Malaria Parasites: The Great Escape

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Parasites of the genus Plasmodium have a complex life cycle. They alternate between their final mosquito host and their intermediate hosts. The parasite can be either extra- or intracellular, depending on the stage of development. By modifying their shape, motility, and metabolic requirements, the parasite adapts to the different environments in their different hosts. The parasite has evolved to escape the multiple immune mechanisms in the host that try to block parasite development at the different stages of their development. In this article, we describe the mechanisms reported thus far that allow the Plasmodium parasite to evade innate and adaptive immune responses.

Keywords: malaria, vaccine, escape mechanism, antibodies, T cells, immunosuppression, antigenic polymorphism, antigenic variation

INTRODUCTION

Malaria, a disease caused by Plasmodium parasites and transmitted by Anopheles mosquitoes, remains one of the most deadly diseases. There are six species able to infect humans, namely, Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, and the zoonotic monkey malaria species Plasmodium knowlesi (1) and Plasmodium cynomolgi (2). Around 300 million cases of clinical malaria are recorded every year. Approximately, half a million deaths occur in Africa and are mainly due to P. falciparum infections (3).

Plasmodium parasites have a complex life cycle. It starts when sporozoites are inoculated into the dermis of the mammalian host by infected mosquitoes. Sporozoites are highly motile and a majority of them migrate from the skin to the capillaries for dissemination by the bloodstream (4, 5). They are retained in the liver where they transmigrate through Kupffer cells and hepatocytes before seeding in final hepatocytes (6, 7). Depending on the species of their mammalian hosts, sporozoites mature in 2–14 days. At maturity, budding vesicles called merosomes are released and are ruptured in the lung circulation where the merozoites are released, ready to infect red blood cells (RBC) (8). During the erythrocytic cycle, a fraction of parasites differentiates into male and female gametocytes which can be taken up during the feeding of an Anopheles mosquito. In the mosquito midgut, ookinetes, generated by the gametocyte fusion, cross the mosquito midgut wall and develop into oocysts. Sporozoites released from mature oocyst then migrate to the salivary gland, ready for the next round of infection during the mosquito’s next blood meal.

Malaria is a disease characterized by fever, headache, chills, sweating, and vomiting (9). Unlike viral or bacterial infections, the main indication of clinical malaria is the recurrent fever which varies between species. This is due to the release of parasite toxins into the bloodstream during the erythrocytic cycles of merozoite egress and reinvasion of erythrocytes. As the infection progresses, the number of RBC decreases and this may lead to severe anemia (10). In addition, RBC containing parasites such as P. falciparum can also sequester in deep tissues by cytoadhering to endothelial cells (11). This may cause organ failure, and is partly responsible for cerebral malaria. All these pathologies can eventually lead to death (9, 12, 13).
In the last two decades, the mortality to malaria has decreased substantially (3). This results from the combined efficacy of prevention measures, such as the use of insecticide-impregnated bed nets, the development and use of rapid and easy to use diagnostic tools, and the potent artemisinin-based combinations therapies against the malaria parasites (14). However, this gain of lives might be temporary. In the recent years, all these interventions have shown some limitations. With the advent of decreased efficacy of artemisinin (15, 16), it is now clear that new drugs and other interventions should be developed (9, 17, 18). New drug families, such as spiroindolones (19, 20) and imidalopiperazines (21, 22) compounds, have shown promising results in phase II clinical trials in the recent years and have a great future ahead. However, a vaccine would be the most important tool in the armamentarium against malaria.

While vaccines have been readily developed for many bacterial and viral infections, there are currently no vaccines to protect against human parasites. The need to develop a vaccine to protect against malaria has been highlighted as early as the identification of the parasite in 1897 (23). There have been two schools of thought for the development an antimalarial vaccine. The first is based on the fact that naturally acquired immunity is often observed under field conditions. However, this immunity requires long period of time to develop. It first targets the disease and then the parasite (24). This immunity has been called premonition or relative immunity. It has been defined by Edmond Sergent in 1935 as “a special type of immunity connected with the persistence of living germs in the organs of the immunized host” (25). In other words, immunity is maintained as long as the host immunity is stimulated by the continuous or repeated parasite exposure. Understanding the mechanisms responsible for this premonition will help to develop a vaccine.

The other approach is based on the Jenner principle of vaccination, which was further exemplified by Louis Pasteur. Instead of letting Nature take its course, this approach uses an offensive target as a formulation to induce an immune response in healthy individuals to protect against a subsequent infection. It might not be surprising, that the first attempt was reported by the same Sergent mentioned above, who was working in the Pasteur Institute in Algiers in Algeria. Sergent was able to partially protect birds from Plasmodium lophurae, an avian malaria parasite, using inactivated sporozoites (26). Decades later, in 1946, the first attempt in human was done by Heidelberg et al. using formalin-inactivated P. vivax-infected blood to immunize volunteers, however no protection was induced (27, 28). Jules Freund took it one step further by inventing the Freund adjuvant and combining the adjuvant with formalin-inactivated-blood infected with P. lophurae or P. knowlesi, a monkey malaria parasite. Freund was more successful in protecting ducks or rhesus monkeys, respectively (29, 30). However, because of its toxic side effects, the adjuvant could not be used in humans. Since then, various approaches and technologies have been used for vaccine development against the malaria parasites. Purified parasite preparation has been used as immunogens. Peptides, recombinant proteins, DNA plasmids, bacterial and viral vectors, and genetically modified malaria parasites, in combination with new adjuvants, have also been used as vaccine delivery systems (31). However, despite having more than thousands of pre-clinical trials in rodent and monkey models and more than 200 trials in humans, very few vaccine candidates have shown vaccine efficacy in human. The subunit vaccine, RTS,S, a chimeric molecule based on a large part of the circumsporozoite (CS) protein, the major surface protein of the sporozoite, fused to the S antigen of the hepatitis B virus, together with a strong adjuvant in the formulation, is the only candidate that has moved to Phase III clinical trials. However its efficacy was at best ~50% against infection or clinical disease (32, 33). So far, only whole-parasite based approaches have repeatedly shown high efficacy (34–36).

**MECHANISMS OF IMMUNITY AGAINST THE MALARIA PARASITE**

The parasite has a complex life cycle. Depending on the stage of development in its mammalian host, the parasite can be extracellular or intracellular. The parasite exists in different forms and shapes, possibly expressing different sets of its ~ 5000 gene pool (37) at a particular time. The parasite also has different localization during development, infecting different cell types. Hence, various innate and adaptive immune mechanisms are involved in parasite control and elimination (38, 39). Thus, for any vaccine development, it is important to know the protective immune mechanisms to induce.

**Immunity to the Pre-Erythrocytic Stage**

During the pre-erythrocytic stage, antibodies can (i) inhibit sporozoite motility in the dermis or in the liver (40), (ii) bind to sporozoite and facilitate phagocytosis by monocytes or macrophages in the spleen or the liver (41), (iii) block sporozoite invasion into hepatocytes by preventing the sporozoite ligand to interact with the hepatocyte receptor(s) (42), and (iv) inhibit sporozoite development inside the hepatocytes (42). Antibodies can also recognize parasite neo-antigens such as heat-shock protein expressed at the surface of infected hepatocytes and induce liver parasite killing through an antibody-dependent cell-mediated mechanism likely involving Kupffer cells or NK cells (43). The production of high levels of antibodies is dependent of CD4 T cell help, preferably by recognizing (an) epitope(s) present in the sporozoite to facilitate boosting of the antibody during natural infection with the parasite. When the parasite is inside the hepatocytes, it can become the target of CD4 or CD8 T cells (44–46). Hepatocytes express MHC Class I and Class II molecules that can be loaded with parasite antigen-derived epitopes following the TAP or the endosomal pathways (44–47). T cells kill the parasite either directly or through the action of cytokines, such as IFN-γ. IFN-γ induces the inducible nitric oxide enzyme to produce nitric oxide which directly kills the liver parasites (48–51). Innate immune mechanisms involving type I interferon pathway induced by the parasite infection and active against late schizonts or against reinfection have been recently uncovered (52–54).

**Immunity against the Erythrocytic Stage**

Adaptive immunity against the blood stage is more complex than in the liver stage. Merozoite-specific antibodies can (i) prevent...
merozoites from invading RBC alone (55–57) or, in conjunction with complement factors, (ii) prevent merozoite egress from RBC, (iii) agglutinate released merozoites, (iv) promote phagocytosis of merozoites, and (v) facilitate clearance of infected RBC (iRBC) by phagocytic cells through a mechanism called antibody cell-dependent inhibition (ADCI) (58). In ADCI, anti-merozoite cryophilic (IgG1 or IgG3) antibodies bind to merozoites and the immune complexes stimulate phagocytes to release cytokines such as TNF-α, which in turn stimulate the phagocytes to produce mediators that lead to the killing of intra-erythrocytic parasites (59, 60). *Plasmodium* parasites express antigens at the surface of iRBC (61). These antigens are mainly encoded by multigene families such as the var (62), stever (63), and rifins gene families (64, 65) for *P. falciparum* or the pir gene family for *P. vivax*, *P. knowlesi*, and the rodent malaria species (66). The antigens they encoded have been implicated in the cytoadherence phenotype to endothelial cells in deep tissues in order to avoid splenic clearance (67, 68). They are also involved in other adverse phenomena, such as rosetting (the binding of an iRBC to non-infected RBC) (69–72) and agglutination (the binding to iRBC to iRBC through bridging by platelets) (73, 74). The cytoadherence abilities of the malaria parasites have been proposed to be responsible for some of the pathologies during malaria infection. Antibodies targeting the surface antigens are thought to act by preventing cytoadherence, promoting iRBC phagocytosis, or iRBC agglutination (74, 75).

Antibodies targeting the parasite toxins could also protect from disease. During infection, multiple parasite toxins are released at the time of iRBC rupture. These toxins include the malaria pigment, a by-product of heme degradation by the parasite (76–78), glycosphatidylinositol (GPI) moieties that are present in many merozoite proteins, a TatD-like DNase (79), a tyrosine-tRNA synthase (80), or lipids extracted from *P. vivax* schizonts (81). Protection from disease by anti-toxins antibodies has been demonstrated experimentally using synthetic glycans mimicking GPI (82).

T cells are also critical effectors in the immunity against blood-stage malaria infections, despite the lack of MHC antigens on the surface of iRBC. First and foremost, blood-stage parasite-specific antibodies secreted by B cells depend on CD4+ T helper cells enhancement for optimal production (39). Cytokines released by CD4 T cells are important for multiplication and maturation of B cells. The cytokines produced by malaria-specific T cells influence the isotype of the antibodies produced (83, 84) and thus possibly affecting the type of antibody-mediated responses induced. It has been shown that ADCI against *P. falciparum* is mediated by human IgG3 (induced by a Th1 response) and antagonized by IgG2 and IgG4 (induced by a Th2 response) (59, 85). Inducing the right isotype is thus important for an antibody-based vaccine. Recent studies have shown that inducing an immune response skewed toward the IgG3 could be achieved through the use of the right adjuvant (86). CD8 T cells were once thought to have only a minimal role in blood-stage immunity (87, 88). However, there is now evidence that these cells can inhibit blood-stage infection (89, 90). In particular, IFNγ-secreting CD8 T cells are important for preventing chronic blood-stage infection in mice (91).

### Immunity against the Sexual Stages

The sexual forms of *Plasmodium* parasites, gametocytes, are also targets of the immunity against the disease. They are targeted by antibodies which can induce complement-mediated killing of the gametocytes in the host blood (92, 93). In the mosquito, antibodies can (i) prevent gamete fusion (94), (ii) induce complement-killing of gametes or ookinetes (95), and (iii) prevent ookinete motility, penetration of the midgut wall and formation of oocyst (96–99). Sexual-stage parasite-specific antibodies depend on CD4 T helper cells for optimal production. However, although antibodies specific for gametes or ookinetes can be produced by vaccination, the humoral immune response cannot be boosted by repeated infections since these forms are not present during infection in the mammalian hosts. Monocytes/macrophages stimulated during infection by the parasites produce cytokines such as TNF-α which in turn stimulates the monocytes/macrophages to produce nitric oxide, a potent inhibitor of gametocytes (100).

### MECHANISMS OF IMMUNE EVASION

To avoid being eliminated by the host immunity, the parasite has developed many escape strategies (Table 1).

### EVASION OF THE COMPLEMENT SYSTEM

The complement system is one of the first innate immune defense mechanisms against pathogens. Many proteins are involved in the activation or the regulation of its lytic activity (101). During the blood-stage, the parasite has developed multiple ways of evading the action of complement. *P. falciparum* merozoites and iRBC bind to the factor H (fH), a complement regulator factor, and its alternatively spliced form fH-like protein 1 through its surface molecule Pf92. In the mosquito, gametes bind the fH through PfGAP50 (102). In both situations, this allows protection against the activation of complement-mediated lysis. In addition, ookinetes express Pfes47, which disrupts the c-Jun N-terminal kinase pathway and prevents mosquito midgut epithelial nitration, making the parasite undetectable by complement system (103).

### ANTIBODY-DEPENDENT ENHANCEMENT OF INFECTION

Antibody-dependent enhancement of infection was first described for viruses (104). Similarly, antibody-dependent enhancement of infection has been described for all stages of parasite development in the mammalian host. It was first reported in the early 1990s that antibodies against the repeat region of the CS protein enhance sporozoite entry and development in hepatocytes for both rodent (42, 43) and human parasites (105). Antibodies against the *P. falciparum* asparagine-rich protein enhance merozoite invasion of RBC in vitro (106). Merozoite-specific antibodies in conjunction with complement can also facilitate RBC invasion (107). Antibody-dependent enhancement of infection was also observed in vivo after immunization with a *Plasmodium berghei* parasite blood-stage preparation.
which led to increased death after challenge (108). In the sexual stage, anti-gamete antibodies were shown to enhance transmission to the mosquito (109).

Antibodies against proteins expressed in one stage of the parasite’s development might mediate an enhancement effect in another stage of the parasite’s development. This was observed for antibodies against two antigens, the CS protein and heat-shock protein 1 (HSP-1) (110). The CS protein is expressed during sporogony in the mosquito and in the subsequent sporozoite stage. Do Rosario et al. showed that sporozoites generated during sporogony in the presence of anti-CS protein antibodies cannot be inhibited by human polyclonal anti-CS (sporozoite CS) protein (111). HSP70-1 is expressed by the liver stage and in the blood stages (43, 110). HSP70-1 antibodies have no effect against sporogony in the presence of anti-CS protein antibodies can—
vary in size and number of repetitive units (115). Such diversity can often occur in regions which can be recognized by antibodies or T cells. Mutations in B epitopes abolish the recognition of

**TABLE 1 | Host immunity and parasite immune escape strategies.**

| Host immunity | Parasite stage | Parasite evasion strategy | Outcome |
|---------------|----------------|--------------------------|---------|
| Complement    | AS, S          | Antibody enhancement invasion and/or growth | Expansion of parasite in host |
| Monocytes/macrophages | AS | Antibody enhancement invasion and/or growth | Avoid recognition by antibody |
| Dendritic cells (DC) | AS | Antibody enhancement invasion and/or growth | Poor or limited B cell memory |
| Antibodies/B cells | Spz, AS, S | Antibody enhancement invasion and/or growth | Poor or no antibody response by inducing immunological tolerance |
| T cells | LS | T cell epitope polymorphism | Avoid T cell recognition prevent T cell priming and activity, and interfere with memory T cell development |
| Hepatocytes | LS | Cellular shelter | Avoid immune surveillance due to intracellular niche |

Spz, sporozoite; LS, liver stage; AS, asexual blood stage; S, sexual stage.

ANTIGENIC POLYMORPHISM

Similar to all other organisms, the parasite is prone to mutation since the replicative machinery is not error free. In its mammalian host, the parasite is haploid and the mutation rate is \(10^{-9}\) mutations per base per generation (113). Since the *Plasmodium* parasites have a 24- to 72-h blood cycle, there is a high probability that mutation can occur and generate different parasite clones. In the mosquito host, the parasite undergoes sexual reproduction where two haploid gametocytes will generate four haploid sporozoite progenies. Recombination does occur in the mosquito stage, and, thus, it will increase the occurrence of gene polymorphism. For some antigens, hundreds of haplotypes have been observed (114). Polymorphism in the coding sequence can be due to point mutations, insertions, or deletions. Interestingly, many *Plasmodium* antigens possess regions of repeats which can vary in size and number of repetitive units (115). Such diversity is, in most cases, the result of immune pressure since mutations often occur in regions which can be recognized by antibodies or T cells. Mutations in B epitopes abolish the recognition of
parasites by antibodies and may lead to the selection of parasites with a different haplotype. This is important for vaccine development since antibody-based vaccine formulation targeting polymorphic epitopes may either have limited efficacy or select for vaccine-resistant parasite. One such example is the vaccine based on the AMA-1 protein, a highly polymorphic antigen (116–118). However, it is possible to envisage that immunogen(s) inducing broadly inhibitory antibodies recognizing multiple variants may circumvent polymorphism. Studies to identify structural conserved constraints in different variants may pave the way for new vaccines against polymorphic antigens (56).

Polymorphism in T cell epitopes may have profound consequences in T cell responses and have shown to limit the efficacy of the RTS,S vaccine (119). T cell epitopes are, in general, 8–11 amino acid (aa) and 11−25 aa in length for CD8 T cell and CD4 T cells, respectively (120). After the digestion of parasite protein in the cytoplasm, CD8 antigenic peptides are generated and transported to endoplasmic reticulum by the transporter antigenic peptide protein in the endoplasmic reticulum where they are loaded onto MHC class I molecules and beta 2-microglobulin. CD4 epitopes are generated in endosomes after phagocytosis and protease digestion where they meet MHC class II. Peptide–MHC complexes are then expressed in the surface. Exogenous antigens can also be presented by cross-presentation by MHC class I (121). These complexes can be recognized by the T cell receptor (122). Mutations in the amino acids that anchor the epitope to the MHC groove can prevent binding of the peptides to the MHC or recognition by the TCR and thus abrogate T cell activity (123, 124). This was demonstrated for the Cs protein (125). Altered peptide ligand (APL) can still bind to the MHC molecules; however, they prevent T cell proliferation, no matter if it was used singly or concurrently with a wild-type peptide. They can also prevent cytokine production (126) or change their production pattern, i.e., from IFN-γ to the immunosuppressive cytokine interleukin-10 (127). These APL can also interfere with induction of memory T cells from naïve T cells (128). This potent mechanism of immune escape is a major hurdle for vaccine development. For any T cell-based vaccine to succeed, a thorough analysis of T cell polymorphism and their effect should be performed.

**ANTIGENIC VARIATION**

Antigenic variation was first described by Neil Brown during *P. knowlesi* chronic relapsing infection in Rhesus monkeys induced by sub-curative drug treatment (129). In an agglutination assay, antibodies produced against different relapse parasite broods agglutinated only schizonts of the immunizing brood but not the other broods. It was further shown that antibodies (130) and spleen could induce antigenic variation in Rhesus monkey infected with a cloned line of *P. knowlesi* (67) or monkey infected with cloned lines of *P. falciparum* (131). The latter experiments suggested that, contrary to antigenic polymorphism where parasites have different alleles and can be categorized as genomic clones, antigenic variation is a phenotypic variation occurring with the same genomic clone of the parasite. The molecular basis of this phenomenon was elucidated with the sequencing of large segments of the *P. falciparum* and *P. knowlesi* genomes and the subsequent complete sequencing of the genome of many *Plasmodium* species. Antigens prone to antigenic variation are often expressed on the surface of iRBC. Examples includes the multigene family, such the var genes (62), the stever gene family, the rifin gene family (63), the surfin gene family (132), the sicavar gene family (133), and the *Plasmodium* interspersed repeats (pir) genes. The var gene family is the most studied and has been shown to be the target of protective anti-blood-stage antibodies (134, 135). It is also involved in many of malaria pathologies due to parasite sequestration resulting from iRBC cytoadherence (136). PiEMP1 proteins, encoded by the var gene family, are highly polymorphic and have different variable domains, called Duffy binding-like domain, which determine their binding specificities to various ligands on endothelial cells such as thrombospondin (137), CD36 (138), ICAM-1/CD54 (139), chondroitin sulfate A (140–142), VCAM-1, E-selectin (143), ovf3 integrin (144), hyaluronic acid (145), PECAM-1/CD31 (146), gC1qR/HABP1/p32 (147), or endothelial protein C receptor (148). PiEMP1 proteins bind also to RBC through complement receptor 1 to form rosettes (149). *P. falciparum* parasites possess ~60 var genes distributed over the 14 chromosomes of the parasite (37). Var genes expression is extremely regulated and only one PiEMP1 type is produced and displayed on the surface of iRBC (150). At each cycle, the parasite switches the expression of the var gene at a rate of 2% *in vitro*, generating new clones with new antigenic and adhesive phenotype (151, 152). Vir proteins, members of a superfamily of the pir multigene superfamly, also mediate cytoadherence of *P. vivax* iRBC to endothelial cells (153), resulting to sequestration of mature iRBC (154). There are ~350 vir genes, and they are also highly polymorphic (155, 156). Stevor and Riffin proteins are involved in cytoadherence processes and are also highly polymorphic (70, 71). In the recent years, it has become clear that some members of these multigene family has a particular role and may be involved in certain pathology. As example, var2CSA mediated the specific cytoadherence of iRBC to placenta (142) and is associated with placental pathology, such as still birth and fetus growth retardation. Vaccination using immunogens based on var2CSA will thus induce inhibitory antibodies, preventing cytoadherence (157, 158) and placental sequestration. Thus defining the role of members of these multigenic families may lead to tailored immune intervention.

**ANTIGENIC DIVERSION**

Antigenic diversification occurs when non-inhibitory anti-parasite antibodies prevent the action of inhibitory antibodies. Antigenic diversification has been observed with the merozoite surface protein (MSP)-1. MSP-1 is a surface protein which binds to glycophorin A, a molecule present on the surface of RBC, and thus is essential for merozoite invasion (159, 160). MSP-1 is cleaved at the time of invasion. Neutralizing antibodies, which block the proteolytic cleavage in the C-terminal part (MSP-1α) of the protein (antibodies), can prevent invasion (152, 161). However, antibodies against the adjacent or overlapping region can block the effect of MSP1α-inhibitory antibodies (blocking antibodies) and thus allowing invasion to occur (162). One possibility to overcome this
EPITOPE MASKING

Epitope masking is the capacity of non-parasite-specific antibodies to prevent parasite-specific inhibitory antibodies to react with their epitopes. During the establishment of an antibody response, IgM precedes IgG production and thus are the first line of humoral response either alone or in combination with complement (164). Malaria-specific IgM have been shown to have efficient inhibitory activity against sporozoite and iRBC (165). However, IgM with different specificities can also bind to PfEMP-1 molecules expressed at the surface of iRBC through their Fc portion (Fc) (166). Non-parasite-specific IgM (NpsIgM) promote rosetting and thus may facilitate sequestration, in order to avoid splenic elimination. NpsIgM binding to the PfEMP-1 VARC2A2, which is involved in the binding of IRBC to chondroitin sulfate in the placenta, appears to protect iRBC from phagocytosis mediated by IgG (167). NpsIgM also binds to the MSP DBLMSP and DBLMSP2 and prevents IgG binding to these molecules by masking epitopes (168). The role of these two molecules in merozoite biology is still unclear but non-specific IgM epitope masking may be important for protecting the parasite against a specific IgG inhibitory response.

SMOKE-SCREEN STRATEGY BY CROSS-REACTIVITY

Smoke-screen strategy is often used by the parasite to divert the antibodies specific for one antigen (antigen A, e.g., an epitope of inhibitory antibodies) to react against another antigen (antigen B, e.g., an epitope of non-inhibitory antibodies) which possess regions homologous to antigen A. Antibody reactivity to segments shared by the two proteins will decrease the amount of antibodies reacting to antigen A and thus reducing efficacy of inhibitory antibodies that can inhibit parasite invasion or development. Many *Plasmodium* antigens contain repeats, and it was shown that cross-reactivity can occur between different blocks of repeats (169). Cross-reactivity has been observed: (i) between different epitopes from the same block of repeats [i.e., within the same block of repeats in the CS protein, or Ring-erythrocyte surface (RESA), or S-antigen or falciparum interspersed repeat antigen (FIRA)]; (ii) between epitopes present in different blocks of the same antigen (i.e., RESA or FIRA), between repeats of different antigens [between the CS protein and the cross-reactive antigen (CRA), between the histidine-rich proteins]. So far, the importance of this mechanism in the immune response evasion has remained uncertain.

DYSREGULATION OF B CELLS

Atypical memory B cells are a population of hypo-responsive memory cells which have been first described in chronic HIV infections (170). They increase in numbers during chronic exposure to malaria and are poor responders to antigen restimulation (171). However, they are able to produce neutralizing antibodies (172). These cells might be important during repeated stimulation due to constant exposure and help to control parasite density. However, in regions with lower exposure or in the absence of reinfection, immunity might wane rapidly.

As mentioned in the previous section, many *Plasmodium* antigens contain repeats. Similar to many viral antigens (173) and haptenated polymers (174), repeats-containing *Plasmodium* antigens can stimulate B cell independently of T cell help. However, this often leads to the production of mainly IgM and limited B cell memory (175). In addition, exposure to antigens containing repeated motifs can result in the suppression of an ongoing T cell response (176, 177).

HOMOLOGIES WITH HOST PROTEINS

Many *Plasmodium* antigens involved in the invasion of host cells have regions which strong homologies with host proteins involved in protein–protein interactions. Thrombospondin type-1 repeat (TSR) domains and von Willebrand factor (vWF)-like A domains are present in the CS protein, thrombospondin-related anonymous protein (TRAP) (178, 179), CS protein TRAP-related protein (CTR) sporozoite surface protein (180), secreted protein with an altered thrombospondin (SPATR) (181), and thrombospondin-related apical merozoite protein (TRAMP) (182). These molecules are involved in different stages of parasite development, in sporozoite and merozoite motility, and in invasion of mosquito midguts, salivary glands, hepatocytes, and RBC. Many merozoite proteins such as MSP-1, MSP-4, MSP-5, MSP-8, and MSP-10 (183), PfRipr (184) and sexual-stage protein, such as P25 or P28 (185, 186), have been found to contain an epidermal growth factor domain that is involved in its binding to their receptor. Due to the homology to the host protein, the induction of antibodies to epitopes contained in these homologous regions is difficult since the host is tolerized against its own proteins. Immunization with immunogens containing these motifs with strong adjuvants could possibly escape immunological tolerance but may have the risk of inducing auto-immunity.

IMMUNOSUPPRESSION

Myeloid cells are essential mediators of an efficient immune response against the malaria parasites. Monocytes, macrophages, and neutrophils phagocytose iRBC, which eventually leads to the elimination of the parasites (187). Phagocytosis by monocytes/macrophages can be mediated through the interactions of PfEMP-1 and CD36 without inducing or increasing a protective pro-inflammatory response (188). However, during infection, phagocytose functions can be diminished after the ingestion of the malaria pigment or hemozoin (the digested product of hemoglobin by the parasites) (189, 190). Pigment-loaded macrophages cannot phagocytose more iRBC, and their capacity to generate radical oxygen intermediates is also reduced (191).

Dendritic cells (DCs) are essential to induce adaptive immune responses (192). Engagement of CD36 and CD51 (the αv integrin
of infection. However, as for the merosomes, these vesicles may not express parasite antigens on their surface. Infection with sporozoites from some human/primate parasites, such as Plasmodium falciparum, was thought to use only one pathway for RBC invasion (237), the mechanisms by which the parasite subverts immune recognition is unknown. Vaccine development against the pre-erythrocytic of P. vivax has been limited, and formulations targeting the liver forms may not work against the hypnozoites.

As mentioned above, during primary sporozoite-induced infection in natural hosts, no cellular immune responses are observed (8, 238–240). The liver is rich in macrophages and Kupffer cells, which can phagocytose emerging hepatic merozoites. To avoid being recognized by phagocytes, merozoites are released inside vesicles called merosomes in to the sinusoid lumen. Merosomes do not express macrophage-recognition signal such as phosphatidyl-serine and thus escape being phagocytosed (8). Merosomes are carried to the lung vasculature where blood circulation is low (241) before releasing their merozoite cargo (242).

Vesicles containing merozoites have been observed inside other cell types other than RBC, such as platelets (243), in macrophages (244), and in DCs (245, 246). It has been shown that merozoites can also divide in the DCs expressing CD317/ tetherin, and eventually initiate new infections (245). It is not yet known how the phagocytosed parasites evade digestion or if a population of merozoites can infect and multiply in these cells. Free merozoite-containing vesicles, called merophores, have also been observed in the lymph circulation during rodent malaria infection (247, 248). This could explain recrudescence or latency of infection. However, as for the merosomes, these vesicles may not express parasite antigens on their surface.
CONCLUSION

Malaria parasites have been interacting with its mammalian hosts for more than 150 million years (249) and have efficiently evolved to survive under the pressure of the host immune system. The parasite has developed numerous immune evasion mechanisms. Since new host immune mechanisms against the parasite are constantly being discovered, it will be of no surprise that new immune escape mechanisms by the parasite will be uncovered. For example, there is still little known on how innate immunity are induced during infection and on how protective epitopes are generated. The ultimate goal of understanding the immune responses to the malaria parasites is the development of vaccines. The selection of antigens and delivery system is governed by the target. Historically, before the whole genome of vaccines. The selection of antigens and delivery system is governed by the target. Historically, before the whole genome was sequenced, the first malarial antigens, that were cloned and sequenced, have been assumed to be good vaccine candidates (250). However, all these antigens are immunodominant and are involved in immune escape. For vaccines to develop in a timely manner, the selection criteria should involve a more stringent GO-NO Go selection based on the analysis of the potential of the vaccine candidate to avoid immune escape. This also calls for an immunological approach to define correlates of protection to guide vaccine development. The development of the experimental human malarial challenge model, where complete sterile protection can be obtained (34, 35), is strong evidence that a vaccine against malaria can be obtained. Together with parasite genetics, the development of the rodent models and the experimental human challenge model would greatly assist in making the critical GO-NO Go decisions and facilitate the development of an efficacious vaccine against malaria in the foreseeable future.

AUTHOR CONTRIBUTIONS

Both the authors listed have made substantial, direct, and intellectual contribution to the work and approved it for publication.

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