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Novel approaches to whole sporozoite vaccination against malaria

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ABSTRACT

The parasitic disease malaria threatens more than 3 billion people worldwide, resulting in more than 200 million clinical cases and almost 600,000 deaths annually. Vaccines remain crucial for prevention and ultimately eradication of infectious diseases and, for malaria, whole sporozoite based immunization has been shown to be the most effective in experimental settings. In addition to immunization with radiation-attenuated sporozoites, chemoprophylaxis and sporozoites (CPS) is a highly efficient strategy to induce sterile protection in humans. Genetically attenuated parasites (GAP) have demonstrated significant protection in rodent studies, and are now being advanced into clinical testing. This review describes the existing pre-clinical and clinical data on CPS and GAP, discusses recent developments and examines how to transform these immunization approaches into vaccine candidates for clinical development.

1. Background

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Malaria is a life-threatening, multi-organ disease caused by blood stage infections with *Plasmodium* parasites, particularly *Plasmodium falciparum*. Disease-limiting immunity that develops after multiple malaria episodes does not prevent parasitemia or transmission [1]. WHO estimates that despite the massive roll-out of conventional control measures over the last decade, malaria causes more than 200 million clinical cases and claims almost 600,000 lives per year, mostly in African children [2]. Further progress will necessitate novel interventions that can reliably interrupt the transmission cycle and achieve elimination of malaria. A potent and long-lasting vaccine is the most promising strategy to achieve this ambitious goal; however, it has proved difficult to develop [3].

Malaria parasites are unicellular eukaryotes with a complex life cycle characterized by developmentally distinct phases of rapid and massive asexual replication in the vertebrate host and the *Anopheles* vector, linked by short but pronounced population bottlenecks during host transitions. One such bottleneck occurs during transmission, when female *Anopheles* mosquitoes inoculate tens to hundreds of motile sporozoites into the human host. Following active penetration into the blood circulation, sporozoites are passively carried to the liver where they rapidly invade hepatocytes. Within a week, a single, clinically silent round of replication from a single *P. falciparum* sporozoite will give rise to 1×10^3 to 5×10^4 invasive forms of the parasite, termed merozoites, which initiate the pathogenic intra-erythrocytic replication cycle and can result in up to 10^{13} parasites at the height of an infection. The small number of naturally transmitted sporozoites in an injected inoculum may explain the apparent lack of acquired pre-erythrocytic immunity even in individuals with substantial anti-blood stage immunity (as evidenced by recurrent asymptomatic and low-density blood stage re-infections) [4,5].

Experimental malaria vaccine approaches using live sporozoites and different strategies to arrest the infection *before*, *during* or *shortly after* liver stage development induce immunity targeting this clinically silent phase of the *Plasmodium* life cycle [6]. The concept is simple: deliver attenuated parasites to induce

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anti-sporozoite and anti-liver stage immune responses capable of reliably preventing progression of a subsequent infection beyond the liver stage and thus completely averting a pathogenic blood stage infection. Instead of trying to mimic 'natural' immunity against malaria parasites as induced in residents of endemic areas, whole sporozoite vaccination aims for 'unnatural' immunity by induction of effective, sterilizing immune responses [5,7,8]. While antibodies are important to target the extracellular sporozoites, cellular responses are likely to be crucial for elimination of intracellular liver stages. During differentiation and replication of liver stages, antigen expression changes significantly [9]. In brief, upon invasion of hepatocytes sporozoites transform inside a parasite induced cellular compartment (parasitophorous vacuole). Parasites then start a process of massive replication, termed schizogony, at the end of which, thousands of daughter cells are formed. These cells are differentiated into invasive blood stage parasites (merozoites) that are released in 'batches' into the blood stream within membranous remnants of their host cells (merosomes). The transformation from sporozoites to merozoites is driven by the expression of a discrete set of genes, including potential antigens. Whole sporozoite immunization therefore exposes the immune system to a wide array of parasite antigens as opposed to a single or limited number of antigens used in subunit vaccine approaches. This is an advantage because it potentially induces more potent, heterogeneous and broader immune responses that can lead to stage- and strain-transcendent immunity [10–13].

There are four approaches to whole organism immunization: radiation-attenuated sporozoites (RAS), chemoprophylaxis and sporozoites (CPS), genetically attenuated parasites (GAP) and chemically attenuated parasites. In this review we will discuss novel insights and ideas principally on CPS and GAP, whereas RAS is discussed in more detail elsewhere in this special issue.

2. Chemoprophylaxis and sporozoites (CPS)

Administration of sporozoites under chemoprophylaxis as an immunization method was first explored in rodent malaria models in the late 1970s–early 1980s using infection under chloroquine (CQ) prophylaxis [14–16]. The rationale is that administration of antimalarials with sporozoites allows parasite infection of the liver but prevents the erythrocytic phase where clinical disease occurs. In the case of CPS with CQ, which selectively kills asexual blood stage parasites but not sporozoites and liver stage parasites, the parasite completes liver stage development and releases infectious merozoites, which initiate the first wave of blood stages before being eliminated by the drug—exposing the immune system to multiple parasite life stages, including those parasites that either failed to enter or complete liver stage development. Though successful in animal models, the efficacy of CPS in humans was not investigated in clinical trials until 2009 when 10 volunteers were immunized 3 times by the bite of 12–15 *P. falciparum*-infected mosquitoes while receiving CQ prophylaxis [17]. This regimen resulted in sterile protection in all 10 volunteers against a controlled human malaria infection (CHMI) by mosquito bites, eight weeks after the final immunization [17]. Further, 4/6 of these 'protected' volunteers re-challenged at 28 months post-immunization remained fully protected—indicating a robust and prolonged immune response [18]. These results have recently been confirmed in a follow-up dose de-escalation study, showing that complete protection is dose-dependent (number of bites) and have been replicated using mefloquine (MQ), a drug with similar structure and activity to CQ, with equivalent results [19,20]. Although subjects are exposed transiently to low levels of blood stage parasitemia during CPS-CQ immunization, protection in humans appears to be heavily based on pre-erythrocytic immunity [6]. While data from animal models suggest CPS is capable of eliciting multi-stage immunity, the inability to monitor a continued parasitemia in human volunteers makes it difficult to examine the full extent of immunity against blood stage parasites [13,21–24].

Despite being logistically challenging to deliver, the dose of parasites required to generate immunity similar to RAS is considerably (orders of magnitudes) lower as just 3×10^{-15} mosquito bites are required for >80% protection [20] compared to the more than 1000 bites required of RAS-infected mosquitoes [25]. These encouraging and consistent results have established CPS as the current benchmark for malaria vaccine development, and have initiated a large effort to delineate immune mechanisms of protection, to generate a first-generation candidate whole-sporozoite vaccine and to optimize the CPS immunization regimen.

2.1. Drugs available for CPS

Using drugs targeting blood stages for CPS immunization has the advantage that full liver stage development can be achieved (as discussed in more detail later). There are many antimalarial drugs available that are candidates for CPS. They vary in efficacy, safety and mechanisms of action but only a handful have been evaluated in animal or clinical studies for use in CPS.

Drugs that suppress blood stage growth can block infections during the first round of replication inside red blood cells. The exact mechanism of one such drug, CQ, remains under debate but most likely acts by disrupting detoxification of heme, a harmful by-product of hemoglobin digestion during the trophozoite phase of blood stage infection [26]. Mefloquine (MQ) is similar to chloroquine in that it is a quinine derivative and also kills trophozoites. In order to block blood stage infection altogether, registered drugs or drug combinations known to act on replicating *Plasmodium* liver stages such as primaquine, sulfadoxine–pyrimethamine, atovaquone–proguanil, doxycycline, azithromycin and clindamycin (with the exception of primaquine, all are also potent inhibitors of asexual blood stages) could be used [27]. Primaquine, pyrimethamine and atovaquone lead to early developmental arrest, whereas the antibiotics azithromycin, clindamycin and presumably, doxycycline allow for an unconstrained production of merozoites that subsequently fail to complete erythrocytic development, due to loss of apicoplast function [28].

Thus the quiver of antimalarials available for potential use in CPS is well stocked. Although only CQ and MQ have been demonstrated effective in humans, alternative protocols have been demonstrated feasible in murine models using primaquine, pyrimethamine, artesunate, azithromycin, clindamycin and piperaquine [24,29–32]. MQ and CQ were shown to be equally effective in humans [19], and rodent studies indicate that while CPS with CQ is more potent than primaquine, antibiotics such as azithromycin induce superior immunity [31]. These findings support the hypothesis that full liver stage development, but not necessarily exposure to blood stages, is advantageous [33]. These studies not only provide greater options for clinical development, but may prove to be essential in understanding the immune mechanisms involved in CPS. Overall, the use of registered drugs with proven chemoprophylactic efficacy for CPS provides a unique platform for rapidly testing experimental whole-parasite vaccine approaches. However, in order to advance CPS from an approach to study anti-malarial immunity into a clinical intervention, further work will be required (Table 1).

3. Genetically attenuated parasites (GAPs)

Parasites can be attenuated by deletion of genes that are necessary at different phases of liver stage developmental progression.

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