

## In search of vaccines against malaria

Malaria has been an enemy of humans for thousands of years and remains a major public health problem in the tropical and subtropical regions of the world. Description of malaria-like symptoms of periodic fever can be found as far back as in Chinese texts (2700 BC), writings from Mesopotamia (2000 BC), Egypt (1570 BC) and Indian Vedic scriptures (600 BC). However, it was only in 1880 that Charles Alphonse Laveran discovered crescent-shaped bodies (*Oscillaria malariae*) in the blood samples of infected individuals as the cause of malaria, followed by Ronald Ross, who in 1897 described the whole sexual cycle on the gut wall of mosquito (*Culex fatigans*) of avian malaria parasite, *Plasmodium relictum*. Both received the Nobel Prize for their discoveries. Malaria-related research has yielded three more Nobel Prizes, including the one in 2015 for the discovery of artemisinin to the Chinese scientist Youyou Tu.

In 2015, 214 million people were infected with malaria and 438,000 deaths were reported worldwide. Encouraged by effective vector control by insecticide spraying and treatment with drugs like chloroquine, an eradication programme was conceived in mid 1950s. Although highly successful in some parts of the world, including India, this programme mostly failed in African countries and was abandoned in 1969, highlighting the location-specific challenges. Following the resistance of the vector to insecticides and due to fast-spreading drug-resistant strains, the disease returned with a vengeance, making it clear that serious malaria control programmes will require better tools and their effective implementation. Eradication of malaria is one of the key stated targets of the United Nations Millennium Development Goals, and with quicker and more reliable ways for diagnosis as well as affordable treatments. It is perhaps the right time to consider malaria eradication agenda as an achievable goal. However, with emerging resistance to currently available malaria drugs, and to insecticides by the vector, it is necessary to be cautious.

It is generally believed that an efficacious vaccine will be a major asset and the most cost-effective intervention in combating malaria. The fact that individuals living in malaria endemic areas develop immunity against the disease and that injection of irradiated sporozoites can protect humans from infection, suggest that it may be possible to develop vaccines against malaria. The malaria parasite is transmitted by an infective female anophel

mosquito and once in the host, it goes through its life cycle and huge multiplication in distinctly different stages. Starting from its first residence in the liver, followed by a complex life cycle in the red blood cells, it slips into a sexual life cycle, where sexually differentiated parasite is taken up by a mosquito during a blood meal from an infected host.

Vaccines against all the three stages of the life cycle of the parasite are being perused. The liver-stage vaccines have the potential to stop malaria infection from going to the blood stages, which are responsible for the clinical disease. Blood-stage vaccine(s) are expected to reduce morbidity and further transition to sexual stages, whereas vaccines(s) against the sexual-stage parasites can block the cycle of transmission, killing the parasite during this stage. In this context, the recent announcement of licensure of a pre-erythrocytic stage vaccine called RTS,S is an exciting and long-awaited development. This vaccine, named as Mosquirix, has been developed jointly by scientists at the Walter Reed Army Institute of Research (WRAIR) and Glaxo Smith Kline (GSK), with continuous support from several funding agencies. The development of RTS,S has taken more than 20 years and at a cost in excess of 400 million dollars, which underscores the scientific and organizational problems encountered in the development of vaccines against complex diseases like malaria, tuberculosis and AIDS.

It is well known that immunization with irradiated liver-stage parasites (sporozoites) can protect the host against malaria infection. It was the identification of the circumsporozoite protein (CS protein) as a key antigen involved in irradiated sporozoites-induced protection that led scientists from GSK and WRAIR to develop a vaccine based on this protein. The CS protein contains an immunodominant tetrapeptide repeat region, in the middle of its structure that was an obvious choice for the design of a subunit vaccine. Constructs based solely on the repeat region or on the flanking regions turned out to be poorly immunogenic. To enhance the immunogenicity of constructs, the highly immunogenic hepatitis virus surface protein (HBsAg) was used as a carrier protein and finally, after many unsuccessful attempts, a CS protein construct that included a C-terminal flanking region along with tetrapeptide repeat region, fused with HBsAg and co-expressed with HBsAg(S) produced stable and immunogenic virus-like particles (VLPs). However, these

VLPs were not immunogenic in alum-based adjuvants. Not deterred by this unexpected setback, the researchers then tried various combinations of novel adjuvants, that were being developed at GSK. Finally, formulations of VLPs in the ASO series of adjuvant provided stable and immunogenic VLPs of RTS,S that comprised only 25% of the fusion protein containing CS protein (RTS; R for repeats, T for T-cell epitopes and S for HBsAg(s)) and the rest 75% made up of HBsAg. Several trials with different ASO adjuvant formulations in different locations established the safety of RTS,S as well as the novel adjuvant formulations. The formulations that provided protection against malaria infection in a controlled human malaria infection (CHMI) model were developed for further trials.

Several safety and phase-II proof-of-concept trials that began in 1992 were finally completed in 2007. They demonstrated that indeed children and infants immunized with RTS,S vaccine formulations were partially protected against malaria. Following these trials of RTS,S vaccine, phase-III clinical trials were planned, involving all stakeholders, including GSK as the sponsor, the scientists represented by the clinical trials partnership committee, and in many ways was an unprecedented exercise in its intent, scope and financial implication. These phase-III trials were conducted in 11 different locations in seven malaria endemic African countries; they lasted over a period of 5 years. However, in the final analysis, in children and infants who received three doses of the vaccine and a booster, the risk of clinical episodes reduced only up to 36% in children and up to 26% in infants over a period of 3–4 years of observation, with no remarkable protection against severe disease in infants. By any standards of prevention of disease by immunization, these are modest results, even for a first-generation vaccine. However, as recommended by the European Medicines Agency, WHO decided to approve this vaccine in July 2015, with the tradename Mosquirix for use in young children in Africa. While questions about its mode of action and concerns about its low efficacy remain, RTS,S is the first ever vaccine against any parasitic disease. Scientists involved in malaria vaccine development and major funding agencies should be encouraged with the licensure of the first ever vaccine against malaria. The story of development and licensing of RTS,S is not only about design of a protein-based subunit vaccine, but also about two important developments relevant to vaccine research in general and malaria vaccine research in particular. First is development of a series of non-alum-based adjuvants for vaccine development. Although GSK has proprietary on these adjuvants, one can hope that these new and safe adjuvants will be made available to other vaccine researchers. The second innovation has been the establishment of the CHMI model as a human infection model for testing candidate malaria vaccines to make early go–no go decisions for their further development. The role of volunteers who will fully agree to be bitten by infective mosquitoes in order to assist in developing malaria vaccines cannot be

over-stated. Establishment of the CHMI model in many parts of the world, including USA, UK, Australia, Holland and Columbia has been a major advancement in the field of malaria vaccine development.

Given that the CS construct constituted only one-fourth in RTS,S, the rest being made up of HBsAg, there is no doubt that continued efforts will yield vaccines with much higher efficacy in the coming years. However, it is noteworthy that a vaccine based on repeat sequence of the CS protein of *Plasmodium vivax*, adjuvanted with ASO1<sub>B</sub> did not show any protection in human volunteers in *P. vivax* CHMI trials conducted recently. Another liver-stage vaccine based on irradiated sporozoites has been reported. Intravenous immunization with five doses of this vaccine called PfSPZ, has shown complete protection in human volunteers, but many challenges remain in conducting trials with this vaccine.

Malaria vaccines against the blood stages and sexual stages of the parasite are also being pursued by several research groups around the world, including India. A team of scientists at the International Centre for Genetic Engineering and Biotechnology, New Delhi has carried out, first of its kind, phase-I clinical trial of a malaria vaccine based on two major blood stage proteins, produced by recombinant methods in India. Similarly, another experimental vaccine against *P. vivax*, the most prevalent of all human malaria cases, is being developed for clinical trials in India. Such indigenous efforts have opened the door for development of vaccines against diseases, for which there are no vaccines yet, including malaria. The need for establishment of the CHMI model in India should be urgently addressed by the regulatory authorities.

Development of RTS,S as the first ever malaria vaccine illustrates the scientific logistic and financial hurdles, not to mention the single-minded devotion of the team that developed the vaccine. Pre-clinical development and conducting field trials for any infectious disease are hugely expensive and can cost up to US\$ 500 million. A recent report published in *Science* (2016, **351**, 16–19) has highlighted that at least ten vaccines against infectious diseases, including chikungunya, SARS and schistosomiasis have been developed, but await clinical trials. Development of vaccines against neglected diseases will certainly need a different narrative, financial support from Government and non-profit funding agencies, pharma industry and a strong political will. With that, and new technological advances, it is certain that novel vaccines will be developed, which will be added as crucial weapons in the control and elimination of infectious diseases, including malaria.

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