

# Global Eradication of Malaria using a DNA and Attenuated Vaccine

## INTRODUCTION

Malaria is a blood infection caused by protozoan parasites of the genus *Plasmodium*, transmitted from human to human by female *Anopheles* mosquitoes. There are four species of malaria parasites, *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale* (Carter, 2002). *P. vivax* and *P. falciparum* are the most commonly encountered malaria parasites (2002). *P. vivax* is found sporadically in some temperate regions while *P. falciparum* remains prevalent in the tropics (2002).

Malaria is an endemic responsible for over a million deaths per year. Therefore, the need for a malaria vaccine is crucial. Currently, there are a wide variety of treatments for those infected with malaria. Determination of treatment is based on a combination of factors, including, the type of malaria infection, the severity of the infection, the age of patient and if she is pregnant, and the location where infection was acquired.

## EPIDEMIOLOGY

Malaria consists of the occurrence, distribution and control of the disease in a population. Climatic factors such as: temperature, rainfall, and humidity are the key determinants that influence the occurrence of malaria and are critical for the malaria life cycle to be completed. Rainfall creates “breeding sites” for mosquitoes to lay their eggs and develop into adulthood. Once in adulthood, the same climate factors contribute to their chances of survival. Warmer temperatures shorten the extent of the extrinsic cycle, which increases the chances of transmission. *Plasmodium falciparum*, predominates in warmer regions (CDC 2004). Warmer climates also increase human contact with *Anopheles* mosquitoes. Human behavior, such as sleeping outdoors without bed nets increases contact with mosquitoes because *Anopheles* mosquitoes are more active between dusk and dawn (CDC).

Malaria is mainly transmitted in tropical and subtropical areas where *Anopheles* mosquitoes can survive and grow, and where malaria parasites can complete their life cycle (CDC). The transmission of malaria occurs in warmer regions closer to the equator. The highest occurrences of transmission are found in Africa and South of the Sahara (CDC).

Biological characteristics and behavioral traits can influence the occurrence of malaria in a population. It has been shown that certain biological characteristics present from birth can protect against certain types of malaria (CDC). Persons who have the sickle cell trait are relatively protected against *P. falciparum* malaria, which has been a leading cause of death in Africa. The sickle cell trait is most frequently found in Africa and in persons of African ancestry than in other populations. Also, persons who are negative for the Duffy blood group are resistant to infection by *Plasmodium vivax*. Human behavior caused by social and economic reasons can also influence the risk of malaria (CDC).

Acquired immunity influences how malaria affects an individual. People who have had repeated attacks of malaria develop a partially protective immunity. These people can still be infected by malaria parasites but do not develop severe disease (CDC). In Africa and south of the Sahara where *P. falciparum* transmission is high, newborns are protected during the first few months of life due to the maternal antibodies transferred to them through the placenta. After these antibodies have decreased, children become vulnerable to disease and death by malaria.

Women who have developed protective immunity against *P. falciparum* tend to lose protection when they become pregnant, and thus both the mother and child become susceptible to malaria.

In many countries of the world malaria has been eliminated. Though, cases of malaria can still occur mainly due to “imported malaria” which occurs when returning travelers come from a country where malaria is prevalent.

Malaria can be a potentially fatal disease if left untreated, early detection can lead to prompt treatment and prevention from further spread of the disease in a community.

### **Current Malaria Treatments**

Infection with malaria parasites results in a variety of symptoms which can be absent, mild, or severe. For less severe cases of malaria, the general treatment involves a series of antimalarial drugs. These drugs can include chloroquine, sulfadoxine-pyrimethamine, mefloquine, atovaquone-proguanil, quinine, and doxycycline. Each of these medications attacks the parasite when it has entered the blood of the host. Primaquine, another drug, can be used to treat the parasite when in the liver stage of its lifecycle within the host. This drug is also used to treat relapse infections.

Currently, the World Health Organization suggests that malaria infections be treated with a combination of at least two drugs since many strains of the parasite have become resistant to the drugs. Thus, in this combination therapy, if the parasite has a resistance to one of the drugs, it will still be treated by the second drug.

In cases of more severe malaria infections, more drastic measures must often be taken to ensure that the patient will survive. Severe cases of malaria can cause abnormal behavior or neurological abnormalities, hemolysis, hemoglobinuria, pulmonary edema, acute respiratory distress syndrome, abnormalities in blood coagulation, and cardiovascular collapse. Once the infection has reached this severity, other concerns arise, including, acute kidney failure, hyperparasitemia, metabolic acidosis, and hyperglycemia. Hence, when a person is recognized with a severe case of malaria, treatment should be immediately started. This treatment includes the administration of drugs, whether they can be taken orally or introduced through an IV. Once a patient is able to take the drugs orally, they are generally switched to an oral medication. For these patients other treatment measures often involve blood transfusions and dialysis until the anemia and acute kidney failure cease.

At this time many doctors over-diagnose malaria. If a patient presents the common symptoms of fever, chills, sweats, headaches, nausea, vomiting, and body aches, the doctor often prescribes a malaria treatment in order to treat a possible illness that if left untreated could develop into a much more severe infection. In many areas of the world the drugs that are used to treat the parasite are over-prescribed and misused. This has led to parasites in certain areas of the world developing resistance to the drugs, making them harder to treat.

The lack for an effective vaccine is mainly because there are different strains of the *Plasmodium* parasite, which encodes for various protective antigens. Not only are there different strains, but the parasite itself undergoes lifecycle changes that produce different antigens for each stage. This in turn makes it difficult to develop a vaccine that would be effective at targeting the different strains as well as recognizing the antigens at various stages of the parasite's life. Another reason for the ineffectiveness of previous vaccines is due to the lack of immune memory that the body manufactures. Immunity for this disease is slow and is lost rather quickly when no longer exposed to parasite antigens. Those who are continuously stimulated by the parasitic antigens tend to acquire strong immunity from the more severe cases of malaria and when

unexposed for sometime, the body loses some immunity naturally. People who have never encountered malaria do not have strong immunity. One major issue is the high cost of multiple treatments for individuals that simulate repeated exposure of the antigens.

## TRANSMISSION

There are many surface proteins that have been the target for vaccines and have had relative success. Again the lack of effectiveness is due to the complexity of the parasite and the lack of a strong immune response. Many vaccines target the different stages of the parasite's life. The most critical stage is the intra-hepatocytic or liver stage. Here the parasite multiplies with little detection by the body, and is released into the blood stream where red blood cells are attacked. Red blood cells suffer severe damage, therefore it is important to prevent this stage of the parasite. The surface protein known as CSP-1, or circumsporozoite protein is presented in the sporozoite or intra-vascular stage when the parasite is introduced into the body. An example of a vaccine is the CSP vaccine which targets this stage. The CSP molecule has two B-cell epitopes which can induce an effective immune response. The efficacy is challenged however, by the versatility of the parasite. Other vaccines include DNA vaccines, attenuated vaccines, and others which target the antigens produced at various stages.

## VACCINE PROPOSAL

Malaria is one of the most severe public health problems worldwide and will continue to be a burden unless an effective vaccine is proposed. The best approach to derive an effective vaccine is to use a combination of DNA, and attenuated vaccine. DNA vaccine will contain the genetic information to produce multiple antigens at various stages of the parasite's life. At this point the known antigens are not always expressed when performed *in vivo*. Of the antigens known to DNA, those that will be used for the vaccine are CSP-1 during the sporozoite stage, liver stage antigen; LSA-1, and merozoite or blood stage antigens; MSP-1. One other antigen present during the sexual stage in *Plasmodium falciparum* is Pfs25.

The DNA vaccine will encode for all of the antigens described and the antigens that are not expressed will be presented by the attenuated form. Many of the antigens, especially the liver and blood stage antigens are highly conserved over all of the strains of malaria that is why the antigens were carefully selected to ensure that any infection from another strain will be covered by immunity obtained by vaccination. Several of the antigens that will be introduced by vaccination are linked to creating protective immunity. Having antigens that are present at the different life stages of the parasite ensures that the body will create immunity at any point of the parasite's life. The parasite is extremely versatile and if the body can recognize the antigens at each stage, parasitic invasion can be defended in any stage at the utmost potential.

The DNA vaccine will induce CD4+ and CD8+ T-cells and will cause them to secrete IFN- $\gamma$ . The prophylactic and attenuated vaccine will both induce an adaptive and innate immune response. DNA vaccine combined with an attenuated vaccine will help create immune memory. DNA vaccines are typically injected intramuscularly and the cells then uptake the DNA fragments. This DNA vaccine will be introduced intramuscularly which will allow the muscle cells to generate copies of the antigens that are naturally produced by the parasite. The antigens produced by the muscle cells will create a continuous response that will help create immune memory that is sustained for a much longer period of time as opposed to other methods.

The attenuated parasite vaccine acts as an adjuvant by also creating immune response. An attenuated parasite is produced by growing the parasite in animal or tissue culture and the environment is controlled to create the least virulent form of the parasite. The parasite can not be given unless it is weakened to ensure the safety of the recipients. An attenuated vaccine can also be created by genetically altering the parasite's DNA so that the virulence factors are not transcribed or inhibited. Not much is known about the genetic makeup of the *Plasmodium* parasite, so the attenuated parasite will be cultured. The advantages to using an attenuated parasite are that the antigens will be present in their natural form, as opposed to the DNA vaccine which can potentially produce antigens which are not exact replicas of the parasite antigens. Another advantage is that the vaccine can be administered orally, which is both cost efficient and still induces an immune response. One downside to using an attenuated vaccine is that the parasite has the potential to revert back to the virulent form, but is unlikely. Another way the parasite can regain its virulence if another parasite is introduced into the host and information is exchanged that allows the attenuated parasite to revert.

Attenuated parasites will be isolated from the cultures at the various life stages so that antigens from each stage are represented. The DNA vaccine will be introduced first to induce a primary immune response followed by the attenuated parasite which will test the effectiveness of the immune response induced by the DNA vaccine. The attenuated parasite will also create an immune response and immunity from the antigens that it contributes. The attenuated parasite will present antigens that were not expressed by the cells that received DNA vaccine. This will ensure that a majority of the antigens will be introduced to the host and an effective immune response will be created that will be retained for a longer period of time. The vaccine may have to be given in several intervals to ensure that immunity has not been lost. The efficacy can be determined by testing the host for specific antibodies that have been produced in response to the foreign antigens introduced.

## **INNATE AND ADAPTIVE IMMUNITY**

The innate response to malaria tries to prevent the parasite from spreading. In order to initiate innate immunity, an appropriate adjuvant formulation is needed to ensure effective presentation of the antigen by dendritic cells.

Natural killer cells (NK) and neutrophils are the first line of defense. NK cells attack the malaria parasite in an attempt to destroy it before it spreads. Macrophages are then activated, and are responsible for the eventual clearing of the parasite by engulfing it through phagocytosis.

Cellular immunity involving cytotoxic T cells attack the malaria parasites during the liver stage development. Cytokines released from lymphocytes help in this process. Cytokines secreted by different leukocyte populations play a direct role in protection. In previous clinical trials, interferon-gamma has been shown to work against liver stage parasite development and activate macrophages to attack blood stage parasites, creating an effective defense system against malaria.

Antibodies, which are part of adaptive immunity, are activated and try to completely wipe out malaria. Antibodies help the cell develop immunity against the parasite by neutralizing it and stopping retard cell development. Antibodies along with macrophages then try to engulf and eliminate malaria during the immunity response.

## **IMMUNITY ASSESSMENT**

In order to demonstrate vaccine efficacy, we propose an ELISA assay to measure the humoral response to the vaccine and flow cytometry to measure the cellular response to the malaria vaccine.

### **Elisa Assay**

An indirect ELISA assay will be used to measure the humoral immune response to the vaccine. This assay measures the amount of antibody produced by B cells.

This assay will be performed on plasma samples withdrawn from the vaccinated person three weeks after the attenuated vaccine is administered. To perform this assay the LSA-1 antigen will be incubated in microtiter plates to allow it to adhere to the bottom of the wells. After rinsing the excess antigen from the plate, the plasma sample will be added. Any IgG in the sample that is specific for LSA-1 will bind to the antigen in the bottom of the microtiter plate. After an incubation period the excess plasma will be rinsed from the wells and an anti-human gamma chain antibody will be added. This antibody will bind any of the IgG which is bound to the LSA-1 antigen. After rinsing away any excess anti-gamma antibody, an enzyme will be added. This enzyme will cleave the substrate bound to the opposite end of the anti-gamma antibody and result in a visible color, the intensity of which will be directly proportional to the amount of antibody the organism has produced.

For this experiment, both a positive and negative control will be used to ensure that the reagents work correctly and are not contaminated. The positive control will add a purified anti-LSA-1 antibody to the wells instead of a plasma sample. The negative control will contain an IgG antibody for a different antigen instead of the plasma sample.

### **Flow Cytometry**

Flow Cytometry will be used to determine the cellular response to the vaccine. This test will allow the amount of CD4 T cells, helper T cells, to be measured.

In this experiment, a serum sample will be prepared. The sample will be incubated with antibodies for specific membrane proteins, including an anti-CD4 antibody as well as an anti-LSA-1 T cell receptor antibody. Each of the antibodies will be supplemented by the chemical addition of a fluorochrome, each of which will fluoresce a different color. During the incubation period, the cells with the CD4 and the LSA-1 T cell receptor will be labeled with the antibodies. Then, the samples will be run through the flow cytometer. As the cells pass through the instrument, those with bound fluorochromes will be excited to higher energy states. As the fluorochromes relax to lower energy states, the cells will fluoresce and a detector will measure the emitted light. The instrument will then sort the cells into groups, those that fluoresced due to the binding of a single antibody, those that fluoresced because both antibodies were bound, and those that did not fluoresce and therefore had no bound antibodies. Only the CD4 cells specific for the LSA-1 antigen will fluoresce both colors. This will allow the amount of helper T cells specific for the LSA-1 antigen to be measured. Knowing the number of malaria specific CD4 cells will in turn allow one to determine if enough CD4 cells are present in the system to initiate a cellular response.

Malaria is a leading cause of death and disease in many developing countries. It serves as a compelling representation of the struggle between humanity and the natural world. The impact

of malaria affect those in Africa and south of the Sahara more than in other parts of the world. The severity and prevalence of malaria are the key determinants of the need for an effective vaccine. Current research has come close to formulating an effective treatment but there is still no solution. Global eradication efforts are still a work in progress, but this ambitious proposal could pave the way towards effective treatment and eradication.

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