

A Proposed Study of the Ebola Subunit Vaccine in Humans

Literature Review

In 1976 a highly fatal disease swept across the Democratic Republic of Congo infecting 318 people and killing 280. This was the first outbreak of Ebola Hemorrhagic fever. This disease continues to be prevalent in the Democratic Republic of Congo, Gabon, Sudan, Uganda and the Ivory Coast. The Ebola virus has an 88 percent mortality rate in humans and non-human primates. However, bats are known to carry the virus without the virus being fatal. Therefore, it is believed that the virus is transferred from bats to humans or to non-human primates. The virus is passed on even further by direct contact with an infected person's bodily secretions such as blood or saliva. Direct contact with objects that have been exposed to infected secretions is also known to pass on the disease. Once the disease is contracted it incubates for 2 to 21 days and then symptoms of weakness, muscle aches, fever, stomach aches, vomiting, and diarrhea start to occur. (CDC, 2002)

The Ebola virus is a filamentous or threadlike virus which appears to be folded up on itself. It is a negative sense RNA virus and has four subtypes: Ebola-Zaire, Ebola-Sudan, Ebola-Ivory Coast, and Ebola-Reston. The virus's genome encodes for glycoprotein spikes which attach to endothelial cells and allow the virus to invade and replicate in these cells. The virus replicates in all tissues in the body and the resultant destruction of the endothelial cells leads to tissue necrosis. The RNA codes for four structural proteins including VP30, VP35, nucleoprotein, and polymerase protein. The RNA also codes for three membrane associated proteins including VP40, glycoprotein, and VP24. Secreted glycoproteins bind to neutrophils and inhibit neutrophil activation. VP35, a structural protein, blocks the transcription of IF3, which is a transcriptional factor that assists in the transcription of interferon genes. Thus, the Ebola virus can replicate undisturbed in the epithelial cells because there are no interferons to inhibit its replication. All of the Ebola glycoproteins reduce the effectiveness of the host adhesion molecules and the MHC class I receptors. The primary receptor for macrophage uptake of antigen is currently unknown and under research. (King, 2007)

The Ebola Virus has no current vaccine available for humans, non-human primates, or any other animal. In earlier experiments scientists used vaccines with formalin-fixed or heat-inactivated virion preparations in non-human primates. However, there were mixed results and using this method for human vaccinations was not pursued because there were concerns that the virus might revert to a wild-type form. (Anderson, Davis, Geisbert, Jahrling, Pushko, Smith, 2002)

One group of scientists vaccinated guinea pigs using recombinant DNA techniques in order to get a higher cytotoxic T lymphocyte response. They used plasmids against the Ebola virus's nucleoprotein, soluble glycoprotein, or glycoprotein. After infection with the virus the guinea pigs did show signs of an increased cellular and humoral immune response. However, the survival period of non-vaccinated guinea pigs

after exposure is between 8 and 14 days and the vaccinated guinea pigs were killed 10 days after the infection. Therefore it is unknown if the guinea pigs were immune to the virus. (Anderson, Davis, Geisbert, Jahrling, Pushko, Smith, 2002)

Another experiment with guinea pigs as the subjects used RNA interference to inhibit the gene expression of the virus. RNA interference is able to stop viral replication. Four small interfering RNAs, or siRNAs, targeted the polymerase gene of the Ebola virus. When the siRNAs were complexed with polyethylenimine and were given before the infection they reduced the plasma viremia levels and partially protected the guinea pigs from death. When the siRNAs were formulated in stable nucleic acid particles and given right after the infection the guinea pigs were completely protected from viremia and death. (Daddario-DiCaprio, Fritz, Geisbert, Geisbert, Hensley, Jahrling, Jeffs, Judge, Kagan, Lee, MacLachlan, McClintock, Phelps, Yu, 2006)

In one study, a bivalent cADVax (GPs/z) vaccine was used on mice. This adenovirus based vaccine vector included the Ebola virus glycoprotein genes. After the mice were vaccinated they showed efficient induction of Ebola virus antibodies and had good cell-mediated immune responses. The mice had full protection from the disease and all the subjects survived a lethal dose of the virus. (Deitz, Dong, Hart, Juompan, Luo, Moore, Pratt, Raja, Swain, Trubey, Wang, Woraratanadharm, Yu, 2006)

There was another experiment that used rodents as their subject. The scientists used the Venezuelan equine encephalitis virus replicon particles which expressed Ebola virus genes that protected the rodents from the virus. They also used a recombinant Vaccinia virus system that expressed the Ebola virus's glycoprotein. This was able to protect the rodents from the hemorrhagic fever caused by the virus. The scientists finally evaluated the rodents when they vaccinated them with a concentrated, gamma-irradiated whole-virion preparation. The rodents in this study did survive after being exposed to the virus; however these methods were not successful when primates were used as subjects. (Anderson, Davis, Geisbert, Jahrling, Pushko, Smith, 2002)

Even if rodents become vaccinated against the Ebola virus, there is still a long way to go before a human vaccine can be produced. A rodent's immune system reacts quite differently to the Ebola virus than a human's or a non-human primate's immune system. The disease is different in rodents because they do not show the disseminated intravascular coagulation found in end-stage lesions that are found in humans and primates. Also, lymphocyte apoptosis is very mild in rodents, where in humans it is a major symptom. (Anderson, Davis, Geisbert, Jahrling, Pushko, Smith, 2002)

There was a study where scientists vaccinated horses with the Venezuelan equine encephalitis virus replicon particles. They then passively transferred the hyper immune equine immune globulin from the horses into monkeys. The monkeys did survive longer than untreated monkeys, but they died eventually from the disease. However, when this method was used on baboons, the baboons did show protection and survived. (Anderson, Davis, Geisbert, Jahrling, Pushko, Smith, 2002)

Another experiment has successfully protected monkeys against the virus by using the recombinant vesicular stomatitis virus. This virus expresses the Ebola virus glycoprotein which was a very effective way of vaccination. The Ebola virus becomes infective and pathogenic only when the glycoprotein is cleaved by cellular endosomal proteases like cathepsin B and L. Therefore, if humans have immunity against the Ebola

virus's glycoprotein, the Ebola virus will be unable to become pathogenic. (Alazard-Dany, Terrangle, Volchkov, 2006)

Treatment for people with an Ebola virus infection can only be symptomatic. There are some antiviral treatments, but none of them seem to have any effect on the final outcome of the disease. There is no known cure for this disease and consequently it kills about 90 percent of the infected individuals. Ebola virus outbreaks have been seen to be self-limiting if the infected individuals are quarantined immediately in order to stop the spread of the disease. (Anderson, Davis, Geisbert, Jahrling, Pushko, Smith, 2002) (Daddario-DiCaprio, Fritz, Geisbert, Geisbert, Hensley, Jahrling, Jeffs, Judge, Kagan, Lee, MacLachlan, McClintock, Phelps, Yu, 2006)

Since the Ebola virus is most seen in African countries it will be beneficial if we use African volunteers for the vaccine trials. This will give these African citizens immunity against a disease that is known to cause outbreaks in the Africa area. (Anderson, Davis, Geisbert, Jahrling, Pushko, Smith, 2002)

We chose this pathogen because the disease causes a huge mortality rate in its victims. With no vaccine and unreliable treatments, it is of great important that a solution to this disease is made. The disease caused by the Ebola virus is traumatic and very painful and we think it is important to eliminate this awful way of dying. There is also a major threat of this virus being used as a biological weapon since it is spread so easily. A vaccine to this virus will reduce this threat enormously. (Anderson, Davis, Geisbert, Jahrling, Pushko, Smith, 2002)

Description of Vaccine

The Zaire Ebola virus (EBOV) is both deadly as well as difficult to vaccinate against. In light of this, we believe it would be best to illicit an immune response to the majority of the viral proteins expressed. Our proposed strategy is to inoculate the patient with a subunit vaccine consisting of the glycoprotein (GP), NP, VP35, VP30, VP24, and VP40 viral proteins. The vaccine will be administered as an injection into the skin in order to get it into secondary lymph tissue. This will incite an IgG response from B cells and cause memory to the specific antigens. It will also incite CD8 T cells through antigen take-up by dendritic cells.

The patient will be inoculated with prepared samples of the surface glycoproteins bound in the envelope. This is the most obvious choice for a vaccine as these proteins are bound to the viral membrane and aid in virus entry into the host cell. To prepare these proteins they will be removed from the membrane and unmasked to allow antibody binding. This method has been shown to provide an efficient way of inhibiting viral entry. Glycoproteins should be the first antigen protein encountered by the immune system and immunity to them will aide in stopping the infection from taking a hold in the body. We cannot rely upon GP as the sole target antigen because this virus produces a soluble form of GP, which can act as a decoy towards IgG antibodies to GP. (Manicassamy, Rong, Rumschlag, Tymen, Wang, Volchkova, Volchkova, 2007)

We will also attempt other methods to ensure that the patient will have every resource available to survive an infection. We will try to illicit an immune response to all of the VP proteins (VP35, VP40, VP30, and VP24) and NP. Two of these proteins, VP35 and VP24, aid the virus by helping it evade the immune response by interfering with

interferon production and sensitivity, respectively. Providing these proteins to the body in order to illicit an immune response is the most dangerous portion of this vaccination procedure, as this will potentially leave the patient temporarily unable to handle viral infections with the same degree of competency. The body will not be able to respond as well to a viral infection because the cells will not undergo apoptosis if infected by virions. The infected cells will also not be targeted by NK cells because they will exhibit protein in the MHC class II receptors. VP40 is a matrix protein that interacts with lipid bilayers to form particles, indicating it is essential for viral construction and budding, as well as VP30 being a transcription factor. All of these proteins are structural proteins found in the ribonucleoprotein complex and matrix within the viral envelope. These proteins can be present on MHC receptors native to the host cell and will target the cell for destruction. It will also exist in the serum when the host cell ruptures due to the viral infection. The VP35 and VP24 specifically will be important to target in the serum as these proteins increase EBOV's ability to infect new host cells through affecting interferon production and sensitivity. By using the VP proteins in a vaccine, protective immunity was developed in mice. (Cervený, Feng, He, Yan, 2007) (Bakken, Bray, Hart, Wilson, 2001) (Bavari, Enterlein, Gamble, Iversen, Kroeker, Muhlberer, Smith, Stein, Swenson, Warfield, 2006)

This vaccine will require the use of adjuvants, which are useful for increasing immune responses. One of the adjuvants used will be Freund's incomplete adjuvant. Freund's incomplete adjuvant is an oil-in-water emulsion that provides a delayed release of antigens. This delayed release will increase the uptake of the antigens by macrophages. Another adjuvant used will be immune stimulatory complexes (ISCOMs). This will first have to be cleared for human vaccines, and if it is, it will allow the antigens to be delivered directly into the cytoplasm of cells and result in a CD8 T cell response.

The innate immune response initially occurs for this virus in the epithelial cells. Here the macrophages take up virions and then begin to secrete cytokines to recruit other monocytes and adhesion molecules to aid neutrophils in entering epithelial tissue. These macrophages will also secrete cytokines that cause inflammation. The resulting dilated blood vessels will cause gaps to form between endothelial cells lining the vessel. This will allow the monocytes to enter the infected tissue. Viral infection of the host cell induces interferon production which stops viral replication. Dendritic cells and macrophages will take up the antigen from the environment and take it to secondary lymph tissue. Here, the dendritic cell showing the antigen on its MHC class I and class II receptor will activate CD8 and CD4 T cells respectively. The T_C cells will lyse cells exhibiting Ebola antigen on the host MHC class I receptors. T_H2 cells will help activate B cells to transform them into antibody secreting plasma cells. The T_H1 cells are active at the site of infection and increase the effect of the macrophages.

While a live attenuated vaccine was out of the question, considering the deadliness of the disease and the possibility of the virus reverting or regaining virulence, we considered an irradiated virus as a vaccine. However, this posed some problems. The largest concern was the vast quantities of live virus that we would have to culture to create the vaccine. This would create a large health concern in the area where the vaccine was being produced. Furthermore, only the GP is known to be exposed on the outside of the viral envelope. While the immune system may remove the envelope and create response to the interior ribonuclearprotein complex and matrix, it is more likely to

illicit an immune response if the proteins are already separated into subunits. (Chen, Mohamadzadeh, Olinger, Pratt, Schmaljohn, 2006)

Upon the introduction of our vaccine into the human component, there is bound to be complications. We anticipate running into problems that center around both the availability and usefulness of the vaccine towards every type of individual. It is necessary to understand that what might work for military personnel and medical staff from the U.S., could easily be ineffective for individuals residing in African countries.

Some individuals are clearly more susceptible to the virus than others. For instance, an individual who resides in the area of transmission most likely has a different immunity level. An individual who is infected with the virus more than once tends to develop a higher immunological defense. Their body has a memory for the virus, and is therefore able to react more quickly and efficiently towards the virus. With this in mind, it is in our best interest to provide a vaccine that is specific to the individuals in which we are immunizing.

Secondly, once we have generated our vaccine, it is necessary to re-examine the dangers that the Ebola virus possesses. With the capability of causing death, it is important that our vaccine minimizes this possibility. The utilization of proteins from the virus in the development of the vaccine should prove to be successful, as it limits the possibilities of the virulent form remaining in the system.

The final problem that we might encounter revolves around the potential for the virus to weaken the effectiveness of our vaccine. In such a case, the virus most likely will work with the advantages of antigenic variation. Scientists are unsure just how quickly the virus can induce antigenic variation. With this we hope that the utilization of a subunit vaccine consisting of the GP, NP, VP35, VP30, VP24, and VP40 will work against the virus by eliminating it right before it enters into host cells. (Nabel, Rollin, Sanchez, Sullivan, Yang. 2000)

Description of Immunity Assessment

The vaccine will be administered by injection to adults between the ages of 18 and 30. It is hoped that these individuals will have the most resilient and healthy immune systems, allowing them to quickly produce the appropriate antibodies. Testing with younger individuals is potentially dangerous because the effects of VP35 and VP24, subunits in our vaccine, are well known to weaken the immune response and allow greater viral replication. While infants are of course protected by the IgA antibodies from their mothers, they are still to vulnerable. To expose them to the harmful anti-interferon agents, VP35 and VP24, would be extremely dangerous.

To ensure that the vaccine is safe for human use, we will first administer the vaccine titer concentrations to NHPs (nonhuman primates). This experiment will be a double blind study using placebos to ensure that the side effects of the vaccine, if any, are minimal.

To ensure that an immune response has occurred, we will use flow cytometry to detect cytotoxic T cells because these T cells specialize in destroying viruses that infect human cells. This prevents the replication of the virus and the spread of the virus to healthy cells. Flow cytometry will also measure the effects of the monocytes,

macrophages, and dendritic cells because these are the primary cells that are infected by the virus.

Flow cytometry, using markers CD3 and MHC Class I, can be used as a way in which to monitor T cell levels at specific points following vaccination. If the vaccine is not working the T cells will be receiving a kill-signal; their levels will decrease rather than rise in response to the pathogen. If the T cells are not activated, the B lymphocytes will not be urged to produce the antigen-specific antibodies at high levels.

This response can be monitored using an indirect ELISA test for IgG levels. IgG is the most abundant antibody in the internal fluids and it has many effector functions that will better destroy the virus. It can directly recruit phagocytic cells and activate the complement system. Each subunit will be tested for individually. Each of the target proteins of Ebola virus (VP 24, VP30, VP35, VP40, GP, and NP) will be used to coat the wells in different assays. Tissue and serum samples from the test subjects will be added to the wells after the viral proteins have attached. Bound IgG can be detected using an anti-IgG that is specific to the Fc region of the test subject (anti-human gamma chain to detect human IgG; anti-primate gamma chain to detect primate IgG; etc.). In these ways, the adaptive response of the test subject can be monitored over time and a quantitative determination of the activation of T and B lymphocyte response to each protein can be made.

If the side effects are determined to be within safe parameters, and the tests for an immune response to the vaccine come back positive, we will then challenge the NHPs with the Zaire Ebola virus, also using a double blind study. We will need couple of weeks to monitor the NHPs after we expose them to the virus in order to give the immune system enough time to make an adaptive response. If all the results are indicative of a successful vaccine, we will proceed to a human study.

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