

Proposal for the Use of Recombinant DNA in a Vaccine Against P. Falciparum

Introduction

Malaria is a dangerous disease that infects humans and causes one million deaths each year. In children under five years of age, it is the fourth leading cause of death beaten only by neonatal causes, acute respiratory infections, and diarrheal diseases. Although malaria affects mainly those in poorer nations, it is still present in the United States with 1,300 cases diagnosed annually (About 1).

People can become infected with malaria after being bitten by a female *Anopheles* mosquito carrying one of four different bacterial parasites: *Plasmodium falciparum*, *P. vivax*, *P. ovale*, or *P. malariae*. Malaria affects human hosts differently based on the species of mosquito that infected the human and the human host itself. *P. falciparum* causes the most severe forms of the disease and can often be fatal. Some malaria infections can be asymptomatic while most people infected display fever, chills, sweating, headaches, and muscle pains. Complications can arise due to malaria and can include cerebral malaria, anemia, and kidney failure, all of which can be fatal to the human host (Biology 1).

Three components must be present in order for a malaria infection to occur: the *Anopheles* mosquito, the malaria parasite, and the human host. The climate can have a great effect on the mosquito, the parasite, and the host and increase or decrease the probability of a successful malaria outbreak. Mosquitoes need standing water in order to reproduce. Thus, in the rainy season, mosquitoes reproduce quickly and multiply in number. It only takes 9-12 days in tropical areas for a mosquito to reach adulthood (Epidemiology 1). Once a mosquito bites an infected host and takes in the parasite, it takes 9-21 days at 77°F for the parasites to complete their cycle in the vector before the parasite can be transmitted, also known as the extrinsic cycle. At warmer temperatures, this time period is shortened and significantly increases the probability that the infection will be passed on. The humans may be affected by warmer temperatures, and cause them to engage in behaviors that will increase their chances for infection such as sleeping outside, sleeping without the mosquito net, or spending time near the water to cool off (Epidemiology 2).

A human can be infected with malaria rather easily. When a human is bitten by an infected mosquito vector, the sporozoites are transferred from the mosquito's saliva into the human's blood. The sporozoites travel through the host's blood until they reach the liver, where they infect and mature from the sporozoite to the merozoite stage in hepatocytes. At this point the parasites begin to target erythrocytes and have developed new receptors including the merozoite surface proteins. When the parasite reaches the blood, another mosquito can bite the human, take the gametophytes in (where they develop into sporozoites), and the cycle starts anew (Biology 1).

Description of Vaccine

It is being proposed that both the blood stage Merozoite Surface Protein (MSP 3) and Circumsporozoite Surface Protein (CSP) should be targeted in order to induce immunity to multiple stages of the parasites development.

The CSP is a highly expressed antigen located on the surface of the malaria sporozoite while MSP3 is one of the many expressed during the merozoite stage. The CS antigen is a good target since it is present during the early asexual stages of malaria infection and MSP3 is one of the proteins consistently found on *P. falciparum* in the later stage.

CS plays a vital role in each stage of the parasites development, such as assisting in the invasion of mosquito salivary glands, facilitating the binding of the sporozoite to liver cells, and inhibiting protein synthesis by the host (Garcia et al., 2006). The CS gene is divided into three regions: both a 5' and 3' non-repeat region and a central tandem repeat region. This repeat region is the target of CS protein antibodies (Hughes, 1990). A B cell epitope located in the repeat region is presented by class II MHC and recognized by helper T cells (Hughes, 1990). Antibodies to the CSP could neutralize the sporozoites before they bind to the basolateral domain of any hepatocytes and cause disease. The vaccine will target the B-cell epitope located in the repeat region of the CS protein. MSP is a highly variable protein and may undergo conformational changes; however this vaccine will target a highly conservative portion of the protein.

MSP3 is one of five known merozoite surface proteins and any similarity in their structures may allow for mild cross reaction (Crabb and Beeson). The introduction of specified protein fragments to the host will increase the number of possible antibodies elicited by the humoral response.

A recombinant vaccine was chosen in response to the tendency of the malaria receptor proteins to change conformation. Using recombinant DNA it is possible to combine the gene sequence for multiple antigens and therefore target their constant regions. It is also possible to target the parasite at two or more different stages in its development. The recombinant DNA for the two proteins will be encapsulated into an adenovirus vector. Adenoviruses are known to be successful vectors due to their high infectivity rate if they are modified to lack the E3 gene. They are able to infect host cells and use the host cell machinery to produce the desired malarial surface proteins without the risk of developing into disease. While an adjuvant will not be used in this vaccine, the adenovirus will replicate inside the host, producing more protein and increasing the exposure time of the immune system to the antigen.

When the vaccine is injected into the patient it will trigger a viral immune response. As the malarial proteins are produced the cell will present them on their MHC I for identification by antigen presenting cells. B and T cells are activated by the presence of the foreign proteins and a mild immune response ensues. Because the proteins are produced intracellularly the cytotoxic lymphocyte response is activated and the B-cells are activated into antibody producing plasma cells. Antibodies are specified to the natural occurring form of the surface proteins and will be more effective in recognizing different

epitopes. Specific memory B and T cells will be circulated for years after the vaccine has been introduced; however, individuals may need to be revaccinated in order to assure lasting immunity. Upon secondary infection the memory will be able to produce a faster and more accurate immune response to the presence of the *P. falciparum*.

The challenge to using MSP is that it is highly variable. While it is believed to be present on all merozoites, the protein undergoes conformational changes as the parasite develops. The genome has been previously mapped out; therefore it is possible to purify the gene sequences for CSP and MSP. Using DNA from the parasite may increase the chances of producing a protein that will have similar recognizable parts to the MSP 3 produced during an actual infection. The vector is easy to produce and store, which lowers cost. However, locating, cloning and inserting each gene into the vector can be costly and time-consuming.

This vaccine is especially useful for pathogens with numerous antigens, because antigens that either do not induce protective immunity or could possibly cause damage to the host can be left out. In order for the vaccine to be effective, the vector must have low virulence so it does not inadvertently infect the host. This is one of the major advantages of utilizing a recombinant vaccine. Also,

The use of both CSP and MSP covers the two different stages, allowing for two chances for the immune system to react and attack the disease. If the immune system is able to develop an immune response to the CSP sporozoite protein it should effectively prevent the host from becoming sick. Unfortunately, the parasite is only in the open for a matter of minutes before invading the liver hepatocytes. If the immune system fails to respond quickly to the CSP, the parasite will develop in the liver cells and the individual will get sick. However, pre-exposure to MSP may decrease the risk of death in the host, and will induce a greater immunity to the disease in the future.

This vaccine induces active immunity. Both B and T cells will be activated with this vaccine and produce memory cells. After the hepatocytes release the merozoites into the blood stream, the vaccine-induced memory T and B cells would be able to recognize MSP and begin the immune response (MVI). Vaccines for viral infections typically last a number of years, however individuals may need to be revaccinated every few years to maintain immunity. Due to the fact that RBC's do not have MHC on their surface it will be necessary for the B-cells to develop into antibody producing plasma cells. The recombinant vaccine allows for the production of the CSP and MSP without the fear of infection by the entire parasite. T-cells may have access to the merozoite during the late hepatocyte phase and before infecting RBCs.

* There has been a correlation to MSP3 and acquired immunity in endemic regions., There is a possibility for cross immunity with other MSP proteins (msp6 mentioned).

Description of Immunity Assessment

The *P. falciparum* vaccine will be administered parenterally. To test the efficacy of the vaccine, an Enzyme-Linked ImmunoSorbent Assay (ELISA) and a Flow Cytometry assay will be performed 4 months after the vaccine has been administered.

In order to conduct the ELISA, a serum sample will be taken from the patient. First, however, a microtiter plate will be coated with a purified sample of MSP-3. After this antigen has sat in the wells for nearly an hour, it will be washed with buffer to remove unattached antigen; then, a solution of powdered milk will be added to cover any sites that have not bound antigen and might potentially bind, nonspecifically, antibody.

At this point, the serum sample from the patient will be added to the wells. The wells furthest to the left will contain a 1:10 dilution, and each of the next 9 columns of wells to the right will be diluted twofold. The 11th column of wells will be a positive control (purified antibody to MSP-3 deliberately added), while the 12th column of rows will be a negative control (no antibody against *P. falciparum* present). After having sat in the wells for a sufficient length of time, the serum will be washed away with buffer.

Next, an enzyme known to convert a chromogenic substrate will be covalently attached to Fc region of anti-human Ig; this complex will be added to the wells and subsequently washed off with buffer after having sat. At this point, the afore-mentioned chromogenic substrate will be added. If antibody against MSP-3 is present, the substrate will bind to the anti-Ig that has bound to the antibody. Wells containing a significant amount of antibody against MSP-3 will now display a color change.

All the above steps will also be repeated using CSP as the antigen to test for antibodies against this antigen as well.

Cytotoxic T cells will be isolated from the malarial host by using the Pan T Cell Isolation Kit by Miltenyi Biotech. In this procedure, cells are exposed to a cocktail of biotin-conjugated antibodies which bind to all non-T cells. These antibodies seek out CD11b, CD45R, DX5, and Ter-119, markers which are expressed on non-T cells. Microbeads seek out the antibody-bound complex, so that the non-T cells can be washed out via a magnetic filtration process. Isolated T cells will be incubated in a drug to block secretion. Isolated T cells will then be fixed in formalin to cross-link specific cytokine IFN- γ and TGF- β , followed by treatment with detergent to permeabilize the cell so that antibodies specific to the aforementioned cytokines may enter the membrane and bind the cytokine.

Next, the mixture will be incubated and washed out of any non-bound antibodies. Antibodies will also be labeled with a fluorescence molecule such as FITC. Cells will be run through the flow cytometer, which will quantify fluorescence expression within each cell. This procedure will be repeated for two samples: 1st, it will be performed with cells that have been exposed to malaria and have not received the vaccination; and 2nd, it will be performed with cells that have had exposure to malaria and have received the vaccination. It is important to test the cells within 7 to 10 days of vaccination to have an active T cell sample. The hypothetical outcome of the flow cytometry should indicate

higher intensity levels of T cell activation within cells that have been given the vaccination. Analysis will be conducted via dot plot and histogram of the fluorescence intensity graphed against cell volume.

Sources:

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