back together in one of two ways. In non-homologous end joining, there is a random insertion and or deletion of nucleotides, which leads to a gene being completely dysfunctional or knocked out. In homologous end joining a donor gene is inserted into the DNA, where the target sequence was cut out. The new gene functions to either replace, act as a tag, or to correct the gene that was targeted. (4)

Significance and Innovation:

High Active Antiviral Therapy (HAART) is the current treatment for HIV/AIDS. It includes a series of pills, which slow the replication process of the HIV/AIDS virus; however, HAART cannot rid the body of the virus so it is a lifetime treatment. The lifetime treatment of the disease greatly correlates with its highly expensive cost, which can reach up to $379,000. The treatment process entails a drug cocktail of multiple pills that must be taken daily. Which cocktail a patient receives is determined by many factors such as, the viral strain, the CD4 count, any other medical issues the patient may have. Figure 4 shows how by not receiving or discontinuing the antiviral therapy, the virus can quickly replicate, opening the door to a whirlwind of issues and infections. The increase in viral load leads to the lysing of copious T-cells, and as a result the immune system is greatly weakened. This weakened immune system makes the patient susceptible to many ailments, which are even harder for the body to fight off because of the already weakened immune system. When taking multiple pills a

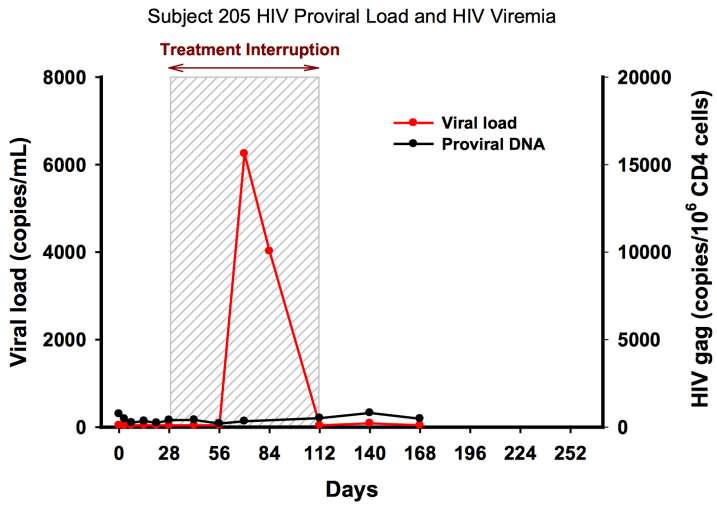

Figure 4. After stopping HAART, patients were subjected to gene therapy HIV treatment. Once the HAART treatment stopped the viral load increased drastically. Once the gene therapy was given the viral load quickly declined to its original value. (12)

day it is not hard for a patient to forget a pill or even multiple pills a week, an event that can greatly impair his or her health. By using gene editing, we can create and infuse patients with CD4 T-cells that do not contain the CCR5 receptor. By infusing patients with the modified CCR5 negative T-cells, we are decreasing the number of available cells for the virus to bind to and replicate in. If the virus cannot replicate than the number of virus particles in the patients body should drastically decrease. Because the modification is permanent in those selected cells, this process should require little to no daily monitoring. As the number of viral particles continues to drop the immune system should strengthen and be able to help fight off the viral infection. Eventually, with the help of gene therapy the body should be able to eradicate the virus. Hopefully as the use of this technology increases and improves, it will become cheaper and widely available to multiple people in a variety of social classes.

Section 2: Experimental System

Objective. To determine if the infusion of CD4 T-cells that are negative for the CCR5 protein, will decrease the viral load of individuals who are infected with the HIV/AIDS virus.

Inserting the Mutation. To produce cells that are negative for the CCR5 receptor, autologous CD4 T-cells will be subjected to electroporation because the process is quick, easy, and it can be applied to multiple cells. During this process, cells will be suspended in a conductive solution, and a small electrical pulse will be generated. The pulse will disturb the phospholipid bilayer of the autologous CD4+ T-cells, which in turn will create small, temporary pores in the cell membrane. The small pores will allow the ZFNs to enter the cell. Once they enter the cell, ZFNs will produce double stranded breaks in the DNA at the region that codes for the CCR5 receptor. Through homologous recombination, a constructed CCR5-delta 32 gene will be inserted into the genome. This new gene, will code for the dysfunctional, misshapen version of the CCR5 surface protein.

The experiment will be performed ex-vivo on a humanized-mouse model. All of the mice models will be infected with the virus and randomly assigned into groups. Each of the groups will be watched until it reaches a predetermined stage in the disease. In other words, the mice will be infected with the virus and observed until their CD4 count reaches a predetermined level. Once the desired level is reached, the group of mice will be infused with the autologous CD4 T-cells that are negative for the CCR5 surface protein. This testing method will show how the treatment affects the viral load at different stages of the disease. In each group, there will be a small percentage of mice that act as a control and do not receive any treatment. All of the mice will be tested every few days to monitor the changes in the viral load and the T-cell count.

Future Goals:

If this treatment method works it can be applied to a multitude of viruses that cause chronic infections that lead to disease. For example, the Hepatitis C virus is the blood borne version of the hepatitis virus that causes chronic hepatitis infection. This chronic infection can lead to liver diseases, which currently kills about 500,000 people each year (8). Through similar gene editing techniques used in the creation of the CCR5 negative CD4 T-cells, we can construct cells that are protected from infection by the Hepatitis virus. After identifying which cells the virus targets and which surface proteins the virus needs to enter those cells, we can use the ZFN nucleases to cut and remove the gene that codes for those proteins. If genes that make people immune to hepatitis C exist, then through homologous recombination, they can be inserted as a replacement gene in the genome. If no such gene exist or cannot be created through current lab

techniques, then non-homologous end joining can be used to knockout the gene that codes for the protein. Either method would result in a dysfunctional gene, and in turn, would either produce cells that have a mutated protein or cells that do not display the protein. This will prevent the virus from binding to the cells; and therefore, prevent the infected individual from developing liver disease. These methods can be used to prevent the development of copious diseases that are caused by chronic viral infections.

Gene editing can also be used to treat different forms of cancer. Research has shown that some viruses can lead to the development of some cancers such as Hodgkin's Lymphoma, Cervical Cancer, and Liver Cancer. These viruses, known as oncoviruses, target healthy cells and mutate them by inserting mutations into their genome, and these mutations lead to the development of cancer. Like all viruses, oncoviruses can only replicate inside cells, and each virus must bind to a specific receptor on a specific cell. For the oncoviruses that cause chronic infection, using ZFNs and other gene editing techniques, the receptors for the viruses can be removed from the cells. If the virus cannot infect the cells, then the cells will not receive the mutation that the virus induces during its replication process, and the cells will not become cancerous.

Another possible implication of this gene editing techniques, though it has some moral and ethical backlash, is the creation of embryos that are immune to common life threatening diseases. The HIV/AIDS virus currently infects about 35 million people worldwide, and about 3.2 million of those individuals infected are under the age of 15 (7). By using the previous techniques to insert the CCR5-delta 32 gene into germ cells, we can create children who are born completely immune to most forms of the HIV/AIDS virus. As gene editing becomes more prevalent and costs for it decreases, this could be extremely beneficial. Individuals, who receive the mutation before birth, would pass that genetic mutation to their offspring, who can then pass the mutation to their offspring. Overtime, this prevention method becomes cheaper than the current treatment for disease. Instead of paying $379,000 in a lifetime to treat a disease, individuals would be able to pay a single price to prevent the disease for generations. For areas that have high HIV/AIDS rate, this process has the opportunity to save many lives. As the number of people who are born immune to the virus increases, the incident rate of the disease should greatly decrease. Theoretically, if this happens then the diseases should eventually be able to be eradicated from the population. Even though there is currently a lot of controversy, about "designer babies" and using gene editing to create children with specific desired traits, using gene editing to give people immunity to lethal infections and diseases that deprecate the quality of life should be considered.

All in all, the use of ZFN and gene editing technology can be used to treat and prevent a variety of diseases that are caused by chronic viral infections. By targeting genes that code for different surface proteins we are able changing the expression of the proteins, and prevent viruses from being able to bind to and replicate in the cells. If the virus cannot replicate, then they cannot cause disease. These processes can be applied to somatic cells and germ cells, allowing people to be immune to specific viral infections before birth. If it is successful and somewhat widely accepted by the general public, this process has the opportunity to save millions of lives worldwide.

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