

Proposal for an Ebola Virus Vaccine

Literature Review:

Ebola Virus is a lethal virus from the RNA virus family Filoviridae. It is a part of a group of Hemorrhagic fevers which contain a range of mild diseases as well as deadly ones like the Ebola virus. This virus is a cause of disease; it affects both humans and non-human primates, such as monkeys, gorillas, and chimpanzees. The natural reservoir or host remained unknown until as of recently when studies indicated that evidence pointed to the fruit bat as a reservoir for the disease.

Ebola was initially recognized in 1976 in Zaire, Africa (Special Pathogens Branch 2005). It was found to be acute, with no carrier state. After the first transmission was successful it was then spread by direct contact with bodily fluids or blood, or contaminated objects such as needles. People at risk during the initial outbreak and in subsequent outbreaks since then have been indigenous people in the affected area, such as Zaire, as well as the health care workers treating these patients. There are many reports of outbreaks in hospitals because the staff was unaware of how the disease spread. This is still a major problem today in the prevention of individuals being infected as well as the spread of it, because health care workers today are for the most part aware of the disease and its symptoms but many are unaware or unable to practice proper safety precautions and use the proper Personal Protective Equipment (PPE) when working with these patients, thereby contracting the disease themselves. The reason PPE is stressed so heavily for these healthcare workers is because the virus is spread through bodily fluids or contaminated objects so without proper hygiene and disinfection the disease will continue to contribute to larger outbreaks.

Signs and symptoms health care workers must look for when working with people in the endemic areas are: fever, headache, joint and muscle aches, sore throat, lethargy, as well as diarrhea, vomiting. Stomach pain, and in some cases red eyes, internal and external bleeding and rashes have all been seen. Since there is no cure for Ebola, hospitals provide symptomatic treatment for the different phases of the disease. This could include fluid replacement as well as the administration of coagulation factors to help bleeding. Current treatment of Ebola has been found most successful when antibodies are administered to a patient in order to neutralize the virus before attachment occur and curb the spread of the virus. Another way to treat Ebola would be to try to block the physiological cascade that the virus triggers rather than target the virus itself. Many researchers are now focusing on this complex, and poorly understood, process. Researchers know that the bleeding in Ebola is caused by coagulopathy, a dysfunction of the blood-clotting system, but it's not quite clear where this starts or how it can be stopped. (Ensenrink). At a meeting in the US led by Tom Geisbert of the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) in Fort Detrick, Maryland, he presented evidence that macrophages, which are a type of white blood cell, play an important role. When infected by the virus, they start expressing a clotting protein called tissue factor (TF) on their surfaces. These molecules attract other clotting molecules from the blood, resulting in localized, harmful blood clots that leave the body vulnerable to bleeding elsewhere.

Outbreaks have been reported throughout Africa since the initial one on 1976. Known outbreaks since then include: 1976 Zaire, and Sudan, 1976 England, 1977 Zaire, 1979 Sudan, 1989 USA, 1990 USA, 1989-90 Philippines, 1992 Italy, 1994 Gabon, 1994 Ivory coast, 1995 Congo(Zaire) 1996 Gabon, South Africa, USA, Philippines, 2001 Uganda, 2001-02 Gabon, Congo, 2002-03 Congo, 2004 Sudan. It was also noted that in all of the outbreaks outside of Africa and the Philippines were due to the virus being introduced into a laboratory setting and humans contracting it there (Special Pathogens Branch 2005). A recent study gathered data to suggest that there was a correlation between each new case of Ebola and the distance from the previous cases. Researchers found a pattern of increasing genetic divergence among virus genotypes with increasing geographic distance. Meaning that the likelihood of the virus already being prevalent in the population there is slim and that there is more evidence pointing to a wave like spread of the virus, such evidence leads researchers to believe that in the future they may be able to predict where the next outbreak will be (Biek).

The Ebola Virus is a group V; negative-sense single stranded RNA virus from the order *Mononegavirales*, family *Filoviridae* and genus *Ebolavirus*. There are currently four identified species: *Reston Ebolavirus*, Sudan Ebolavirus, Ivory Coast Ebolavirus and Zaire Ebolavirus. The Ebola virus has a thread-like structure much like the structure of a filovirus. The virions it contains vary in shape, but mostly resemble the letter U or number 6. They are also circular, coiled and branched shapes. The virions usually possess a diameter size of about 80nm and on average are about 100nm long. Found in the center of the virion is a nucleocapsid which forms by the viral genomic RNA complex containing envelope glycoprotein, GP, nucleoprotein, NP and two matrix proteins, VP24 and VP40. Ebola also contains nonstructural proteins to include VP30 and VP35 and viral polymerase. The Ebola virus enhances two gene products, soluble 60 to 70-kDa protein, sGP and a full length 150- to 170-kDa protein (GP) that inserts into the viral membrane through transcriptional editing.

Each virion contains one molecule of single stranded negative-sense RNA with a total of about 18,960 nucleotides in length. It codes for seven structural proteins and one nonstructural protein with a gene order of 3' - leader - NP - VP35 - VP40 - GP/sGP - VP30 - VP24 - L - trailer - 5'. The genomic material is not infectious because the viral RNA polymerase is necessary for transcription of the viral genome into messenger RNA as well as replication of the viral genome.

The Ebola virus is extremely efficient in causing disease within its host. The virus is transmitted through any contact with bodily fluids, including saliva and feces, of an infected host. Ebola begins its rapid replication in the epithelial cells. From there, it enters dendritic cells, monocytes and macrophages located around the initial site of infection. The virus causes a decrease in the production of Interferon alpha from dendritic cells. $INF\alpha$ aids in the cells ability to present MHC-I and the activation of Natural Killer cells to kill infected cells. Infected macrophages have been found to secrete increased levels of IL-1B, IL-6, IL-8 and $TNF\alpha$. The cytokines released by these cells spark the general symptoms of fever and malaise. They also result in the increased permeability of endothelial cells. From here the virus is able to move into the vasculature, where it gains access to the rest of the body. Typically a strong immune response is not initiated. Once the virus reaches the endothelial cells of the blood vessels it causes damage and ultimately apoptosis of those cells. This is what causes the

characteristic rash of early onset. As more and more endothelial cells are destroyed the vessels become even more permeable and leaky. Internal organs are also infected, which ultimately causes internal bleeding. This is why the disease is referred to as a hemorrhagic fever. The target organs are the liver, spleen, kidneys and lungs. Necrosis of these organs is widespread and the ending stages of disease. Destruction of the endothelial cells is thought to bring on Disseminated Intravascular Coagulation or DIC. The blood will begin to coagulate throughout the body. At this stage the virion level in the blood is so high that all bodily surfaces and fluids become highly infectious. From start of infection to death can only last 7 days, which is why protective immunity is so important.

Vaccine Proposal:

The type of vaccine we chose to utilize in our Ebola virus vaccine is a type of subunit vaccine. Subunit vaccines use only components of a bacterium or virus to elicit a response. These vaccines do not contain the actual pathogen itself. Subunit vaccines are currently available for diseases such as; human papilloma virus, parvovirus, rotavirus, typhoid, and hepatitis B. The specific type of vaccine is called Virus like Particles vaccine or (VLP). Virus Like Particles are released from the ectopic expression of the glycoprotein and we will use the matrix VP40 to elicit a response to the VLP's (Warfield). The VLP's we will be using in our vaccine will be morphologically similar to the Ebola virus but will contain no genetic information in it so it will not be possible for the individual to be infected with the actual virus. Using these particles will also provide us with an immune response without unwanted responses such as suppression or activation of unwanted cells. Our vaccine will first activate innate and adaptive immunity followed by humoral and cell-mediated immunity in the future.

The chosen method for vaccination will be an injection of a virus-like particle. This is basically the envelope of the protein with both the genomic and other interior proteins removed. This prevents any chance of a pathogenic particle being introduced into a population and possibly causing an outbreak. It is, although, a prime vehicle for inducing immunity. We have chosen this versus, a live attenuated or even killed virus due to the dangerous aspects of working with the virus. It is a level 4 as a biosafety hazard. This means few, specialized individuals would be able to work with this type of vaccine. There is also the potential for an actual outbreak to occur from a live attenuated vaccine. The use of a VLP vaccine will also allow health professionals in third world communities to have access to and distribute it amongst their communities.

The Ebola virus both exhibits and secretes certain proteins that are able to serve antigenic purposes. Upon researching the structure and products of this virus, three separate antigens have been chosen to initiate a protective immune response of a host treated with our vaccine. We have chosen Viral Envelop Glycoproteins 1 and 2, and VP40 (structural envelope protein).

It is likely that the first antigen that is presented to the body during Ebola infection are the glycoproteins expressed on the viral envelope. These glycoproteins though still under research, they are known to play an important role in cellular entry. Entry is extremely important to Ebola, as it needs the inside of the cell to carry out replication and assembly of new virion. GPO is originally formed and then cleaved into the two forms of glycoproteins on the surface, GP1 and GP2. Another important

structural protein in the Ebola virus is VP40. This protein is responsible for forming the coat of the virus, as well as the budding and assembly of new viral particles. It is one of the most highly expressed proteins within the virion.

The use of these three particular antigens will ultimately allow us to elicit an immune response resulting in the protection on an individual in case of future exposure to the virus.

Innate Immune Response:

The Ebola virus has become very efficient in manipulating the immune response to add to its virulence. By vaccinating before infection, we will be able to build memory of both T and B cells to combat future exposure to the virus. Our subjects will be inoculated with the VLP in a muscle. The first response of the innate immunity will be the activation of complement. Complement fragments, such as C3b will help to opsonize the VLP. This, in turn, will aid the macrophages in both identifying and engulfing the VLP. Once the macrophage has been activated it will begin to release IL-1, IL-6, IL-8, IL-12 and TNF α . All of these cytokines and chemokines will result in the increase of vascular permeability and the influx of other leukocytes to the area. These leukocytes will also contain dendritic cells which will also come in contact with the VLP and engulf it. Once engulfed our chosen antigens will be presented in MHC class I and II. This is the first step towards initiating an adaptive immune response. (Pahram 2005)

Adaptive Immune Response:

The innate immune response will set up for an adaptive response to begin. The antigen presenting cells, APC, will travel back to the regional lymph node where large supplies of naïve T cells are kept. The APC, in this case, a dendritic cell presenting our GP1 and GP2 on its MHC I and II. It will secrete CCL18 which will attract naïve T cells and begin the process of activation. Once a naïve T cell is found that attaches to the presented antigen it will be fully activated and begin to proliferate. Both T helper cells and cytotoxic T cells will be activated. The T helper cells also help macrophages and B cells in the immune response. Cytotoxic T cells will be targeting cells presenting the GP antigens in their MHC once they travel to infected tissue. They contain different proteins, such as granzymes and perforins that will poke holes in infected host cells and ultimately lead to apoptosis. Fully activated T cells will play a role in generating T cell memory. This memory will allow a quick response to the Ebola virus at the time of an infection. This quick response will aid in stopping the spread of infection and hopefully the survival of the patient.

A B cell response will also be crucial to the efficacy of the vaccine. Naïve B cells will come into contact with the chosen antigen in the lymph node or via the presentation of dendritic cells. It has been shown in Ebola survivors that a strong B cell response was occurred against the VP40 antigen. The naïve B cell carrying the IgM receptor will bind to the antigen, and along with the help of a CD4 T cell causes the nucleus to be signaled and therefore activating the B cell. This causes the proliferation of this cell. The B cell will also undergo somatic hypermutation and affinity maturation to assure the strongest affinity for the antigen. The cells will then differentiate into plasma cells secreting IgM. Later on they will switch isotype to IgG which is the predominate antibody of the blood. These antibodies will play important role in neutralizing the virus before it can attach and

enter endothelial cells, ultimately causing the hemorrhagic conditions of the virus. Again, with activation of B cells, memory cells will be formed protecting the individual from future infections. (Pahram 2005)

There are several major problems that we encounter when working with the Ebola virus. Ebola virus is one of the most lethal types of viruses present to date and has mortality rates of up to 90% (1). This brings us to our first problem, which is locating individuals to conduct the research on. We are unable to create our own test population because the risk of infecting these individuals with Ebola is much too great. That means that our test population must be derived from an outbreak population. In most cases, outbreaks occur in central Africa, with the most recent cases in the Republic of the Congo. When working with infected individuals it is vital that the researchers are completely protected from contracting the virus themselves. Ebola is extremely contagious and especially dangerous. This virus does not illicit a big immune response in the host and this raises another problem for our study . Many times the affected are unaware of their condition until it is much too late (1). Recognition of the disease is going to be a major factor because the virus needs to be identified before death occurs. Another major concern with Ebola is the fact that it contains four strains; Ebola Zaire, Ebola Sudan, Ebola Reston, and Ebola Tai (2). This is a problem when creating a vaccine because although we may create a successful vaccine for one strain, the individual may still be infected by one of the other three strains in the future. We plan to create a vaccine that is successful against all four strains of Ebola to avoid this issue.

Immunity Assessment:

Our test group will consist of 20 people from a region in Africa where the disease is endemic. Our trial will span a 28 day period initially. We will perform serological testing of these individuals to determine the levels of antibodies present including IgG and IgM before vaccination to ensure they have not previously been infected and achieved immunity. We will then administer one dose of the VLP vaccine given intramuscularly or IM within the first two weeks of serological testing, and two weeks after the vaccine is given we will follow up with another round of blood tests to determine the level of the immune response. Depending on the amount and type of antibodies and T cells present we will determine if another round of vaccination is necessary, and continue with a monthly administration from there. However, regardless of the number of doses give we will follow up in three month intervals for the first 12 months of serological testing to determine if there was any cell memory induced. The tests chosen will be an ELISA test and flow cytometry.

The testing will involve inoculating a test population with a vaccine for the virus and testing for antibodies, both before and after the vaccine is administered, using the ELISA test. The ELISA test will consist of an enzyme that will be chemically linked to the antibody (IgG and IgM). The antigen, VP40, will be attached to the wells of the plate that will be utilized for the absorption of a protein. The antigen and the antibody will then bind to each other and unbound material will be washed away. A color change will be present when binding is detected, making it easily identifiable. We will also utilize flow

cytometry to measure any type of T-cell memory increase due to the vaccine (Sullivan). A cell suspension from the patient will be needed containing CD8 T cells in order to perform the flow cytometry. Tetramer MHC (class I) of the appropriate class, allotype, and peptide-binding specificity loaded with the specific peptide and tagged with a fluorochrome. The cells will be incubated with the labeled tetramer MHC-peptide and washed. The flow cytometer then analyzes the incubated cells. A vibrating nozzle located on the flow cytometer works to break the cells down to droplets. Each individual droplet contains one cell. The cells will then pass through the cytometer and the laser beam in a fine stream of liquid. The amount of fluorescence on each cell is quantified and converted to electronic signals. These electrical signals can be recorded and plotted and displayed in the form of a histogram of fluorescence intensity versus cell number.

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