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**Abstract**

An antibody has been discovered through a variety of techniques that may prevent the influenza B virus. The antibody is called 46B8 and functions to neutralize strain B. The influenza B strain is a major part of the flu season, so this antibody could have major benefits. 46B8 is an IgG antibody that interacts with hemagglutinin located on the surface of the influenza B virus. It neutralizes the influenza B virus in a variety of ways. First, 46B8 inhibits the conformational change of the HA1 and HA2 subunits of hemagglutinin at low pH. This locks the hemagglutinin protein in the original confirmation and inhibits the function of the pathogen. Secondly, the antibody is capable of inducing antibody-dependent cytotoxicity (ADCC) (Chai et al., 2017). This is imperative because it causes the lysis of cells and ultimately leads to the death of infected cells. Overall, monoclonal antibody 46B8 was found capable of completely inhibiting the influenza B strain in mice after administration at 24 and 48 hours post infection. In addition, it was shown to have protection after administration at 72 hours, which is significant because Tamiflu has a threshold of only 48 hours. 46B8 and Tamiflu were also administered together and proved to show optimal results. 46B8 is limited to mice at this time, and has not been approved for human use (Chai et al., 2017).

**Introduction**

The influenza virus commonly known as the flu is a respiratory illness that affects people greatly each year. While the flu affects people of all ages, some people are more susceptible to the influenza virus than others (Center for Disease Control and Prevention, 2018). Young children and people over 65 have a higher chance of contracting the flu. In addition, people who are pregnant or have other medical conditions such as heart disease or diabetes are more likely to get the flu. The infection may spread through droplets that someone may come in contact with in two different ways. A person may touch an inanimate object contaminated with the strain or may come in contact with someone who is sneezing or coughing and spreading the virus (CDC, 2018).

Each year there are between 9.2 million and 35.6 million flu illnesses in the United States and 140,000 to 710,000 hospitalizations annually since 2010 (CDC, 2018). Sadly, there are 12,000-15,000 deaths each year due to this infectious disease (CDC, 2018). In Oklahoma, there have been 3,560 hospitalizations and 168 deaths reported since September of 2017 (OK FluView, 2018). There are four types of these microbes, influenza type A, B, C and D (CDC, 2018). The influenza type A and B strains are the viruses that cause the flu epidemic annually. Unfortunately, these pathogens can change genetically each year and therefore the can be contracted again because the body has not made new antibodies for the new form of the virus. This is why the CDC recommends getting the flu vaccination yearly (CDC, 2018). Luckily, there is much research going on in order to prevent the flu. Recently an antibody has been created in order to block influenza B (Chai et al., 2017). The antibody is called 46B8 and has been shown to prevent the influenza B virus in mice. 46B8 has been show to have greater success at preventing the flu over Tamiflu (Chai et al., 2017).

**Recent Progress**

The influenza B virus causes over half of the flu cases each year, and contributes up to 52% of the child mortality rate. This virus is found in Victoria and Yamagata lineages and contains two surface proteins called hemagglutinin (HA) and neuraminidase. Antibodies have two regions. The Fab domain binds to the antigen and the Fc region binds to the cell. Both of these binding regions have desired antiviral effects. The Fab region can directly block the microbe while the Fc region recruits immune cells to come and fight off the infection by using complement-dependent cytotoxicity (CDC) or antibody-dependent cytotoxicity (ADCC). The antibody 46B8 is a human IgG antibody that focuses on interacting with the hemagglutinin protein. The hemagglutinin protein of the pathogen is very important to the overall function of the microbe. It allows the virus to attach to the host cell, it undergoes a change in confirmation, and aids in the exiting of viral particles in infected cells (Chai et al., 2017).

In order to find an antibody that will fight against the pathogen, researches isolated blood from donors who were vaccinated 7 days prior. Then the researchers added the influenza B virus hemagglutinin protein in order to elicit an immune response and make monoclonal antibodies against the infection. This mixture was then transplanted into mice with severe combined immunodeficiency. Plasmablasts specific to the microbe were isolated from the splenic cells of the mice. These were cloned and then screened by Enzyme-linked immunosorbent assay (ELISA). Researchers then selected for antibodies that would bind to hemagglutinin variants from Yagamata, Victoria, and previously encountered influenza B strains. Researches found 99 monoclonal antibodies that could bind to hemagglutinin from each lineage. Three of these monoclonal antibodies bound to hemagglutinin of both Yagamata and Victoria lineages but two were not able to bind to a specific virus called the B/Wisconsin/1/2010 virus. These monoclonal antibodies included 46B8, 34B5, and 33F8. Luckily, 46B8 was successful against each lineage, and successful against all eleven of the influenza strains tested (Chai et al., 2017).

The mechanism of how 46B8 works was tested. Researches found that this monoclonal antibody allows the virus to attach to cell surface receptors and did not show hemagglutination-inhibition. In contrast, monoclonal antibody 34B5 did show hemagglutination-inhibition when tested against B/Victoria/504/2000. Since the researchers knew it did not block the virus from attaching to the cell surface receptors, they tested to see if 46B8 blocked hemagglutinin in other ways, such as blocking membrane fusion or maturation of hemagglutinin. Researchers used a Western blot technique with trypsin-mediated hemagglutinin and discovered that 46B8 does not inhibit the activation of hemagglutinin. A cell-cell fusion assay was used to see if 46B8 was capable of blocking membrane fusion of hemagglutinin. Researches found that 46B8 blocked hemagglutinin entirely by inhibiting the conformational changes at low pH needed to happen in hemagglutinin. To further test this, a conformational change assay was used involving 34B5 and 46B8. Researches assumed that 34B5 probably binds to the HA head, so it was used to detect if the subunit of HA1 was present. The separation of the HA1 subunit from the HA2 subunit was influenced by diothiothreitol treatment. As the researchers assumed, 34B5 was unable to bind after diothiothreitol was used in accompany with low pH. The researchers statement that 46B8 inhibited the conformational change at low pH during membrane fusion was further proven because the binding of 34B5 returned when in the presence of 46B8 (Chai et al., 2017).

Prior to this study, it was found that the Fc region of monoclonal antibodies in the lab could provide inhibition against the influenza A virus through ADCC. ADCC is started when the Fc domain binds to receptors on effector cells, from there a signal cascade is started which ultimately causes lysis of the cells. Researchers wanted to find out if 46B8 possessed the same quality, so they performed two tests. First they measured the amount of expression of natural killer cells and second they measured the amount of lactate dehydrogenase (LDH) released during cell lysis. A positive control was used called Ceuximab that mediates ADCC and a negative control was used called Ritixuimab. Researchers found that 46B8 showed similar results as Ceuximab, and that 46B8 could induce ADCC. Next, the researchers wanted to know if 46B8 was also capable of inducing CDC. CDC causes the lyses of cells by complement on opsonized cells. 46B8 or Rituximab, a negative control, were used to coat A549 cells. A complement was added and the cells were incubated. The positive control that was used included WIL2-S cells and were also coated with Rituximab and exposed to complement. The cells were allowed to lyse and ATP was detected in live cells (cells that were not lysed) using a luminescent substrate. Rituximab did not lyse A549 cells but was successful in lysing the WIL2-S cells. In conclusion, this antibody does not induce CDC on A549 cells with the B/Brisbane/60/2008 virus (Chai et al., 2017).

Researchers decided to test the efficacy of 46B8 with and without Tamiflu in mice. The researchers tested five influenza B virus strains from each of the two lineages and the ancestral strains from the years 1966-2010. The mice were given a dose of the viruses and administered 46B8 or IgG, which was used as a control, at 24, 48, or 72 hours post infection. The control resulted in mortality of each mouse by days 9-12, with an exception of mice infected with B/Massachusetts/3/1996. All of the mice treated with 46B8 at 24 or 48 hours had a 100% survival rate with an exception for mice infected B/Russia/1/1969. Those mice affected were protected by 60% 48 hours post treatment. 46B8 proved to even show protection given 72 hours after infection against B/Brisbane/60/2008, B/Victoria/504/2000, and B/Massachusetts/3/1966 strains. Tamiflu has a window of only 48 hours, but 46B8 was proven to be effective at 72 hours post infection. In addition, 46B8-treated mice were shown to gain weight back quicker than Tamiflu-treated mice (Chai et al., 2017).

**Discussion**

The new results seem valid that 46B8 could be used as a treatment for influenza B virus over Tamiflu. In addition, researchers compared the use of Tamiflu and 46B8 administered alone versus in combination. The results showed that administering the two together could have increased benefits over administering the two alone. Researchers found that 46B8 neutralizes the pathogen by the binding of hemagglutinin. It can also induce ADCC and is successful at lysing cells in the lab. Additionally, this antibody was able to prevent the dissociation of HA1 subunit from HA2 because it inhibits the conformational change of HA at low pH. Several other neutralizing monoclonal antibodies are being tested such as HC45, HC63, and H6M9. HC45 prevents the influenza A strain form attaching to cell-surface receptors. HC63 also prevents viral attachment and H5M9 fights against the influenza A virus and also inhibits the confirmation change at low pH as does 46B8 (Chai et al., 2017). 46B8 is beneficial in mice, but the question still remains, will it be as successful in humans as it is in mice? Monoclonal antibody 46B8 may hold the future for new treatment for the influenza B virus and may provide a gateway for research on new antibodies that result in the same or even greater benefit.

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