

Vaccine for malaria – closer to reality

- Dr. Parul R. Sheth

Today malaria is a major public health problem in India. It is a mosquito-borne disease caused by a parasite. Malaria kills approximately 900,000 people a year worldwide, most of them children living in sub-Saharan Africa. Almost half the world's population is at a risk of getting infected with malaria, especially those who travel to affected areas.

Preventive measures, early diagnosis and treatment measures have helped contain malaria. It is true that the using bed nets or killing mosquitoes with insecticides and other control measures have cut malaria rates to half in some countries. And yet an insecticide-resistant and treatment-resistant strain of malarial parasites is increasingly problematic.

Eradicating the disease has today become a priority for scientists and health officials around the world including India. Developing an effective and protective vaccine is the key step to get rid of the malaria menace.

The culprit

A single-celled protozoan parasite, Plasmodium causes malaria. The parasite is transmitted to humans by infected female Anopheles mosquitoes when they bite. When an infected mosquito bites, it injects a tiny quantity of saliva depositing the parasite in the body. An infected mosquito is capable of delivering up to 100 malaria parasites in a single bite.

The malaria parasite Plasmodium was first identified in 1880. Ronald Ross, a scientist from UK was given the Nobel Prize for his discovery on the role of mosquitoes in causing malaria in 1898. Once in the body, Plasmodium has a complex life cycle. The parasite invades red blood cells and divides inside them, increasing up to 30-fold. The parasite makes the red cells sticky so they adhere to blood vessel walls.

Where the parasites are most concentrated will determine the worst effects of the disease, like kidney failure or brain damage. The parasite finds ways to mutate, develop resistance to drugs and unfortunately it is constantly mutating or changing. It tries to evade medicines that are given to kill it. What is needed is a lifetime protection against malaria. And the only hope is a vaccine against the disease. However, the problem with vaccine preparation lies in the fact that there are many different strains of malaria.

Parasite species

There are four types of malaria, each of which is caused by a different species of Plasmodium. The four protozoans that cause malaria are *P.falciparum*, *P.vivax*, *P.ovale* and *P.malariae*. The most common type of malaria as of seen today is the one that is caused by *P.falciparum* and *P.vivax*. The malaria parasite takes many forms over the

course of its lifetime, ingeniously escaping the body's defenses. These parasites have an extraordinary capacity to evade the immune systems.

The life cycle of Plasmodium circles from mosquito to man and back to mosquito. A mosquito dribbles the malaria parasite into the skin with its saliva while enjoying a blood meal. After a couple of cycles in and out of the red blood cells, the billions of parasites make human blood their home. A mosquito bite can again transport the parasite into another person. The show begins all over again.

Life cycle of the malaria parasite

The female Anopheles mosquito injects parasites in the form of sporozoites into the bloodstream while it bites. These quickly travel to the liver within 3-6 minutes and invade liver cells. Over 5 to 16 days, the sporozoites reproduce producing tens of thousands of merozoites. After a week or so the cells burst and merozoites attack more red blood cells. However, some remain dormant for longer time in the liver causing relapses weeks or months later.

The merozoites leave the liver cells and re-enter the bloodstream, beginning a cycle of invasion of red blood cells, asexual replication and release of newly formed merozoites from the red blood cells repeatedly over one to three days. This multiplication can result in thousands of parasite-infected cells in the host bloodstream, resulting in illness and complications of malaria that can last for months if not treated.

Some of the merozoite-infected blood cells leave the cycle of asexual multiplication. Instead of replicating, some merozoites in the infected blood cells develop into sexual forms of the parasite, called male and female gametocytes that circulate in the bloodstream. These are picked up by a mosquito when it bites its host.

In the mosquito gut, the infected human blood cells burst, releasing the gametocytes, which develop further into mature sex cells called gametes. Male and female gametes fuse to form diploid zygotes, which develop into actively moving ookinetes that burrow into the mosquito mid-gut wall and form oocysts.

The oocysts grow and divide giving rise to thousands of sporozoites, which travel to the salivary glands of the mosquito. The cycle of infection in humans begin when this infected mosquito bites and takes a blood-meal thus injecting sporozoites from the saliva into the human blood stream.

Need for malaria vaccine

Preventing infection is especially important because resistance to anti-malarial drugs is a growing problem. A malaria vaccine, even of moderate efficacy, could make a huge impact. At present there is no approved vaccine to protect against the condition. But research work is in progress and there have been several approaches to the making of an effective malaria vaccine, which can aide in malaria eradication saving millions of lives across the globe.

Vaccines work by introducing a killed or weakened version of a disease into the body, where the immune system spots it and cranks out antibodies against it. Then, if a wild strain of the pathogen comes along later; one that has the power to sicken or kill, the body is ready for it.

For years scientists have been hunting for an effective vaccine against malaria. The answer may lie within the body's own immune system. Malaria vaccine formulations must contain antigens or proteins that serve as targets for the immune system. Varied antigens targets express at different stages of the malaria parasite's life cycle.

The basic road map to malaria vaccine apparently is simple. However, the genetic diversity between the strains of parasite makes certain antigens immunologically distinct. Also, these parasites have learned to be immune to the antigens thus complicating the process of evaluating which antigens to use in vaccine formulations.

The immune response

It is speculated that the ultimate vaccine for malaria can have components from the multiple stages of the malaria parasite's life cycle. For instance, antibodies can target the sporozoites, which are free swimming. However, for the antibody to be effective, it must be of very high efficacy and very specific. This is because it has to act instantly before the parasite hides inside the liver cells. In addition, every sporozoite must be counteracted since a single sporozoite can become 40,000 merozoites and continue multiplying in the blood stage. Furthermore, the antibody level must be very high to achieve long lasting protection since immunologic boosting via natural infection is limited.

During the liver stage, the parasite matures within the liver cells. A cell-mediated immunity can work well at this stage. Stimulation of lymphocytes; both CD4 plus CD8 T- cells can target and destroy the infected liver cells thus wiping out the developing parasites. The fact that the liver cell antigens can be exposed for longer times, boosting with exposure to natural infection can strengthen the vaccine's immune response.

The malaria parasite hides within blood cells ó erythrocytes during the blood stage. Here the immune response is dependent on antibody-associated process and boosting with natural infection is possible.

The immune response to the sexual stages depends on the transfer of the host's antibody into the mosquito during the blood meal. The human antibody then neutralizes the sexual stages before they have the opportunity to mate and develop into sporozoites.

As with vaccines targeting the pre-erythrocytic stage, this strategy would depend on very high antibody titers.

Problems faced

Malaria parasites have a complex life cycle, and there is poor understanding of the complex immune response to malaria infection. The parasites are also genetically complex, producing thousands of potential antigens. Unlike the diseases for which there

have been the current effective vaccines, exposure to malaria parasites does not confer lifelong protection. Acquired immunity only partially protects against future disease, and malaria infection can persist for months without symptoms of disease.

The malaria parasite is very good at evading the immune response. Natural immunity is usually very effective but it is unable to destroy the parasites completely; they can continue growing even in people with immunity. Unfortunately the parasite gets into human cells quickly and hides within the cells; literally hiding from the immune system.

Once inside the cells, certain proteins appear on the parasite's surface. Although vaccines can be designed to recognise these proteins, the different parasites having different protein coats and varied signals create problems. Also, in malarial areas, immunity builds up through early life, as children encounter and develop resistance to different strains of parasite. Only as an adult one may be more or less immune to malaria.

The whole malaria parasite cannot be used to make a vaccine. Perhaps some parts of it used in subunit vaccines, can mobilize defenses. Vaccines may stimulate antibody production, but may have little impact on T-cells and antibodies on their own cannot get rid of the parasite. And even if the Plasmodium-specific T cells are generated, they may not give protection or the immune response may be short-lived.

An ideal malaria vaccine should therefore require as few doses, should be cheap to make and administer. It should be long-lasting, preferably life-long and should have protection against all parasite strains. An ideal vaccine would be a combination; working against multiple stages. The vaccine would have to be stable in different temperatures and simple to administer.

Vaccines in development

Sporozoite and liver stage (pre-erythrocytic stage)

- Vaccine inducing antibodies against the sporozoite main coat protein, circumsporozoite protein (RTS,S) is the most successful vaccine (GSK Biologicals)
- Vaccine inducing T-cell response such as DNA or viral vector vaccines that encode pre-erythrocytic antigens recognized by T cells (Oxford)

Blood stage

- Vaccines that induce antibody responses. There are many potential targets, on or released by the merozoites as they attach to red blood cells. Many vaccines aim at MSP-1 (merozoite surface protein-1). Other targets include MSP-2, -3, -4 and -5, AMA-1, and GLURP
- Vaccines producing antibodies the malaria targets on the red blood cells. These are much less developed than the ones targeting merozoites
- T-cell based vaccines yet remain theoretical

Sexual stage

- Vaccines could block disease transmission. These mostly induce antibodies that the mosquito takes up when it bites, and block parasite development in the insect's stomach ó e.g. Pfs25 (US National Institute of Allergy and Infectious Diseases)

Combination trials ó are carried out using vaccines against several stages.

Vaccine research - current status

More than a dozen vaccine candidates are now in clinical development, and one, GlaxoSmithKline Biologicals (GSKBio) RTS,S, is in Phase III clinical testing; the first malaria vaccine candidate, which has a promising safety profile to advance this far. Results from this trial should be available in late 2012. This vaccine has been in development since the mid-1980s and has advanced as far as clinical trials thanks to a unique public-private partnership of GSKBio, the Program for Appropriate Technology in Health (PATH) Malaria Vaccine Initiative (MVI), and African and other research organizations, with funding support from the Bill and Melinda Gates Foundation.

The global malaria vaccine community has laid out a blueprint for developing by 2025 a malaria vaccine with protective efficacy of more than 80 percent against clinical disease and with protection lasting for many years without a booster immunization. It is anticipated that, to achieve the level and duration of protection required to achieve the 2025 goal, a vaccine that is more potent and efficacious than RTS,S is likely required.

A new avenue of attack has been recently put forward by the Program for Appropriate Technology in Health (PATH) and Malaria Vaccine Initiative (MVI), funded by the Bill and Melinda Gates foundation in collaboration with the John Hopkins Bloomberg School of Public Health and the Sabin Vaccine Institute. Called a transmission-blocking vaccine (TBV), it is aimed not at protecting individuals from the disease but at preventing mosquitoes that carry it from spreading it. The vaccine is directed against the form of the malaria parasite that is injected by mosquitoes.

Human clinical trials using another new approach to malaria vaccine research are in action at the Sanaria Inc., the Maryland-based biotechnology firm. The research is dependent on mosquitoes. Bioengineers have been growing millions of mosquitoes in a sterile environment, letting them feed on malaria-infected blood, irradiating them, extracting the disease-causing parasites and storing them for use in vaccines. The vaccine is unique among other candidates in that it uses the entire parasite and not just parts of it. The body recognises the malaria parasite as a foreign material; it goes to the liver, where a lot of the immune response is generated, but does not develop into a disease because the mosquito was irradiated.

The vaccine, called FMP2.1/AS02A, was developed as part of longstanding research collaboration between the Walter Reed Army Institute of Research (WRAIR) GlaxoSmithKline Biologicals (GSK). The vaccine consists of a form of the AMA-1 protein, invented and manufactured by WRAIR, and the AS02 Adjuvant System,

developed and manufactured by GSK. The Adjuvant System is a compound that boosts the immune response to the vaccine. Previous studies in the U.S. and in Mali already have found the vaccine to be safe and to produce strong immune responses in adults.

The vaccine, based on a single strain of the *P.falciparum* malaria parasite; the most common and deadliest form of the parasite found in Africa targets malaria in the blood stage.

Recently, the research team led by Dr. Shigeto Yoshida at the Jichi Medical University in Japan, have developed a genetically modified - transgenic Anopheles mosquito having the Leishmania vaccine within its saliva. The blood-sucking transmitter turns into a flying vaccinator and its bite raises antibodies in humans.

The Indian contribution

Scientist Dr. Chetan Chitnis and his colleagues at The International Centre for Genetic Engineering and Biotechnology (ICGEB) in New Delhi, India along with Bharat Biotech International Limited (BBIL) are developing a *P. vivax* vaccine candidate, PvRII (*P. vivax* Region II).

The PvRII vaccine candidate is based on the Duffy binding protein (Duffy blood group antigen), the sole invasion pathway for this parasite. This protein enables *P. vivax* to bind to receptors on the surface of the red blood cells, in essence, opening doors to invade the cells. Because the vaccine is designed to thwart invasion of red blood cells, it may be able to prevent disease caused by *P. vivax*. The protein is about 150 kilo-Daltons (about 150,000 times heavier than a hydrogen atom) the binding region is narrowed down to about 40 kilo-Daltons. The protein is divided into six different regions, and region II is found to be the binding domain. That information is the key factor to the creation of a potential vaccine.

Vaccines targeting both types of parasite are of immense importance in India. *P. vivax* is the most widespread malaria species occurring throughout Asia, South America and to a lesser extent, Africa. In areas of Africa there are places where 95 per cent of people may not be infected with *P. vivax* for genetic reasons.

Globally, *P. vivax* causes 70 million to 80 million cases of malaria each year and at least half the cases of malaria in India. In fact, over 80 per cent in some areas suffer from *P. vivax*. *P. vivax* malaria has been on the rise due to the parasite's increased resistance to anti-malarial drugs. Although *P. vivax* is rarely fatal, it can cause serious disease and affect the overall health and productivity of infected people, thereby affecting social and economic development as well. As a general rule, every individual in a moderately *P. vivax*-endemic area can expect to experience from 10 to 30 or more episodes of malaria in his or her lifetime.

A focus on *P. vivax* is important more so as there is an international emphasis on eradicating *P. vivax* altogether after global players including the Roll Back Malaria partnership created an eradication roadmap.

P. vivax is not the only focus of the ICGEB malaria team's work. They are also looking at potential antigens for *P. falciparum*, the most deadly species of malaria

parasite. *P. falciparum* is far more complex than *P. vivax*, because there are hundreds of different mechanisms believed to be used by the parasites to invade the body. As a result, the researchers are combining two different fragments. But this approach is unusual, because most groups are testing one antigen at a time.

All in all the priority goal of the ICGEB organisation is to develop a vaccine for *P. vivax* vaccine PvRII based on Duffy binding protein, another *P. vivax* vaccine based on MSP-1 and a *P. falciparum* vaccine JAIVAC-1, which is a combination of PfF2 (EBA175) and PfMSP1(19) and get set for the Phase I clinical trials.

The good thing is that now there is funding for malaria vaccine development and more people are doing this at a global level. ICGEB and BBIL are being funded by numerous national and international partners, including the Government of India's Department of Biotechnology, the World Health Organization's Tropical Disease Research Division, and the Indo-US Vaccine Action Program, an alliance between the Department of Biotechnology and the US National Institutes of Health and the PATH MVI based in Bethesda MD, USA. In addition, the project involves technical collaboration with GlaxoSmithKline Biologicals, which is based in Rixensart, Belgium.

Hope for the near future

Hopefully, other successful candidate vaccines will follow the suit. And a highly efficacious malaria vaccine would emerge saving hundreds of lives. An effective malaria vaccine can complement existing interventions, such as insecticide-treated bed nets, indoor residual spraying, and effective drug therapies, to help prevent death due to malaria.
