

The development of a DNA vaccine for Ebola Virus- Zaire

This proposal is to request funding for the development of a necessary vaccine against the Zaire strain of Ebola Virus. The purpose of this vaccine is to control the outbreak and spread of the deadly Ebola virus in the regions of western Africa and to aid in the development of function vaccines for other Ebola strains.

Ebola virus, named after the Ebola River in central Africa, is found primarily in rainforests in both Africa and the Western Pacific. While mostly sporadic outbreaks occur in these areas, a number of cases have been reported elsewhere. The virus is spread through person-to-person contact via bodily secretions, contaminated blood, and close physical contact. This includes soiled linens and clothing, and transmission is still possible once the carrier has become deceased. Infection can also come from the handling of infected gorillas, antelopes, and chimpanzees, regardless of whether they were dead or alive. This occurs mostly in Africa; however, handling of these animals has contributed to infections in places outside of Africa.

The infection resulting from Ebola virus results in severe hemorrhagic disease that leads to mortality rates of up to 90%. Clinical symptoms appear after two to twenty-one days starting with high fever, chills, malaise, myalgia. The disease is distinguished by generalized fluid distribution problems, hypotension, coagulation disorders, and bleeding tendency resulting in sudden and severe shock. The infection with the virus involves necrosis of the liver, spleen, kidney, lymph nodes, testes, and ovaries due to the replication of the virus within the parenchymal cells.

Epidemiology

The medical community has recognized Ebola since its initial known outbreak in 1976, occurring in the Democratic Republic of Congo and Southern Sudan. Since then it has had repeated outbreaks in the Democratic Republic of Congo typically resulting in increasing

mortality rates and incidence. The most recent epidemic, resulting in the greatest incidence (425 cases and 224 deaths), took place in Uganda, which neighbors the DRC. For all epidemics, the death rate is greater than 50%, indicating the importance of eradicating the pathogen and the necessity for a vaccine. (CDC site)

The transmission of Ebola in epidemics is, in most cases, associated with hospitals, which serve as an added opportunity for movement to new hosts. The pathogen is most commonly transmitted via close contact with the body fluids of an ill patient. Although this seems to be the most prevalent route of transmission, cases have been identified showing that this alone does not cause disease in all persons involved. Many times the person must have multiple exposures to the pathogen before illness, and evidence shows that increased exposures is directly related to severity of illness. Less common routes of transmission are the use of the same materials, handling of the deceased, sharing meals, sharing sleeping quarters or sleeping mat, and airborne transmission has a minor probable role. These routes have shown to have slightly decreased mortality rates. (CDC site)

It is difficult to control Ebola outbreaks due to its ability to easily transmit to both those living with infected people and to health care workers via treating infected patients. The lack of historical data before 1976 makes outbreak control even more difficult; preventing us from understanding how it could spread to distant countries/provinces. Also, the disease spreads very quickly and early detection in infected persons is difficult due to the lack of medical funding and differing cultural beliefs. Continuous civil war and bureaucratic factors often make the ability to deliver proper health care to those in rural and civilized areas near to impossible. From a global viewpoint, if the pathogen were given the means to travel to more populated areas it could do insurmountable damage. (Ebola book).

Ebola Physiology

The Ebola virus has an intricate physiology that is responsible for both its high virulence and difficulty in developing a functional vaccine. It belongs to the *Filoviridae* family of viruses that are characterized by the presence of seven proteins: NP, VP35, VP40, GP, L, VP30, and VP24. The NPs and VPs form the main body of the virus while the GPs are used for attachment to host cells that initiate infection. L and VP35 proteins serve as an anchor for the nucleoprotein to keep it in place. Although all of these proteins are involved with the pathogenicity of the Ebola virus, our focus will be on the Ebola glycoproteins.

The Ebola virus uses two different forms of its glycoprotein to induce a double dose of infection. One form is secreted by the virus and inhibits the hosts' inflammatory response. The other form remains attached to the virus and binds to endothelial cells lining the blood vessels and enables the virus to infect and damage them. Amino acid sequences associated with the glycoproteins have an immunosuppressive domain that inhibit NK cells, decrease phagocytosis and block the transformation of lymphocytes into larger cells. These factors are the reason why Ebola glycoproteins are considered to be major viral antigens, thus making them a good target for a vaccine.

Current research suggests that host microtubules are an important part of viral infection and replication. VP40 is a membrane associated matrix protein that interacts with the host cells microtubules and catalyzes the polymerization of tubulin. VP40 not only anchors the virus to the host, but also induces budding of the virus for replication. This protein is a strong virulence factor for the Ebola virus and should be the main target for a vaccine. By disabling the VP40 protein the virus would lose most of its ability to bind host cells and replicate.

Ebola Virus Mechanism

Eight proteins are encoded in the Ebola virus genome, but the functions of these proteins in infection are still unknown. Two glycoproteins, an envelope glycoprotein, and a nonstructural secretory glycoprotein are the major components of Ebola virus pathogenicity. The envelope glycoprotein upon binding to the receptor of a host cell fuses into the host cell's membrane, while the secretory glycoprotein is released from the infected cells. Upon infection with the Ebola virus, the virus stimulates antibody production that enhances the virus infection. These virus antibodies activate the complement pathway resulting in facilitated entry for the virus. The complement protein C1q controls the augmentation of infection through the antibody-dependent enhancement (ADE). The suggested mechanism of complement enhancement is that couple of monomeric IgG antibodies bind to specific epitopes on the glycoprotein that are in close proximity which permits the binding of C1 to the Fc region of the antibodies. This complex then binds C1q ligands on the cell surface leading to the binding of more viruses to virus-specific receptor or endocytosis. These C1q ligands have been found in immune cells such as: monocytes, macrophages, and endothelial cells. These cells are targeted by the Ebola virus and are involved in the pathogenesis of the virus (1).

Due to their role of initiating the innate immunity the virus uses these immune cells to its advantage. The virus can replicate in dendritic cells without activating the secretion of cytokine and chemokines. The infected dendritic cells are not able to mature and alert mediators of adaptive immune responses, which results in the lack of response from natural killer cells, T and B cells and therefore allows the rapid spread and growth of the Ebola virus. The early initiation of immune responses is thought to be linked to the survival of the host because of the survival of

the virus upon the early initiation of innate proinflammatory responses (3). In addition to replicating within dendritic cells the virus also replicates within the mononuclear phagocytic cells. The virus-induced activation to these cells is thought to cause the vascular instability and the dysregulation. The activation leads to the production of active mediator molecules, which occurs early upon infection with the virus. The molecules that are produced are proinflammatory cytokines and chemokines (2).

Lastly, the Ebola virus also interferes with the responses of the host's interferon due to the VP35 protein found on the virus. Interferons that are regulated through transcription factors excrete antiviral, cell growth-inhibitory, and immunoregulatory activities. The interferons activate a cascade of events that leads to the inhibition of viral replication through the restraint of protein synthesis. The VP35 protein aids the synthesis of viral polypeptides and therefore counteracts the antiviral response by the interferon

Vaccine Development

The development of a vaccine for Ebola is imperative to prevent unnecessary deaths. Recently, clinical trials were conducted that tested the effectiveness of a DNA vaccine against Ebola. This DNA vaccine consisted of three plasmids that encoded glycoproteins of two strains: Zaire, Sudan/Gulu and the nucleoprotein of the Zaire strain. Healthy volunteers between the ages of 18 and 44 were recruited. The vaccine was given to volunteers in a series of three injections. The volunteers' health was monitored closely after each injection was administered. Initially, the volunteers were given a DNA/plasmid vaccine followed by an anti-replication adenovirus vaccine to prevent the plasmids from replicating. Last, a DNA/rAd booster was given to provoke an immune response by attracting nearby monocytes. Humoral and T-cell responses were measured by ELISA and ELISPOT. Researchers discovered that the majority of

participants generated a sufficient amount of specific antibodies to glycoproteins of the Zaire and Sudan/Gulu strain. Only 87.5% of the participants generated specific antibodies for the nucleoproteins of the Zaire strain. However, neutralizing antibodies were not detected in any of the participants. Cellular immunity appeared to have a weaker response than humoral immunity. The frequency of CD4+ T-cells of both glycoproteins was 100%, which appeared a month after the last injection. However, it took twice as long for the Zaire nucleoprotein to produce a strong response to CD4+ T-cells. Unfortunately, the CD8+ T-cell response was not frequent in any strain. All strains did develop some CD8+ T-cells, but the frequency was less than 25%. The participants only experienced minor side effects to the vaccine and there were no casualties (Martin et.al.).

CD8+ T cells are highly significant to the immune response of Ebola because it stimulates the formation of cytotoxic T-cells. In an earlier study, mice were utilized to identify epitopes of MHC class I complexes. Initially, these mice were given a DNA/rAd booster to enhance cellular immunity. The mice were then injected with a DNA plasmid vaccine containing nucleoproteins of the Zaire-Mayinga strain. The Zaire-Mayinga peptides were tagged with fluorescent markers to identify them within a cross-linked peptide library. Lastly, a recombinant adenovirus was injected to prevent Ebola from replicating in the mice. Twenty four peptide pools were monitored; however, only four pools tested positive for the adhesion of viral peptides to the epitopes of CD8+ T-cells. NP279-288 and NP388-396 were the common links between the pools. These specific peptides were able to stimulate the production of IFN- γ from CD8+ T-cells. Further testing identified restricted epitopes that were compatible to the nucleoprotein sequence bound to CD8+ T-cells. Unfortunately, the bonding affinity was not high enough to

stimulate a sufficient cellular response in humans; therefore, additional research is required (Simmons, G et. al.).

Vaccine Assessment

Initially, trial vaccinations will be administered in mice so that safety can be better assessed prior to testing in human patients. However, once safety is proven in mice, human testing will commence. Inoculations will be given intramuscularly to elicit both T-cell and B-cell response and the further production of the glycoprotein antibody by skeletal muscle cells. The vaccine will be given to patients in a series of three inoculations, spacing injections at least 21 days apart, and patients will randomly be placed into three groups, placebo, 2-mg or 4-mg injections. Prior to the series, patients will be examined for immune competency so that patient safety and experiment accuracy will be maintained (Martin et al. 1268).

Blood serum samples will be begin on the 24th day after the primary injection and repeated every 2 weeks for 12 months. Although it is hoped for those inoculated with the vaccine to maintain a cellular memory to GpZaire with only one series of vaccination, the expectation is that a “booster” may be required to maintain immunity.

The vaccine will be evaluated by both Flow Cytometry and ELISA from blood serum samples. These tests will assist in determining the effectiveness of the vaccine and if/when it should be released to the general public for use. The use of ELISA and Flow cytometry in conjunction will prove the activation of both T-cells and B-cells, as well as give specific T-cell activation information.

Flow Cytometry

Flow Cytometry will be used to quantify the antigen specific T cells for Gp Zaire found on the surface of the Ebola Virus. This procedure is conducted by running a sample of the patient's blood in the flow cytometer, which will quantify the relative number of specific antigen binding molecules after they have been tagged by a fluorochrome-labeled antibody on the specific molecules. The cytometer does so by passing the sample through UV light causing those molecules tagged with the fluorochrome to emit a visible light that can be detected by a photomultiplier tube within the machine. Thus, the cytometer quantifies the amount of emitted light, which, in turn, relates to the amount of antigen-specific cells.

A two color fluorescence can be conducted by quantifying the intensity of the fluorochrome-tagged antibody of the vertical axis and the other on the horizontal axis. This will then show the molecules that bind to neither antigen, to both, and then to only one of the antigens. By using different antigens, we can quantify specific B cells and CD+8 T cells; this is necessary given the differences between the two cells. The amount of specific B cells can be measured by using the actual antigen in the experiment. This would then have the fluorochrome antibody bound to the antigen, which would be bound to the B cell.

In order to quantify antigen-specific CD+8 another complex must be used. Antigen-specific CD+8 T cells do not bind the actual antigen but instead bind to antigen that is already

bound to MHC-I complex. Therefore, in order to measure the relative amounts of antigen-specific CD+8 cells a tetramer of MHC-I must be used with the peptide specificity for Gp Zaire and labeled with the fluorochrome antibody. The tetramers are more sensitive and therefore allow the antigen-specific T cells to be detected. Quantifying antigen specific T cells is usually difficult due to low numbers unless an immune response has been activated. Therefore, if the vaccine is producing the expected immune response for creating memory cells and specific cytotoxic cells, the flow cytometry will help determine the efficiency of the vaccine.

ELISA

The ELISA test, or Enzyme-Linked Immunosorbent Assay, will be the main test of B cell response to the Ebola Zaire glycoprotein vaccine. This assay is run by, initially purifying a sample of antigen, in this case GpZaire, and coating a plastic multiwell plate with the preparation. In addition to the preparation, milk will be blocked onto the well to prevent the non-specific binding of the patient's antibody to the plastic. Lastly, in order to determine a relative amount of serum antibody, each well will contain an increasing amount of antigen to which the antibody may bind. As you will see, the antigen-antibody complex will produce a color change based on quantity (Janeway, 690).

Once the multiwell plate is prepared, the patient's serum will be added to the wells to cause an antigen-antibody complex formation. This, however, is not noticeable in color change and another step is required to produce the effect. Development of an antigen to the human Gp Zaire antigen covalently bonded to a color-producing enzyme gives us that capability. Injecting the human Gp Zaire antigen into a non-human organism, such as a rabbit, produces the anti-human GP Zaire antigen, and by purifying the antibodies made by such animal we have the

required marker for complex formation. This is added into the multiwell plate and gives a color change with differing intensity, based on relative amounts of the antigen-antibodies complex in each plate (Janeway, 690).

This assay will allow for the speedy assessment of individual patients immune response to the vaccine. Although it will not tell us how a patient will react once exposed to Ebola, we can infer that because a patient is producing the required antibodies to the Gp for the Zaire strain, B cell activation is occurring thus producing memory cells to the antigen. B cell activation and memory cell formation to Ebola will then reduce the adaptive response time and give the patient an added defense against this fast-acting pathogen (Janeway, 690).

The choice to use a DNA vaccine for the Ebola virus was based on a number of supporting factors from previous vaccine research. A project conducted by the Virology Division of the United States Army Medical Research Institute of Infection Disease, headed by Lorna Vanderzanden, showed DNA viruses expressing either Ebola glycoprotein (GP) or nucleocapsid protein (NP) injected into mice provided protection from the virus. Mice were first vaccinated four different times with an initial 0.5 μ g dose and three additional 1.5 μ g doses of the GP vaccine at four-week intervals. All mice survived exposure to the virus following the vaccine regime (Vanderzanden et al. 136). The same level of protection was found with the NP vaccine (Vanderzanden et al. 134). Both elicited humoral and cellular immune responses. However, the same experiment performed by Thomas Geisbert using non-human primates, specifically macaques, yielded completely opposite results. The same GP and NP vaccine regiment was used based on Vanderzanden's methodology. Only one of the twenty-six primates used survived; the others died by the ninth day after exposure to the virus. This discrepancy has

been attributed to a different disease pathway in rodents versus primates (Geisbert et al. 505). However, a different pathway should imply the use of a different vaccine method, so the effectiveness of a GP vaccine is still applicable. Another study, performed by Nancy Sullivan of the National Institutes of Health, involving another GP vaccine used in conjunction with an adenovirus, which was tested on macaques, proved more effective. Subjects received three injections over a twelve-week period, at four-week intervals. All subjects vaccinated saw increased antibody levels, and once exposed to a wild-type Ebola virus, survived infection (Sullivan et al. 607).

The successful outcomes of the research above support the choice of a DNA vaccine. Furthermore, Ebola is too deadly of a vaccine to administer in an attenuated form. Should the virus revert to a virulent form, the results would be devastating. This holds especially true for Africa, the endemic location of the virus, where a portion of the population has a compromised immune system due to HIV. An inactivated Ebola virus vaccine, while plausible, only induces a humoral response when protection against Ebola requires the additional cellular response. A DNA vaccine avoids the potential infection of the patient, and it has been shown to induce both humoral and cellular immune responses in other trials, not just with Ebola. The DNA vaccine also allows for the selection of virulent genes found in all strains of Ebola, granting a broader base of protection.

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