

A Proposal for a Live Attenuated West Nile Virus Vaccine for Humans

Literature Review

Introduction

West Nile Virus is part of the *Flavivirus* genus. In 1937 West Nile Virus was first isolated from Uganda's West Nile province. *Culex* mosquitoes are what transmit West Nile Virus to the reservoir host, these are avian. The mosquitoes also transmit West Nile Virus to horses and humans, which are dead end hosts. West Nile Virus infection in humans is usually asymptomatic, sometimes it produces a moderate febrile disease, or it can cause West Nile fever. West Nile Virus infections have become more severe, causing neurological disease and deaths mainly in the elderly (Chappell, 2006). Included in these neurological diseases are West Nile meningitis, West Nile encephalitis, and West Nile meningoencephalitis. West Nile fever's signs and symptoms are aches, fever, tiredness, headache, and sometimes a rash. Some healthy people that are infected have been sick for weeks but most of the time it lasts for a couple of days. Meningitis is when the meninges have inflammation. Encephalitis is when the brain has inflammation. Meningoencephalitis is when the membrane around the brain and the brain itself has inflammation (CDC, 2004).

Pathogen Structure

West Nile Virus "is a small, enveloped virus with single-stranded, positive sense 11-kb RNA genome, which encodes a single polyprotein precursor." One important enzyme is NS2B-NS3, which is a viral protease. This protease is conserved within the *Flavavirus* genus. The protease is necessary for viral replication (Chappell, 2006). Another important part of the pathogen structure is the WNV E DIII protein, which is thought to recognize and attach to the cellular receptor (Chu, 2007).

Protective Immune Response

To look into the effects of West Nile Virus on the immune system a mice model was used. In this model the mice were injected with the domain III of West Nile Virus's E protein (WNV E DIII) and also WNV E DIII protein with CpG adjuvant. The mice that were injected with either of these produced anti-WNV E DIII antibodies. IL-2, IL-4, IL-6, and IL- γ were released as an immune response. IgG2a and IgG1 were also produced. IFN γ and IL-2 which are T cell helper 1 cytokines were produced at higher levels than T cell helper 2 cytokines. T cell production is usually associated with cell-mediated immunity (Chu, 2007). IgM and IgA are also produced by the immune system when there is an infection with West Nile Virus.

Epidemiology

Everyone is at risk that lives in areas where West Nile Virus is found. West Nile Virus has been found in Africa, Western Asia, Europe, Russia, Australia, the Middle East, and North America. In 1999 West Nile Virus was introduced into New York. Since then West Nile Virus has moved across North America. People that are over 50 years old have the greatest risk of severe disease. It has infected more than 19,000 people and caused over 700 deaths (Chappell, 2006).

Previous or Current Vaccines

There are no antiviral medications or vaccines for the prevention and treatment of West Nile

Virus infection in humans (Chappell, 2006). The WNV E DIII protein is thought to be a good option for a recombinant subunit vaccine. When using the mice models it was shown that the mice neutralizing antibodies that were produced prevented West Nile Virus infection in other mice that were injected with the antibodies. There are two types of horse vaccines for West Nile Virus that are currently in use. The first one is a recombinant vaccine which uses canarypox virus to produce antigens to West Nile Virus. The second is an inactivated West Nile Virus vaccine (Chu, 2007).

Human Vaccine Proposal:

West Nile virus is a flavivirus transmitted by the bite of a mosquito. West Nile has the ability to cause many severe symptoms in humans as well as horses. Some of these signs include febrile symptoms, nausea, headache, encephalitis, and in severe cases, even death. Since West Nile is a rather emerging disease, only a small number of plans for treatment have been tested. It has been only recently that major changes have occurred in the research of this arbo-virus (Dauphin, 2006).

Developing a vaccine will provide the most efficient means for protection about this potentially deadly virus. Since proliferation of the arbo-virus has increased in the past century, researchers are starting to make finding a vaccine a higher priority. West Nile encephalitis is also becoming a problem for the equine business as well. In clinical studies, it has been shown that West Nile has many similarities to other mosquito-based diseases such as Yellow Fever and Japanese encephalitis. Since there is already an effective vaccine for Yellow Fever, the vaccine for West Nile should be basically similar if it has the same infection route and symptoms. The proposed vaccine for the West Nile virus is therefore a live attenuated virus. The conventional method of producing a live attenuated vaccine is basically recurring virus passage in cell culture. This experimental process causes a significant difficulty in creating the most effective mix of attenuation and the right amount of immunogenicity (Monath, 2001).

Precise methods of West Nile replication and sites of replication immediately after the bite of an infected mosquito are unfortunately not known but primary replication is hypothesized to occur at the site of infection in the skin then travel to lymph nodes. Depending on how virulent the strain of West Nile is or how susceptible the host is, the secondary point of replication may move to the central nervous system. From serological tests, healthy individuals who were infected show extreme decreases in the virus after about four days after symptoms occurred. This is due to the production of the IgM antibody and the effectiveness of the macrophages in the immune system. Immunocompromised persons, on the other hand, can have West Nile in their blood for up to and over a month. (Live Attenuated, 2006) There are a few justifications as to why the elderly are so susceptible to the fatal symptoms of West Nile. First of all, there could be increased viral entrance into the central Nervous System. The virus is likely to be interrelated with the level of virulence and duration of the infection and symptoms (Monath, 2001).

Research studies show that the E protein on the membrane envelope is the main virulence factor in West Nile Virus. This E protein regulates attachment to other cells for infection, and also is the mediator that helps cross the blood-brain barrier that ultimately affects the central nervous system causing encephalitis. How this happens specifically is still not known. The changes that

occur in the brain as a result of the pathogenicity of virus occur for many reasons. First of all, the virus reaches the brain, passes through the blood-brain barrier and eventually proliferates in the neuronal cells. They also interact with glial cells, which directly impact neuronal cells through nutrition, signaling, and protection. The disease can readily dissolve into the brain causing encephalitis. Parts of the brain that are affected by this swelling include the thalamus, medulla, portions of the brain stem, and spinal cord, or any other place where nodules of glial cells are in abundance. When West Nile infects one of these nodules of glial cells, it can result in major degeneration of neurons (Campbell, 2002).

Within these glial nodules, T cells, bearing the CD8 membrane receptor, predominate. T-lymphocytes with CD4 are there in lesser density than the CD8 cells. B-lymphocytes are for the most part found in areas where inflammation is occurring on the tissues surrounding the blood vessels. Natural immunological responses to West Nile from healthy individuals have been seen through the production of the antibody IgM. The viral-specific antibody may interfere with the proliferation of the viral infection by interfering with the E protein on the cell surface. This induces structural rearrangements internally. The E protein would generally allow attachment and fusion of the virus (Campbell, 2002).

Vaccines that are live-attenuated have the advantage of hasty immunization following a single dose. Live attenuated vaccines also produce a memory in the host that boosts the immune system if ever exposed a second time. They are also very durable in that once administered, they last a long time. Quick commencement of an immune response and immunity after only one dose is a key characteristic for a West Nile vaccine. A live vaccine will resemble a natural disease or infection that includes all parts of the immune system. The result is much milder than a real run of the disease, including a cytokine environment and T helper cell course comparable to that brought about by natural infection. Replication of the virus inside the cell is though processing the antigen through the MHC class 1 pathway. This ultimately is the mechanism that produces a long-lasting and durable cellular immunity (Monath, 2001).

The live attenuated model for the vaccine employs the ideas used for a Yellow fever virus vaccine. Yellow fever is a similar arbo-virus transmitted by mosquitoes. The virion of the yellow fever is neutralized and contains epitopes that have high affinity for cytotoxic T cells. The protein encapsulation is that of West Nile virus. It will contain the E proteins sued for attachment, recognition, and ultimately virulence. This will allow the body to resultantly produce IgM and antibodies specific to the West Nile virus. The IgM antibodies produce the memory needed to eradicate if ever the host is infected with it (Monath, 2001). Major locations for replication are going to be skin and the lymph tissues, since this is the general mechanistic approach of the real West Nile virus (Monath, 2006).

T cell memory

Antigen-specific T cells are activated by the antigens used in an immunization. They proliferate and differentiate, and large numbers become effector cells and travel through the peripheral tissue to confront the pathogen. CD8 T memory cells can be divided into two subgroups. The first group resembles the effector cells made during the primary response; they lack the L-selectin and CCR7 receptors and express receptors for migration into tissue with inflammation.

These memory T (also known as T_{EM}) cells can produce IFN- γ or IL-4, or discharge pre-stored perforin upon re-exposure to the antigen. The second subgroup expresses L-selectin and CCR7 and lacks the immediate effector function, but can excrete IL-2. These memory cells, also known as T_{CM}, are more central, remaining in the lymph nodes, and have a low activation threshold. Once activated in secondary lymphoid organs, T_{CM} proliferate and can differentiate into effector cells (Shrestha, 2004).

The immune response to West Nile Virus shows that the antibodies are responsible for ending the viremia, but T cells are necessary for clearing the infected tissues. It has been shown, through studying CD8 deficient mice, that the virus persists longer within the host if CD8 cells are not present to destroy infected cells, thus causing a persistent infection (Shrestha, 2004). When a CD8 cell encounters a flavivirus-infected cell, it kills, proliferates and releases inflammatory cytokines. T cells are thought to be protective against West Nile Virus, but their complete role in the control and recovery process is still unclear.

Other studies have shown that the potential for CD8 cells to kill infected neurons can be seen as either favorable or unfavorable with respect to mortality based on the inoculation doses. The damage that can be done to the Central Nervous system is not reversible. "Thus, it remains unclear under what circumstances CD8+ T cells protect against disseminated infection or contribute to the pathogenesis of WNV-related neurological disease (Shrestha, 2004)."

T cell Measurements

Flow Cytometry is used to measure T cell memory level in immunized individuals. The measurements would be taken from the individuals in the study 14 days after immunization and compared to a control group. T cells can be activated 4 days after immunization, but would not be measurable until 7-10 days after immunization. (Decker, 2007.)

Side Effects

There is no current human West Nile Virus vaccine; thus, the side effects of the current West Nile Vaccine for horses were examined. The only noted side effect was a reaction in the tissue at the site of the vaccine (Monath, 2006).

Cellular Immune Response

The adaptive immune response activation of T cells plays a major role in the control and elimination of the West Nile Virus from the body. CD8 T cells are activated through the presentation of viral antigens on MHC I in combination with co-stimulatory signals through APC. CD4 cells are activated through MHC II and by co-stimulatory signals from APC. The CD4 T cells do not play as large of a role in this viral infection because it is an intracellular pathogen; the CD4 T cells play a larger role in extracellular pathogens. Once activated, the CD8 cells can proliferate and differentiate into effector cells and different groups of T memory cells. Once an activated CD8 cell encounters an infected cell, it can induce the cell to go through apoptosis. "As for encephalitic flaviviruses, potent Tc cell responses in the spleen are observed after peripheral infections, with unusual extensive cross-reactivities on target cells infected with

a wide spectrum of heterologous flaviviruses (Wang, 2003).” It has also been observed that exposure to flavivirus infection leads to an increase in the expression of MHC I in mammalian cells.

Studies have concluded that IFN- γ plays a roll in virus control, but little information is available on the function of IFN- γ . IFN- γ is produced by NK cells, $\gamma\delta$ T cells, and CD8 T cells, and typically inhibits virus proliferation through different methods. Those methods include antiviral inhibitory activity, polarization and activation of T helper cell responses, enhanced MHC I expression and presentation, and activation of phagocytic myeloid cells (Shrestha, 2006). $\gamma\delta$ T cells and their ability to regulate immune responses connected to inflammation is not really understood, but studies have shown that they are important in regulating inflammation of the Central Nervous System. It has also been seen that $\gamma\delta$ T cells aid “in disease recovery through the Fas/Fas ligand-induced apoptosis of encephalitogenic T cells and a quick resolution of inflammation in the CNS is essential to prevent permanent damage to the CNS resulting in chronic disease (Ponomarev, 2005).” Upon exposure to West Nile Virus after vaccination antibodies will be made to control the viremia, but T cells will play a crucial role in killing infected cells so that a persistent infection does not occur.

Timing:

The vaccine will be administered once on an annual basis. Because the immune memory of the vaccine is unknown, it would be best to administer a booster on an annual basis. Since its longevity has not been determined, a booster is a protective measure to insure immunity is maintained throughout the life of the individual. There is evidence that supports the annual booster. The horse vaccine for West Nile Virus that is already in use is administered on an annual basis (West 2007). This is reinforced by the fact that IgG immunoglobulin has been present in patient blood for up to a year after an immune response has occurred to West Nile Virus (Prince 2005). Once more research has been preformed on the longevity of the memory T and B cells to West Nile Virus, a more accurate time frame can be determined for the proper administration of the booster or if it is necessary at all.

To determine the efficacy of the vaccine, ELISA test will be administered to patient serum. The ELISA test will aid in determining whether humoral immunity has occurred as a result of the vaccine. IgG is the most common and longest lasting immunoglobulin of the B cells, it will be used for the ELISA test. To perform the ELISA, rabbit anti-human IgG will coat the mictotiter wells. The Fc of the anti-Ig will have an enzyme present that will convert chromogenic substrate to a colored product. This reaction will take place once the enzyme and substrate have come in contact. The more color present in the wells will denote a larger number of IgG specific antibodies in the wells and patient plasma. Controls will also be tested along with the sample as a means to compare the ELISA results. (ELISA 2004)

Isotypes:

Three immunoglobulins will be produced as a result of the West Nile Virus vaccine. The three immunoglobulins produced are IgM, IgA, and IgG. IgM is the first immunoglobulin secreted by the B cells. IgM secreting cells will undergo somatic hypermutation, isotype switching, which

will change yield plasma cells that will secrete IgA and IgG. IgM secreting cells can undergo the same process to yield memory B cells. IgA aids in the immune response to West Nile Virus much like IgG, both neutralize the virus by blocking its binding site. (Parham 2000)

Criteria for Vaccine:

Certain groups will best benefit from the vaccine. The elderly are at high risk for the development of encephalitis due to the West Nile Virus and a compromised immune system. Young children are also in the high risk category, also due to the likelihood of developing fatal encephalitis. Although it would be recommended for a child to receive the vaccine, it would be advisable that children under the age of five not receive the vaccine due to possible side effects. Another group that is high risk for contracting West Nile Virus is individuals that who spend extended periods of time in areas that put them in contact with mosquitoes. (CDC 2006)

Groups of individuals that should avoid the vaccine are pregnant and/or nursing women. There may be birth defects as a result of administering the vaccine to pregnant women. Birth defects such as lacking limbs and skull deformities have occurred in foals who were exposed to the vaccine while in the womb of the mare. West Nile Virus has also been known to have been transferred from the mother to infant through breast milk. Other groups of individuals that may have problems with the vaccine are immuno-compromised individuals. (CDC 2006)

Side Effects

There is no current human West Nile Virus vaccine; thus, the side effects of the current West Nile Vaccine for horses were examined. The only noted side effect was a reaction in the tissue at the site of the vaccine. However, there have been online reports of encephalitis cases as well as mares bearing deformed foals when they received the vaccine early in pregnancy (<http://lost-foals-group.4t.com/photo6.html>).

Kinetics

Innate Response:

After inoculation of the vaccine, the first response would be by the innate immunity at the site of inoculation from the foreign antigen. The macrophages are activated, which then release the cytokines and chemokines. The cytokines and chemokines yield the inflammatory response. This will draw leukocytes to the site of inoculation. TNFalpha will be the main cytokine that will increase the cellular adhesion molecule expression, to aid in the attraction of circulating leukocytes to the site of infection. Also, the cytokine IL-6 will act on the liver to start producing acute-phase proteins, which will bind to the bacterial antigen and activate complement. The complement cascade will initiate the use of complement components to help in opsonization and increase circulation. This allows for T cells and dendritic cells to enter the site and bind the antigen. The dendritic cells bind the antigen and are able to migrate to the lymph node through the use of cytokines (IL-1/IL-6/TNFa). T cells are activated as a result of binding to the antigen-presenting cells in the draining lymph node via class II MHC, activating the adaptive immune response (Parham 2000).

B-cell Response:

As the circulating B cells pass through the T cell zones interactions take place with the Th2 cells, whose T cell receptors screen the peptides presented by the class II MHC molecules on the surface of the B cell. When a B cell presents the specific antigen recognized by the Th2 cell, the adhesive interactions are strengthened and the B cell becomes trapped by the T cell. The T-cell interactions with the B cell cause the synthesis of CD40 ligand. The CD40 ligand corresponds to the receptor molecule CD40, which drives the resting B cell into a cycle of cell division. Once the B cell has matured and migrated to the secondary lymphoid organ, there are means to furthering Immunoglobulin diversity. The means for immunoglobulin diversity is somatic hypermutation, affinity maturation and isotype switching. Hypermutation affects the variable region of the immunoglobulin. Affinity maturation is a result of the mutant immunoglobulin molecules being better suited to bind antigen. If this does not occur than the B cell dies. To change the constant region of the immunoglobulin isotype switching takes place. Once these means for immunoglobulin diversity have taken place than new IgA and IgG will have been yielded to combat West Nile Virus due to their ability to neutralize the virus' binding sites. (Parham 2000)

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