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# Tissue-specific cellular immune responses to malaria pre-erythrocytic stages

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Complete and long-lasting protective immunity against malaria can be achieved through vaccination with invasive live attenuated *Plasmodium* sporozoites, the motile stage inoculated in the host skin during a mosquito bite. Protective immunity relies primarily on effector $CD8^+$ T cells targeting the parasite in the liver. Understanding the tissue-specific features of the immune response is emerging as a vital requirement for understanding protective immunity. The small parasite inoculum, the scarcity of infected cells and the tolerogenic properties of the liver represent hurdles for the establishment of protective immunity in endemic areas. In this review, we discuss recent advances on liver-specific features of immunity including innate recognition of malaria pre-erythrocytic stages, $CD8^+$ T cell interactions with infected hepatocytes, antigen presentation for effective $CD8^+$ T cell responses and generation of liver-resident memory $CD8^+$ T cells. A better understanding of the factors involved in the induction and maintenance of effector $CD8^+$ T cell immunity against malaria pre-erythrocytic stages is crucial for the development of an effective vaccine targeting the initial phase of malaria infection.

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## Introduction

Malaria, caused by *Plasmodium* parasites, is one of the leading causes of mortality and morbidity in resource poor areas worldwide. Notwithstanding global control and elimination efforts, >400,000 people still die annually due to malaria (http://www.who.int/malaria/publications/world-malaria-report-2016/report/en). A highly efficacious malaria vaccine remains elusive. *Plasmodium* sporozoites are injected in the host skin by a female infected *Anopheles* mosquito. These sporozoites travel to the liver, invade hepatocytes and develop into exo-erythrocytic forms (EEF), which generate thousands of blood stage parasites. Targeting the malaria pre-erythrocytic stage is an ideal and attractive strategy for malaria vaccination. Inhibiting liver infection and development of malaria parasites can prevent both the disease-causing blood stages and the transmissible sexual stages.

Humans, rhesus monkeys and mice exposed to multiple doses of γ-radiation-attenuated sporozoites (RAS), the gold standard vaccine for malaria, can be fully protected against normal sporozoite challenge (reviewed in [1]). Alternative attenuation strategies, such as genetically attenuated parasites (GAP) or chemoprophylaxis with sporozoite infection (CPS), also induce sterile protection (reviewed in [2]). Whilst the use of attenuated parasites is a feasible approach for vaccination, they demand production of large quantities of infected mosquitoes that is not easily scalable to mass vaccination in poor settings. But, if we can discover the important features of a protective immune response, we can replicate these phenotypes by sub-unit vaccination. RTS,S/AS01, the most advanced malaria sub-unit vaccine candidate to date, is based on the circumsporozoite protein (CSP), the surface coat antigen of sporozoites. Yet, despite being designed to elicit different arms of the immune response, RTS,S/AS01 only provides partial protection in malaria-naïve and experienced individuals [3•].

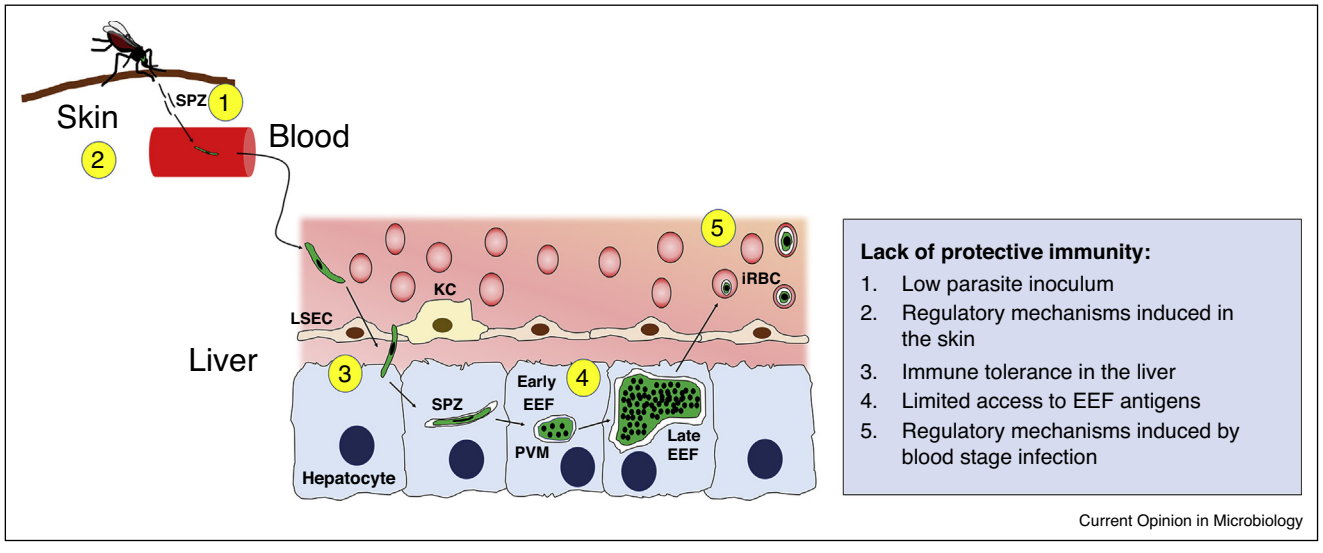
In rodent models and rhesus monkeys, protection conferred by RAS vaccination is largely dependent on effector $CD8^+$ T cells (reviewed in [4]). Depletion of $CD8^+$ T cells prior to challenge of immunised mice and rhesus monkeys consistently abrogated protection [5,6]. *Plasmodium falciparum* (*Pf*) RAS vaccination of humans induces high numbers of sporozoite-specific $CD8^+$ T cells producing IFN-γ [7]. Understanding the key features of host–parasite interactions and the induction of innate and adaptive immune responses, particularly parasite-specific $CD8^+$ T cells, is crucial for informing the development of an effective next generation malaria vaccine.

## Immunisation with attenuated parasites versus natural infections: numbers matter

Despite repeated infections, individuals in endemic areas do not develop sterilising protection and those surviving episodes of childhood malaria remain vulnerable to intermittent infections [8]. Several possibilities, including the small number of parasites naturally transmitted by mosquitoes or the down-regulation of immunity by malaria blood infection, can explain the reasons behind the contrasting outcomes with those experimentally vaccinated with attenuated sporozoites (Figure 1).

In mice, only ~20–50 *Plasmodium yoelii* (*Py*) or *Plasmodium berghei* (*Pb*) sporozoites are inoculated in the host skin during an infective bite and only a small fraction invades and develops inside hepatocytes (reviewed in [9]). $CD8^+$ T cell responses to CSP and sporozoites following *Py* and *Pf* RAS immunisation, respectively, are dependent on antigen dose so low inoculum equates to poor $CD8^+$ T cell responses [7,10]. In the *Py* model, $CD8^+$ T cell responses are not readily increased by repeated immunisation [11,12]. To achieve sterile protection in humans, more than 1000 *Pf* infective bites (*Pf* RAS) are required [13]; this amount corresponds to almost ten years of exposure to *Pf* in a high malaria transmission area [14] but administered in a much shorter period. Sterile protection can also be achieved by the intravenous inoculation of ~700,000 *Pf* RAS [7]. To protect humans under CPS, fewer *Pf* infective bites (~40) or cryopreserved sporozoites (~150,000) are needed [15,16•]. For CPS, the host is exposed to both pre-erythrocytic and blood stage (transient parasitemia) antigens, and sterile protection is observed only against a *Pf* sporozoite challenge [17]. Comparable to findings in humans, CPS induces efficient protection against *Pb* sporozoite infection, but not against blood stage challenge. This sterilising protection is abolished after depletion of $CD8^+$ T cells and is not affected by the lack of mature B cells [18,19]. In contrast, CPS vaccination not only induces high levels of antigen-experienced $CD8^+$ T cells but also targets blood stages of *Py* and *P. chabaudi* (*Pc*) [18,20–22]. In common, the two species used in these studies cause an acute parasitemia that can be naturally controlled by non-vaccinated hosts, indicating a lower stringency for the immune-control of the blood stage infection in comparison to the *Pb* and *Pf* lethal strains. Late arresting *Py* GAPs have been shown to provide superior protective immunity, suggesting a role of mid/late EEFs antigens in protection, and similar to RAS and CPS, sterile protection is dependent on the immunising dose of attenuated sporozoites [23]. These data suggest that exposure to a broad antigenic repertoire, including antigens shared between EEFs and blood stages, improves protection against the pre-erythrocytic stages. Additionally, the absence or the rapid clearance of

**Figure 1**




Potential factors contributing to the lack of protective immunity during natural *Plasmodium* infection. Under natural transmission conditions, only a few sporozoites are injected by an infected mosquito into the host skin. The motile sporozoites enter the blood stream by traversing a dermal capillary, are transported to the liver and traverse across liver sinusoidal endothelial cells (LSEC) or Kupffer cells (KC) to reach hepatocytes. Sporozoites invade hepatocytes inside a vacuole, where they replicate into thousands of merozoites, which once released into the bloodstream invade erythrocytes and initiate the blood stage infection. A combination of factors concurs to the lack of protective immunity in naturally exposed individuals. Infected mosquitoes inject very low numbers of sporozoites (1). Dermal inoculation is associated with immune regulatory mechanisms (2). The liver environment is prone to immune tolerance (3). The membrane of the parasitophorous vacuole limits diffusion of parasite liver stage antigens and exposure to the immune system (4). The blood stage infection that follows complete parasite development in the liver has immunosuppressive effects on liver stage immunity (5).



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