"Recombinant DNA Vaccine (JPKS007) against Ebola for developing countries in Africa"

Introduction:

Ebola virus from the Filoviridae family consists of four distinct serotypes: Zaire, Sudan, Cote d'Ivoire and Reston. It is believed that Ebola is a zoonotic virus with the natural reservoir seemingly resides in the rain forests of the African continent and Western Pacific. Fruit bats and dogs are possible reservoir although there has been no direct proof, despite the World Health Organization's (WHO) considerable effort. Ebola virus causes Ebola Hemorrhagic fever (EHF), a febrile hemorrhagic illness, which causes 50-90% death of all cases. It is often exemplified by sudden onset of fever, intense weakness, muscle pain, headache and sore throat followed by vomiting, diarrhea, rash, impaired kidney, liver dysfunction, internal and external bleeding. Because Ebola has a high mortality rate and since no vaccine or treatment available, it has been classified as a Biosafety Level 4 by the CDC (www.who.int).

Clinical Features/Transmission:

Ebola virus has shown to have thread-like characteristics of a filovirus. It varies in shape and may appear to be circular, branched or coiled. In the center of the virion is a nucleocapsid formed by RNA complexes with nucleocapsid-associated nucleoprotein (NP) and nonstructural proteins (VP30, VP35). Ebola has a single transmembrane glycoprotein (GP) present on the outer viral envelope, derived from the host membrane that is crucial in mediating the virus' entry into the cell. The viral proteins VP24 and VP40 are located between the envelope and nucleocapsid. The virus also secretes sGP that is produced in abundance early in the infection and are specific for different cell types (Rao *et al.* 2002).

The Ebola virus is transmitted by direct contact with bodily fluids, skin or mucus membrane contact of infected persons with an incubation period of two to 21 days. In the early stages of infection, Ebola might not be highly contagious. As the illness progress, vomiting, diarrhea and bleeding present extreme biohazard and has a high probability of being transmitted to another human (www.who.int).

The mechanism of viral entry into the host cells is still not yet known but it is safe to assume that the glycoprotein spike on the outer envelope contribute to the process. As soon as the virus is inside the host cell, the virion will activate and release its genetic material using the host's replication machinery and resources. As replication progress, the cell will eventually rupture and bursts allowing the virus to spread and infect neighboring cells. However, in order for a successful viral infection Ebola must first avoid the immune system. One of the ways to accomplish this is by inhibiting IFN activities. VP24 impedes IFN-α/ß and γ signaling thus preventing the formation of interferon induced antiviral immune response. The nonstructural protein, VP35, stops the transcription of interferon transcription factor 3 which stimulates the formation of IFN-α/ß. Interferon assist in the immune response by preventing viral replication within the host cells (www.cdc.gov).

Ebola is not limited to impoverished countries; globalization permitted the spread of this virus to other parts of the world even those with advance medical technologies. However, most outbreaks have occurred in hospital conditions, where basic sanitation and proper hygiene is not common. Since Ebola is not transmitted airborne, it is often spread within the hospital settings with infected Ebola patients. In modern medical facilities with proper hygiene, disposable needles and barrier nursing techniques, it is rarely spread in a large scale. Nevertheless, in isolated settings such as small villages in Africa, it is highly transmissible due to direct contact with bodily fluid of the infected or deceased individuals (www.cdc.gov).

Pathogen Strains:

Zaire Ebolavirus

Ebola Zaire Virus (EBO-Z) is an enveloped, nonsegmented negative-strand RNA virus from the *Filoviridae* family which has a 90-100% mortality rate and is the most prevalent out of all the Ebola strains. EBO-Z causes severe hemorrhagic fever in both humans and nonhuman primates. It first emerged in 1976 in Yambuku, northern part of Zaire when a 44-year old schoolteacher, Lokela, with a high fever returned from his trip north of Zaire. His case was first diagnosed as another case of malaria and he was given a shot. But weeks later, his symptoms progressed to uncontrollable vomiting, bloody diarrhea and trouble breathing which eventually led to internal bleeding. The patient died only 14 days after the onset of the symptoms. Shortly after, a large number of patients began arriving with similar symptoms. They believed that the initial transmission is from a reused needle utilized from Lokela's case without sterilization (www.cdc.gov).

Sudan Ebolavirus

The Sudan Ebolavirus was the second Ebola outbreak reported in 1976. It originated among factory cotton workers in Nzara, Sudan where a worker was exposed to Ebola antigen in the cotton factory. Another case involved a nightclub also in Nzara, Sudan. Health officials investigated and searched the cotton factory to determine a possible natural reservoir of the virus but they weren't able to find anything. As for the second case, the local hospital tried to treat the infected patient but they were unsuccessful. Due to unsafe protocols and the nurses did not utilize sterilization of the medical tools; the entire hospital eventually became infected. Sudan Ebolavirus has a 50-60% mortality rate compared to Zaire (www.cdc.gov).

Reston Ebolavirus

Initial outbreak was first discovered in 1989 in a group of crab-eating monkeys that were imported from the Philippines to Reston, Virginia. This particular strain of Ebola virus is highly lethal to monkeys but there has been no report on any human casualties. A high rate of Simian Hemorrhagic Fever (SHF) was prevalent in monkeys which attributed to their deaths (www.cdc.gov).

Ivory Coast Ebolavirus

Two corpses of chimpanzees were discovered in Cote d'Ivore, Africa. The autopsy did on the chimpanzees have shown blood in their lungs. Tissue samples were taken and it was confirmed that the chimpanzees had shown results similar from Ebola infected humans in

Zaire and Sudan. It is believed that the source of contamination was from the meat of infected Colobus monkeys, which the chimpanzees preyed on (www.cdc.gov).

Treatment/Prevention:

Patient serum is tested to detect specific antigens and/or genes of the virus. Infected individuals are then isolated from other patients to prevent further transmission. The treatment, however, is primarily supportive as there is no Ebola human vaccine available. The treatment include noninvasive procedure, balancing patient's electrolytes via intravenous fluids or oral rehydration, replacing the host's coagulation factor to slow down the bleeding, maintaining oxygen and blood levels as well as treating secondary infection (www.who.int).

Isolation is not the only preventive method health officials and scientists have used to inhibit the spread of this disease. Various vaccines conducted on monkeys and guinea pigs have been developed. Much of those work focused on the development and use of recombinant viral GP or NP as the pathogen, concentrating on GP as the target for eliciting neutralizing response from the immune system (Rao *et al.* 2002). Sullivan *et al*. (2000) used DNA constructs on guinea pigs and macaques that coded for combinations of glycoproteins and nucleoproteins from different strains. The entire test animals used who survived a lethal challenge of Ebola with the aid of their vaccine. Another vaccine proposed by Parren *et al.* (2002) was performed on guinea pigs. This vaccine, KZ52, have shown remarkable results in animal model. It is composed of an IgG neutralizing monoclonal antibodies that protected guinea pigs from Ebola virus by reducing plasma viremia [the presence of virus in the blood]. Interestingly enough, it has been found that people infected with Ebola virus are intensely viremic (Parren et al. 2002). Consequently if this vaccine was to be administered to an infected person, it may slow down the onset of the disease. Research on VP30, a nucleocapsid-associated Ebola-specific transcription factor has also been conducted to assist in the development of more vaccine. The initiation of Ebola virus replication requires the presence of VP30 in stabilizing the growing mRNA strand (Hartlieb *et al.* 2007). If we were to develop a vaccine that inhibits the production of VP30, or at least slow down Ebola virus transcription by preventing the binding of VP30 to the mRNA strand, then we might have a chance in slowing down the spread of the virus to other parts of the body that it could infect. A CAdVaxE (GPs/z) vaccine, including the SEBOV and ZEBOV glycoprotein (GP) was tested on mice. It was found to induce both humoral and cell-mediated (CMI) responses. They produce anti-EBOV GP serum antibody and EBOV specific CMI responses. The studies showed 100% protective immune response in mice (Danher *et al*. 2006).

The African continent has suffered enough, with famine, civil war, insufficient resources and diseases such as malaria. Ebola is another disease that is plaguing their country. Without an effective vaccine, it will be a disaster if this virus escapes the isolated regions of Africa and spreads to other parts of the world. The immediate onset of Ebola virus in infected persons inhibits our chance of saving them. Its high mortality rate also thwarts our effort in helping them. It seems that the only treatment we can afford them is to either slow down the infection or isolation to prevent the spread of the disease to another

person. In our battle against Ebola, it seems that the current treatment is to watch those infected people die. It is our responsibility to aid those in need of help and alleviate their suffering, which is why we chose to make a vaccine against this deadly disease.

Human Vaccine Proposal:

We will be using a recombinant DNA vaccine that encodes for the Zaire and Sudan strains of GP and VP40 proteins. Three different doses will be administered roughly a month apart, each containing a segment of Zaire and Sudan GP and VP40 proteins. DNA vaccines use segments of DNA (or plasmids) to induce immune responses. The DNA recombinant vaccine will be taken up by muscle cells then utilize native machinery to create the antigens it codes for. These peptide fragments will then be broken down by the cell and presented on MHC receptors. CD8 cells specific to the Ebola peptides will recognize the antigens presented on MHC I receptors and subsequently kill the infected cells as well as replicate itself. Th2 cells will recognize the antigens presented on MHCII receptors then present these antigens to B cells and secrete cytokines that cause B cell maturation. These B cells will be specific to the Ebola proteins and create a lasting immune response via antibodies. While naturally occurring DNA can be used, it is more efficient to induce alterations in the genome in order to increase the magnitude of invoked responses. VP40 (of Zaire and Sudan strain) is the most lethal component of the Ebola virion. However, VP40 alone is not effective in eliciting the onset of the disease. GP protein is needed for the virus to infect the cells. We will incorporate the Ebola (Zaire and Sudan strain) GP, as it is a prolific envelope protein and thus will be one of the first parts of Ebola that an immune response will encounter. This should create specific antigen immunity to these proteins while also creating a wide range of protection against Zaire and Sudan Ebola strains. Using partial DNA also limits the chances of accidental side effects; naked DNA will not be able to revert to a virulent form.

Innate Immunity

The innate immune response will have no lasting effect upon the presented Ebola antigens. Some of the constructs will be engulfed through phagocytosis as well as will induce a typical inflammatory response due to the foreign nature of the vaccine. This inflammation includes the production of certain cytokines that will influence the type of antibodies produced in adaptive immunity. The actual virus typically activates only this innate response, killing the infected individual before adaptive immunity is activated. The virus proves evasive because its glycoproteins form heterotrimers on the envelope causing a shifting variety of epitopes, delaying the immune system's ability to react to the disease (Reed et al. 2007).

Adaptive Immunity

The vaccine will be taken into muscle cells, where native cellular machinery will transcribe and translate it into the target protein antigens. These antigens will then be broken down by the cell and processed to be presented on MHC receptors. Th2 cells will respond to presentation on MHCII receptors and CD8 cells respond to MHCI presentation. Once Th2 cells bind to the antigen on MHCII it will initiate the activation of B cells and thus, eventually, the production of Ebola-specific antibodies. Due to the

specific cytokines produced upon entry of the vaccine, the antibodies created will be of type IgG. These antigen specific antibodies will provide a lasting immune memory against the two Ebola proteins. CD8 cells specific to the antigens presented on MHCI will bind to the infected cells and kill them without help from the helper cells, while also cloning themselves in preparation for future infections. In this manner two memory cells will be produced: Ebola-specific B and CD8 cells.

The vaccine will be tested on primates since they are mostly similar in cellular function to humans. It would be preferable to test the vaccine on humans. However, due to ethical problems, it will be appropriate to use primates as subjects first before it is to be administered to humans. Once antibodies from Ebola virus GP and VP40 protein has been isolated in primates and the efficiency of the vaccine has been determined, the proposed vaccine can then be administered to human subject to test its efficacy in human population.

Immunity Assessment:

After the administration of the proposed vaccine (JPKS007) with DNA recombinant encoding GP and VP40 protein. Blood serum from each test subject will be collected to test for any immune response. By comparing the results of the tests and the level of immunity the subject elicit, the efficacy of the vaccine in cellular and humoral immunity can be assayed.

ELISA (Enzyme-linked Immunosorbent Assay)

Humoral immunity is composed of the production of B-cells, subsequently the production of antibodies to an invading pathogen. Immunoglobulin G will be the focus of our vaccine as the main antibodies produced upon initial infection. IgG can be detected using ELISA, and since IgG is the antibody mostly produced by the body it will be the target of our assay.

Blood serum will be collected from primates and an indirect ELISA will be performed to detect IgG production in the serum. The glycoprotein (GP) binding region of the virus will be used as the antigen which will bind to the antibodies. The initial step of the assay is to bind (DNA recombinant encoding) GP protein to the microtiter plate. Serum samples from the subject to be tested for Ebola-specific IgG will be added afterwards and allow it to bind to the GP protein. An anti-Ig will then be added, which will bind to the Fc region of the antibody. The anti-Ig that is bound to the Fc region will have an enzyme, which will convert a chromogenic substrate to a colored product once the binding of the enzyme and substrate occurred. The level of color intensity will be used as an indicator of the specificity of the antibody and the amount of specific antibody production in the sample. Another assay must be performed as a comparison so a negative control will be conducted. The negative control will be run by adding another antibody that will not bind to the antigen or by excluding the antigen altogether. The same assay will be performed on a human serum to test the amount of Ebola-specific antibody in the serum, hence the efficacy of the vaccine.

Flow Cytometry
A viral infection such as that of Ebola will trigger a cellular response, which is mainly the production of T-cells particularly CD8 T-cells. Th cell levels will also be determined since Th cells are needed to aid in the activation of Ebola-specific B cells (antibodies). This step is not completely necessary, as IgG levels will also be measured – however the level of Th2 cells may be of interest for further studies. Flow cytometry is used to measure the amount of cells based on the markers they present. Common flourochrome such as FITC (fluorescein isothiocyanate, green) are added to assay the amount of cytokine produced by Ebola-specific CD8 T-cells in the serum. Blood serum drawn from the subject is used and all red blood cells are isolated via centrifugation. FITC-labeled CD8 T-cells specific for Ebola (GP and VP40) structure are then incorporated for flow cytometry analysis. The negative control portion of the study is attained by including an isotype and antibody for the same species but bind to different antigen. Ebola-specific CD8 T-cells and antibodies are incubated then proceed through the flow cytometer. As the cells pass through the detector, the amount of fluorescence is detected and quantified. A fluorescence-activated cell sorter utilizes the fluorescent signals emitted by each cells to separate them into groups based on the fluorescence-labeled antibody bound to them. Since flow cytometry works by analyzing each individual cells that passes through the detector, the number of CD8 T-cells present in the serum can then be calculated using this technique. The recorded number of Ebola-specific CD8 T-cells can be use to identify whether the vaccine initiated a cellular response against the Ebola antigen.

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