IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 19, Issue 3 Ser. 2 (May. – June. 2024), PP 27-31
www.iosrjournals.org

Creating an Experimental Vaccine to Prevent Microcephaly Caused By the Zika Virus

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ABSTRACT
I noticed that a vaccine for Zika hadn't been discovered yet, even with mRNA vaccine technology having been an area of interest in the scientific community since the beginning of the pandemic, so I made the goal of my project to create a blueprint of a potential mRNA vaccine for the Zika virus. I researched possible ways of approaching this vaccine by looking at Zika's connection with viruses in other successful mRNA virus vaccines, such as the COVID-19 vaccine, and the recently developed mRNA flu vaccine. I also looked at previous proposed Zika vaccine models. I found out that mRNA vaccines work by using the mRNA of the virus proteins to create fake proteins that will latch onto host cells. Though these proteins pose no health risk, the body recognizes them as a threat, and fights against them, creating antibodies. These antibodies are shown to be remembered by the body when an actual infection happens. I also found out that the Zika virus affects fetus brains by binding to a receptor called AXL, which is responsible for brain cell proliferation and survival. Once Zika gets into one of these glial cells, it is able to affect the rest of the brain as it can travel axonally. This is why I am aiming to create an mRNA vaccine that contains the RNA of the protein Zika uses to bind with the AXL receptor. If this vaccine is used, it should be able to also help pregnant mothers, as the antibodies produced by the mothers would cross the placental barrier, and therefore make the baby more resistant to microcephaly.

Date of Submission: 26-06-2024 Date of Acceptance: 05-07-2024

I. INTRODUCTION

When a virus attacks the body, it attaches itself to a cell and injects its DNA or RNA into it, and makes the cell into what is essentially a virus cloning factory. Usually, it takes a couple days for the immune system to recognize that the virus factories exist in the body and fight against them, which is why we get sick. When the body fights these factories, it attaches antibodies to the viruses, making them unable to latch onto cells. Both free floating viruses and those attached to cells can be targeted by antibodies. These antibodies mark the viruses they are attached to for destruction .

What mRNA vaccines do is isolate the protein that the virus latches onto cells with in mRNA form, and then go into the body and tell the cells in the body to make this protein. When the proteins are formed, they start acting like they would if they were part of the actual virus and start latching onto host cells and floating through the bloodstream (Komaroff, 2021). The body recognizes these proteins as "non-self" and perceives these proteins the same way it would an actual viral invasion, and thinks it's under attack. In response to this "attack," the body creates antibodies for the proteins. These antibodies are remembered by the body for future infections, and when it is truly infected with the virus, it is easier for the body to fight against it.

The first reported case of the Zika virus was in Brazil in May 2015 (*Zika Virus*, 2014). The disease is spread by the bite of the Aedes Albopictus and Aedes Aegypti mosquitoes, both of which lay eggs in stagnant water.
Symptoms of this disease include fever, red eyes and joint pain, but most people infected with the virus don't end up getting sick at all. The biggest issue with the Zika virus is that it can linger in a person's semen and blood for months.
During this timespan, the virus can be transmitted sexually (Vanegas et al., 2021). This means that it can be accidentally transferred to an unborn baby.
Zika causes severe fetal brain defects, the most common being microcephaly, a condition that signifies incomplete brain development.
Microcephaly is a condition in which a baby's head is abnormally small, meaning that the brain is abnormally small as well. Microcephaly can occur because the baby's brain stopped growing early, or didn't develop properly during pregnancy. Microcephaly can also be classified as severe microcephaly, where a baby's head is much, much smaller than expected.
Severe microcephaly can occur because a baby's brain started to develop correctly and was damaged during the pregnancy, or had not developed properly at all during pregnancy. Microcephaly greatly decreases quality of life

for the child in most cases. Problems linked to microcephaly include seizures, problems with movement and balance, difficulty swallowing, and hearing and vision problems. Microcephaly can also cause developmental delay, such as problems with speech, sitting and walking, and intellectual disability, which is the decreased ability to function and learn in daily life (*Microcephaly | Birth Defects*, 2016).

Microcephaly often occurs in the children of people affected by Zika. This is because Zika virus contains two proteins called NS4A and NS4B (Klaitong & Smith, 2021). NS4A plays a role in viral replication, and NS4B interacts with other nonstructural proteins and binds viral DNA. When Zika infects a fetus, it is able to easily travel to the developing brain. There, it interacts with AXL receptors, which regulate cell proliferation and survival.

The virus then injects its DNA into the developing glial cells, which disturbs the proliferation and differentiation of the oligodendrocyte progenitor cells and leads to a complete stop in oligodendrocyte development. This leads to microcephaly.

The NS4A protein also causes mutations in the Ankle2 gene, which is responsible for fetal brain development. When this gene is mutated, it slows down the overall development of the fetus's brain and disturbs the production of the Ankle2 protein. Zika contains two proteins called NS4A and NS4B, and these proteins interact with Ankle2 and cause the cell development in the fetus's brain to slow and for these cells to mutate (Link et al., 2019).

The NS4A and NS4B proteins also interact with the Ankle2 protein. Ankle2 is a protein that is associated with microcephaly in humans, and is known to interact with this Zika protein. The loss of Ankle2 reduces brain volume and impacts the function of the Par complex, which is to regulate cell polarity. This causes the cell development in the fetus's brain to slow.

Certain types of cells in the fetus are eliminated completely (Link et al., 2019).

As of 2024, there are multiple mRNA vaccines for Zika under development and testing, but none work in the way that I've proposed. One old proposed Zika vaccine was a DNA vaccine, and DNA vectors encoding 2 viral proteins called PRM and E protein were prepared from a Brazilian Zika virus strain. It was tested on mice intramuscularly, and 3 weeks later the mice were found to have antibodies to the E protein, which could block infection. While this vaccine never made it to human use, due to vaccine approaches to similarly built viruses, a blueprint vaccine for Zika can be created.

The COVID-19 vaccine was the very first successful and widely used mRNA vaccine. It was created in a very short span of time to respond to a global crisis. Coronavirus uses a protein on its surface to attach to and enter cells.

Antibodies that bind to the protein can block COVID from attaching to cells.

Covid mRNA vaccines teach the body to create these antibodies, since the mRNA in the vaccine teaches cells to produce proteins that are on the actual Coronavirus and stimulate an immune reaction in the body (Trafton, 2020).

The COVID-19 mRNA vaccines inspired the experimental Influenza mRNA vaccine. The new experimental mRNA flu vaccine guards against all 20 known subtypes of influenza. This approach makes it a multivalent vaccine, which is a vaccine that is able to protect a person against several diseases, or in this case, several strains of a single pathogen. It even gives the patient a vague sense of immunity toward new, mutated strains of the flu. It uses the same mRNA technology as the Pfizer and Moderna COVID-19 vaccines. This vaccine, when injected into recipients, starts producing copies of the protein the flu uses to latch onto host cells, called hemagglutinin, for all 20 hemagglutinin subtypes (H1-H18 for Influenza A, and two for influenza B) (Hohl, 2021).

On average, a traditional vaccine takes five to ten years to develop. At the fastest speed, they take around 2 years, but mRNA vaccines are way faster to develop. For example, the COVID-19 vaccine was released less than ten months after the genetic sequence of the virus was published. This is because mRNA vaccines are extremely versatile and malleable, due to the fact that the mRNA in question can be easily manipulated. This means that with mRNA vaccines, the treatments for many diseases can be generated rapidly. mRNA vaccines are thought of as a viable possible treatment to some types of cancers, since they can be easily modified to suit a patient's specific needs. Traditionally, vaccines contain either killed or weakened forms of a virus or bacteria, and are meant to provoke an immune response in the body. Once the body knows how to fight the weakened or dead pathogen, it will remember how to fight said pathogen if it is ever infected for real.

However, instead of containing a virus or a viral protein, mRNA vaccines simply carry the genetic sequence for the viral protein. This leads the body, much like it does when infected by a virus, to create copies of the viral protein, stimulating the immune system to respond without infecting it, since there is no actual pathogen in the vaccine. The vaccine is easily synthesized because once developers know the sequence of the viral protein they need to target, they simply have to find the genetic code for it.

mRNA vaccines are also significantly easier and faster to produce than traditional vaccines. This is because it does not require the use of cell cultures, unlike traditional vaccines. Because of its fast reaction time, the risk of contamination is lower than what was observed with other complex vaccine making processes (Prazeres et al.). As opposed to traditional vaccines, where a big factory is required to make the protein or virus as well as the time to grow them, mRNA vaccines need no such thing. If mRNA is injected into a person, the person's body turns into the factory, and creates the proteins (that are harmless) to stimulate the immune response on its own (Trafton).

However, one negative to mRNA vaccines is that they can break down at high temperatures, which is why they need to be stored at around -70 degrees celsius. mRNA vaccines can be made more stable by adding stabilizers that remove water from the vaccine through lyophilization, which has allowed some mRNA vaccines to be stored in a refrigerator instead of a freezer (Sousa Rosa et al., 2021).

II. RESULTS

When a virus attacks the body, it will attach itself to a cell and inject its DNA or RNA into it, and make the cell essentially into a virus cloning factory. Usually, it takes a couple days for the immune system to recognize that the virus factories exist and fight against them, which is why we get sick. When the body fights these factories, it attaches antibodies to the viruses, making them unable to latch onto cells, as well as marking them for destruction. What mRNA vaccines do is isolate the protein that the virus latches onto cells with in mRNA form, and then go into the body and tell cells to make this protein. When the proteins do what the virus makes them do, which is latch onto host cells, the body thinks it's actually under attack and creates antibodies for them. These antibodies are remembered by the body for future infections. It's important to note that the vaccine does not have to replicate the protein that latches onto other cells, but rather can stimulate the body into making antibodies by replicating any protein on the surface of the virus.

Coronavirus uses a protein on its surface to attach to and enter cells. Antibodies that bind to protein can block COVID from attaching to cells. Covid mRNA vaccines teach the body to create these antibodies, since the mRNA in the vaccine teaches cells to produce the spike proteins that cover the actual Coronavirus and give it its name. The mRNA is wrapped around a layer of fat particles that protect it and help it get taken up by cells in the immune system called dendritic cells, where the mRNA stays in the cytoplasm and doesn't interact with anything, but waits with other mRNA molecules to create enzymes that the body needs.

When ribosomes read the vaccine mRNA, pieces of the viral surface spike protein are made, which are then displayed on the surface of the dendritic cell. The dendritic cell travels to a nearby lymph node, where it presents the surface proteins to other cells of the immune system. Helper T cells train B cells to make antibodies that will fit perfectly on the surface protein of the virus, and cytotoxic T cells can kill virus infected cells.

The new experimental mRNA flu vaccine guards against all 20 known subtypes of influenza. This approach makes it a multivalent vaccine, and gives the patient a vague sense of immunity toward even new, mutated strains of the flu. It uses the same mRNA technology as the Pfizer and Moderna COVID-19 vaccines. This vaccine, when injected into recipients, starts producing copies of the protein the flu uses to latch onto host cells, called hemagglutinin, for all 20 hemagglutinin subtypes (H1-H18 for Influenza A, and two for influenza B).Tests in animals show that the vaccine works correctly and that it greatly reduced illness symptoms and protected patients from death, even when the animals were exposed to mutated flu strains. This means that the vaccine gives people a baseline level of immune memory to diverse flu strains, so that mutated flu strains will also be somewhat protected against. Rather than providing complete immunity, the vaccine stimulates a memory immune response that can quickly be retrieved by the body and adapted to new flu strains.

One old proposed Zika vaccine was a DNA vaccine, and DNA vectors encoding 2 viral proteins called PRM and E protein were prepared from a Brazilian Zika virus strain. It was tested on mice intramuscularly, and 3 weeks later the mice were found to have antibodies to the E protein, which could block infection. Then these immunized mice were injected intravenously with 100 plaque forming units of Zika virus (Brazilian or Puerto Rican). No Zika was detected in the immunized animals who had received the DNA vaccine. Animals that weren't inoculated or received DNA vectors alone had high levels of Zika in their blood. This showed that a single dose of Zika virus DNA vaccine prevents infection in mice

III. DISCUSSION

Zika affects the brain in both fetuses and adults by latching onto AXL receptors, using them as an entry point into host cells. The viral envelope protein E binds to the AXL receptor, and allows the virus to inject its DNA into the glial cells, which disturbs the proliferation and differentiation of the oligodendrocyte progenitor cells and leads to a complete stop in oligodendrocyte development.

Interaction with the AXL receptor has also been shown to enhance the replication of the virus in host cells, making it more efficient and allowing the virus to wreak more havoc on the body. The activation of this receptor can also dampen the host's immune response.

The AXL receptor signaling pathway can suppress the production of interferons, which are important antiviral molecules. This allows Zika to evade the host's defenses and establish a successful infection.This eventually leads to microcephaly. My vaccine targets the AXL receptor by using a protein mRNA to replicate the Zika virus surface E protein, and inducing a fake immune response to it in order to create antibodies. Replicating the protein E targets the AXL receptor because Zika's E protein is what binds to AXL receptors in the brain. With this logic, the E proteins created by the mRNA vaccine should be able to stage a fake viral Zika infection for the body to fight against and formulate antibodies for.

My final outline should work, as this is the way the COVID-19 vaccine was created, and the COVID vaccine proved to be very successful. My final outline might not work because the Zika virus might have required a more multivalent approach, similar to the flu mRNA vaccine I researched. The Zika virus does bind to multiple receptors in the brain, and though the AXL receptor is the most important receptor, it isn't the only one. There aren't any studies that completely overlap with my project or its findings, which is why it has potential to one day become an actual Zika vaccine.

There are a few receptors on the surface of glial cells that are useful for the Zika virus to get into the host cell. Once Zika crosses the placenta, AXL receptors (which reside in the glial cell), however, which are responsible for stimulation of cell proliferation and survival, are the most important receptors for the Zika virus to gain entry into the glial cells and replicate. This hurts the production of oligodendrocytes in the fetal brain, which are the cells responsible for the myelination of neurons. If neurons are not myelinated, then they become faulty and cannot send signals to each other efficiently. In the brain developmental stage, the shortage of oligodendrocytes can lead to brain disorders such as microcephaly in babies. This means that my vaccine needs to mainly target this receptor by carrying the mRNA for a protein that will bind with the AXL receptor. If the vaccine is administered to the mother, then the baby will get the antibodies produced by the mother's body through the mother's bloodstream, making it harder for Zika to invade the fetus's brain and cause microcephaly. Zika gets into the AXL receptor by using a protein on its surface called the E protein, which attaches to a ligand that bridges the gap between the receptor and the virus.

Once it gets into the host cell, it injects it with Zika DNA, and turns that cell into a Zika-producing factory. My vaccine will have the RNA code to create the protein on the Zika virus's surface (the E protein). This will help it to stimulate a fake immune response in the body, so that the body will be able to create antibodies for future Zika infections. If injected into a pregnant woman, then the antibodies can also cross the placental barrier, immunizing the fetus as well. This will reduce the fetus's risk of microcephaly, as it will have the antibodies required to fight the virus while it is in the womb.

IV. MATERIALS AND METHODS

This project was done through extensive research. I outlined the steps and goals of my project and then did the research necessary to complete them.

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