

Approaches Towards a Malaria Vaccine

Franz Hartmann*

Faculty of Medicine, Pomeranian Medical University Szczecin, Poland

*Corresponding author: Franz Hartmann, Faculty of Medicine, Pomeranian Medical University Szczecin, Poland. Email: Franz_1997@hotmail.de

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Introduction

Malaria is a vector-borne parasitic disease with a significant contribution to the public health burden in many tropical regions of Africa, Asia and South America. In 2017 alone, close to 220 million cases of malaria were recorded worldwide, with Sub-Saharan Africa and India contributing almost 80% of the malaria burden [1]. The causative agent of malaria are protozoan Apicomplexan parasites of the genus *Plasmodium* transmitted by Anopheles mosquitoes. Importantly however, only five out of 170

Plasmodium spp. are pathogenic to humans. Among the different species of *Plasmodium*, *P. falciparum* is among the most prevalent and causes the highest mortality rates in humans. Mortality rates of malaria are highest in children under the age of 5 due to the lack of strong protective immune responses. Importantly, the current major emerging problem in the public health sector is the continued emergence of parasite resistance to anti-malaria drugs [1,2]. In humans, the life cycle of the parasite (Figure 1) begins with the entrance of sporozoites during the blood meal of an infected Anopheles mosquito. Sporozoites undergo development in liver hepatocyte cells and subsequently emerge as merozoites (liver stage).

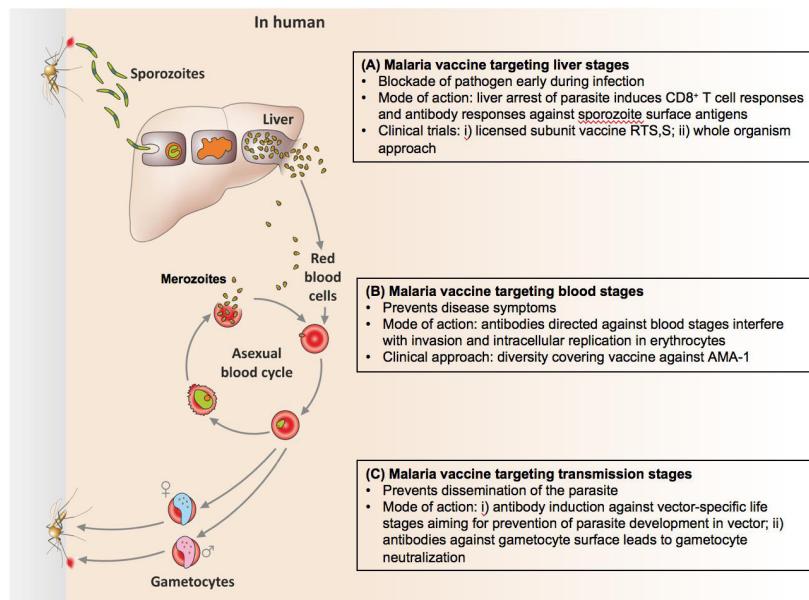


Figure 1: Schematic overview of the malaria life cycle in humans and the different vaccine approaches targeting different life stages. (A): Malaria vaccine targeting the liver stages, the sporozoites; (B): Malaria vaccine targeting the blood stages, the merozoites; (C): Malaria vaccine targeting transmission stages, the gametocytes.

Ten to 100 sporozoites are typically transmitted with mosquito saliva during a blood meal and while sporozoites exhibit low infectivity, up to 5 mosquito bites of a *Plasmodium*-infected mosquito would be necessary to infect a human host [3]. About one week following infection 10,000 – 30,000 merozoites are generated and released into the blood where they infect erythrocytes. Merozoites can then enter healthy red blood cells, where they can undergo continuous cycles of red blood cell infection and rupture, causing the typical periodic symptomatology of malaria every 24–72 hours. Some merozoites emerging from ruptured red blood cells can further develop into the sexual stage of the parasite (gametocytes), which are infectious for *Anopheles* mosquitoes. Sexual reproduction in the mosquito vector and production of sporozoites takes days to weeks, dependent on the temperature of the environment [4]. The clinical picture is characterized by anaemia due to the massive infestation of erythrocytes and fever induced by haemoglobin released by bursting erythrocytes. Most serious progression, induced by *P. falciparum* only, is the cerebral malaria. Here, infected erythrocytes adhere to the blood endothelium and might lead to occlusions of vessels supporting brain maintenance. The disease is contained predominantly by the following measures: comprehensive usage of insecticide-saturated bed nets, targeted in house insecticidal spray disinfection, diagnosis, chemotherapy and monitoring of sick people [5]. However, effective control of malaria would further require the development and clinical application of an effective vaccine to supersede these measures.

Feasibility of A Malaria Vaccine

So far, no malaria vaccine displaying full protection is available for the millions of people living in endemic areas despite the fact that as far back as 1969 it has been demonstrated that protection against *Plasmodium* infection is possible via the application of attenuated parasites [6]. Importantly however, a recombinant protein-based vaccine is currently licensed, albeit with insufficient efficacy. In these early vaccination studies R. Nussenzweig showed that radioactively-attenuated sporozoites had only very limited capacities for development and resulted in more than 90% protection against challenge infection in mice [6]. A few years later D. Clyde demonstrated similar effects in humans [7]. He demonstrated that volunteers, who received radioactively-attenuated sporozoites, were up to 93% protected against a real infection and that the protection lasted more than 10 months. In addition, he showed that the induced protection was transferable to infections with other *Plasmodium* species. Both results from the early 70th have thus demonstrated that a protective immune response is indeed achievable. The experimental approaches of R. Nussenzweig and D. Clyde were a promising beginning for vaccine development against malaria. Efficacy of any potential malaria vaccine or an anti-malaria drug has *a priori* to be tested in human propositi exposed to *Plasmodium*-infected mosquitoes. Only when high efficacy in initial experimental clinical trials can

be demonstrated, can the new vaccine or drug be further verified in field studies in endemic areas [8]. The different vaccine approaches concentrate on the different life stages of the parasite in the human host [9]. State-of-the-art research currently concentrates on the following approaches (Figure 1): (A) a vaccine targeting the liver stages, in which the mosquito-derived sporozoites further develop; (B) a vaccine targeting the blood stages (merozoites); (C) a vaccine targeting the gametocytes (sexual blood stages).

Vaccine targeting the liver stages

A vaccine targeting the early life stages, the sporozoites and liver stages, would prevent the infection in its initial phase (Figure 1). For this purpose, the vaccine should lead to a prolonged resting time of the pathogens in the liver, the so-called liver arrest. Such an extended time of *Plasmodium* in the liver exposes the parasite to the host immune system for a prolonged period of time and should thus lead to the induction and maintenance of a more efficient immune response. This vaccine approach should lead to the development of effective cytotoxic T cell responses targeting *Plasmodium*-infected liver cells. In parallel, this vaccine induces effective antibody responses against surface proteins of sporozoites. Via binding of host antibodies to the surface antigens of sporozoites, their invasion into liver cells are hindered or at least aggravated. Consequently, a vaccine against sporozoites and early liver stages prevents infection. However, sporozoites replicate extremely fast and a single surviving sporozoite would be sufficient to initiate the blood phase of the disease. Therefore, a vaccine based on sporozoites and liver stages would have to display close to 100% efficacy.

The experimental approaches to achieve *Plasmodium* liver arrest center around a subunit vaccine, harnessing a repetitive region (R) of the circumsporozoite surface antigen (Csp) of sporozoites in combination with several T cell epitopes (T) of the Csp antigen. Both parts are fused to a hepatitis B surface antigen (S), constituting the RTS, S subunit vaccine [10]. RTS, S has been tested in different countries in Africa with protection of up to 56% in infants against clinical episodes of malaria [11]. In a follow-up double-blind study in 11 locations in 7 countries in Africa this vaccine has been tested in breastfed babies and infants. A protection against clinical symptoms of malaria has been achieved in about 27% of the babies and about 46% of toddlers depending on the region [12]. Although RTS, S does not induce a 100% protection, it is currently the first and only vaccine licensed against human malaria.

Due to the comparatively low efficacy of a subunit vaccine in which only single components of the pathogen are utilized, a whole organism approach has been revisited in parallel, as already described in 1973 by D. Clyde [7,10]. The following approaches are applied: i) radiation-attenuated sporozoites; ii)

application of infectious sporozoites combined with an anti-malaria chemotherapy; iii) genetically-modified sporozoites. Treatment with a high concentration of $1,35 \times 10^5$ radiation-attenuated sporozoites to 6 individuals induced a 100% protection by intravenous application only [13]. In parallel, exposure of people to infectious sporozoites of 45 infected mosquitoes in combination with the application of the chemotherapeutic chloroquine led to a 100% protection against challenge infection [10,14]. In this approach, 4 out of the 10-tested individuals were fully protected up to 28 months after treatment. The idea behind this combinatory approach is to induce antibody responses against liver stages and blood stages as the immune system is exposed to the fully-infectious pathogen developing in the liver before being eliminated during the blood phase by the chemotherapeutic. During this initial unchecked replication of *Plasmodium* in the liver 80% of *Plasmodium* proteins are presented to the immune system, meaning, hundreds of additional *Plasmodium* antigens are presented to the immune system in contrast to the radiation-attenuated sporozoite vaccine rendering the combinatory vaccine more effective [10]. Subsequent approaches utilize genetically-modified sporozoites by constructing *Plasmodium* deletion mutants missing essential genes for transcriptional regulation during the liver phase [15]. The hope is to generate sporozoites, which stop their development in the liver, but capture the positive elements of the fully-infectious sporozoites/chemotherapeutic vaccine as the immune system will get access to all proteins of the liver phase. A hurdle of these promising approaches is that they are directed against whole sporozoites and to accomplish the successful application of such approaches, appropriate facilities housing state-of-the-art insectariums are needed to generate the huge amounts of sporozoites required.

Vaccine targeting the blood stages

An alternative starting point for a vaccine is the blood stage of *Plasmodium* parasites - the non-sexual life stage replicating in erythrocytes (Figure 1). The mode of action followed with this approach is the induction of antibody responses against merozoites, the life stage released by erythrocytes after having replicated in erythrocytes and invading neighbouring erythrocytes. Here, the variability of the blood stage antigens of *Plasmodium* have to be taken into account to induce an effective antibody response. The concept of this approach is to prevent the development of disease by prohibiting the invasion and replication process within healthy erythrocytes. Experimental approaches using a “virus vector vaccine” in combination with recombinant single merozoites surface proteins such as MSP-1 and AMA-1 have so far not been significantly effective, despite potent cellular responses and only a mild antibody response was detected [5]. Application of the surface protein AMA-1 as recombinantly-expressed bacterial protein in combination with an adjuvant has previously been tested

in clinical phase II trials with children aged 1-6 years in Mali [16]. Here, despite a significant efficacy of the vaccine during the first 240 days after treatment, there was a drastic decline observed thereafter. The decline in efficacy of this approach possibly resulted from the antigen variability of this protein. The genetic variability of single *Plasmodium* proteins is an additional hurdle in the development of a vaccine against malaria and in particular the blood stage antigens of *Plasmodium* exhibit a high variability due to the constant selection pressure induced by the immune system. To encounter the diversity of variable blood stage protein diversity covering vaccines of AMA-1 are in clinical trials [17].

Vaccine targeting transmission stages

A transmission blocking vaccine would also target the *Plasmodium* blood stages, however, its primary targets would be the sexual stages of the parasite, namely the gametocytes. By targeting gametocytes, the development of the parasite in the mosquito following uptake of a blood meal would be prevented. Here, the progression of *Plasmodium* in the mosquito and subsequently the production of sporozoites will be interrupted. Two modes of actions are anticipated (Figure 1): i) induction of antibodies against parasite stages developing in the vector. Thereby, antibodies against the vector stages would be transferred to the mosquito during a blood meal and interfere with *Plasmodium* development in the vector, hindering sporozoite production; ii) the vaccine is targeted against gamete surface proteins and thereby leads to the neutralization of gametocytes in the blood of infected individuals. As a consequence, the transmission blocking vaccine does not protect against an existing infection but impedes the dissemination of the disease. The lead compound tested as a potential transmission blocking vaccine is a surface protein, s25, expressed on the ookinete developmental stage in the mosquito vector [18]. The function of this protein is associated with attachment to the mosquito gut. Antibodies against *P. falciparum* s25 have been demonstrated to prevent the development of infection in *Anopheles* mosquitoes [19]. One potential issue with this vaccine approach is the low immunogenicity of the antigen, as well as the lack of natural exposure of humans to the antigen, as target proteins are only expressed in the mosquito developmental stages. Thus, there is no potential for natural boosting of the human immune response after vaccination. However, the blockade of transmission of *Plasmodium* between the human and mosquito host is assumed to be crucial to render malaria extinct [5].

In conclusion, there is currently no fully-protective vaccine against malaria available. Despite intensive research this is probably due to the fact that *Plasmodium* parasites reveal an extremely complex life cycle, exhibit diverse antigens on different life stages and exert intricate immune evasion mechanisms. Thus, the overall

challenges are considerably higher in contrast to bacterial or viral pathogens. However, a first vaccine against malaria, RTS,S is licenced although its protection is only limited. Other approaches such as whole organism vaccines are in the pipeline and give hope that finally vaccine-induced fully protective immune responses are achievable.

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