INTERACTION OF PFEMP1 WITH THE HUMAN IMMUNE SYSTEM AND THE PROSPECT OF PfEMP1-BASED VACCINE FOR MALARIA

by

Hussein Magale

A Thesis Submitted to the Faculty of the

DEPARTMENT OF CELLULAR AND MOLECULAR MEDICINE

In Partial Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

2016

STATEMENT BY AUTHOR

The thesis titled interaction of PfEMP1 with the human immune system and the prospect of PfEMP1-based vaccine for Malaria prepared by Hussein Magale has been submitted in partial fulfillment of requirements for a master's degree at the University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that an accurate acknowledgement of the source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Hussein Magale

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

David Elliott, PhD
Assistant professor
Cellular and Molecular Medicine

April 18th 2016
Date

Paul St. John, PhD
Associate professor
Cellular and Molecular Medicine

April 18th 2016
Date

Acknowledgements

The author would like to thank Dr. David Elliott, Dr. Lonnie Lybarger, Dr. Paul St. John and Dr. Kacey Ernst for their input and guidance in preparation of this review.

Table of Contents

Page

List of Figures

Abstract

Malaria is a leading cause of death in some developing countries. The malaria parasite has been around for over a century, and has coevolved with humans. Coming up with an effective vaccine for *P. falciparum* will save millions of lives and reduce the morbidity and mortality of malaria globally. Understanding the role of exported parasite proteins *i.e* PfEMP1 a virulence factor and major cause of malarial pathogenesis, has been of great interest to vaccine researchers in the last decade. The focus of this review is to provide a literature review on PfEMP1s, their interaction with the human immune system, and their role in helping *P. falciparum* parasite to evade the immune system. This review will primarily focus on the intra-erythrocytic stage, which is the stage that results in the symptoms of malaria. A review is necessary to understand the antigenic variation of PfEMP1s, and how PfEMP1s challenge the different arms of the immune response, both the innate and adaptive. This review is unique in touching on the major parts of the immune system's interaction with the PfEMP1 antigen. Furthermore, the review explores the discussion of future research and therapeutic opportunities based on our knowledge of PfEMP1 antigens.

Introduction

Malaria overview and epidemiology

Malaria is a life-threatening mosquito-borne infection. The World Health Organization (WHO) considers malaria to be the most important parasitic infectious disease (1). Forty percent of the world's population is at risk of contracting malaria, predominantly in developing countries (2). Sub-Saharan Africa bears the highest burden of mortality and morbidity resulting from malaria (2). There are more than 214 million acute cases of malaria reported globally last each year, 2015, resulting in about half a million deaths, and 88% of those deaths occur in young children under the age of five residing in Africa (1). Malaria accounts for 10% of the continent's overall disease burden (3). The most vulnerable group for malaria are children under five who have not developed immunity towards malaria (4), and pregnant women whose immunity has been decreased and who are also more attractive to the mosquito (5). About 1500 cases of malaria are diagnosed every year in United States, and the majority of these cases are immigrants and travelers returning from malaria-endemic regions, especially sub-Saharan Africa and South Asia (6).

Malaria is an ancient disease that has been around for million of years (7). Some estimates place *Plasmodium falciparum* (*P. falciparum*) to be greater than 100,000 years old and as old as humans (8), suggesting that the human immune system and parasite have coevolved. Malaria in humans was first identified between the 1898 and 1900 by Italian scientists Giovanni Battista Grassi, Amico Bignami, Giuseppe Bastianelli, Angelo Celli, Camillo Golgi and Ettore Marchiafava (7). Although much work

has been done towards developing a vaccine for malaria, especially in the last 10 years, there remains no protective vaccine (6).

Causes and transmission of Malaria

Malaria is caused by a unicellular obligate intracellular parasite belonging to the Apicomplexa phylum of the *Plasmodium* genus (9). Obligate parasites are parasites that cannot live outside their host. There are over 150 species of *Plasmodium,* but there are only four species that are known to cause malaria in immune competent individuals; *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae* and *Plasmodium ovale* (10). There is a fifth species of *Plasmodium* that also causes malaria in primates, *Plasmodium knowlesi* (11), that has been recently reported to cause malaria in humans, but it remains unclear if it can cause malaria through the natural route, transmitted from human to human by the mosquito (12). *P*. *falciparum* is known to be the deadliest form of the malaria parasite and causes more than 90% of the malarial deaths globally (13).

Malaria is thought to be transmitted only by the female *Anopheles* mosquito (14), making it the primary vector for malaria. There are many mosquito species belonging to the *anopheles* genus reported to transmit malaria (15). *Anopheles gambiae* is the major vector of *P*. *falciparum* in sub-Saharan Africa and it is thought to be the most efficient vector causing malaria (16). Little is known about the adaptation of the plasmodium species to different vectors (17). Since malaria originated from Africa (9), it is thought that the disease became global as humans migrated to different continents. There are many

species of anopheles that transmit malaria and they vary in their vectorial capacity for multiple factors including vector competence and feeding preferences (18).

Treatment of uncomplicated and severe Malaria

The severity of disease dictates the approach to any therapy for malaria caused by *P. falciparum* infection. A clinician has to distinguish the type of malarial infection (uncomplicated or severe malarial infection) before any diagnosis is made. Uncomplicated malaria (UM) involves symptomatic parasitemia of less than 5% without observations of any vital organ dysfunction. These patients have the ability to take oral medications (19). Treatment of UM consists of oral therapy with combination of two antimalarial agents per WHO recommendation. The combination strategy of two drugs has been encouraged by the WHO to forestall the development of antimalarial drug resistance and also to protect the effectiveness of the working antimalarial agents (20). A clinician has to be up-to-date on the local antimalarial drug resistance patterns, government treatment guidelines, tolerability, availability, and gametocidal activity (effect on gametocytes, sexual stage) before finalizing the therapeutic agents to administrate for UM (21). For instance, in Sub-Saharan Africa, where *P. falciparum* strains that are chloroquine-resistant have been reported, antimalarial agents should not be administrated without knowledge of the prevalence of drug resistance (22). WHO recommends the first-line of treatment for UM in areas with chloroquine-resistant *P. falciparum* should include: artemisinin-derivative combinations, atovaquone-proguanil (malarone), quinine-based regimen in combination with doxycycline or clindamycin and mefloquine in combination with artesunate or doxycycline (23).

Severe malaria is an acute malaria that presents with major signs of organ dysfunction and/or high levels of parasitemia (24). Patients with severe malaria present with some of the following symptoms; impaired consciousness, multiple convulsions/coma, acidosis, shock, pulmonary edema, significant malarial anemia, hypoglycemia, and/or hyperparasitemia (25)(26). In malaria-endemic regions, children under the age of five, pregnant women, and immunocompromised individuals are at higher risk of progressing to severe malaria after *P. falciparum* infection. Also, travelers to malaria-endemic regions with no previous exposure to malaria parasites are at higher risk to progressing to severe malaria if infected with *P. falciparum* (27). Since the risk of death from severe malaria is higher during the first 24 hours of illness, patients should be treated with pre-referral dose (if coming from rural malaria-endemic regions) with intramuscular or intrarectal therapy and admitted to an acute care facility (24). A single intramasuclar artesunate dose has been shown to reduce mortality of *P. falciparum* severe malaria among children under the age of five as demonstrated in randomized clinical trials done in Ghana and Tanzania (28). In places where artesimisin is not readily available, WHO recommends the use of parental administration (IV or intramuscular) of quinine or quinidine, with regular cardiac monitoring (24).

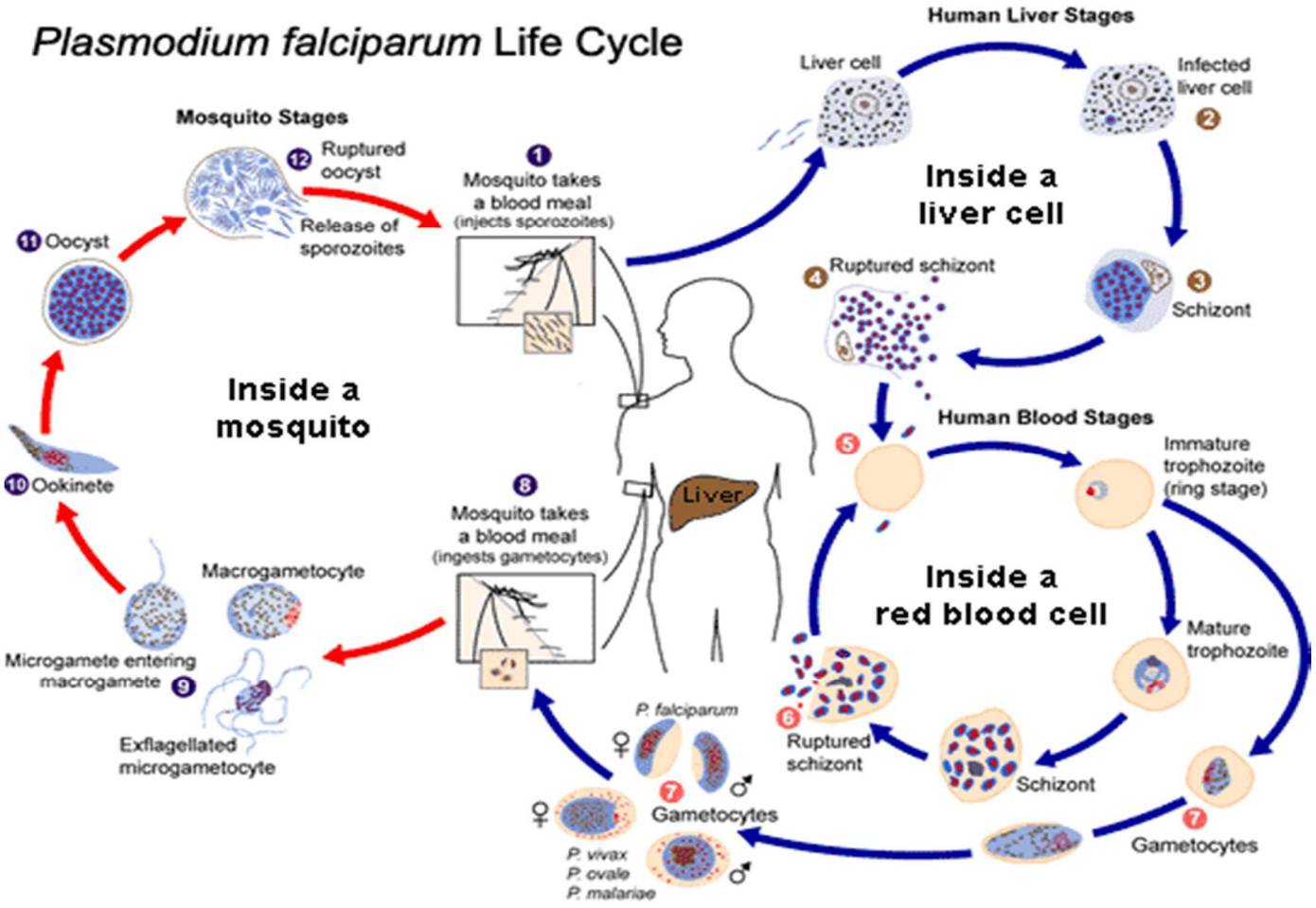
Plasmodium falciparum life cycle

The malaria parasite presents a complex life cycle involving the *Anopheles* mosquito and the vertebrate host. Like many other protozoa, plasmodia passes through a number of stages in the course of their two-host life cycle (29). The mosquito is the definitive host for malaria parasite, meaning this is where it completes the sexual stages

of its life cycle. Figure 1 shows the simplified life cycle of *P. falciparum*. Briefly, the cycle begins when an infected mosquito bites a human and injects the *Plasmodium* sporozoites. Some of the sporozoites injected find a blood vessel within 30-60 minutes and travel to the liver and invade the hepatocytes (30)(31). The parasites replicate in hepatocytes as schizonts, asexual replication that continues until several thousands *merozoites* are produced. The *meroziotes* rupture and are released into the circulation to infect red blood cells (RBC) (32). *P. falciparum* and *P. malariae* are not known to cause clinical latency during malarial infection in humans. However, *P. vivax* and *p. ovale* can relapse and reactivate after several months or even years after the initialinfection (33). For *P. ovale* and *P. vivax,* some of the sporozoites at the liver stage do not undergo asexual reproduction immediately, but enter a dormant phase known as hypnozoite (34). The hypnozoite can reactivate later and establish infection by undergoing schizogony, resulting in relapse. Therefore, the primary infection of malaria may result in repeated bouts of illness depending on the species (35).

Most clinical presentations from *P. falciparum* malaria occur during the 48-hour asexual development in the RBC (36). In the RBC, the parasite takes over and remodels the RBC by exporting proteins to the membrane surface of the RBC (37). *P. falciparum* has been reported to export more than 400 proteins to the infected RBC membrane surface, of which more than 100 have no known function in malaria pathogenesis ((38). One important family of exported *P. falciparum* proteins is the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1). PfEMP1 proteins are mostly expressed on the surface of the RBC during the late erythrocytic stage of *P. falciparum* infection and are associated with “knobs” on the surface of the infected RBC (39). The

knob is a nanoscale protrusion on the infected RBC surface which has been shown to act as a site for PfEMP1 anchorage (40). The pathogenesis of severe malaria is largely attributed to the ability of *P. falciparum* to remodel the host's RBC surface by exporting PfEMP1 proteins, which change the rigidity and adhesiveness of the infected RBC to the endothelium (36)(41).



A diagram showing *Plasmodium falciparum* life cycle showing the parasite in the mosquito, hepatocyte and in the RBC. Adopted from CDC.gov (30).

Plasmodium falciparum Erythrocyte Membrane Proteins 1 (PfEMP1)

PfEMP1s are a family of proteins that are encoded by the hyper-variable *var* genes (43). Each *P*. *falciparum* parasite's genome contains approximately 50-60 *var* genes distributed in clusters across chromosome 14 (41). Based on the conserved upstream sequences (UPS) of coding regions, *var* genes are grouped into four; Groups A, B, C and E (or UPS A, B, C and E) (44)(45). Group A *var* genes are confined to the subtelomeric regions of the chromosome, and have been shown by *in vivo* studies to be involved in the pathogenesis of severe malaria (46). Group B *var* genes occur both within the central regions of the chromosome and in the subtelomeric regions of the chromosome, whereas Group C *var* genes are only found within the chromosome bodies (43). UPSE is particularly unique in that it contains the unique var2csa which encodes for PfEMP1 variants shown to be involved in placental malaria pathogenesis (47) . In the *P. falciparum* clone 3D7, a lab strain, sequencing has shown that two-thirds of the *var* genes are located in the subtelomeric regions of the 14 chromosomes, with the remainder occurring in the central regions of the chromosome (48).

PfEMP1s are large proteins with a highly variable molecular weight ranging from 200-350kDa (49). The PfEMP1-encoding family of *var* genes have a two-exon structure; exon I and II, connected by a single highly conserved intron. Exon I codes for the polymorphic sequences forming the extracellular domains and begins with an N-terminal segment (NTS), and is followed by a succession of Duffy Binding Like (DBL) repeats and followed by cysteine rich inter-domain regions (CIDR) domains (43). Exon II is semi-conserved and encodes for the intracellular components of PfEMP1s (43). The most variable part of the protein is the NTS followed by segments composed of three

domain types: DBL-domains, CIDRs and C2 domain (50). Figure 2 depicts the schematic *var* genes locus and PfEMP1 protein (43).

Figure 2. A diagram showing the organization of *var* gene locus and schematic representation of PfEMP1 protein.
Abbreviations: DBL= Duffy-binding like; CIDR= Cys rich inter-domain region; NTS=N-terminal segment; ATS= Acidic terminal segment. Modified Claessens et al. 2014 (43).

PfEMP1 and infected RBC remodeling

To understand the impact of PfEMP1 proteins on remodeling and take-over of the infected RBC, it is important to start with the function of the RBC. The human RBC has been described as a sack lacking the many cellular organelles, that has been evolutionary tailored to perform the specific tasks of transporting oxygen and carbon dioxide (37). During its terminal differentiation, the RBC loses its nucleus and ability to synthesize new proteins (51). The RBC membrane is what allows it to undertake the journey of millions of kilometers transitioning in the circulation during its 120-day life span (37). To cope with the stress and with the forces in circulation, the RBC has a specialized cytoskeleton that provides mechanical stability and flexibility. The RBC

membrane also has the ability to undergo remarkable deformities without fragmentation, illustrated by how an RBC with an 8μm diameter can transit through 1-2μm inter-endothelial slits that separate the splenic cords and sinuses (52). The RBC membrane ability to deform is owed to the RBC membrane cytoskeleton which has a fibrous polypeptide, spectrin, composed of two isomers (alpha and beta) forming a loosely wound helix (53). The two alpa-beta helices of spectrin form a single tetramer and take part in the 'breathing or squeezing' mechanism whereby spectrin tetramer association and dissociation imposed by shear forces allows the membrane to accommodate the distortion that it suffers in the circulation (54).

P. falciparum exports membrane proteins such as PfEMP1, to the RBC membrane. This results in profound structural and morphological changes to the RBC that alter their physical properties and impair circulation *in vivo* (55). In contrast to normal uninfected RBC, the parasitized RBC is rigid and adheres to host endothelium and other varieties of uninfected cells. This can result in sequestration (56), which is mediated by PfEMP1 variants, when a parasitized RBC bind to several different endothelial receptors, including CD36, thrombospondin, ICAM-1, E- selectin, P-selectin and Chondroitin Sulphate A (57)(58)(59)(60). The adherence of the infected RBC to the vascular endothelium is thought to be mediated by the PfEMP1s (61). The increased adhesiveness and rigidity of *P. falciparum*-infected RBC to the vascular lining, allowing the parasite to avoid splenic clearance (62), and plays a major role in the pathogenesis of malaria (63). Morphological changes of infected RBC include the appearance of thousands of small protrusions called knobs (64). PfEMP1s are anchored at the RBC skeleton by knobs, a macromolecular complex consisting of a knob-associated

histidine-rich protein (KAHRP) (65). Apart from interacting with PfEMP1, KAHRP protein also interacts with the RBC cyto-skeletal proteins such as spectrin, actin and ankyrin and has been shown to be involved in making the parasitized erythrocyte membrane become more rigid and adhere to the endothelium (66). PfEMP1s are concentrated on the exterior surface of the knobs and alter the adhesiveness of the infected RBC (64). A gene knock-out study finds that without the knobs, PfEMP1 cannot form adhesive interactions of sufficient strength that can withstand disruption by forces of blood flow (65).

Antigenic variation of PfEMP1

Following invasion into the RBC, *P. falciparum* makes over the biology of the host cell, changing the infected RBC morphology and modifying its ability to adhere to the endothelium with the aid of virulence antigen, PfEMP1. Even though the function of the PfEMP1 proteins is well studied during the asexual cycle in the RBC, *var* gene expression is not limited to the blood stage (48). PfEMP1 proteins have been shown to be expressed in the early stages of RBC infection by gametocytes, the transmittable form of *P. falciparum* parasite that is infective to mosquitoes (67). Also, PfEMP1 tryptic fragments has been reported in sporozoites in the mosquito salivary gland (68). Therefore, PfEMP1s are involved in different stages of the *P*. *falciparum* life cycle and may have multistage functions including aiding in cyto-adhesion, transmission, and other functions (48).

PfEMP1s present the most extreme forms of clonal variation, creating a phenomena known as “mutually exclusive expression,” also referred to as mono-allelic

expression, where a single *var* gene is transcribed, while all other *var* genes are silenced (69). PfEMP1 has been shown to undergo clonal variation in *in vitro* studies (70)(71). The persistence of the *P. falciparum* parasite during the blood stage proliferation in the RBC depends on the successive expression of the variant PfEMP1 molecules on the surface of the infected RBC (69). PfEMP1s are encoded by 50-60 distinct clonally variant *var* genes, but with DNA recombination and gene conversions it is possible for *P. falciparum* to generate a virtually unlimited repertoire of PfEMP1s variants (72). Only one of these *var* genes are expressed at a given time by the *P. falciparum* parasite in the RBC (73). *In vitro* studies have shown that parental clones of *P. falciparum* give rise to clones that differ in their agglutination from a clone-specific antisera, and result in antigenically distinct forms of PfEMP1s (74).

Mutually exclusive expression is not unique to *P. falciparum* but has been shown in several other organisms including imprinting and VSG expression in mammals (75)(76). The regulation of the mammalian immunoglobulin (Ig) heavy-chain genes expressed in B cells encoding for the cell surface receptors (B cell receptors) are analogous to the *P. falciparum* exported surface PfEMP1s in that the genes encoding the B cell receptors are expressed in a mutually-exclusive manner, leading to the paradigm of one B cell having one unique receptor, also commonly referred to as the allelic exclusion during B cell development (77). However, the allelic exclusion in mammals is ensured by the negative feedback inhibition mediated by the production of functional protein/receptor (77). In *P. falciparum*, the regulation of PfEMP1 expression is rather a mono-allelic exclusion since it depends solely on the noncoding elements at

each *var* gene (transcriptional regulatory elements) and has been shown to be independent of the production of a functional PfEMP1 (78).

Several potential mechanisms have been put forward to explain the mutually exclusive expression resulting in the antigenic variation of PfEMP1s. Epigenetic factors, such as silencing of *var* genes through histone modifications, are hypothesized to account for the regulation of *var* gene expression. Another model postulates that the expression of *var* genes is controlled at the level of transcription initiation (69) and that a *var*-specific, sub-nuclear expression site exists in the p. *faciparum* nucleus which limits access to only a single gene at a time, resulting in mutually exclusive expression (79).

PfEMP1 mediated pathogenesis- Cerebral and Placental malaria

One of the primary functions of the PfEMP1 as a virulence factor for *P. falciparum* is to mediate the sequestration of infected RBC in the micro-vasculature (80). Sequestration refers to the adherence of the infected RBCs containing the late stages of the *P. falciparum* (especially trophozoites and schizonts) to the endothelium of capillaries and venules (81). Sequestration favors the development of the parasite in the RBC by escaping from the immune surveillance of the spleen (65). PfEMP1 proteins also mediate another pathogenic phenomenon, erythrocyte rosetting, which involves the adhesion of infected RBC to uninfected RBCs, forming a complex “flower-like” structure around a central infected RBC (82). The phenomena of erythrocyte rosetting in the vasculature can result in impaired vascular blood flow and disruption of local oxygen delivery, leading to the death of the host (83). This consequence of killing the host by *P.*

falciparum is unintended by the parasite since it hinders the dissemination of malarial infection to the population.

One of the most malignant forms of malarial infection, cerebral malaria, can result from the massive sequestration of uninfected and infected RBC in the brain microvasculature. PfEMP1 proteins have been shown to mediate the adhesion of infected RBC to the brain's microvascular endothelial cells, resulting in severe forms of clinical cerebral malaria (84) by causing obstruction in thebrain capillaries. Studies have identified several human endothelial receptors that participate in the PfEMP1-mediated adherence. CD36 and Intra-Cellular Adhesion Molecule-1 (ICAM-1) are the most commonly reported endothelial human receptors from clinical isolates causing cerebral malaria (85).

Pregnancy is an event of immunological tolerance where the mother's immune system has to tolerate and accept the implantation of the fetal allograft in her uterus (86). However, *P. falciparum* takes advantage of this tolerance and puts pregnant women at increased risk for malarial infection, because the parasite can bind and adhere to the trophoblastic villous epithelium and sequester in the placenta (87). The sequestration in the placenta results from the parasite binding to chondroitin sulfate A (CSA), a glycosaminoglycan molecule that is not found in other tissue beds, but *P. falciparum* parasite has tropism for CSA (87). Several studies reported that cytoadhesion of the infected RBC to CSA is mediated by clonally variant PfEMP1 (encoded by *var2csa*) in the placental inter-villous space (87)(88). In malaria-endemic regions, pregnancy-associated malaria (PAM) is highly concentrated in primigravid women, which suggests that there is a protective immunity that is developed by the

multigravid women (89). Maternal malaria has been associated with poor fetal outcomes including stillbirths, abortion and low birth weight babies (90)

Innate immunity

This section of the review focuses on the interaction between the innate immune responses and PfEMP1 antigen (schematic representation shown in figure 3). The innate immune system forms the first line of defense against invading pathogens. The innate immune system recognizes foreign invaders through pathogen-associated molecular patterns (PAMPs) (91). Innate immune recognition depends on a limited number of receptors that are germline-encoded, and develops no immunological memory even after encountering a pathogen several times (92). The innate immune system recognizes conserved molecular patterns of microbes from repeating structural motifs indicating non-self, such as lipopolysaccharides of gram-negative bacteria, bacterial CpG DNA, mannans, and glycans (92). The innate immune system has different components: the epithelial barriers, the different innate cells, and complement system. Even though innate immunity lacks specificity (relative to adaptive immunity), it has cells with receptors such as the Toll-like receptor (TLRs), NOD-like receptors and RIG-like receptors to distinguish self from non-self that are selected over evolutionary time to sense pathogenic microbes and activate innate immunity (93). The two main functions of the innate immune response are to prevent infection by pathogenic microorganisms, and if the infection has occurred, to keep it at “bay” until the adaptive immune response is fully activated (94).

Natural Killer Cells and PfEMP1

Natural killer (NK) cells are effector lymphocytes that are part of the innate immune system. NK cells have both cytotoxicity and cytokine-secretion effector functions to control the development of the pathogenic pathways and avoid tissue damage (95). Even though NK cells are non-specific in their antigenic recognition, NKs have the ability to discriminate target cells from the other "self" cells through the phenomenon known as the "missing-self" hypothesis, where target cells are deficient in Major Histocompatibility Complex (MHC) I protein (96).

NK cells are widespread in the lymphoid and non-lymphoid organs, and comprises about 2-18% of the human peripheral blood lymphocytes, and were shown to be important in controlling the blood stage development of *P*. *falciparum* in the RBC (96). NK cell-mediated inhibition of the growth of the asexual blood stages of *P*. *falciparum* has been shown in *in vitro* studies, where in the presence of a serum from semi-immune individuals, NK cells destroy the parasitized erythrocytes through direct cytotoxity (97). The molecular mechanism underlying how NK cells distinguish between infected RBC and uninfected RBC is not well understood but the physical interaction between the NK and parasitized erythrocyte was reported to be the prerequisite in addition to the release of inflammatory cytokines, interferon-gamma (IFN-γ) (98). Infected RBCs expressing PfEMP1 proteins are susceptible to NK lysis through the interaction of the NK receptors NKp30 immunoglobulin and NKp46 immunoglobulin fusion protein with PfEMP1 DBL-1 alpha subdomain (99). This interaction between the NK cells and PfEMP1 proteins on parasitized RBCs is a specific and functional one,

since uninfected RBC do not express PfEMP1 with DBL-1alpha that can lead NK cells to produce perforin and granzyme B, resulting in the lysis of the infected RBC (97). The protection of NK cells against *P*. *falciparum* parasites is one that comes with its own drawback, and is a “double-edged sword”. While NK cells play a role in providing immune-mediated control of PfEMP1-expressing RBCs, it has also been shown that NKs contribute to the pathogenesis of cerebral malaria (100). Even though the precise mechanism of how NK cells lead to the pathogenicity of cerebral malaria is not clear, a large body of work suggests that pro-inflammatory responses through the cytokines released, such as tumor necrosis factor (TNF) and IFN-γ, play a role that can lead to both systemic and local inflammation (100)(101). In addition, NK lysis receptors —the natural cytotoxicity receptors (NCRs) NKp30 and NKp46 immunoglobulin fusion proteins, have been shown to interact with PfEMP1 via DBL-1α domain(99). Heterogeneity of the sequence of the PfEMP1 DBL-1α domain has been reported between strains, suggesting that NK cells can only play a selective role in the fight against *P*. f*alciparum* (99), suggesting their immune response will be lost as new variants of PfEMP1 is expressed. Another study has reported that PfEMP1s suppress the early production of the host IFN-γ released by human mononuclear cells including NK cells (102), postulating a possible strategy of how *P*. f*alciparum* uses PfEMP1 to evade the host’s immune responses.

Complement system

The complement system is part of innate immunity, and is composed of a diverse set of plasma proteins that react with one another to opsonize pathogens and induce a

series of inflammatory responses to fight infections (103). Some of the complement proteins are proteases that are activated by proteolytic cleavage, and have to be kept in their inactive form as zymogens in the body fluid and tissues to prevent uncontrolled complement activation (103). There are three pathways through which the complement system can be activated: classical pathway, which can be triggered directly by the pathogen, or indirectly by an antibody binding to the pathogen surface; mannose binding (MB) lectin pathway; and the alternative pathway, which provides an amplification loop from the other two pathways (103).

Studies have reported the protective roles of the complement system against *P. falciparum,* especially the classical pathway (104). Both human and animal models have shown evidence of complement activation, leading to the reduction of complement factors in the serum including C1, C2, C3, C4 and C1q levels during malarial infection (105)(106). The most likely mechanism of the complement system that is activated during malarial infection is through the classical pathway by the formation of antibody-immune complexes containing *P*. *falciparum* merozoites (107). However, *in vitro* studies have shown evidence, both direct and indirect, that the other complement pathways, lectin and alternative pathways, can also be activated during *P. falciparum* infection (108). MB-lectin, which is an acute-phase reactant and the first component of the lectin pathway, was shown to increase in the serum at the early phases of *P*. *falciparum* malarial infection (109), demonstrating that the lectin pathway is also activated. One study has pointed out that having deficiency in MBL, and thereby not being able to activate the lectin pathway of the complement system, was not associated with a greater risk of becoming susceptible to acquiring severe malaria or cerebral malaria

(110), suggesting that the other two pathways could compensate for the deficiently in the MBL pathway. However, other studies pointed out that even though *P. falciparum*-infected RBC can activate the complement system, the infected RBC is resistant to the lysis because of the complement-regulatory proteins on the RBC surface (111)(112).

PfEMP1 antigens on the surface of parasitized erythrocytes have been shown to interact with the complement receptor 1 (CR1), CD35, a complement regulatory protein found on variety of cells including RBCs (113). The PfEMP1s expressed on infected RBCs interact with other uninfected RBCs, causing rosetting of uninfected RBC to infected RBC, contributing to vascular blockage and severe malaria pathogenesis (114). *P. falciparum* PfEMP1s binding to CR1 on uninfected erythrocytes is facilitated by the PfEMP1's most N-terminal domain (DBL-alpha) (114). Furthermore, experiments have shown that erythrocytes deficient in CR1 have reduced rosetting (115). Populations living in malaria-endemic regions possess polymorphism of the CR1 receptor, and one study done in Papua New Guinea shows that it has been associated with protection against severe malaria by two-thirds (116).

Neutrophils and Macrophages

Both neutrophils and macrophages are part of the innate immune system that initiates an immune response once a micro-pathogen breaches the epithelial barrier, by phagocytosis of the microbe, aiding in infection control or prevention (117). Neutrophils are the immune cells with the highest percentage present in the blood and are considered to be part of the first line of defense against acute infection, and their involvement in *P. falciparum* parasite infection are reported (118). As shown in *in vitro*

studies, the mechanism by which activated neutrophils suppress the growth of asexual blood stages of *P. falciparum* is through the release of oxygen radicals (119)(120). There was no direct link of PfEMP1 and neutrophil interaction reported at the time of this literature review.

On the other hand, macrophages (referred as monocytes when in the blood) have been shown to be crucial to control malarial infection because of their ability to phagocytize infected RBC, which limits the parasite density in the absence of opsonizing *P. falciparum*-specific antibodies (121). Macrophages possess pattern recognition receptors (PRRs) such as the Toll-like receptors (TLRs) and the scavenger receptor, CD36 which plays an important role in immune regulation and inflammatory responses (122). CD36 is a membrane glycoprotein expressed on variety of cells including macrophages/monocytes, and it helps the macrophage in the recognition of pathogens (123). Macrophage CD36 has been shown to aid in recognition and internalization of non-opsonized *P. falciparum*-infected RBC (124), a critical component of the host immune mechanism to fight *P. falciparum* infection. That is why the African populations in malaria-endemic regions have a high frequency of mutations resulting polymorphism in their CD36 receptors (125). However, if those mutations of the CD36 receptors are deleterious, it can result in CD36 deficiency which has been associated with susceptibility to severe and cerebral malaria (126).

P. falciparum infected RBCs with PfEMP1 proteins on their surface which are not opsonized by the complement system or antibodies are recognized and phagocytized by macrophages via the interaction of the PfEMP1 antigen to the CD36 receptor on the macrophage surface (124). CD36 interacts with PfEMP1 via the first CIDR1 domain

after the first DBL domain of PfEMP1 (127). A gene knock down study showed that macrophages lacking CD36 receptors in mice phagocytized 80% less parasitized RBCs compared to the wild-type macrophages with no deficiency in their CD36 receptors (128). A similar study on rats showed that rats lacking macrophages CD36 phagocytized significantly less *P. falciparum*-infected RBC than the wild type, indicating the importance of CD36 and PfEMP1 interaction for macrophage uptake (121).

Dendritic cells

Dendritic cells are known to form the boundary between innate and adaptive immunity. Dendritic cells form heterogeneous cell populations and are mostly found in peripheral tissues, specifically at the sites of interface with the environment (skin and mucosae) (129). In the peripheral tissues, dendritic cells uptake both self and non-self antigens, internalize the antigens and proteolytically process them into peptides which are loaded into MHC I and MHC II molecules expressed on the dendritic cell surface. This process of antigen uptake, degradation of the antigen and loading the antigen on MHC molecules is known as antigen presentation. Dendritic cells are central to the induction of adaptive immunity by serving as the most potent professional antigen-presenting cells (APC) (130). Dendritic cells are known for their ability to present antigens to CD4 (+) and CD8 (+) T cells, forming by far the most potent stimulator of naïve T cells (131). Unlike other APC such as B cells and macrophages, dendritic cells are the only APCs that can provide all the signals required to activate a naïve T cell, and stimulate all the arms of the adaptive immune responses including aiding in antibody production and activation of different types of T cells: CD8, CD4 helpers and T regulatory cells (132). As they encounter antigens/receive a stimulus, dendritic cells

become more efficient in antigen uptake, inhibit further endocytosis and increase their intracellular transport and degradation, and intracellular trafficking of MHC molecules (133). As dendritic cells mature, their antigen presentation capacity function is augmented, their MHC molecules half-life increases, and expression of T cell co-stimulatory molecules also rises (134). Mature dendritic cells migrate to the secondary lymphoid organs to initiate adaptive immune responses (135).

The role of dendritic cells in protection against *P. falciparum* infection is hotly debated, and their role in induction of protection (either through naturally or by vaccination) against blood-stage development of *P*. *falciparum* parasites is largely unknown (136). It has been postulated that *P. falciparum* blood stage infection can suppress CD8 (+) T cells against the liver stages parasites by affecting dendritic cell maturation, cytokine secretion and their capacity to initiate new immune responses (137). The question that begs to be asked is, how can *P. falciparum* asexual blood stage parasites suppress immune response at the liver stage, which precedes the blood stage cycle of *P. falciparum*? It has been shown in an *in vitro* study that *P. falciparum*-infected RBCs adhere to immature dendritic cells and inhibit their maturation, and invert the interleukin (IL)-12/IL-10 secretion pattern (138). Production of cytokines such as IL-10 and IL-12 by dendritic cells during their maturation process can affect the dendritic cell's induction of either Th1 or Th2 immune response (see CD4 (+) T cell section for more on Th1 and Th2). It is thought that dendritic cells secrete IL-12 to stimulate Th1 immune response whereas secretion of IL-10 will block the dendritic cell's maturation process by down regulating MHC-II expression and interfering with up-regulation of co-stimulation molecules (required for naïve T cell activation) and IL-12 production (139).

Blockade of Th1 activation will subsequently impair the ability of CD8 (+) T cells to kill infected hepatocytes.. This *P. falciparum* strategy of dendritic cell maturation suppression has been speculated to aid in malaria transmission because of the lack of protective immunity at the liver stage of the parasite (137).

P. falciparum PfEMP1 proteins have been shown to interact with immature dendritic cells in the blood even though their interaction is not well defined. Immature dendritic cells express the CD36 and CD54 (intercellular adhesion molecule 1, ICAM-1) receptors on their cell surface, glycoproteins that were both shown to interact and adhere to *P. falciparum*-infected RBC with PfEMP1 protein variants on their surface (58). CD36 on immature dendritic cell interacts with PfEMP1 *via* its CIDR-1α domain while ICAM-1 binds to DBLβ domain of PfEMP1 (140)(141). It has been reported that interaction of immature dendritic cells with PfEMP1 suppresses dendritic cell maturation and subsequently reduces their ability to stimulate T cells (142). The adhesion of PfEMP1 to immature dendritic cells is not by chance and provides a selective advantage for the parasite to evade the host immune system (142), indicating that *P. falciparum's* strategy is to delay the host's immune response by modulating the function of the antigen-presenting cells. On the other hand, some studies have reported that the inhibition of dendritic cell maturation is independent of PfEMP1 interaction, but depends on the dose of the infected RBC based on the finding that intact infected RBC interacted closely with dendritic cells, and were phagocytized to similar extent regardless of the level or PfEMP1 variants expressed (143). However, the latter interpretation is based on the interaction between CD36 and infected RBC lacking PfEMP1 interaction could be more complex and further study is recommended (143)

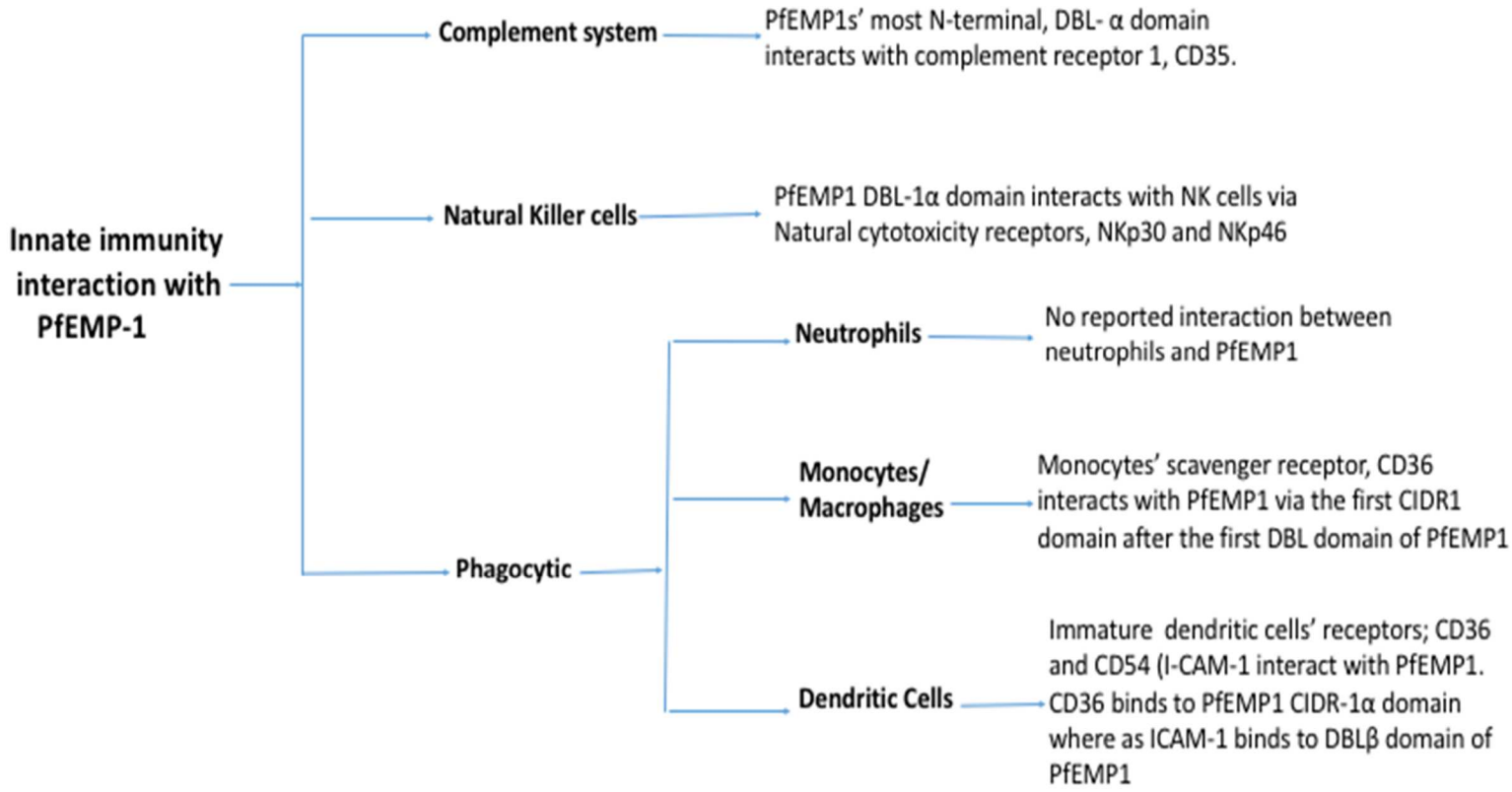


Figure 3. Shows schematic representation of the innate immune systems' interaction with Plasmodium *falciparum* PfEMP1 antigen.

Abbreviations: pfEMP1, Plasmodium falciparum Erythrocyte Membrane Protein-1; DBL; Duffy binding-like domain of PfEMP1; NK, natural killer cells; CD36, cluster of differentiation 36; CIDR1, cysteine-rich interdomain region; CD54, cluster of differentiation 54; ICAM-1, Intercellular adhesion molecule 1 .

Adaptive immunity

Adaptive or acquired immune responses are generated by antigen-specific lymphocytes. Lymphocytes are a subset of the white blood cells that carry clonally-distributed cell-surface receptors to recognize specific antigens. The two main classes of lymphocytes are B lymphocytes (B cells) and T lymphocytes (T cells), which mediate humoral and cell-mediated immunity, respectively (145). Humoral immunity involves antibody-mediated immune responses, whereas cell-mediated immunity does not involve antibodies, but rather is carried out by immune cells such as phagocytes (*i.e.,* neutrophils, macrophages, and dendritic cells), and antigen-specific T lymphocytes (CD4 (+) Th1 and CD8 (+) T cells). An important aspect of the adaptive immune system is the development of immunological memory after encountering a pathogen. Immunological memory involves remembering the specific adaptive responses generated towards an antigen, and making a greater and more rapid response in subsequent encounters towards the same antigen/pathogen (146). Adaptive immunity is as essential as innate immunity in providing effective host defense, and many immunodeficiency syndromes are associated with lacking part(s) of the adaptive immune response (147). The ability of lymphocytes to recognize virtually all pathogens specifically and to provide protection against reinfection is based on the concept of clonal selection of lymphocytes bearing antigen-specific receptors (148).

This section of the review will focus on the interaction of different arms of the adaptive immune system towards *P. falciparum*, with particular focus on the PfEMP1 antigen (summarized in figure 4). It is thought that severe malaria is more prevalent in children in regions with stable transmission of *P. falciparum* and not in adults, because

older people have developed adaptive immunity from constant exposure to natural malaria (149). Protection against *P. falciparum* can take years or decades, and probably never results in sterile immunity, *i.e.,* complete protection (149). This is part of the reason why adults in malaria-endemic regions experience only sporadic parasitic episodes, and rarely develop severe malaria (150). When naïve individuals (*i.e.,* those who have never been exposed to malaria) of any age get infected with *P. falciparum,* it is always symptomatic and the clinical symptoms of malarial infection are observed at even very low levels of parasitemia (150). Human adaptive immunity has been shown to predominantly target the blood stage of *P. falciparum* lifecycle in the RBC, and specifically against PfEMP1 antigens (151). Therefore, it is important to understand how naturally-acquired adaptive immunity develops, to inform efforts to induce artificial adaptive immunity (through vaccination) towards the PfEMP1 antigen.

The role of the spleen in malaria

The spleen is a fist-sized organ located under the ribcage in the upper left quadrant of the abdomen and serves as the body's largest filter of blood. Unlike the primary lymphoid organs, such as the bone marrow and the thymus, where lymphocytes are generated, the spleen is a secondary lymphoid organ, where adaptive immune responses are initiated. The spleen is a complex organ is adapted to selectively filter and destroy the senescent (damaged or old) RBC (144). The micro-anatomical zones and microcirculations of the spleen are adapted to performing different functions such as induction of adaptive immune responses, recycling of iron, as well as destroying senescent erythrocytes and pathogens including *P. falciparum* (145).

The spleen has a trabecular structure formed by the red and white pulps. The red pulp, which forms the largest portion of the spleen, 70-80% of the spleen volume, has sinuses and cords which are open spaces that are populated with active macrophages (146). The specialized reticular meshwork of the red pulp gives the spleen its unique mechanical ability to filter blood and remove old and abnormal RBCs. The splenic macrophages not only play a role in the recognition and uptake of pathogens in the circulation, but also fight bacteria by competing for the iron (147). On the other hand, the white pulp is a lymphoid tissue with B and T lymphocyte compartments (T and B cell zones). The specific establishment and maintenance of the correct compartments in the white pulp is controlled by specific chemokine receptors of the T and B cells, which allows the establishment of specific zones in the white pulp (147)(144). In the T-cell zone (also referred as periarteriolar lymphoid sheath, PALS), T cells interact with antigen presenting cells such as DC cells and collaborate with B cells to make specific antibodies. While in the B-cell zone (also known B cell follicles), clonal expansion of the activated B cell takes place, which leads to isotype class switch and somatic hypermutation (147)(148) (see the B cell section). The distinctive functions of the red and white pulp clearly points to the spleen's involvement and ability to mount both innate and adaptive immune responses.

The important protective role that the spleen plays against *P. falciparum* infection has been shown both in rodent and human studies (149)(150). During the asexual replication of *P. falciparum* parasite, the spleen is the main organ that establishes immune response in order to clear the parasite (150). Splenectomized patients have invariably shown increased parasetemia during *P. falciparum* infection regardless of the

antimalarial drugs administrated (151), suggesting the crucial role that the spleen plays in *P. falciparum* clearance. The spleen's involvement and fight against *P. falciparum* infection can also be noticed from the morphological changes the spleen undergoes, the most apparent being the enlargement of the spleen, splenomegaly, which is used as a clinical indicator for malaria (152). The spleen removes from the circulation RBCs that are less deformable such as those infected with *P. falciparum* (145)(153), and RBC sensitized by IgG (coated with IgG immunoglobulin) during acute *P. falciparum* infection (154). In addition to the spleen's role in removing old, deformed, or parasitized RBCs from circulation, the spleen has been shown to selectively extract the parasites from the RBC but leaves the RBC intact to proceed to the circulation through the mechanism known as "pitting," which is important for dead parasite removal from the RBC following malarial treatment (155)(156).

Despite the protective roles that the spleen plays to clear *P. falciparum* from circulation, the parasite has developed a mechanism to evade splenic clearance by sequestration of infected RBC to the endothelium. The process of sequestration is thought to be mediated by the PfEMP1 antigen (discussed in the PfEMP1 section). The major role of PfEMP1 in parasite sequestration in the vasculature to avoid splenic passage not only speaks to its importance as a virulence factor for *P. falciparum,* but also its role in immune evasion.

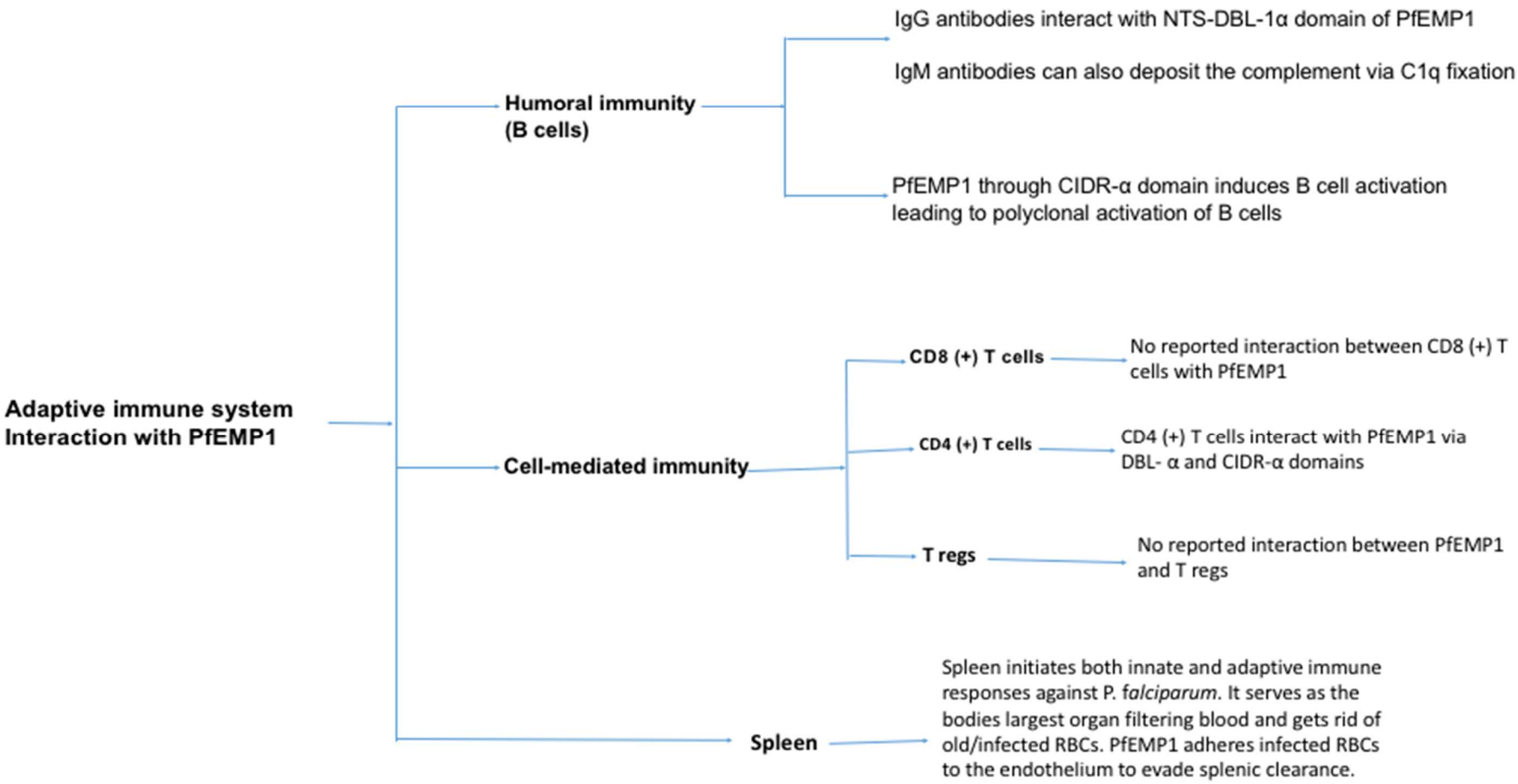


Figure 4. Shows schematic representation of the adaptive immune systems' interaction with Plasmodium *falciparum* PfEMP1 antigen.

Abbreviations: PfEMP1, Plasmodium falciparum Erythrocyte Membrane Protein-1; C1q, the first subcomponent of the C1 complex of the classical pathway of complement activation; IgG, immunoglobulin G; IgM, immunoglobulin M; DBL; Duffy binding-like domain of PfEMP1; CIDR1, cysteine-rich interdomain region; T regs, regulatory T cells; RBC, red blood cells

Humoral Immunity

B lymphocytes (B cells)

B cells perform the humoral function of the adaptive immune response by their ability to make antibodies. B cells undergo several developmental stages and checkpoints to become mature cells with a functional B cell receptor (BCR), the antigen-recognition molecules of B cells (known as an Immunoglobulin; Ig) (166). B cells produce antibodies as both surface receptors and secreted molecules, and each B cell produces a single species of antibody, a single specificity (147). One of the developmental mechanisms that generates the diversity in BCR is somatic hypermutation, a process wherein B cells undergo point mutations in their BCR genes, within regions that encode the antigen-recognition domain, and keep mutating until the BCR that binds best to the antigen is selected. When a memory or a naïve B cell is activated with the aid of a CD4 (+) T cell helper cell, the B cell proliferates and differentiates into an antibody-secreting effector cell, known as a plasma cell (167). Secreted antibodies can bind to pathogens and other toxic products in the extracellular spaces of the host's body, and antibody production is thought to be the major function of effector B cells (plasma cells) in adaptive immunity (168). The first antibody isotype secreted by B cells in the humoral adaptive response is IgM, and its presence in the serumindicates acute infection (169). B cells undergo class-switching with aid from activated T helper cells, after which the B cell switch from IgM to different isotypes, such as IgG, IgA, or IgE (170).

Although their specific mechanisms of protection are not well understood, antibodies are thought to prevent *P. falciparum* blood-stage replication by blocking RBC

invasion and preventing high-density parasitemia (171). It has been demonstrated that passive transfer of immune serum (purified IgG) to infected monkeys substantially modified the course of infection by reducing parasitemia, demonstrating the protective roles that IgG antibodies could play in malarial infection (172). Another study showed that immunoglobulin (specifically IgG) purified from the sera of adult Gambians clinically immune to *P. falciparum* was able to reduce parasitemia when transfused to infected children from Gambia and East Africa (173). In an *in vitro* study, antibodies have been shown to block *P. falciparum* from invading the RBC, targeting the merozoite stage before it invades the uninfected RBC (174). In addition, it has been demonstrated that human antibodies promote complement deposition on the *merozoite* through C1q fixation, which results in the activation of the classical pathway of the complement system (171). Other studies have shown the role of cytophilic antibodies to opsonize and coat, allowing phagocytic cells to recognize and engulf *P. falciparum* parasites (175).

PfEMP1 antigens form a major target for humoral immune responses to combat *P. falciparum* infection. Several studies have indicated that development of antibodies against PfEMP1 is a key component in acquisition of clinical immunity against *P. falciparum* malaria (157)(158). A study has shown both serum and purified IgG were able to agglutinate parasitized erythrocytes with PfEMP1 antigens on their surface (159) . The antibody response against PfEMP1 has been shown to be dependent on the overall parasite biomass and the different proportions of the parasites' PfEMP1 variants expressed (160).

Individuals living in malaria-endemic regions develop specific antibodies recognizing variant PfEMP1 —resulting in a variant-specific protection against malaria (161)(162). In a large prospective study, it was shown Kenyan children developed a repertoire of anti-PfEMP1 antibodies that agglutinates infected RBC in a variant-specific manner. The PfEMP1 variants expressed during clinical episodes of malaria were less likely to be recognized by a child's own pre-existing antibodies, and clinical immunity was associated with developing new anti-PfEMP1 antibodies against the PfEMP1 variants (161). In addition, pre-existing anti-PfEMP1 antibodies have been shown to cross-react with other variants of PfEMP1, and the levels of cross-reactivity can differ depending on the groups (Group A, B or C) of PfEMP1(159). Another study on Ghanaian children showed that those with higher levels of plasma IgG to variant PfEMP1s before the malaria peak season were protected against severe malaria episodes compared to the naïve children (162). Taken together, having anti-PfEMP1 antibodies can make an individual immune either through cross-reactive, strain-transcending antibodies to a conserved PfEMP1 domain, or immunity could rely on a large pool of antibodies against PfEMP1 variants acquired over time (163)(164)(162).

Much of the protective effect of anti-PfEMP antibodies against *P. falciparum* is thought to be due to their ability to block PfEMP1-mediated sequestering and rosetting in the vasculature (165)(166)(167). Studies have demonstrated antibodies recognizing the PfEMP1 NTS-DBL-1α domain, which is shown to be central in mediating rosetting with uninfected RBC (168)(169), disrupts rosetting of uninfected RBC to infected RBC in *in vitro* studies and protects against PfEMP1 mediated sequestration *in vivo* (170)(171).

Another body of work suggests that antibodies can even dislodge already sequestered infected RBC, forcing them into circulation(172), which would lead to splenic clearance.

Humoral immune memory against *P.* f*alciparum* is credited to having long-lived antibody produced by plasma cells and memory B cells (173). Memory B cells (MBCs) have the ability to respond, proliferate, and differentiate more rapidly into effector cells (plasma cells) in subsequent infection than inexperienced naïve B cells (174). An analysis has shown the magnitude of circulating MBC from individuals living in malaria-endemic regions is in the same range as childhood vaccination-induced memory cells against tetanus toxoid in the same population (175). However, the induction and maintenance of long-term malaria-specific MBC responses remains a challenge, if the individual leaves the malaria-endemic region (176). These studies cite the prolonged non-exposure periods of individuals diminishes the prevalence and magnitude of malaria-specific MBCs (173), partly because of the lack of periodic reinfection that is thought to be required for maintaining acquired immunity (reviewed in (177)). One study in Thailand found that the magnitude of malaria-specific MBCs was lower in adults that had no *P. falciparum* and P.*vivax* clinical episodes for the last 6 years compared to those with 1-3 episodes of malaria (178). A similar study on Kenyan children has also found that malaria-specific MBCs were higher in constantly exposed children, compared to those that experienced prolonged non-exposure (7 years) of *P. falciparum* (174).

PfEMP1 CIDR-α domain expressed on infected RBCs has been reported to induce activation and alteration of B cell responses (173). *In vitro* studies have shown that *P. falciparum* infected RBC with PfEMP1 on their surface adhere (through the CIDR-α) to B cells from non-immune donors and cause B cell activation, proliferation,

and cytokine production (179)(180). It has been postulated that PfEMP1 activation of B cells is not *via* the interaction of BCR with PfEMP1 CIDR-α domain but through the B cell TLR signaling, specifically intracellular (endosomal) TLR 7, 9 and surface expressed TLR 10 of the B cell (181). It has been reported that the CIDR-α region of PfEMP1 is a key mediator in causing polyclonal B cell activation (PBA) (180).PBA is when different B cells (of different antigen specificity) get activated by the same antigen inducing the proliferation of multiple of B cell clones without collaboration of antigen-specific Th cells (182). Although the role of PBA in *P. falciparum* infection is somewhat controversial, the antibodies produced through PBA are not only parasite-specific, but can react with unrelated antigens or self-antigens (183)(184). PfEMP1 antigens serving as polyclonal activators of B cells could lead to B cell dysfunction and B cell exhaustion during *P. falciparum* infection, which aids the parasite's survival (185).

Cell-mediated immunity and PfEMP1

For this part of the review, the T cells considered are CD4 (+) T cells, CD8 (+) T cells and regulatory T cells. T cells undergo development in the thymus (so that self and auto-reactive T cells do not make to the periphery) and acquire unique cell surface receptors (known as T cell receptors, TCR) that recognize specific antigens (186). T cells occur in large numbers in the blood, lymph, and secondary lymphoid organs such as lymph nodes (187). Each T cell responds to a specific antigen, and its response during the first encounter with the antigen ensures a more rapid response occurs on subsequent encounters of the same antigen (188). Naïve T cells need three signals to get activated: the TCR has to recognize antigen presented in the context of MHC I or

MHC II, receive co-stimulation signals from the APC (dendritic cell) and the cytokine signal from the APC directing which type of effector T cell will be formed such as Th1, Th2 or Th17 (189).

One of the biggest challenges to acquisition of cellular immunity against *P. falciparum* is the RBC's lack of MHC molecules. All nucleated cells have MHC I molecules on their cell surface, whereas APC also have MHC II molecules. The main function of the MHC molecules is to bind peptides, MHC II for exogenous- and MHC I for endogenous-derived peptides, and the MHC molecules are displayed on their cell surface for appropriate T cell recognition (190). MHC molecules are highly polymorphic, which allows them to present diverse antigens to T cells (190). Since the RBC lacks MHC, the RBC cannot initiate direct T cell-mediated immune responses.

CD8 (+) T cells

The invasion of hepatocytes by *Plasmodium* sporozoites is a prerequisite to establish infection. But the molecular mechanism underlying sporozoite invasion remains largely unknown (191) . However, CD81, a protein of the tetraspanin superfamily (also a known receptor for hepatitis C virus) which is a hepatocyte surface protein has been shown to be required for *Plasmodium* sporozoite invasion into the hepatocytes (192). CD8 (+) cytotoxic T lymphocytes (CTL) have been shown to be important for protecting and eliminating *P. falciparum* that invades and replicates within the hepatocytes (193). Considerable data suggests a protective role for CTL against pre-erythrocytic stage of *P. falciparum* parasites (194)(195). So far, several epitopes of *P. falciparum* antigens that CTL can recognize were identified: circumsporozoite protein

(CSP), thrombosponding related adhesion protein, liver-stage antigen 1 (LSA-1), Pfs16, and sporozoite threonine and asparagine-rich protein (196). Individuals immunized with irradiated *sporozoites* or naturally exposed individuals were also shown to generate CTLs against liver-stage parasites (195).

CD4 (+) T cells

CD4 T cells are categorized into two major subsets depending on their pattern of the cytokine production: T helper 1 cells (Th1) and T helper 2 (Th2). It is thought that Th1 cells produce IL-2, IFN-γ and tumor necrosis factor (TNF), whereas Th2 cells secrete IL-4, IL-5, IL-6 and IL-10 (197). Generally, Th1 cells are responsible for aiding in cell-mediated immunity such as activating a macrophage that has already ingested an antigen, whereas the Th2 cells aid in regulating humoral immune response such as helping B cells produce antibodies, especially IgG1, IgA, and IgE (198). Malaria-specific CD4 T cells are activated when a malaria antigen is recognized in the context of MHC molecules on antigen presenting cells such as dendritic cells (199) and macrophages (200).

The precise role of CD4 T cells in providing immunity against *P. falciparum* is not well understood (201). Although both Th1 and Th2 cells may provide protection to *P. falciparum* at different time/stages of the infection (201), a study done in 15 Gabonese patients has shown that there was a shift from a Th2 cell response (decrease of frequency of IL-4- and IL-13 producing CD4 (+) T cells) to a more pronounced Th1 cell-driven immune responses; frequency of T cells producing IFN- γ and IL-2 cytokines were not significantly altered (202). Even though the Gabonese patients were given

antimalarial drugs, the study suggests that the shift to a Th1 immune responses during acute malaria infection has been associated with parasite clearance. A study has shown that children that were infected with *P. falciparum* of milder malaria had higher levels of IL-12 in their plasma (Th1 function), compared to children who suffered severe malaria, and also the high levels of IL-12 was associated with lower parasitemia levels (203).

The sequential accumulation of antibodies against PfEMP1 variants, which contributes to immunity against *P. falciparum* requires specific CD4 T cell help. It is also thought that exposure to *P. falciparum* induces CD4 T cell responses to the conserved and variant regions of PfEMP1 antigen (204). It has been shown that the magnitude of the CD4 T cells to peptide pools representing variant PfEMP1 domains was significantly higher in the malaria-exposed donors (from Benin) when compared to the UK malaria-unexposed individuals (204). A similar study has shown CD4 T cell responses to different domains of PfEMP1 such as DBL-α and CIDR-α domain, and those responses were significantly higher in malaria exposed donors compared to European donors (205). In addition, another study postulated the role of CD4 T cell response against DBL-α domain to be protective for future *P. falciparum* malarial episodes (206). These findings imply that effective responses to PfEMP1 variants are gained over a period of years, and it is unclear how one can gain a full repertoire of memory CD4 T cells recognizing PfEMP1s.

T regulatory cells (Tregs)

Tregs are key regulators of immune responses, controlling the initial immune cell activation, proliferation, differentiation, and effector functions of other cells (207).

However, the mechanism by which Tregs regulate the cellular immune responses is not well understood (207). Tregs have been shown to suppress both protective and pathological adaptive immune responses during *P. falciparum* malarial infection (208). Consequences from the elevated Tregs during malarial infection remain undetermined, but several studies have indicated it depends on the stage of infection (208)(209). A study on malaria-naïve volunteers bitten by *P. falciparum* sporozoite-infected mosquitoes, found the up-regulation of FOXP3 gene (a transcription factor for Treg development and function) and induction of T cells with regulatory function during the blood stage infection (210).

Additional work needs to be done on the specific direct interaction of PfEMP1 antigens with Tregs (211). One study found that *P. falciparum* can induce Foxp3hi CD4 T cells independently from the PfEMP1s expressed on the RBC surface, and that soluble parasite components (of about the size of 20 nm) are responsible for the induction of Tregs (211).

Vaccine studies on PfEMP1

The blood stage of P. falciparum is a good target for malaria vaccine, since all the symptoms of malaria occur during this stage. Specifically, there has been a major effort in the past few years to design a PfEMP1-based vaccine for *P. falciparum* malaria (212). Studies on animal models have shown that PfEMP1 recombinants can stimulate strong immune responses. For instance, *Aotus* monkeys that were immunized with the PfEMP1 CIDRα domain were protected against the lethal challenge of homologous *P. falciparum* strains, but not heterogeneous parasite strains (213). The monkeys were

susceptible to heterogeneous parasite strains, likely because it is unfeasible to incorporate all PfEMP1 variants in a single vaccine. Also, another study that has used an *in vivo* rat model to study *P. falciparum* PfEMP1-mediated sequestration, demonstrated that rats immunized with NTS-DBLα domains induced protective antibodies that reduced infected RBC sequestration (214). Furthermore, mice immunized with CIDRα domain of PfEMP1 developed antibodies capable of agglutinating *P. falciparum* parasitized erythrocytes using various parasite lines (214). Taken together, all these studies suggest that PfEMP1-based vaccine can illicit antibodies that are protective against *P. falciparum* malaria.

Synthesis and future work

Making the case for PfEMP1 vaccine

Evidence has been presented in this review that *P. falciparum* PfEMP1 antigen interacts with both the human innate and adaptive immune system. The different arms of the immune system that interact with (target) PfEMP1 indicate its importance during *P. falciparum* infection and the need for the immune system to target PfEMP1 to control malarial infection. Elsewhere, it has been noted that the most important antigen that the immune system targets appears to be PfEMP1 in *P. falciparum* infection (215). A key question is why will the immune system spend so much energy to elicit immune responses (innate and adaptive responses) to recognize and target PfEMP1, if its clearance is not related to controlling the infection? or why is the strong immune responses against PfEMP1 are largely ineffective? This provides the basis for the

rationale for why PfEMP1 should be investigated and studied as a vaccine candidate for the blood stages of *P. falciparum*. Even though the immune system does interact with PfEMP1, this does not mean that it can combat the different variants of PfEMP1. As extensively discussed in this review, PfEMP1 challenges the immune system to recognize its antigenicity, suppress immune response initiation against PfEMP1, and evade immune response, to aid *P. falciparum* survival.

PfEMP1 involvement as major player of *P. falciparum* pathogenesis, including cerebral and placental malaria, speaks loudly to the need for PfEMP1-based vaccine. As discussed in the cerebral and placental sections of the review, PfEMP1 mediates attachment of infected RBCs to brain microvascular and placental inter-villous spaces, resulting in the pathogenesis of cerebral and placental malaria, respectively. A PfEMP1 variant, VAR2CSA, is a vaccine candidate to protect against pregnancy-associated malaria. Antibodies generated by immunization with a full length or a single domain of VAR2CSA inhibited the adhesion of infected RBC to CSA (molecule thought to mediate placental sequestration) (216). This finding suggests that PfEMP1 vaccine-induced antibodies have protective roles *in vivo* against controlling placental *P. falciparum* infection. On the other hand, it is not by chance that older children in African populations living in regions where there is stable *P. falciparum* transmission rarely acquire cerebral malaria. Protection against cerebral malaria has also been largely attributed to acquiring a larger pool of antibodies recognizing PfEMP1. One aspect of the pathogenesis of cerebral malaria is infected RBC sequestering in the cerebral microvasculature. This sequestration phenomena is mediated by PfEMP1 DBL-β domain binding to endothelium ICAM-1, as discussed in the cerebral malaria section of this review.

PfEMP1-based vaccine-induced antibodies blocking this interaction could also prevent cerebral infected RBC sequestration. Even if a vaccine for PfEMP1 does not reduce all cases of malaria it could reduce cerebral malaria specifically.

Currently there is no effective vaccine for *P. falciparum* malaria targeting the blood stage of the parasite, the stage that presents the clinical symptoms of malaria. The only licensed vaccine for *P*. f*alciparum* targets the pre-erythrocytic antigen, the circumsporozoite protein (CSP). The RTS,S vaccine is a recombinant protein-based vaccine that stimulates CTL response. It is worth mentioning that RTS,S vaccine efficacy is about 25-35%, protecting against *P. falciparum (215).* One of the arguments against having RTS,S as the only vaccine for malaria is that it only targets liver stage (pre-erythrocytic stage). A single sporozoite that successfully infects a liver cell, a hepatocyte, can lead to the production of ~10,000 infectious merozoites (217) to infect RBC. Having a PfEMP1 vaccine targeting the blood stage in addition to the RTS,S vaccine will equip the immune system of individuals living in malaria-endemic areas to combat *P. falciparum* infections.

Overcoming the antigenic variation PfEMP1

One of the biggest challenges to design a PfEMP1-based vaccine is accommodating for the enormous diversity the PfEMP1 antigens present during *P. falciparum* infections. As discussed in the PfEMP1 section of this review, PfEMP1 antigens possess a high level of sequence diversity and each variant is different in sequences even within a single *P. falciparum* isolate and between different isolates.

How can a PfEMP1 vaccine be designed that can recognize all variants of PfEMP1 during *P. falciparum* infection to generate immune responses?

One approach that can be taken to overcome PfEMP1 antigenic diversity is to design a multivalent vaccine that includes multiple PfEMP1 variants in order to generate a broad repertoire of antibodies (i.e. IgG). The PfEMP1 domains selected should cover the majority of antigenic diversity seen in the PfEMP1 populations. There is precedent in designing multivalent vaccines for pathogens that present antigenic diversity as seen in *Streptococcus pneumonia* and *Neisseria meningitides* (218) which induce immune responses to the most prevalence types seen in a population. As pointed out in the PfEMP1 sections, individuals living in malaria-endemic regions with a broader pool of antibodies that recognize PfEMP1 variants were protected against severe malaria. Also, a recent study finds there is a limited number of PfEMP1 antigens that cause severe malaria (219). As a result, a survey (sequence analysis) can be carried out in the population living in malaria-endemic regions, to determine the most prevalent variants of PfEMP1 in order to come up with a multivalent vaccine. The same way that there is a new vaccine for the influenza virus every year, the same approach can be used to carry out an annual surveillance that can be adapted for *P. falciparum* PfEMP1 antigens.

Another approach that can be employed to overcome antigenic diversity of PfEMP1 is to target the conserved epitopes of PfEMP1 antigens. Targeting conserved regions of PfEMP1 will allow the PfEMP1-based vaccine to induce antibodies that can cross-react with different PfEMP1 variants. This could involve targeting conserved regions of PfEMP1 such as the DBLα domain which has been shown to be involved in rosetting (discussed in PfEMP1 section). PfMP1-based vaccine can elicit memory

antibodies against PfEMP1 DBLα domain and could inhibit PfEMP1-mediated infected RBC rosetting with uninfected RBCs. Immunization-induced antibodies elicited by recombinant PfEMP1 DBLα domain disrupted preformed rosetting and also significant reduction in infected RBC sequestration in DBLα-immunized animal models (220)(221). Even naturally acquired antibodies against PfEMP1 have been shown to cross-react with different PfEMP1, likely mediated by the conserved regions of PfEMP1. A recent study done in the Netherlands, where naïve individuals were challenged with *P. falciparum,* generated cross-reactive antibodies recognizing PfEMP1 variants from different parasite genomes (222). Further research needs to be done to identify conserved PfEMP1 domains that could be used as vaccine targets to overcome the antigenic diversity of PfEMP1 variants.

Conclusion

One of the central obstacles to developing a vaccine for *P. falciparum* malaria is the lack to acquire sterilizing immunity after natural infection. But, recent progress in malaria vaccine development has spurred new hopes that it is realistic to design a PfEMP1-based vaccine for *P. falciparum*. A PfEMP1 vaccine should be able to generate protective immune responses involving both innate and adaptive immunity. The innate immune responses will be important to keep the infection at bay until the adaptive immune system is fully activated. Since the major immunity acquired naturally against PfEMP1 are antibodies (memory IgG), an ideal vaccine should focus on generating such responses. However, the cell-mediated immunity is equally important because the humoral immunity requires the collaboration and aid of immune cells (*i.e.*, T helper

cells). Understanding the mechanism and target of human immunity in fighting against *P. falciparum* is essential to develop a highly effective vaccine for malaria. Tools have to be developed to measure how natural immunity is acquired by exposed individuals living in regions where there is stable transmission of malaria. As discussed in this review, studies are beginning to identify specific variants of PfEMP1 that are linked to severe malarial pathogenesis. However, there is still a major gap in our knowledge in understanding PfEMP1 variants; what variants are common in what population and how each variant may contribute to malaria pathogenesis. Finally, developing better strategies to overcome the antigenic variation of PfEMP1 will provide new opportunities to develop PfEMP1-based vaccine for *P. falciparum* malaria.

References

1. WHO | Water-related Diseases [Internet]. WHO. [cited 2015 Dec 21]. Available from: http://www.who.int/water_sanitation_health/diseases/malaria/en/

2. Akande TM, Musa IO. Review Article: Epidemiology of Malaria in Africa. Afr J Clin Exp Microbiol. 2005;6(2):107–11.

3. WHO | Malaria [Internet]. WHO. [cited 2015 Dec 21]. Available from: http://www.who.int/malaria/en/

4. Pediatric Malaria: Background, Etiology, Epidemiology. 2015 Sep 28 [cited 2016 Jan 3]; Available from: http://emedicine.medscape.com/article/998942-overview

5. Himeidan YE, Elbashir MI, Adam I. Attractiveness of pregnant women to the malaria vector, Anopheles arabiensis, in Sudan. Ann Trop Med Parasitol. 2004 Sep;98(6):631–3.

6. Prevention C-C for DC and. CDC - Malaria - About Malaria [Internet]. [cited 2016 Jan 3]. Available from: http://www.cdc.gov/malaria/about/index.html

7. Cox FE. History of the discovery of the malaria parasites and their vectors. Parasit Vectors. 2010 Feb 1;3:5.

8. Mu J, Duan J, Makova KD, Joy DA, Huynh CQ, Branch OH, et al. Chromosome-wide SNPs reveal an ancient origin for Plasmodium falciparum. Nature. 2002 Jul 18;418(6895):323–6.

9. Liu W, Li Y, Learn GH, Rudicell RS, Robertson JD, Keele BF, et al. Origin of the human malaria parasite Plasmodium falciparum in gorillas. Nature. 2010 Sep 23;467(7314):420–5.

10. Kantele A, Jokiranta TS. Review of Cases With the Emerging Fifth Human Malaria Parasite, Plasmodium knowlesi. Clin Infect Dis. 2011 Jun 1;52(11):1356–62.

11. Kantele A, Jokiranta S. [Plasmodium knowlesi--the fifth species causing human malaria]. Duodecim Lääketieteellinen Aikakauskirja. 2010;126(4):427–34.

12. White NJ. Plasmodium knowlesi: The Fifth Human Malaria Parasite. Clin Infect Dis. 2008 Jan 15;46(2):172–3.

13. Deshmukh AS, Srivastava S, Dhar SK. Plasmodium falciparum: epigenetic control of var gene regulation and disease. Subcell Biochem. 2013;61:659–82.

14. Singh B, Daneshvar C. Human Infections and Detection of Plasmodium knowlesi. Clin Microbiol Rev. 2013 Apr 1;26(2):165–84.

15. Tsy J-MLP, Duchemin J-B, Marrama L, Rabarison P, Goff GL, Rajaonarivelo V, et al. Distribution of the species of the Anopheles gambiae complex and first evidence of Anopheles merus as a malaria vector in Madagascar. Malar J. 2003 Oct 8;2(1):33.

16. Holt RA, Subramanian GM, Halpern A, Sutton GG, Charlab R, Nusskern DR, et al. The genome sequence of the malaria mosquito Anopheles gambiae. Science. 2002 Oct 4;298(5591):129–49.

17. Molina-Cruz A, Canepa GE, Kamath N, Pavlovic NV, Mu J, Ramphul UN, et al. Plasmodium evasion of mosquito immunity and global malaria transmission: The lock-and-key theory. Proc Natl Acad Sci U S A. 2015 Dec 8;112(49):15178–83.

18. Afrane YA, Little TJ, Lawson BW, Githeko AK, Yan G. Deforestation and Vectorial Capacity of Anopheles gambiae Giles Mosquitoes in Malaria Transmission, Kenya. Emerg Infect Dis. 2008 Oct;14(10):1533–8.

19. Deen JL, von Seidlein L, Dondorp A. Therapy of uncomplicated malaria in children: a review of treatment principles, essential drugs and current recommendations. Trop Med Int Health TM IH. 2008 Sep;13(9):1111–30.

20. WHO | Treatment [Internet]. WHO. [cited 2016 Feb 25]. Available from: http://www.who.int/malaria/publications/treatment/en/

21. Prevention C-C for DC and. CDC - Malaria - Diagnosis & Treatment (United States) - Treatment (U.S.) - Guidelines for Clinicians (Part 2) [Internet]. [cited 2016 Feb 25]. Available from: http://www.cdc.gov/malaria/diagnosis_treatment/clinicians2.html

22. Sidhu ABS, Verdier-Pinard D, Fidock DA. Chloroquine resistance in Plasmodium falciparum malaria parasites conferred by pfcrt mutations. Science. 2002 Oct 4;298(5591):210–3.

23. WHO | Guidelines for the treatment of malaria. Third edition [Internet]. WHO. [cited 2016 Feb 24]. Available from: http://www.who.int/malaria/publications/atoz/9789241549127/en/

24. White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA, Dondorp AM. Malaria. Lancet Lond Engl. 2014 Feb 22;383(9918):723–35.

25. White NJ. The treatment of malaria. N Engl J Med. 1996 Sep 12;335(11):800–6.

26. Crawley J, Chu C, Mtove G, Nosten F. Malaria in children. Lancet Lond Engl. 2010 Apr 24;375(9724):1468–81.

27. Mali S, Steele S, Slutsker L, Arguin PM, Centers for Disease Control and Prevention (CDC). Malaria surveillance--United States, 2006. Morb Mortal Wkly Rep Surveill Summ Wash DC 2002. 2008 Jun 20;57(5):24–39.

28. Gomes MF, Faiz MA, Gyapong JO, Warsame M, Agbenyega T, Babiker A, et al. Pre-referral rectal artesunate to prevent death and disability in severe malaria: a placebo-controlled trial. Lancet Lond Engl. 2009 Feb 14;373(9663):557–66.

29. Crutcher JM, Hoffman SL. Malaria. In: Baron S, editor. Medical Microbiology [Internet]. 4th ed. Galveston (TX): University of Texas Medical Branch at Galveston; 1996 [cited 2015 Dec 22]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK8584/

30. Plasmodium Falciparum - Malaria [Internet]. [cited 2016 Apr 28]. Available from: http://www.parasitesinhumans.org/plasmodium-falciparum-malaria.html

31. Yamauchi LM, Coppi A, Snounou G, Sinnis P. Plasmodium sporozoites trickle out of the injection site. Cell Microbiol. 2007 May 1;9(5):1215–22.

32. Siciliano G, Alano P. Enlightening the malaria parasite life cycle: bioluminescent Plasmodium in fundamental and applied research. Front Microbiol [Internet]. 2015 May 11 [cited 2015 Dec 22];6. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4426725/

33. Lover AA. Epidemiology of Latency and Relapse in Plasmodium vivax Malaria. ArXiv150502072 Q-Bio Stat [Internet]. 2015 May 8 [cited 2016 Jan 4]; Available from: http://arxiv.org/abs/1505.02072

34. Imwong M, Snounou G, Pukrittayakamee S, Tanomsing N, Kim JR, Nandy A, et al. Relapses of Plasmodium vivax Infection Usually Result from Activation of Heterologous Hypnozoites. J Infect Dis. 2007 Apr 1;195(7):927–33.

35. Information NC for B, Pike USNL of M 8600 R, MD B, Usa 20894. TREATMENT OF UNCOMPLICATED MALARIA CAUSED BY P. VIVAX, P. OVALE, P. MALARIAE OR P. KNOWLESI. 2015 [cited 2016 Jan 10]; Available from: http://www.ncbi.nlm.nih.gov/books/NBK294428/

36. Mbengue A, Yam XY, Braun-Breton C. Human erythrocyte remodelling during Plasmodium falciparum malaria parasite growth and egress. Br J Haematol. 2012 Apr;157(2):171–9.

37. Maier AG, Cooke BM, Cowman AF, Tilley L. Malaria parasite proteins that remodel the host erythrocyte. Nat Rev Microbiol. 2009 May;7(5):341+.

38. Heiber A, Kruse F, Pick C, Grüring C, Flemming S, Oberli A, et al. Identification of New PNEPs Indicates a Substantial Non-PEXEL Exportome and Underpins Common Features in Plasmodium falciparum Protein Export. PLoS Pathog. 2013 Aug 8;9(8):e1003546.

39. Horrocks P, Pinches RA, Chakravorty SJ, Papakrivos J, Christodoulou Z, Kyes SA, et al. PfEMP1 expression is reduced on the surface of knobless Plasmodium falciparum infected erythrocytes. J Cell Sci. 2005 Jun 1;118(Pt 11):2507–18.

40. Desai SA, Miller LH. Malaria: Protein-export pathway illuminated. Nature. 2014 Jul 31;511(7511):541–2.

41. Fried M, Wendler JP, Mutabingwa TK, Duffy PE. Mass spectrometric analysis of Plasmodium falciparum erythrocyte membrane protein-1 variants expressed by placental malaria parasites. Proteomics. 2004 Apr;4(4):1086–93.

42. White NJ. Antimalarial drug resistance. J Clin Invest. 2004 Apr 15;113(8):1084–92.

43. Claessens A, Hamilton WL, Kekre M, Otto TD, Faizullabhoy A, Rayner JC, et al. Generation of Antigenic Diversity in Plasmodium falciparum by Structured Rearrangement of Var Genes During Mitosis. PLoS Genet. 2014 Dec 18;10(12):e1004812.

44. Rottmann M, Lavstsen T, Mugasa JP, Kaestli M, Jensen ATR, Müller D, et al. Differential Expression of var Gene Groups Is Associated with Morbidity Caused by Plasmodium falciparum Infection in Tanzanian Children. Infect Immun. 2006 Jul;74(7):3904–11.

45. Rask TS, Hansen DA, Theander TG, Gorm Pedersen A, Lavstsen T. Plasmodium falciparum Erythrocyte Membrane Protein 1 Diversity in Seven Genomes – Divide and Conquer. PLoS Comput Biol. 2010 Sep 16;6(9):e1000933.

46. Smith JD, Rowe JA, Higgins MK, Lavstsen T. Malaria's Deadly Grip: Cytoadhesion of Plasmodium falciparum Infected Erythrocytes. Cell Microbiol [Internet]. 2013 Dec [cited 2015 Dec 25];15(12). Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3836831/

47. Fried M, Wendler JP, Mutabingwa TK, Duffy PE. Mass spectrometric analysis of Plasmodium falciparum erythrocyte membrane protein-1 variants expressed by placental malaria parasites. Proteomics. 2004 Apr;4(4):1086–93.

48. Kyes SA, Kraemer SM, Smith JD. Antigenic Variation in Plasmodium falciparum: Gene Organization and Regulation of the var Multigene Family. Eukaryot Cell. 2007 Sep 1;6(9):1511–20.

49. Magowan C, Wollish W, Anderson L, Leech J. Cytoadherence by Plasmodium falciparum-infected erythrocytes is correlated with the expression of a family of variable proteins on infected erythrocytes. J Exp Med. 1988 Oct 1;168(4):1307–20.

50. Smith JD, Subramanian G, Gamain B, Baruch DI, Miller LH. Classification of adhesive domains in the Plasmodium falciparum erythrocyte membrane protein 1 family. Mol Biochem Parasitol. 2000 Oct;110(2):293–310.

51. Dzierzak E, Philipsen S. Erythropoiesis: Development and Differentiation. Cold Spring Harb Perspect Med. 2013 Apr 1;3(4):a011601.

52. An X, Mohandas N. Disorders of red cell membrane. Br J Haematol. 2008 May;141(3):367–75.

53. An X, Guo X, Zhang X, Baines AJ, Debnath G, Moyo D, et al. Conformational Stabilities of the Structural Repeats of Erythroid Spectrin and Their Functional Implications. J Biol Chem. 2006 Apr 14;281(15):10527–32.

54. An X, Lecomte MC, Chasis JA, Mohandas N, Gratzer W. Shear-Response of the Spectrin Dimer-Tetramer Equilibrium in the Red Blood Cell Membrane. J Biol Chem. 2002 Aug 30;277(35):31796–800.

55. Cooke BM, Mohandas N, Coppel RL. The malaria-infected red blood cell: structural and functional changes. Adv Parasitol. 2001;50:1–86.

56. Trossaert M, Dieye A, Dieye Y, Sarthou JL. [Cytoadherence of Plasmodium falciparum and complications of malaria]. Dakar Méd. 1991;36(2):192–7.

57. Rowe JA, Claessens A, Corrigan RA, Arman M. Adhesion of Plasmodium falciparum-infected erythrocytes to human cells: molecular mechanisms and therapeutic implications. Expert Rev Mol Med [Internet]. 2009 May [cited 2015 Dec 28];11. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2878476/

58. Barnwell JW, Asch AS, Nachman RL, Yamaya M, Aikawa M, Ingravallo P. A human 88-kD membrane glycoprotein (CD36) functions in vitro as a receptor for a cytoadherence ligand on Plasmodium falciparum-infected erythrocytes. J Clin Invest. 1989 Sep;84(3):765–72.

59. Coppel RL, Cooke BM, Magowan C, Narla M. Malaria and the erythrocyte. Curr Opin Hematol. 1998 Mar;5(2):132–8.

60. Berendt AR, McDowall A, Craig AG, Bates PA, Sternberg MJ, Marsh K, et al. The binding site on ICAM-1 for Plasmodium falciparum-infected erythrocytes overlaps, but is distinct from, the LFA-1-binding site. Cell. 1992 Jan 10;68(1):71–81.

61. Identification of a strain-specific malarial antigen exposed on the surface of Plasmodium falciparum-infected erythrocytes. J Exp Med. 1984 Jun 1;159(6):1567–75.

62. Raventos-Suarez C, Kaul DK, Macaluso F, Nagel RL. Membrane knobs are required for the microcirculatory obstruction induced by Plasmodium falciparum-infected erythrocytes. Proc Natl Acad Sci U S A. 1985 Jun;82(11):3829–33.

63. Maier AG, Rug M, O'Neill MT, Brown M, Chakravorty S, Szestak T, et al. Exported Proteins Required for Virulence and Rigidity of Plasmodium falciparum-Infected Human Erythrocytes. Cell. 2008 Jul 11;134(1):48–61.

64. Kilejian A. Characterization of a protein correlated with the production of knob-like protrusions on membranes of erythrocytes infected with Plasmodium falciparum. Proc Natl Acad Sci U S A. 1979 Sep;76(9):4650–3.

65. Crabb BS, Cooke BM, Reeder JC, Waller RF, Caruana SR, Davern KM, et al. Targeted gene disruption shows that knobs enable malaria-infected red cells to cytoadhere under physiological shear stress. Cell. 1997 Apr 18;89(2):287–96.

66. Pei X, An X, Guo X, Tarnawski M, Coppel R, Mohandas N. Structural and Functional Studies of Interaction between Plasmodium falciparum Knob-associated Histidine-rich Protein (KAHRP) and Erythrocyte Spectrin. J Biol Chem. 2005 Sep 2;280(35):31166–71.

67. Hayward RE, Tiwari B, Piper KP, Baruch DI, Day KP. Virulence and transmission success of the malarial parasite Plasmodium falciparum. Proc Natl Acad Sci. 1999 Apr 13;96(8):4563–8.

68. Florens L, Washburn MP, Raine JD, Anthony RM, Grainger M, Haynes JD, et al. A proteomic view of the Plasmodium falciparum life cycle. Nature. 2002 Oct 3;419(6906):520–6.

69. Scherf A, Lopez-Rubio JJ, Riviere L. Antigenic variation in Plasmodium falciparum. Annu Rev Microbiol. 2008;62:445–70.

70. Biggs BA, Goozé L, Wycherley K, Wollish W, Southwell B, Leech JH, et al. Antigenic variation in Plasmodium falciparum. Proc Natl Acad Sci U S A. 1991 Oct 15;88(20):9171–4.

71. Roberts DJ, Craig AG, Berendt AR, Pinches R, Nash G, Marsh K, et al. Rapid switching to multiple antigenic and adhesive phenotypes in malaria. Nature. 1992 Jun 25;357(6380):689–92.

72. Freitas-Junior LH, Bottius E, Pirrit LA, Deitsch KW, Scheidig C, Guinet F, et al. Frequent ectopic recombination of virulence factor genes in telomeric chromosome clusters of P. falciparum. Nature. 2000 Oct 26;407(6807):1018–22.

73. Biggs BA, Anders RF, Dillon HE, Davern KM, Martin M, Petersen C, et al. Adherence of infected erythrocytes to venular endothelium selects for antigenic variants of Plasmodium falciparum. J Immunol Baltim Md 1950. 1992 Sep 15;149(6):2047–54.

74. Biggs BA, Goozé L, Wycherley K, Wollish W, Southwell B, Leech JH, et al. Antigenic variation in Plasmodium falciparum. Proc Natl Acad Sci U S A. 1991 Oct 15;88(20):9171–4.

75. Okamoto I, Otte AP, Allis CD, Reinberg D, Heard E. Epigenetic dynamics of imprinted X inactivation during early mouse development. Science. 2004 Jan 30;303(5658):644–9.

76. Borst P, Ulbert S. Control of VSG gene expression sites. Mol Biochem Parasitol. 2001 Apr 25;114(1):17–27.

77. Dzikowski R, Frank M, Deitsch K. Mutually Exclusive Expression of Virulence Genes by Malaria Parasites Is Regulated Independently of Antigen Production. PLoS Pathog [Internet]. 2006 Mar [cited 2016 Jan 2];2(3). Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1386720/

78. Gannoun-Zaki L, Jost A, Mu J, Deitsch KW, Wellems TE. A silenced Plasmodium falciparum var promoter can be activated in vivo through spontaneous deletion of a silencing element in the intron. Eukaryot Cell. 2005 Feb;4(2):490–2.

79. Merrick CJ, Duraisingh MT. Heterochromatin-mediated control of virulence gene expression. Mol Microbiol. 2006 Nov;62(3):612–20.

80. Baruch DI, Gormely JA, Ma C, Howard RJ, Pasloske BL. Plasmodium falciparum erythrocyte membrane protein 1 is a parasitized erythrocyte receptor for adherence to CD36, thrombospondin, and intercellular adhesion molecule 1. Proc Natl Acad Sci U S A. 1996 Apr 16;93(8):3497–502.

81. David PH, Hommel M, Miller LH, Udeinya IJ, Oligino LD. Parasite sequestration in Plasmodium falciparum malaria: spleen and antibody modulation of cytoadherence of infected erythrocytes. Proc Natl Acad Sci U S A. 1983 Aug;80(16):5075–9.

82. Chen Q, Barragan A, Fernandez V, Sundström A, Schlichtherle M, Sahlén A, et al. Identification of Plasmodium falciparum Erythrocyte Membrane Protein 1 (PfEMP1) as the Rosetting Ligand of the Malaria Parasite P. falciparum. J Exp Med. 1998 Jan 5;187(1):15–23.

83. Miller LH, Good MF, Milon G. Malaria pathogenesis. Science. 1994 Jun 24;264(5167):1878–83.

84. Tembo DL, Nyoni B, Murikoli RV, Mukaka M, Milner DA, Berriman M, et al. Differential PfEMP1 Expression Is Associated with Cerebral Malaria Pathology. PLoS Pathog [Internet]. 2014 Dec 4 [cited 2016 Jan 3];10(12). Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4256257/

85. Chaiyaroj SC, Angkasekwinai P, Buranakiti A, Looareesuwan S, Rogerson SJ, Brown GV. Cytoadherence characteristics of Plasmodium falciparum isolates from Thailand: evidence for chondroitin sulfate a as a cytoadherence receptor. Am J Trop Med Hyg. 1996 Jul;55(1):76–80.

86. Robertson SA, Seamark RF, Guilbert LJ, Wegmann TG. The role of cytokines in gestation. Crit Rev Immunol. 1994;14(3-4):239–92.

87. Fried M, Duffy PE. Adherence of Plasmodium falciparum to chondroitin sulfate A in the human placenta. Science. 1996 Jun 7;272(5267):1502–4.

88. Salanti A, Staalsoe T, Lavstsen T, Jensen ATR, Sowa MPK, Arnot DE, et al. Selective upregulation of a single distinctly structured var gene in chondroitin sulphate A-adhering Plasmodium falciparum involved in pregnancy-associated malaria. Mol Microbiol. 2003 Jul;49(1):179–91.

89. Brabin BJ. An analysis of malaria in pregnancy in Africa. Bull World Health Organ. 1983;61(6):1005–16.

90. McGregor IA. Epidemiology, malaria and pregnancy. Am J Trop Med Hyg. 1984 Jul;33(4):517–25.

91. Janeway CA. Approaching the Asymptote? Evolution and Revolution in Immunology. Cold Spring Harb Symp Quant Biol. 1989 Jan 1;54:1–13.

92. Janeway CA, Medzhitov R. Innate immune recognition. Annu Rev Immunol. 2002;20:197–216.

93. Charles A Janeway J, Travers P, Walport M, Shlomchik MJ. Receptors of the innate immune system. 2001 [cited 2016 Jan 11]; Available from: http://www.ncbi.nlm.nih.gov/books/NBK27129/

94. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Innate Immunity. 2002 [cited 2016 Jan 11]; Available from: http://www.ncbi.nlm.nih.gov/books/NBK26846/

95. Trinchieri G. Biology of natural killer cells. Adv Immunol. 1989;47:187–376.

96. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. Nat Immunol. 2008 May;9(5):503+.

97. Mavoungou E, Luty AJF, Kremsner PG. Natural killer (NK) cell-mediated cytolysis of Plasmodium falciparum-infected human red blood cells in vitro. Eur Cytokine Netw. 2003 Sep;14(3):134–42.

98. Korbel DS, Newman KC, Almeida CR, Davis DM, Riley EM. Heterogeneous Human NK Cell Responses to Plasmodium falciparum-Infected Erythrocytes. J Immunol. 2005 Dec 1;175(11):7466–73.

99. Mavoungou E, Held J, Mewono L, Kremsner PG. A Duffy Binding—Like Domain Is Involved in the NKp30-Mediated Recognition of Plasmodium falciparum—Parasitized Erythrocytes by Natural Killer Cells. J Infect Dis. 2007 May 15;195(10):1521–31.

100. Hansen DS, Bernard NJ, Nie CQ, Schofield L. NK Cells Stimulate Recruitment of CXCR3+ T Cells to the Brain during Plasmodium berghei-Mediated Cerebral Malaria. J Immunol. 2007 May 1;178(9):5779–88.

101. Amani V, Vigário AM, Belnoue E, Marussig M, Fonseca L, Mazier D, et al. Involvement of IFN-gamma receptor-medicated signaling in pathology and anti-malarial immunity induced by Plasmodium berghei infection. Eur J Immunol. 2000 Jun;30(6):1646–55.

102. D'Ombrain MC, Voss TS, Maier AG, Pearce JA, Hansen DS, Cowman AF, et al. Plasmodium falciparum erythrocyte membrane protein-1 specifically suppresses early production of host interferon-gamma. Cell Host Microbe. 2007 Aug 16;2(2):130–8.

103. Charles A Janeway J, Travers P, Walport M, Shlomchik MJ. The complement system and innate immunity. 2001 [cited 2016 Jan 7]; Available from: http://www.ncbi.nlm.nih.gov/books/NBK27100/

104. Phanuphak P, Hanvanich M, Sakulramrung R, Moollaor P, Sitprija V, Phanthumkosol D. Complement changes in falciparum malaria infection. Clin Exp Immunol. 1985 Mar;59(3):571–6.

105. Dulaney AD. Complement activity of human serum with especial reference to malaria. Fed Proc. 1947;6(1):425.

106. Greenwood BM, Brueton MJ. Complement activation in children with acute malaria. Clin Exp Immunol. 1974 Oct;18(2):267–72.

107. Stoute JA, Odindo AO, Owuor BO, Mibei EK, Opollo MO, Waitumbi JN. Loss of Red Blood Cell–Complement Regulatory Proteins and Increased Levels of Circulating Immune Complexes Are Associated with Severe Malarial Anemia. J Infect Dis. 2003 Feb 1;187(3):522–5.

108. Biryukov S, Stoute JA. Complement activation in malaria: friend or foe? Trends Mol Med. 2014 May;20(5):293–301.

109. Thiel S, Holmskov U, Hviid L, Laursen SB, Jensenius JC. The concentration of the C-type lectin, mannan-binding protein, in human plasma increases during an acute phase response. Clin Exp Immunol. 1992 Oct;90(1):31–5.

110. Bellamy R, Ruwende C, McAdam KP, Thursz M, Sumiya M, Summerfield J, et al. Mannose binding protein deficiency is not associated with malaria, hepatitis B carriage nor tuberculosis in Africans. QJM Mon J Assoc Physicians. 1998 Jan;91(1):13–8.

111. Kawamoto Y, Kojima K, Hitsumoto Y, Okada H, Holers VM, Miyama A. The serum resistance of malaria-infected erythrocytes. Immunology. 1997 May;91(1):7–12.

112. Wiesner J, Jomaa H, Wilhelm M, Tony H-P, Kremsner PG, Horrocks P, et al. Host cell factor CD59 restricts complement lysis of Plasmodium falciparum-infected erythrocytes. Eur J Immunol. 1997 Oct 1;27(10):2708–13.

113. Rowe JA, Claessens A, Corrigan RA, Arman M. Adhesion of Plasmodium falciparum-infected erythrocytes to human cells: molecular mechanisms and therapeutic implications. Expert Rev Mol Med [Internet]. 2009 May [cited 2016 Jan 7];11. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2878476/

114. Rowe JA, Moulds JM, Newbold CI, Miller LH. P. falciparum rosetting mediated by a parasite-variant erythrocyte membrane protein and complement-receptor 1. Nature. 1997 Jul 17;388(6639):292–5.

115. Rowe JA, Rogerson SJ, Raza A, Moulds JM, Kazatchkine MD, Marsh K, et al. Mapping of the region of complement receptor (CR) 1 required for Plasmodium falciparum rosetting and demonstration of the importance of CR1 in rosetting in field isolates. J Immunol Baltim Md 1950. 2000 Dec 1;165(11):6341–6.

116. Cockburn IA, Mackinnon MJ, O'Donnell A, Allen SJ, Moulds JM, Baisor M, et al. A human complement receptor 1 polymorphism that reduces Plasmodium falciparum rosetting confers protection against severe malaria. Proc Natl Acad Sci U S A. 2004 Jan 6;101(1):272–7.

117. Silva MT. When two is better than one: macrophages and neutrophils work in concert in innate immunity as complementary and cooperative partners of a myeloid phagocyte system. J Leukoc Biol. 2010 Jan 1;87(1):93–106.

118. Kumaratilake LM, Ferrante A, Robinson BS, Jaeger T, Poulos A. Enhancement of neutrophil-mediated killing of Plasmodium falciparum asexual blood forms by fatty acids: importance of fatty acid structure. Infect Immun. 1997 Oct;65(10):4152–7.

119. Nnalue NA, Friedman MJ. Evidence for a neutrophil-mediated protective response in malaria. Parasite Immunol. 1988 Jan;10(1):47–58.

120. Smalley ME, Abdalla S, Brown J. The distribution of Plasmodium falciparum in the peripheral blood and bone marrow of Gambian children. Trans R Soc Trop Med Hyg. 1981;75(1):103–5.

121. Serghides L, Smith TG, Patel SN, Kain KC. CD36 and malaria: friends or foes? Trends Parasitol. 2003 Oct;19(10):461–9.

122. Silverstein RL, Febbraio M. CD36, a Scavenger Receptor Involved in Immunity, Metabolism, Angiogenesis, and Behavior. Sci Signal. 2009 May 26;2(72):re3.

123. Ren Y, Silverstein RL, Allen J, Savill J. CD36 gene transfer confers capacity for phagocytosis of cells undergoing apoptosis. J Exp Med. 1995 May 1;181(5):1857–62.

124. McGilvray ID, Serghides L, Kapus A, Rotstein OD, Kain KC. Nonopsonic monocyte/macrophage phagocytosis of Plasmodium falciparum-parasitized erythrocytes: a role for CD36 in malarial clearance. Blood. 2000 Nov 1;96(9):3231–40.

125. Ren Y, Ren Y. Peroxisome Proliferator-Activator Receptor : A Link between Macrophage CD36 and Inflammation in Malaria Infection, Peroxisome Proliferator-Activator Receptor : A Link between Macrophage CD36 and Inflammation in Malaria Infection. PPAR Res PPAR Res. 2012 Jan 11;2012, 2012:e640769.

126. Aitman TJ, Cooper LD, Norsworthy PJ, Wahid FN, Gray JK, Curtis BR, et al. Malaria susceptibility and CD36 mutation. Nature. 2000 Jun 29;405(6790):1015–6.

127. Baruch DI, Ma XC, Pasloske B, Howard RJ, Miller LH. CD36 peptides that block cytoadherence define the CD36 binding region for Plasmodium falciparum-infected erythrocytes. Blood. 1999 Sep 15;94(6):2121–7.

128. Patel SN, Serghides L, Smith TG, Febbraio M, Silverstein RL, Kurtz TW, et al. CD36 Mediates the Phagocytosis of Plasmodium falciparum–Infected Erythrocytes by Rodent Macrophages. J Infect Dis. 2004 Jan 15;189(2):204–13.

129. Guermonprez P, Valladeau J, Zitvogel L, Théry C, Amigorena S. Antigen presentation and T cell stimulation by dendritic cells. Annu Rev Immunol. 2002;20:621–67.

130. Steinman RM, Pope M. Exploiting dendritic cells to improve vaccine efficacy. J Clin Invest. 2002 Jun;109(12):1519–26.

131. Charles A Janeway J, Travers P, Walport M, Shlomchik MJ. The production of armed effector T cells. 2001 [cited 2016 Jan 9]; Available from: http://www.ncbi.nlm.nih.gov/books/NBK27118/

132. Charles A Janeway J, Travers P, Walport M, Shlomchik MJ. T Cell-Mediated Immunity. 2001 [cited 2016 Jan 9]; Available from: http://www.ncbi.nlm.nih.gov/books/NBK10762/

133. Théry C, Amigorena S. The cell biology of antigen presentation in dendritic cells. Curr Opin Immunol. 2001 Feb;13(1):45–51.

134. Rescigno M, Borrow P. The Host-Pathogen Interaction. Cell. 2001 Oct 8;106(3):267–70.

135. Guermonprez P, Valladeau J, Zitvogel L, Théry C, Amigorena S. Antigen Presentation and T Cell Stimulation by Dendritic Cells. Annu Rev Immunol. 2002;20(1):621–67.

136. Pouniotis DS, Proudfoot O, Bogdanoska V, Apostolopoulos V, Fifis T, Plebanski M. Dendritic Cells Induce Immunity and Long-Lasting Protection against Blood-Stage Malaria despite an In Vitro Parasite-Induced Maturation Defect. Infect Immun. 2004 Sep;72(9):5331–9.

137. Ocaña-Morgner C, Mota MM, Rodriguez A. Malaria Blood Stage Suppression of Liver Stage Immunity by Dendritic Cells. J Exp Med. 2003 Jan 20;197(2):143–51.

138. Ocaña-Morgner C, Mota MM, Rodriguez A. Malaria Blood Stage Suppression of Liver Stage Immunity by Dendritic Cells. J Exp Med. 2003 Jan 20;197(2):143–51.

139. Corinti S, Albanesi C, la Sala A, Pastore S, Girolomoni G. Regulatory activity of autocrine IL-10 on dendritic cell functions. J Immunol Baltim Md 1950. 2001 Apr 1;166(7):4312–8.

140. Brown A, Turner L, Christoffersen S, Andrews KA, Szestak T, Zhao Y, et al. Molecular Architecture of a Complex between an Adhesion Protein from the Malaria Parasite and Intracellular Adhesion Molecule 1. J Biol Chem. 2013 Feb 22;288(8):5992–6003.

141. Mo M, Lee HC, Kotaka M, Niang M, Gao X, Iyer JK, et al. The C-Terminal Segment of the Cysteine-Rich Interdomain of Plasmodium falciparum Erythrocyte Membrane Protein 1 Determines CD36 Binding and Elicits Antibodies That Inhibit Adhesion of Parasite-Infected Erythrocytes. Infect Immun. 2008 May 1;76(5):1837–47.

142. Urban BC, Ferguson DJP, Pain A, Willcox N, Plebanski M, Austyn JM, et al. Plasmodium falciparum-infected erythrocytes modulate the maturation of dendritic cells. Nature. 1999 Jul 1;400(6739):73–7.

143. Elliott SR, Spurck TP, Dodin JM, Maier AG, Voss TS, Yosaatmadja F, et al. Inhibition of Dendritic Cell Maturation by Malaria Is Dose Dependent and Does Not Require Plasmodium falciparum Erythrocyte Membrane Protein 1. Infect Immun. 2007 Jul 1;75(7):3621–32.

144. Del Portillo HA, Ferrer M, Brugat T, Martin-Jaular L, Langhorne J, Lacerda MVG. The role of the spleen in malaria. Cell Microbiol. 2012 Mar;14(3):343–55.

145. Mebius RE, Kraal G. Structure and function of the spleen. Nat Rev Immunol. 2005 Aug;5(8):606–16.

146. Cadman ET, Abdallah AY, Voisine C, Sponaas A-M, Corran P, Lamb T, et al. Alterations of Splenic Architecture in Malaria Are Induced Independently of Toll-Like Receptors 2, 4, and 9 or MyD88 and May Affect Antibody Affinity. Infect Immun. 2008 Sep 1;76(9):3924–31.

147. Mebius RE, Kraal G. Structure and function of the spleen. Nat Rev Immunol. 2005 Aug;5(8):606–16.

148. Cesta MF. Normal Structure, Function, and Histology of the Spleen. Toxicol Pathol. 2006 Aug 1;34(5):455–65.

149. Demar M, Legrand E, Hommel D, Esterre P, Carme B. Plasmodium falciparum malaria in splenectomized patients: two case reports in French Guiana and a literature review. Am J Trop Med Hyg. 2004 Sep;71(3):290–3.

150. Engwerda CR, Beattie L, Amante FH. The importance of the spleen in malaria. Trends Parasitol. 2005 Feb;21(2):75–80.

151. Bachmann A, Esser C, Petter M, Predehl S, von Kalckreuth V, Schmiedel S, et al. Absence of Erythrocyte Sequestration and Lack of Multicopy Gene Family Expression in Plasmodium falciparum from a Splenectomized Malaria Patient. PLoS ONE. 2009 Oct 14;4(10):e7459.

152. Snow RW, Omumbo JA, Lowe B, Molyneux CS, Obiero JO, Palmer A, et al. Relation between severe malaria morbidity in children and level of Plasmodium falciparum transmission in Africa. Lancet Lond Engl. 1997 Jun 7;349(9066):1650–4.

153. Looareesuwan S, Ho M, Wattanagoon Y, White NJ, Warrell DA, Bunnag D, et al. Dynamic alteration in splenic function during acute falciparum malaria. N Engl J Med. 1987 Sep 10;317(11):675–9.

154. Ho M, White NJ, Looareesuwan S, Wattanagoon Y, Lee SH, Walport MJ, et al. Splenic Fc receptor function in host defense and anemia in acute Plasmodium falciparum malaria. J Infect Dis. 1990 Mar;161(3):555–61.

155. Schnitzer B, Sodeman T, Mead ML, Contacos PG. Pitting function of the spleen in malaria: ultrastructural observations. Science. 1972 Jul 14;177(4044):175–7.

156. Anyona SB, Schrier SL, Gichuki CW, Waitumbi JN. Pitting of malaria parasites and spherocyte formation. Malar J. 2006;5:64.

157. Bull PC, Lowe BS, Kortok M, Molyneux CS, Newbold CI, Marsh K. Parasite antigens on the infected red cell surface are targets for naturally acquired immunity to malaria. Nat Med. 1998 Mar;4(3):358–60.

158. Kinyanjui SM, Bull P, Newbold CI, Marsh K. Kinetics of Antibody Responses to Plasmodium falciparum–Infected Erythrocyte Variant Surface Antigens. J Infect Dis. 2003 Feb 15;187(4):667–74.

159. Krause DR, Gatton ML, Frankland S, Eisen DP, Good MF, Tilley L, et al. Characterization of the Antibody Response against Plasmodium falciparum Erythrocyte Membrane Protein 1 in Human Volunteers. Infect Immun. 2007 Dec 1;75(12):5967–73.

160. Krause DR, Gatton ML, Frankland S, Eisen DP, Good MF, Tilley L, et al. Characterization of the Antibody Response against Plasmodium falciparum Erythrocyte Membrane Protein 1 in Human Volunteers. Infect Immun. 2007 Dec 1;75(12):5967–73.

161. Bull PC, Lowe BS, Kortok M, Molyneux CS, Newbold CI, Marsh K. Parasite antigens on the infected red cell surface are targets for naturally acquired immunity to malaria. Nat Med. 1998 Mar;4(3):358–60.

162. Dodoo D, Staalsoe T, Giha H, Kurtzhals JAL, Akanmori BD, Koram K, et al. Antibodies to Variant Antigens on the Surfaces of Infected Erythrocytes Are Associated with Protection from Malaria in Ghanaian Children. Infect Immun. 2001 Jun;69(6):3713–8.

163. Avril M, Cartwright MM, Hathaway MJ, Smith JD. Induction of strain-transcendent antibodies to placental-type isolates with VAR2CSA DBL3 or DBL5 recombinant proteins. Malar J. 2011 Feb 11;10(1):36.

164. Giha HA, Staalsoe T, Dodoo D, Elhassan IM, Roper C, Satti GMH, et al. Nine-Year Longitudinal Study of Antibodies to Variant Antigens on the Surface of Plasmodium falciparum-Infected Erythrocytes. Infect Immun. 1999 Aug;67(8):4092–8.

165. Chan J-A, Howell KB, Reiling L, Ataide R, Mackintosh CL, Fowkes FJI, et al. Targets of antibodies against Plasmodium falciparum–infected erythrocytes in malaria immunity. J Clin Invest. 2012 Sep 4;122(9):3227–38.

166. Angeletti D, Albrecht L, Wahlgren M, Moll K. Analysis of antibody induction upon immunization with distinct NTS-DBL1α-domains of PfEMP1 from rosetting Plasmodium falciparum parasites. Malar J. 2013 Jan 24;12:32.

167. Angeletti D, Albrecht L, Blomqvist K, Quintana M del P, Akhter T, Bächle SM, et al. Plasmodium falciparum Rosetting Epitopes Converge in the SD3-Loop of PfEMP1-DBL1α. PLoS ONE [Internet]. 2012 Dec 5 [cited 2016 Jan 17];7(12). Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3515580/

168. Vogt AM, Barragan A, Chen Q, Kironde F, Spillmann D, Wahlgren M. Heparan sulfate on endothelial cells mediates the binding ofPlasmodium falciparum–infected erythrocytes via the DBL1α domain of PfEMP1. Blood. 2003 Mar 15;101(6):2405–11.

169. Chen Q, Heddini A, Barragan A, Fernandez V, Pearce SFA, Wahlgren M. The Semiconserved Head Structure of Plasmodium falciparum Erythrocyte Membrane Protein 1 Mediates Binding to Multiple Independent Host Receptors. J Exp Med. 2000 Jul 3;192(1):1–10.

170. Baruch DI, Pasloske BL, Singh HB, Bi X, Ma XC, Feldman M, et al. Cloning the P. falciparum gene encoding PfEMP1, a malarial variant antigen and adherence receptor on the surface of parasitized human erythrocytes. Cell. 1995 Jul 14;82(1):77–87.

171. Plasmodium falciparum erythrocyte rosetting is mediated by promiscuous lectin-like interactions. J Exp Med. 1992 Nov 1;176(5):1311–7.

172. Vogt AM, Pettersson F, Moll K, Jonsson C, Normark J, Ribacke U, et al. Release of Sequestered Malaria Parasites upon Injection of a Glycosaminoglycan. PLoS Pathog [Internet]. 2006 Sep [cited 2016 Jan 17];2(9). Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1579244/

173. Scholzen A, Sauerwein RW. How malaria modulates memory: activation and dysregulation of B cells in Plasmodium infection. Trends Parasitol. 2013 May;29(5):252–62.

174. Tangye SG, Tarlinton DM. Memory B cells: effectors of long-lived immune responses. Eur J Immunol. 2009 Aug;39(8):2065–75.

175. Nogaro SI, Hafalla JC, Walther B, Remarque EJ, Tetteh KKA, Conway DJ, et al. The breadth, but not the magnitude, of circulating memory B cell responses to P. falciparum increases with age/exposure in an area of low transmission. PloS One. 2011;6(10):e25582.

176. Dorfman JR, Bejon P, Ndungu FM, Langhorne J, Kortok MM, Lowe BS, et al. B cell memory to 3 Plasmodium falciparum blood-stage antigens in a malaria-endemic area. J Infect Dis. 2005 May 15;191(10):1623–30.

177. Langhorne J, Ndungu FM, Sponaas A-M, Marsh K. Immunity to malaria: more questions than answers. Nat Immunol. 2008 Jul;9(7):725–32.

178. Wipasa J, Suphavilai C, Okell LC, Cook J, Corran PH, Thaikla K, et al. Long-lived antibody and B Cell memory responses to the human malaria parasites, Plasmodium falciparum and Plasmodium vivax. PLoS Pathog. 2010 Feb;6(2):e1000770.

179. Donati D, Mok B, Chêne A, Xu H, Thangarajh M, Glas R, et al. Increased B cell survival and preferential activation of the memory compartment by a malaria polyclonal B cell activator. J Immunol Baltim Md 1950. 2006 Sep 1;177(5):3035–44.

180. Donati D, Zhang LP, Chen Q, Chêne A, Flick K, Nyström M, et al. Identification of a Polyclonal B-Cell Activator in Plasmodium falciparum. Infect Immun. 2004 Sep;72(9):5412–8.

181. Simone O, Bejarano MT, Pierce SK, Antonaci S, Wahlgren M, Troye-Blomberg M, et al. TLRs innate immunereceptors and Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) CIDR1α-driven human polyclonal B-cell activation. Acta Trop. 2011 Aug;119(2-3):144–50.

182. Montes CL, Acosta-Rodríguez EV, Merino MC, Bermejo DA, Gruppi A. Polyclonal B cell activation in infections: infectious agents' devilry or defense mechanism of the host? J Leukoc Biol. 2007 Nov 1;82(5):1027–32.

183. Zanini GM, De Moura Carvalho LJ, Brahimi K, De Souza-Passos LF, Guimarães SJ, Da Silva Machado E, et al. Sera of patients with systemic lupus erythematosus react with plasmodial antigens and can inhibit the in vitro growth of Plasmodium falciparum. Autoimmunity. 2009 Sep;42(6):545–52.

184. Fesel C, Goulart LF, Neto AS, Coelho A, Fontes CJF, Braga EM, et al. Increased polyclonal immunoglobulin reactivity toward human and bacterial proteins is

associated with clinical protection in human Plasmodium infection. Malar J. 2005 Jan 20;4(1):5.

185. Simone O, Bejarano MT, Pierce SK, Antonaci S, Wahlgren M, Troye-Blomberg M, et al. TLRs innate immunereceptors and Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) CIDR1α-driven human polyclonal B-cell activation. Acta Trop. 2011 Aug;119(2-3):144–50.

186. Charles A Janeway J, Travers P, Walport M, Shlomchik MJ. Generation of lymphocytes in bone marrow and thymus. 2001 [cited 2016 Jan 18]; Available from: http://www.ncbi.nlm.nih.gov/books/NBK27123/

187. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Lymphocytes and the Cellular Basis of Adaptive Immunity. 2002 [cited 2016 Jan 17]; Available from: http://www.ncbi.nlm.nih.gov/books/NBK26921/

188. Charles A Janeway J, Travers P, Walport M, Shlomchik MJ. General properties of armed effector T cells. 2001 [cited 2016 Jan 17]; Available from: http://www.ncbi.nlm.nih.gov/books/NBK27149/

189. Charles A Janeway J, Travers P, Walport M, Shlomchik MJ. The production of armed effector T cells. 2001 [cited 2016 Jan 17]; Available from: http://www.ncbi.nlm.nih.gov/books/NBK27118/

190. Charles A Janeway J, Travers P, Walport M, Shlomchik MJ. The major histocompatibility complex and its functions. 2001 [cited 2016 Jan 17]; Available from: http://www.ncbi.nlm.nih.gov/books/NBK27156/

191. Rathore D, Sacci JB, Vega P de la, McCutchan TF. Binding and Invasion of Liver Cells by Plasmodium falciparum Sporozoites ESSENTIAL INVOLVEMENT OF THE AMINO TERMINUS OF CIRCUMSPOROZOITE PROTEIN. J Biol Chem. 2002 Mar 1;277(9):7092–8.

192. Yalaoui S, Zougbédé S, Charrin S, Silvie O, Arduise C, Farhati K, et al. Hepatocyte Permissiveness to Plasmodium Infection Is Conveyed by a Short and Structurally Conserved Region of the CD81 Large Extracellular Domain. PLOS Pathog. 2008 Feb 29;4(2):e1000010.

193. Overstreet MG, Cockburn IA, Zavala F. Protective CD8+ T cells against Plasmodium liver stages: immunobiology of an “unnatural” immune response. Immunol Rev. 2008 Oct;225:272–83.

194. Sedegah M, Sim BK, Mason C, Nutman T, Malik A, Roberts C, et al. Naturally acquired CD8+ cytotoxic T lymphocytes against the Plasmodium falciparum circumsporozoite protein. J Immunol Baltim Md 1950. 1992 Aug 1;149(3):966–71.

195. Doolan DL, Hoffman SL, Southwood S, Wentworth PA, Sidney J, Chesnut RW, et al. Degenerate Cytotoxic T Cell Epitopes from P. falciparum Restricted by Multiple HLA-A and HLA-B Supertype Alleles. Immunity. 1997 Jan 7;7(1):97–112.

196. Aidoo M, Lalvani A, Gilbert SC, Hu JT, Daubersies P, Hurt N, et al. Cytotoxic T-Lymphocyte Epitopes for HLA-B53 and Other HLA Types in the Malaria Vaccine Candidate Liver-Stage Antigen 3. Infect Immun. 2000 Jan;68(1):227–32.

197. Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. Nature. 1996 Oct 31;383(6603):787–93.

198. Janeway CA. The co-receptor function of CD4. Semin Immunol. 1991 May;3(3):153–60.

199. Bruña-Romero O, Rodriguez A. Dendritic cells can initiate protective immune responses against malaria. Infect Immun. 2001 Aug;69(8):5173–6.

200. Chemtai AK, Hamers-Casterman C, Hamers R, De Baetselier P. T cell-mediated immunity in murine malaria. II. Induction of protective immunity to P. chabaudi by antigen-fed macrophages and antigen-educated lymphocytes. Parasite Immunol. 1984 Sep;6(5):469–80.

201. Wipasa J, Elliott S, Xu H, Good MF. Immunity to asexual blood stage malaria and vaccine approaches. Immunol Cell Biol. 2002 Oct;80(5):401–14.

202. Winkler S, Willheim M, Baier K, Schmid D, Aichelburg A, Graninger W, et al. Reciprocal Regulation of Th1- and Th2-Cytokine-Producing T Cells during Clearance of Parasitemia in Plasmodium falciparum Malaria. Infect Immun. 1998 Dec;66(12):6040–4.

203. Luty AJF, Perkins DJ, Lell B, Schmidt-Ott R, Lehman LG, Luckner D, et al. Low Interleukin-12 Activity in Severe Plasmodium falciparum Malaria. Infect Immun. 2000 Jul;68(7):3909–15.

204. Sanni LA, Allsopp CE, Reubsaet L, Sanni A, Newbold C, Chauhan VS, et al. Cellular responses to Plasmodium falciparum erythrocyte membrane protein-1: use of relatively conserved synthetic peptide pools to determine CD4 T cell responses in malaria-exposed individuals in Benin, West Africa. Malar J. 2002 Apr 26;1(1):1–8.

205. Allsopp CEM, Sanni LA, Reubsaet L, Ndungu F, Newbold C, Mwangi T, et al. CD4 T Cell Responses to a Variant Antigen of the Malaria Parasite Plasmodium falciparum, Erythrocyte Membrane Protein-1, in Individuals Living in Malaria-Endemic Areas. J Infect Dis. 2002 Mar 15;185(6):812–9.

206. Gitau EN, Tuju J, Karanja H, Stevenson L, Requena P, Kimani E, et al. CD4+ T Cell Responses to the Plasmodium falciparum Erythrocyte Membrane Protein 1 in Children with Mild Malaria. J Immunol Author Choice. 2014 Feb 15;192(4):1753–61.

207. Sojka DK, Huang Y-H, Fowell DJ. Mechanisms of regulatory T-cell suppression – a diverse arsenal for a moving target. Immunology. 2008 May;124(1):13–22.

208. Scholzen A, Minigo G, Plebanski M. Heroes or villains? T regulatory cells in malaria infection. Trends Parasitol. 2010 Jan;26(1):16–25.

209. Hansen DS, Schofield L. Natural Regulatory T Cells in Malaria: Host or Parasite Allies? PLoS Pathog [Internet]. 2010 Apr [cited 2016 Jan 18];6(4). Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2861684/

210. Walther M, Tongren JE, Andrews L, Korbel D, King E, Fletcher H, et al. Upregulation of TGF-beta, FOXP3, and CD4+CD25+ regulatory T cells correlates with more rapid parasite growth in human malaria infection. Immunity. 2005 Sep;23(3):287–96.

211. Scholzen A, Cooke BM, Plebanski M. Plasmodium falciparum induces Foxp3hi CD4 T cells independent of surface PfEMP1 expression via small soluble parasite components. Front Microbiol [Internet]. 2014 May 1 [cited 2016 Jan 18];5. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4013457/

212. Barry AE, Arnott A. Strategies for Designing and Monitoring Malaria Vaccines Targeting Diverse Antigens. Front Immunol [Internet]. 2014 Jul 28 [cited 2016 Mar 14];5. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4112938/

213. Ghumra A, Khunrae P, Ataide R, Raza A, Rogerson SJ, Higgins MK, et al. Immunisation with Recombinant PfEMP1 Domains Elicits Functional Rosette-Inhibiting and Phagocytosis-Inducing Antibodies to Plasmodium falciparum. PLOS ONE. 2011 Jan 31;6(1):e16414.

214. Ahuja S, Pettersson F, Moll K, Jonsson C, Wahlgren M, Chen Q. Induction of cross-reactive immune responses to NTS-DBL-1α/x of PfEMP1 and in vivo protection on challenge with Plasmodium falciparum. Vaccine. 2006 Aug 28;24(35–36):6140–54.

215. Chan J-A, Fowkes FJI, Beeson JG. Surface antigens of Plasmodium falciparum-infected erythrocytes as immune targets and malaria vaccine candidates. Cell Mol Life Sci. 2014;71(19):3633–57.

216. Tutterrow YL, Salanti A, Avril M, Smith JD, Pagano IS, Ako S, et al. High Avidity Antibodies to Full-Length VAR2CSA Correlate with Absence of Placental Malaria. PLOS ONE. 2012 Jun 26;7(6):e40049.

217. Nelson KE. Infectious Disease Epidemiology: Theory and Practice. Jones & Bartlett Learning; 2005. 772 p.

218. Arora R. Medicinal Plant Biotechnology. CABI; 2010. 388 p.

219. Avril M, Tripathi AK, Brazier AJ, Andisi C, Janes JH, Soma VL, et al. A restricted subset of var genes mediates adherence of Plasmodium falciparum-infected erythrocytes to brain endothelial cells. Proc Natl Acad Sci U S A. 2012 Jun 26;109(26):E1782–90.

220. Moll K, Pettersson F, Vogt AM, Jonsson C, Rasti N, Ahuja S, et al. Generation of Cross-Protective Antibodies against Plasmodium falciparum Sequestration by Immunization with an Erythrocyte Membrane Protein 1-Duffy Binding-Like 1α Domain. Infect Immun. 2007 Jan 1;75(1):211–9.

221. Chen Q, Pettersson F, Vogt AM, Schmidt B, Ahuja S, Liljeström P, et al. Immunization with PfEMP1-DBL1α generates antibodies that disrupt rosettes and protect against the sequestration of Plasmodium falciparum-infected erythrocytes. Vaccine. 2004 Jul 29;22(21–22):2701–12.

222. Turner L, Wang CW, Lavstsen T, Mwakalinga SB, Sauerwein RW, Hermsen CC, et al. Antibodies against PfEMP1, RIFIN, MSP3 and GLURP Are Acquired during Controlled Plasmodium falciparum Malaria Infections in Naïve Volunteers. PLOS ONE. 2011 Dec 12;6(12):e29025.