

Current Challenges and Status of Vaccines against Malaria

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Introduction

Infectious diseases are an important concern in public health. Although with the help of new technology and advanced knowledge of biotechnology and pharmacy, nowadays, most infectious diseases are eradicated, treated or are under control, still, some of them such as HIV, tuberculosis and malaria generate big burdens on public health and annually a considerable number of people around the world suffer from them and a substantial number eventually die of these diseases. Malaria, a disease which can be transmitted to people of all ages, is caused by parasites of the species *Plasmodium* that are spread from person to person through the bites of infected mosquitoes. Levine et al. (2004, 861) indicate that there are four types of human malaria: *Plasmodium falciparum*, *P.vivax*, *P.malariae*, and *P.ovale*, among which *P. falciparum* and *P.vivax* are the most common and particularly *P. falciparum* is by far the most deadly type of malaria infection. The Plasmodium parasite is spread to humans by the bite of an infected female Anopheles mosquito. According to WHO (2007), about 40% of the world's population, and specifically those who live in third world countries, are at risk of getting malaria. Of this large number at risk, Kristoff (2007, 1989), WHO (2007) and CDC (2007) reveal that more than 500 million individuals become severely ill every year with

malaria, 1-3 millions of whom die from the effects of the disease. In Africa, where 20% of childhood deaths are due to the effects of malaria, the disease is a major concern. An African child has on average between 1.6 and 5.4 episodes of malaria fever every year; in every minute two children die because of malaria in Africa. Moreover, CDC (2007) reveals that malaria is the leading cause of disease and death worldwide, particularly in developing countries of Africa. However, Asia, Latin America, the Middle East and parts of Europe are also affected. People who travel from malaria-free regions to areas where malaria transmission is common are highly at risk because they have little or no immunity against malaria. Unfortunately, throughout history, *plasmodium* species causing malaria have gained resistance to antimicrobial drugs, and also the anopheles mosquito has built up resistance to insecticides used against them. These facts have brought the control and eradication of the disease under a serious challenge. Since the beginning of the vaccinology era, efforts have been made to formulate a vaccine to trigger the plasmodium and generate immunity against it in human body. This essay provides a review of the status of vaccines against malaria by viewing the clinical trial phases and some under-study candidate vaccines formulated against malaria.

Because of the extremely complicated life cycle of *falciparum* malaria, the production of vaccine has been problematic. According to MVT (2007) and Levine et al. (2004, 875) the sporozoites, which are parasites that feed on an individual, are injected into blood stream by the mosquito. Within blood they travel directly to the liver where they mature for about 6 days. The rapid division of sporozoites produces tens of thousands of asexual stage parasites called merozoites that burst from the liver cells and invade red blood cells. Here they again multiply 10-fold before they burst from red blood cells to go on and infect other red blood cells. This cyclic and massive increase in parasite load gives rise to a clinical disease that is recognized as malaria. Thus, scientists thought of formulating a vaccine against this dreadful disease. MVI (2007) indicates that, in such a case, an ideal vaccine against malaria would be the one that prevents all infection by enabling the immune system to destroy all parasites, whether they are swimming freely in the blood, or when they are in the liver- even when they are in red blood cells. Of course, with current vaccinology art and science, this level of

protection and creating a sterile condition would be extremely difficult or even technically impossible to achieve. Therefore, many vaccinology scientists focused their efforts on creating a vaccine that at least limits the capability and ability of parasites to successfully infect large numbers of red blood cells. Certainly, this would not prevent the infection itself, but rather reduces the severity of the disease, and in some degree, prevents malaria deaths.

According to Kilama (2007, 1), malaria vaccine development has traveled a long and hard path. In recent years, well enough about the parasite and its biology and infectivity has been known and advanced biotechnology helps employ this knowledge in producing a promising vaccine against malaria. Over the last few decades, many anti-malarial vaccines have been formulated and gone under clinical trial. But none has passed all four clinical trial phases to be licensed and used for public immunization. But happily, the clinical trials currently under way are providing an unprecedented depth of understanding of this killer disease along with potential means of taming it. Kilama (2007, 2) continues that an ideal malaria vaccine should have these characteristics.

1. be highly effective in all ages including infants, children and adults
2. be safe and not have very severe side effects
3. grant long term immunity without using multiple boosters.
4. be easy to administer
5. not interfere with other childhood vaccines
6. be easy and cheap to manufacture

Clinical trials of malaria vaccine differ from that of other vaccines due mainly to the force and level of infection seen in malaria, and the ability to safely assess the preliminary efficacy of the vaccine in volunteers who are normally not exposed to the disease.

Several Phases of Clinical Trials of Malaria Vaccine

Phase 1 of the malaria vaccine trial includes about 100 people and lasts up to one year. The main purpose of the phase 1 clinical trial is to assess the safety of the product in humans and identify the possible common adverse effects. It also evaluates the vaccine's ability to

produce an immune response and may be used to determine an appropriate dosage for further trials. Because of the danger associated with the disease, usually phase 1 of malaria vaccine trial is trialed in non-endemic countries (phase 1a), and if the safety and immunogenicity of the vaccine are approved then the vaccine is trialed in endemic countries (phase 1b), first in adults and if proved safe, in children.

Phase 2 of the malaria vaccine is trialed once the product has been proven safe. In this phase, hundreds or thousands of people will be recruited to participate in the trials which last for two or more years. This phase, as well, has been trialed as phase 2a and phase 2b. In phase 2a, some malaria-naïve volunteers in non-endemic countries are vaccinated and then exposed to malaria-carrying mosquitoes to see how long it will take for them to be infected by the parasite. As soon as the person is infected, he or she will be treated by appropriate antibiotics effective against the strain of the parasite. So phase 2a will give a preliminary indication of the vaccine's efficacy before it is taken to phase 2b in endemic countries. Safety, immunogenicity, composition, dose, schedule and preliminary efficacy in adults, children and infants are assessed in phase 2 of malaria vaccine trials. If phase 2 of the malaria vaccine was not successful, then it will be reformulated and go to phase 1 again. But, if phase 2 was successful, then it will go to phase 3 clinical trials.

Phase 3 of the malaria vaccine trial is conducted to assess the efficacy and safety of the vaccine in tens of thousand of volunteers. Phase 3 should be long enough in order for the governments and health authorities to make sure that the vaccine works in varied conditions, such as different malaria transmission patterns and different strains, before it is distributed to the public. This phase will last at least 5 years, and unfortunately, so far, most of the anti-malarial vaccines have failed to successfully pass phase 2 and enter phase 3, mainly because of the nature of the parasite and its interaction with the human host. But, in the past two years as Kilama (2007, 6) indicates, two of the candidate vaccines have reached phase 2b, with several others reaching phase 2a.

Having outlined the malaria vaccine trial process, this paper now reviews some of the vaccines that already formulated against malaria.

As was discussed in introduction, *Plasmodium falciparum* has a complicated life cycle showing different stages of development and invasion, such as: mosquito stage, asexual blood-stage, liver stage, pre-erythrocytic stage and erythrocytic stage. A lot of research has been conducted to formulate vaccines to trigger the parasite in one of these stages; but so far none of them could be licensed for public use.

Asexual Blood-Stage Malaria Vaccine

One type of vaccine that has already been formulated and has gone into clinical trial is the asexual blood-stage malaria vaccine. Genton and Reed (2007, 467) indicate that the complex biology of the plasmodium parasite, its wide antigenic diversity and its immune avoidance strategies make vaccine development against malaria demanding. Furthermore, immunity generated against malaria is usually species- or strain-specific and is short-lived, but there is evidence that supports the feasibility of malaria vaccines including antigens from the asexual blood-stage. It is really convincing that non-immune volunteers who were repeatedly challenged and cured with blood-stage parasites at very low doses developed immunity to subsequent exposures, and demonstrated reduction of parasite density in Papua New Guinean children in a phase 2b trial using a multi-component asexual blood-stage vaccine. This type of vaccine can essentially prevent current high morbidity and mortality caused by malaria by reducing the load of parasites in the blood or eliminating parasites circulating freely in the blood stream. However, the pressure induced by this vaccine and the collected evidence of wide-ranging antigenic diversity of blood-stage proteins create a difficult challenge to vaccine researchers, so far.

Moreover, according to Graves and Gelband (2007), four types of malaria vaccines, SPf66 and MSP/RESA vaccines, that are essentially designed against the asexual stages of the *Plasmodium* parasite, and CS-NANP and RTS,S vaccines designed against the sporozoite stages, have been tested in randomized controlled trials in endemic areas. However, the results are not that encouraging. No protection by SPf66 vaccines against *Plasmodium*

falciparum in Africa was evident, and also the result was really low in other regions. It means that further studies about SPf66, vaccine or perhaps its reformulation, might be needed to show its efficacy. Not enough evidence was available to confirm the efficacy of CS-NANP; however, the RTS,S and MSP/RESA vaccines showed very promising results. The result of MSP/RESA also includes the other main allelic form of MSP2. During the trial, it has been recognized that chemotherapy during the MSP/RESA trial may reduce the efficacy of the vaccine, and therefore, special consideration should be planned in regard to this issue, to not only achieve a good result of vaccination, but also to reduce the disadvantageous effects of exogenous factors on the vaccine. Moreover, addressing this issue, Levine et al. (2004, 878) also point out that the antibodies produced against MSP1 vaccine produce encouraging result by working in different ways: neutralizing of merozoites by agglutination and opsonization, preventing the invasion of RBC and inhibiting the growth of parasites into RBCs. Similarly, Graves and Gelband (2006) point out that, in a trial of asexual-blood stage vaccine against malaria, the MSP/RESA, which is also known as combination B, vaccine shows a promising result as a way to reduce the severity of malaria occurrences. Generally, it can be stated that MSP/RESA vaccine designed against malaria is a promising vaccine because of its ability to fight with the parasite in many various ways; further studies on this vaccine will provide the way for development, the full-scale preparation and application of this vaccine in a large scale and eventually in the public. It is worth noting that any pretreatment condition with anti-malaria drugs during a vaccine trial makes the interpretation of the results difficult, particularly when the sample size is small. The results show that asexual blood-stage vaccines may show an important role in protection against parasites and are worth further development and study.

In order to boost the efficacy of the asexual blood-stage vaccine designed against malaria, Hui and Hashimoto (2007, 6600) experimented on an adjuvant with the vaccine. It has been known that the efficacy of a vaccine adjuvant is influenced by the host immunological environment, related to their immunopotentiating mechanism. Interleukin-6 is a cytokine with a broad range of biological activities on both immune and non-immune system cells. In fact, some other adjuvants already used in vaccinology, such as MPL-SE, CFA/IFA, are dependent

to interleukin-6 for their efficacy. Hence, interleukin-6 can stimulate antigen-specific cellular responses; and the immunogenicity of the asexual blood-stage vaccine can be highly boosted by IL-6, and some other adjuvants that are dependent to interleukin-6. The important issue in the formulation of blood-stage vaccines against malaria is the fact that immunity in this stage highly depends on the presence of antibodies and CD4 T cells. So, any vaccines used or formulated for this stage should have the capability of inducing immune system to generate both humoral and cell mediated immunity.

Pre-erythrocytic Stage Malaria Vaccine

The other type of vaccine which has already been designed against malaria is pre-erythrocytic stage malaria vaccine. Mikolajczak et al. (2007, 461) explain that pre-erythrocytic radiation-attenuated parasite vaccine has been created and trialed. It is now well understood that this vaccine induces a long lasting sterile protection. However, some concerns regarding its production and delivery and inherent safety concerns prevented the large scale production and application of this vaccine. But recent advances in genetic engineering and initiatives in the production of live-attenuated malaria vaccine, will overcome roadblocks that presently prevent its large scale production and application. Basically, the development of the pre-erythrocytic subunit vaccine has been based on the circumsporozoites protein and another related protein called thrombospondin; however, so far, even the most advanced circumsporozoite protein-based vaccines confer very limited protection against malaria in endemic areas. Considering above mentioned specificity of the pre-erythrocytic subunit vaccine, efforts on the production of anti-malarial vaccine should focus on two major areas: development of a safe, live-attenuated sporozoite vaccine with its rapid testing in malaria endemic areas, and identification of some antigens that generate sterilizing immune responses induced by vaccination with whole parasites. The current knowledge on sporozoites model paves the way for rapid testing of pre-erythrocytic vaccine candidates, and hopefully in the production and large-scale application of a safe type this of vaccine for the public.

Similarly, Druilhe and Barnwell (2007) state that vaccines, which are formulated against pre-erythrocytic stages of malaria by radiation-attenuated sporozoites, have been the most promising and the most effective intervention tools designed and formulated against malaria

over four decades ago. But the inherent safety concerns and many other issues resulted in failure of the full scale production of a live-attenuated pre-erythrocytic subunit vaccine and its clinical implication for the public. This has already dissuaded some scientists from continuing the research in this field; however, as Druilhe and Barnwell (2007) discuss, some scientists started to think about formulating another pre-erythrocytic stage malaria vaccine based on an understanding of the principle of attenuated sporozoite immunity in humans. Therefore, applying these, hopefully, leads to the discovery of a critical immunogen and renews the promise of pre-erythrocytic subunit vaccines because the pre-erythrocytic stage malaria vaccine invokes and induces different cells in the immune system to generate immunity and it is this that gives this type of vaccine its unique characteristic. It is the requirement of the vaccines which are formulated against the pre-erythrocytic stage or in other words, liver stage to induce immune system to generate sufficient level of CD4 and CD8 T cells to invade the parasite and eliminate the infection before reaching the erythrocytes and causing more severe symptoms, damage and consequently death.

In fact, it has been seen that, in the case of pre-erythrocytic stage malaria vaccine, CD8+ has an important role in the induction of immunity. Addressing this issue, Kuk (2007, 329) states that CD8+ T lymphocytes play a vital and significant role in protection against the pre-erythrocytic stages of malaria, and it is this property of CD8+ that encouraged scientists to think about many vaccine strategies which focus on CD8+ T cell response. Meanwhile, the maintenance and development of memory CD8+ T cell responses are very closely related to the function of CD4+ T cells along with interleukins (IL-4, IL-7, IL-15 and IL-2). CD4+ T cells, in fact, has a triple role in the immune response to malaria parasites: first, by activating B cells to produce high levels of anti-malarial antibodies; second, by reinforcing the induction of CD8+ T cell responses; and third, by preventing the development of liver stage parasites. Although much is known about CD8+ T and CD4+ T cell responses, still cross-action process of these cells and other factors contributing to their response during the malaria cycle is yet to be determined. But the good news is that the understanding of the fact that CD8+ and CD4+ T lymphocytes have a very significant and critical role in protection against pre-erythrocytic

stage of malaria is a sufficient enough sign for the scientists to design a type or types of vaccine against this stage of malaria using this knowledge.

Erythrocytic Stage Malaria Vaccine

Another type of vaccine that is currently under trial has focused on the erythrocytic stage of the malaria parasites. Villard (2007, 645) works on the use of alpha-helical coiled coil domains of some proteins that are expected to be present in the parasite during the erythrocytic stage. The synthetic peptides are expected to be structurally similar to the native epitopes. Actually, so far, 95 chemically synthesized peptides have been distinctively recognized by human sera although in various prevalence. Each of these peptides generates type-specific antibody reaction when used in immunization trials, and there was no cross-reaction of these antibodies for other types of peptides. In depth studies show that the selected peptides assume to have partial or high alpha-helical content. Therefore, the use of bioinformatics and chemical synthesis approach in this regard, can lead to the identification of some molecules that specifically target biological active antibodies, and eventually formulation a novel vaccine against malaria in the erythrocytic stage.

Mosquito-Stage Malaria Vaccine

Another promising vaccine already formulated against malaria is a mosquito-stage vaccine which is commonly known as transmission-blocking vaccine. According to Miura et al. (2007, 107), mosquito-stage malaria vaccines are designed to generate an immune response in human hosts, as a result of which the parasite's growth into mosquito vector and the eventual transmission of the parasites will be blocked. In fact, Proteins present on the surface of gametes, such as Pfs48/45 and Pfs230 in *P. falciparum*, are expressed by parasites while still within the vertebrate host and exposed on the free-living gamete. Saul (2007, 479) and Levine et al. (2004, 887) reveal that naturally occurring antibodies directed against these proteins can be found in people living in endemic areas. Moreover, some other transmission-blocking targets, for example the related P25 and P28 proteins, were found to be expressed later during the subsequent parasite development cycle. Therefore, the mosquito-stage vaccine or

transmission-blocking vaccine aims at preventing the transmission of the infection from one person to another, by preventing infection in the mosquito vector. This would be a very beneficial vaccine because it generates herd immunity and affects the course of infection in vaccinated individuals; it is also able to kill the target outside the vaccinated person; basically, it protects mosquitoes by a passive immunization process induced by antibodies taken by the mosquitoes' food source (human blood). The basis of this mosquito-stage vaccine is four antigens in *plasmodium falciparum* and their orthologs in other species. In fact, these antigens are cysteine-rich motifs in gamete protein Pfs230, Pfs48/45, and EGF (epidermal growth factor) domains of zygote proteins Pfs25 and Pfs28 Ramjaneet et al. (2007, 894), Eksi et al. (2006, 998) & Schaijk et al. (2006, 216). These antigens were discovered about two decades ago by blocking monoclonal antibodies and by surface labeling experiments with gametes and zygotes. But, now, with advanced biotechnology and bioinformatics and recognition of the whole malaria genome sequence, chances are that better antigens and protein targets can be found for the development of a mosquito-stage vaccine, which would be very practical in fighting this deadly disease and reducing its tremendous morbidity and mortality.

Generally, the production of vaccines against malaria is difficult because this parasite has a very complicated life cycle. The rapid amplification of the parasite, upon entering the human body, poses the great problem in this regard. New sophisticated protein-based method of vaccine development also failed, so far, to develop a vaccine against malaria because different proteins are expressed by the parasite in different stages and the immunogenicity of the antigen depends on the stage; but nowadays, there are hopes the DNA vaccinology be able to overcome this problem and help formulate a vaccine against this deadly disease,

Conclusion:

Malaria causes 300-500 million clinical cases and 1-3 million deaths per year. The biggest death toll of the disease occurs in African children of less than 5 years of age. The prevalent resistance of parasites to conventional anti-malarial drugs and resistance of anopheles mosquitoes to available insecticides have made the development of an effective malaria

vaccine a global priority. An ideal malaria vaccine should induce naturally acquired immunity in communities where malaria is endemic. Several vaccines have been formulated and gone under clinical trial but, so far, none could be licensed for the public use, because of inherent safety concerns and low immunogenicity. Also, the highly complicated life cycle of malaria parasites poses a great challenge in the development of vaccine. Many vaccine scientists and researchers have attempted to formulate vaccines that target the parasite in one of the stages of the parasite life cycle, such as: mosquito-stage vaccine or transmission-blocking vaccine, asexual-blood stage vaccine, pre-erythrocytic stage vaccine and erythrocytic stage vaccine. Moreover, progress towards a successful vaccine has been slow, due to the extensive polymorphism of potential target antigens and the failure of most vaccines to elicit long-lived immunological memory in the host.

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