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Molecular Machinery of Malaria Infection: Insights into Host-parasite Interactions and Therapeutic Targets

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Malaria continues to be a main global health issues, with millions of people affected each year. Understanding the molecular machinery behind malaria infection is crucial for the development of effective therapeutic interventions. This review aims to discuss the lifecycle of the malaria parasite, highlighting the molecular mechanisms of invasion, immune evasion, and sequestration. Furthermore, we delve into the intricate signaling pathways and molecular factors that contribute to malaria-induced immune dysregulation and disease progression. Finally, we explore potential therapeutic targets, including drug resistance mechanisms and novel strategies for intervention. By unraveling the molecular machinery of malaria infection, we hope to provide valuable insights for the development of targeted therapies and the eventual eradication of this devastating disease.

Keywords: Malaria; molecular mechanisms; host-parasite interactions; pathogenesis.

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1. INTRODUCTION

Malaria is a common and debilitating tropical disease caused by Plasmodium species. It is transmitted through the bites of female Anopheles mosquitoes that are infected [1]. The protozoan parasites that cause malaria consist of various species, such as Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, and Plasmodium knowlesi [2,3]. Plasmodium falciparum and Plasmodium vivax pose a serious challenge to global health [4].

In 2020 and 2021, malaria prevalence increased, as reported by the WHO. In 2020, there were 245 million cases and 625,000 deaths. The following year, these numbers rose to 247 million cases and 619,000 deaths in 85 countries where malaria is endemic [5]. According to the 2023 WHO malaria report, the incident also increased, with a total of 249 million reported cases of malaria in 2022. These cases resulted in 608,000 deaths across 85 countries where malaria is endemic. The highest number of cases, specifically 233 million, was reported in the WHO African Region [6]. The report above clearly indicates that malaria remains a major global health issue, causing significant mortality, morbidity, and socioeconomic burden each year.

The World Health Organization (WHO) adopted the Global Technical Strategy for Malaria 2016-2030 [7] during the World Health Assembly in 2015 [8]. The strategy centers on universal access to malaria testing and treatment, accelerating towards elimination when possible, enhancing surveillance efforts, promoting ongoing research and innovation, and making investments in infrastructure and capacity-building. The Global Technical Strategy [7] aims to speed up progress towards eliminating malaria. By 2030, the goal is to reduce malaria cases and deaths globally by at least 90% and achieve elimination in at least 35 countries. According to the latest data, it seems that the initial milestones have not been achieved. By 2020 and 2025, the intermediate targets are to reduce the disease burden globally by at least 40% and 75%, respectively, and achieve elimination in at least 10 and 20 countries.

One of the major obstacles to malaria eradication today is the emergence of species of malaria that are resistant to antimalarial drugs. Currently, there is no completely effective vaccine available for malaria, and the disease is becoming resistant to first-line antimalarial drugs [9]. Throughout history, various treatments have been used for malaria, such as quinine, mepacrine, chloroquine, sulfadoxine-pyrimethamine, and mefloquine. However, one major challenge that all of these treatments have faced is the development of resistance [10]. Artemisinin-based combination therapies (ACTs) are globally recommended as the initial treatment for uncomplicated P. falciparum malaria [11]. Unfortunately, the major concerns are the emergence and spread of artemisinin resistance in P. falciparum, as well as the declining efficacy of ACTs. This issue was first reported in the Greater Mekong sub-region in 2009 and has more recently been observed in Papua New Guinea, Guyana, and sub-Saharan Africa [12-14].

Identifying potent molecular markers for drug resistance is a crucial tool in determining the global emergence and spread of antimalarial drug resistance. The most commonly studied molecular markers for antimalarial drug resistance are the Pfmdr1 gene (P. falciparum multidrug resistance 1), the Pfcrt gene (P. falciparum chloroquine resistance transporter), and kelch13 propeller region (Pfk13) gene single nucleotide polymorphisms (SNPs) [15,16]. The pfmdr1 gene, which is located on chromosome 5, is linked to parasite vulnerability to several antimalarial drugs such as chloroquine, lumefantrine, amodiaquine, meoquine, quinine, and artemisinin [17,18]. The Pfmdr1 gene is associated with multidrug-resistant phenotypes. Specifically, the amino-terminal mutations N86Y and Y184F, as well as the three carboxyl-terminal mutations S1034C, N1042D, and D1246Y, have been identified. These mutations are known to enhance resistance to chloroquine and impact the sensitivities of malaria parasites to several drugs, including mefloquine, amodiaquine, quinine, and halofantrine [19]. Mutations in the pfcrt gene on chromosome 7, which are responsible for encoding a digestive vacuole transmembrane protein, have been found to be associated with drug resistance in P. falciparum [20]. Polymorphisms in the P. falciparum chloroquine resistance transporter gene (Pfcrt) have been found to be associated with resistance to chloroquine and amodiaquine. These polymorphisms occur at codon positions 72 to 76, 97, 220, 271, 326, 356, and 371, and have been associated both in vivo and in vitro [21].

2. LIFECYCLE OF THE MALARIA PARASITE

The malaria parasite has both sexual and asexual reproduction. The malaria sexual reproduction cycle begins when certain trophozoites mature into male and female sexual progeny called gametocytes [22]. These gametocytes are essential for transmitting malaria infection from the mammalian host to the mosquito. After an Anopheles mosquito bites an infected host, mature gametocytes are consumed and transferred into the mosquito's midgut. In this location, the gametocytes change into fertile gametes, which then progress to the next stage where zygotes transform into mobile and invasive ookinetes [23]. These ookinetes then transform into oocysts within the midgut basal lamina. Once the oocysts reach maturity, they release sporozoites that migrate to the mosquito's salivary gland. When the mosquito bites another healthy mammalian host, the parasite is transmitted, thus allowing the cycle to continue [24].

Once sporozoites enter the bloodstream and invade hepatocytes, initiating asexual replication [25]. During hepatic stage, the infected hepatocytes rupture, leading to the release of numerous merozoites. In specific cases of P. vivax and P. ovale infections, certain merozoites transform into dormant hypnozoites. These hypnozoites remain within hepatocytes for extended periods, ranging from several months to up to four years, before becoming active and multiplying to initiate a new phase of erythrocytic infection [26]. During this new infection phase, merozoites interact with red blood cells (RBCs) as shown Fig. 1. They attach to and deform the surface of the host cell membrane, allowing them to enter the RBCs for the second round of asexual reproduction. This process is facilitated by the parasite-induced reorganization of the erythrocyte cytoskeleton. P. vivax and P. ovale specifically target younger erythrocytes, while P. falciparum and P. knowlesi invade erythrocytes of all ages. In contrast, P. malariae shows a preference for aging or senescent erythrocytes. Once inside the RBCs, the merozoites undergo replication to form trophozoites and then schizonts. These schizonts rupture the RBCs, releasing merozoites that go on to invade fresh RBCs, perpetuating the cycle of asexual replication [27].

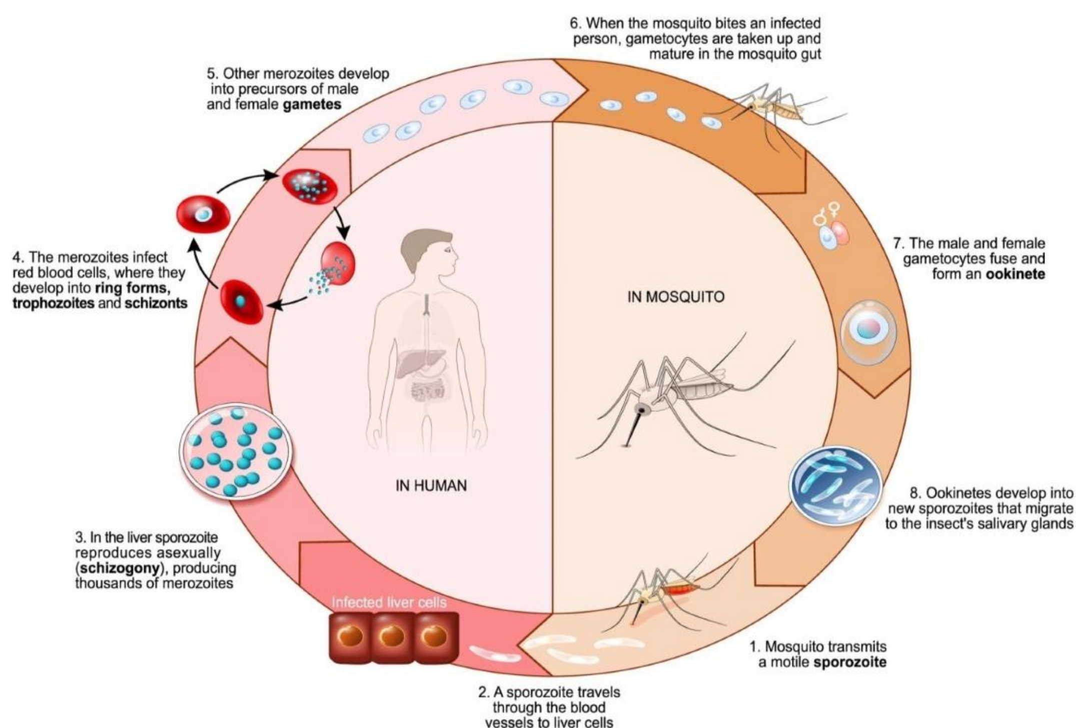

Fig. 1. Life cycle of malaria

3. GENETIC DIVERSITY AND MULTIPLICITY OF MALARIA INFECTIONS

Genetic diversity is the term used to describe allelic richness. Multiplicity of infection (MOI), also known as complexity of infection (COI), refers to the number of different parasite strains infecting a single host. Understanding the level of genetic diversity and the extent of MOI is crucial for studying malaria epidemiological patterns, transmission intensity, host immune system response, and parasite virulence. This information is important for developing an effective anti-malarial vaccine and evaluating the effectiveness of malaria control measures [28].

The prevalence of infections characterized by multiple genetically distinct parasite strains is a significant obstacle to global malaria control and elimination [29]. In areas with high rates of malaria, many individuals are infected with multiple parasite clones [28]. This can have both positive and negative effects in the fight against malaria. On one hand, carrying multiple distinct parasite clones can enhance the development of immunity to different strains. However, due to intense competition within the host, hosting multiple distinct parasite clones can also lead to increased production of gametocytes and the emergence of highly virulent and drug-resistant parasite strains [30].

The PCR-based genotyping of genes with high diversity, such as merozoite surface protein 1 (msp1, PF3D7_0930300), merozoite surface protein 2 (msp2, PF3D7_0206800), and glutamate-rich protein (glurp, PF3D7_1035300), is the most common and widely used tool for estimating P. falciparum diversity [31]. These markers are especially useful for determining the multiplicity of infection (MOI), which is a measure of the effectiveness of intervention programs. Additionally, msp-1 and msp-2 typing is widely used in anti-malarial drug efficacy trials to distinguish recrudescent parasites from new infections [32,33].

The use of PCR to determine the number of repeat length variants at the highly diverse msp-1 and msp-2 gene loci is valuable for measuring MOI and transmission in a population. While conventional PCR [34] may miss some minor parasite populations that can be detected by more sensitive tools like SNP typing, it remains a cost-effective and efficient method for genotyping different parasite clones [35]. In many recent studies on parasite diversity, especially in resource-limited settings that cannot afford more expensive genotyping tools, msp genotyping followed by agarose gel electrophoresis is still the preferred analysis method [36,37].

4. MECHANISMS OF PARASITE INVASION INTO HOST ERYTHROCYTE

The apical end of a polarized cell, known as merozoite, houses various organelles and structures that play a role in invasion upon contact with erythrocytes. These structures include micronemes and rhoptries. Micronemes contain adhesins that are involved in binding to erythrocytes. Rhoptries, on the other hand, are released after the initial engagement with the host cell. They facilitate the invasion process and form the parasitophorous vacuole, where merozoites replicate and give rise to daughter cells. Recent evidence suggests that micronemes may exist in different forms, depending on the adhesins they store. This allows for a highly organized release program. Additionally, rhoptries, which have a club-like shape, are divided into subdomains within the parasite. This may enable the temporal release of various factors [38,39]. In addition, the dense granule organelles may contain different subgroups. There is a specific subset of organelles called exonemes that release the protease subtilisin 1 (SUB1) into the parasitophorous vacuole. This protease then processes various parasite proteins, aiding in the egress of merozoites [40]. The process of erythrocyte invasion is a complex series of steps. After engaging with the erythrocyte, the merozoite undergoes a reorientation as shown Fig. 2. This is followed by the creation of a tight or moving junction, and the final invasion and closing of the parasitophorous vacuole. Throughout this process, there are numerous interactions between parasite host proteins, secretion of invasion-related parasite organelles, and the formation of a single parasitic vacuole that serves as the habitat for the growth of the erythrocyte invasion parasite [41]. Merozoite surface proteins (MSPs) may be involved in the initial phase of invasion, where the merozoite first interacts with the erythrocyte. MSP1, along with several peripheral proteins, forms a complex on the merozoite surface, and there is evidence supporting its role in invasion. However, recent findings indicate that merozoites without MSP1 expression are still capable of invasion, suggesting that it may not be essential for this process [42]. Specific proteins, such as apical

membrane antigen-1 (AMA-1) and the components of the high molecular weight rhoptry (RHOPH) complex, are responsible for sensing the apical orientation of the merozoites with the erythrocyte surface [43]. Apical membrane antigen-1 (AMA-1) is a transmembrane protein found on the surface of merozoites. It is believed to play a role in the invasion of erythrocytes and hepatocytes by the parasites [44].

Duffy binding-like family (DBL) another family of adhesive ligands [45,46] and It is composed of adhesion molecules that play a crucial role in forming junctions between the apical end of the merozoites and the erythrocyte surface. Proteins in this family, such as erythrocyte-binding antigen-175 (EBA175), are similar to P. vivax DBL. These proteins contain one or more DBL domains, which have cysteine residues associated with erythrocyte binding. EBA-175 binds to glycophorin A (the RBC receptor) on the erythrocyte surface through a silaic acid-dependent invasion pathway [47,48]. Additionally, the binding antigen of erythrocyte binding-like family (BAEBL) is a membrane protein that belongs to the erythrocyte binding-like protein family. It is crucial for the invasion of red blood cells by merozoites and the invasion of mosquito salivary glands by sporozoites. BAEBL binds to erythrocytes in a manner that depends on heparin sulfate (HS), which plays a role in the invasion process of merozoites. It has been observed that heparin can inhibit both of these binding pathways. Further research into the mechanisms of heparin's inhibitory effects will contribute to the development of new anti-malarial drugs that can effectively block invasion [49].

Once inside the red blood cells, the parasites undergo various stages of development, including the ring stage, trophozoite stage, and schizont stage. These stages ultimately lead to the production of 16-32 fully developed merozoites.

5. IMMUNE EVASION STRATEGIES BY MALARIA PARASITE

When an infected mosquito bites a human, it transmits approximately 100-200 sporozoites into the skin. While the body's immune system destroys most of these parasites, a few are still able to establish a successful infection even at very low numbers. The skin serves as the first barrier that parasites encounter when they are transmitted into a vertebrate host [50,51]. To achieve successful passage, sporozoites possess specialized mechanical proteins. Studies have demonstrated that the transmission of sporozoites lacking SPECT-1 (a crucial sporozoite microneme protein for cell transversal) and SPECT-2 (also known as perforin-like protein 1 or PLP1) is hindered in the

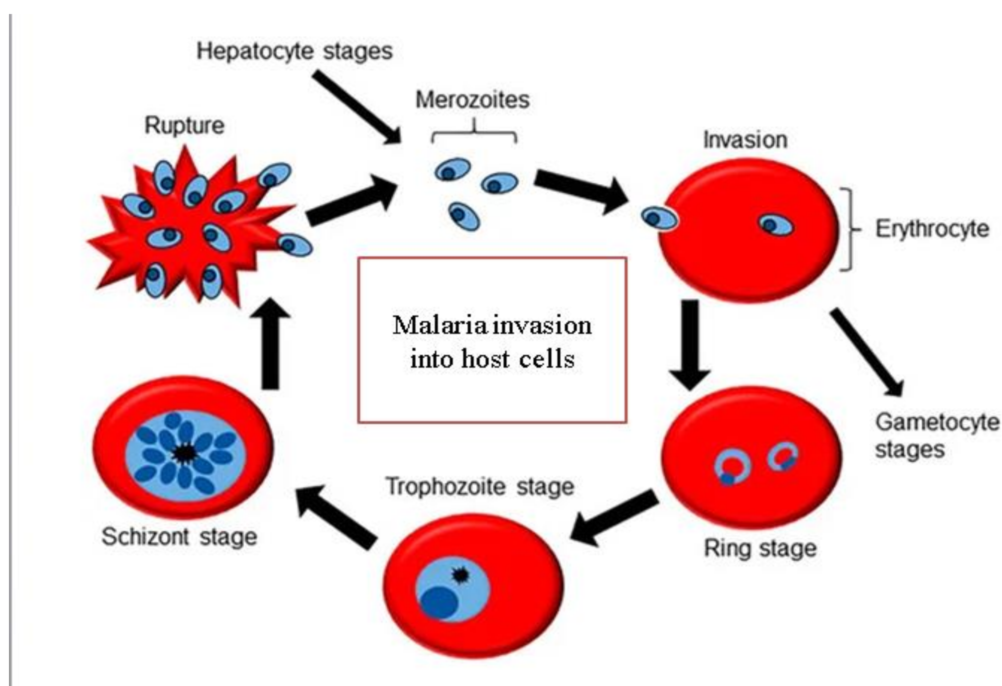

Fig. 2. Malaria invasions to host erythrocytes process

dermis and is cleared by phagocytes, preventing their progression. These proteins are essential for cell transversal and migration to the liver [52]. Sporozoites can traverse different types of cells, including immune cells. The transversal of immune cells can deactivate the immune cell defenses and hinder the clearance of sporozoites (exocytosis) before they cross the barrier [53]. Another protein that plays a role in the motility of sporozoites is called TRAP (Thrombospondin-Related Anonymous Protein). TRAP is present on both the micronemes and the surface of sporozoites. It facilitates the interaction between the parasite and host molecules on the surface, enabling the sporozoites to glide and exit the dermis. In addition, TRAP can attach to specific motifs of sulfated glycoconjugates, assisting in the recognition and entry into hepatocytes [54]. However, some sporozoites may enter the lymphatic system, where they can be identified and eliminated by immune cells like dendritic cells (DCs) [55].

Once the sporozoites enter the circulatory system, they quickly reach the sinusoidal cavity of the liver. This stage, known as the exoerythrocytic (liver) stage, is considered asymptomatic. This is because the liver is an immunoprivileged organ that is effectively shielded from a robust immune response [56]. Once the sporozoites enter the bloodstream, they rapidly reach the sinusoidal cavity of the liver. This particular stage is referred to as the exoerythrocytic (liver) stage and is considered asymptomatic. This is because the liver, being an immunoprivileged organ, is effectively protected from a strong immune response [57]. Type I interferons are potent inflammatory cytokines that are known to inhibit growth of exoerythrocytic forms [58]. Certain cells play a role in exerting anti-parasitic effects, such as Natural Killer and Natural Killer T cells (NK, NKT), and γδT cells. These cells primarily inhibit parasite growth by secreting interferon, which includes both type I interferons and IFN-γ [50]. Other molecules, such as hepcidin, have been associated with the growth inhibition of exoerythrocytic phases [59].

To invade hepatocytes, sporozoites must cross a barrier consisting of endothelial cells (ECs) and immune phagocytic cells known as Kupffer Cells (KCs) [60]. Sporozoites will have to interact with these resident cells in order to establish a successful infection [61]. When sporozoites reach the liver, they are attracted to sulfated molecules present in ECs and KCs. This interaction is primarily mediated by the circumsporozoite protein (CSP) and sulfated heparan sulfate proteoglycans (HSPGs) on the surface of host cells. Other molecules involved in this process include P39 and CD38. Previous studies using intravital and electron microscopy have suggested that KCs, rather than ECs, are the preferred route used by sporozoites [62]. The study demonstrates that in a mice model, sporozoites can pass through KCs and influence their cytokine profile. This results in the suppression of Th1 cytokines (TNF-α, IL-6, and MCP-1) and the enhancement of Th2 cytokines (IL-10), which ensures a safe passage [63]. Furthermore, CSP has the ability to interact with LRP-1 (low-density lipoprotein receptor-related protein) and proteoglycans found on the KC surface. This interaction leads to an increase in intracellular cAMP/EPAC levels and effectively inhibits the formation of reactive oxygen species (ROS). These ROS, which are produced as a natural byproduct during environmental stress, have the potential to cause cellular damage and even kill the parasite [64]. In some cases, the parasite forces the KC to undergo apoptosis. Finally, sporozoites may also have a negative impact on the antigen presentation capacity of KCs that have reduced expression of MHC class I and IL-12 [65]. After successfully penetrating the sinusoidal cell layer, sporozoites enter the hepatocytes and begin to develop within the liver. Sporozoites actively invade the host cells (hepatocytes) by using the cholesterol uptake pathway, which sets them apart from other microorganisms that rely on the host cells' phagocytic activity for invasion. In certain instances, the parasite induces apoptosis in the KCs. Additionally, sporozoites can impair the antigen-presentation ability of KCs, leading to reduced expression of MHC-class I and IL-12 [66]. Furthermore, the released CSP facilitates parasite development by suppressing the NF-κB signaling pathway [67] and upregulating host heme oxygenase-1 (HO-1). This, in turn, enhances parasite development in the liver by modulating the host's inflammatory response [68]. The infection of hepatocytes with sporozoites also disrupts the mTOR pathway. This leads to changes in the levels of proteins involved in cell survival, proliferation, autophagy, anabolism, and cell growth [69]. After invasion into the ultimate hepatocyte, sporozoites become encapsulated within a parasitophorous vacuole (PVM). This vacuole physically separates the sporozoites from the host cytoplasm, preventing them from being degraded by the endocytic/lysosome system. By isolating the

parasitophorous vacuole, the sporozoites are protected from cell intrinsic defenses like apoptosis and selective autophagy [70]. The transition of parasites from liver stage merozoites to initiate blood stage is an important step in immune evasion. To start the blood stage, liver stage parasites must leave the hepatocytes through hepatic spaces where they come into contact with resident phagocytic cells like KCs and DCs. Merozoites protect themselves from being killed by liver phagocytes by surrounding themselves with membranes derived from the host, known as merosomes.After invading the final hepatocyte, sporozoites are enclosed in a parasitophorous vacuole (PVM). This vacuole physically separates the sporozoites from the host cytoplasm, preventing them from being degraded by the endocytic/lysosome system. By isolating the parasitophorous vacuole, the sporozoites are protected from cell intrinsic defenses like apoptosis and selective autophagy [71]. Merosomes are formed when infected hepatocytes release bud-like structures that are able to avoid detection by phagocytes, thereby initiating the blood stage. During this stage, merozoites, which are released from hepatocytes, invade red blood cells (RBCs) and transform into ring-shaped, young, and mature trophozoites through a process called schizogony. Each trophozoite undergoes schizogony, resulting in the production of six to 32 daughter clones. These clones are then released into the bloodstream, where they can invade new RBCs [72]. The intracellular survival is a basic immune escape mechanism used by parasites to avoid direct interaction with immune cells. In addition, red blood cells (RBCs) do not express MHC class I molecules on their surface, allowing them to evade recognition by CD8+ T cells. [73]. Parasites have developed a strategy called rosette formation to evade clearance from the body. Rosettes occur when infected red blood cells cluster together with uninfected ones, allowing the parasites to bind to red blood cell epitopes and avoid detection by the immune system. The formation of rosettes is influenced by blood type, with parasites that bind to blood type A being more virulent than those that bind to blood type O, as they have a greater ability to form rosettes [74].

Generally, evasion mechanisms can be divided into two main strategies. One of these strategies involves the expression of variable antigenic proteins on the parasite's surface during different stages of its life cycle. This helps the parasites disguise themselves from the host's immune system [75]. The evasion of immune clearance is due to highly polymorphic proteins that mediate antigenic variation. These proteins change and adapt to the host immune response, which promotes long-lasting infections [55]. The second is sequestration, which is mediated by genes products of the *PfEMP-1, Var, Rifin* [7], and *Stevor* multigene families [76]. These proteins facilitate the adherence of infected red blood cells (iRBCs) to vascular endothelium, thereby evading clearance mechanisms and sequestering them within the microvasculature of different organs. Additionally, they exploit host factors such as platelets and inflammation, which can induce the agglutination of uninfected red blood cells with iRBCs, promoting the suitable microenvironment for sequestration [77].

6. SIGNALING PATHWAYS AND MOLECULAR PLAYERS IN THE HOST-PARASITE INTERACTION

The molecular characterization of host cell invasion by Plasmodium has historically posed significant challenges. Invasion is a swift process, and the isolation and maintenance of invasive merozoites for in vitro studies are technically demanding. Nonetheless, advancements in genetic and biochemical tools have enabled a thorough examination of the functions of key proteins and signaling molecules, especially in the invasion of human red blood cells by merozoites of P. falciparum, the species responsible for the majority of malaria-related deaths. Recent research has underscored the critical involvement of the second messenger cAMP in the signaling pathways underlying this process [78,79]. cAMP is fundamental to a huge range of signal transduction processes, from human metabolism [80] to the behaviour of social amoeba [81], its role in malaria parasites was previously unclear. In this review, we delineate the recently elucidated critical role of cAMP-dependent signaling in red blood cell invasion.

6.1 cAMP Signalling

Adenylyl cyclases (ACs) are enzymes that synthesize cAMP, whereas phosphodiesterases (PDEs) are enzymes that break down cAMP. In the Plasmodium genome, there are two ACs (ACα and ACβ) and four PDEs (PDEα, PDEβ, PDEγ, and PDEδ). The transcription of ACα only occurs during the sexual and pre-erythrocytic life cycle stages [82]. While ACβ expression is primarily limited to mature intra-erythrocytic

parasites (schizonts) as they approach egress, this suggests that ACβ is the crucial source of cAMP production in blood stage parasites. The transcription of ACα only occurs during the sexual and pre-erythrocytic life cycle stages [83,84]. PDEγ and PDEδ are expressed in mature gametocyte and mosquito stages. These enzymes are believed to have roles that depend on their ability to hydrolyze cGMP [85]. Both PDEα and PDEβ are transcribed to the highest extent in mature blood stage schizonts. An important distinction between these enzyme isoforms is that PDEα exclusively hydrolyses cGMP [86] while PDEβ is a dual-specific PDE able to hydrolyse together cGMP and cAMP as Fig. 3 shows below [79]. PDEβ is the only PDE responsible for regulating cAMP levels in asexual blood stage parasites. It is also the essential PDE during this clinically significant stage in the P. falciparum life cycle [79,87]. The primary function of cAMP in multicellular organisms is carried out through the activity of the cAMP-dependent protein kinase (PKA). This enzyme is composed of two protein subunits: a regulatory subunit (PKAr) and a catalytic subunit (PKAc). When PKAr binds to PKAc, it inhibits the kinase activity. However, when cAMP binds to PKAr, it causes dissociation from PKAc, thereby relieving the inhibition and enabling PKAc to phosphorylate protein substrates [88]. Although PKA is commonly believed to be the primary driver of cAMP-dependent signaling in P. falciparum, the parasite genome also contains a protein known as Epac (exchange protein activated by cAMP). This protein is equipped with cyclic nucleotide-binding domains, as predicted [89].

6.2 cAMP is Critical for Merozoite Attack of RBCs

The invasion of red blood cells (RBCs) by P. falciparum merozoites is a complex process that occurs after the production and maturation of a new generation of daughter merozoites within an infected RBC. Before invading the next RBC, these merozoites must first exit their current host RBC through a process called egress, which is dependent on cGMP [90], releasing of proteins from apical secretory organelles, specifically exonemes, micronemes, and rhoptries, is essential for egress and invasion. Within seconds, free merozoites tightly bind to a target red blood cell (RBC), simultaneously pulling the host RBC membrane around themselves. This process is facilitated by an actinomyosin motor, which propels the merozoites into the cell [91]. The role of cAMP signalling in invasion was first proposed based on the observation that pharmacological inhibitors of mammalian cAMP regulatory and responsive proteins could disrupt invasion [89] and phosphoproteome data signifying that PKA is very active in mature schizonts [92]. Recent advancements in genetic techniques have enabled a more comprehensive analysis of cAMP signaling [93] that allowed robust, inducible disruption of ACβ , PKAc [78] and PDEβ [79] in *P. falciparum*.

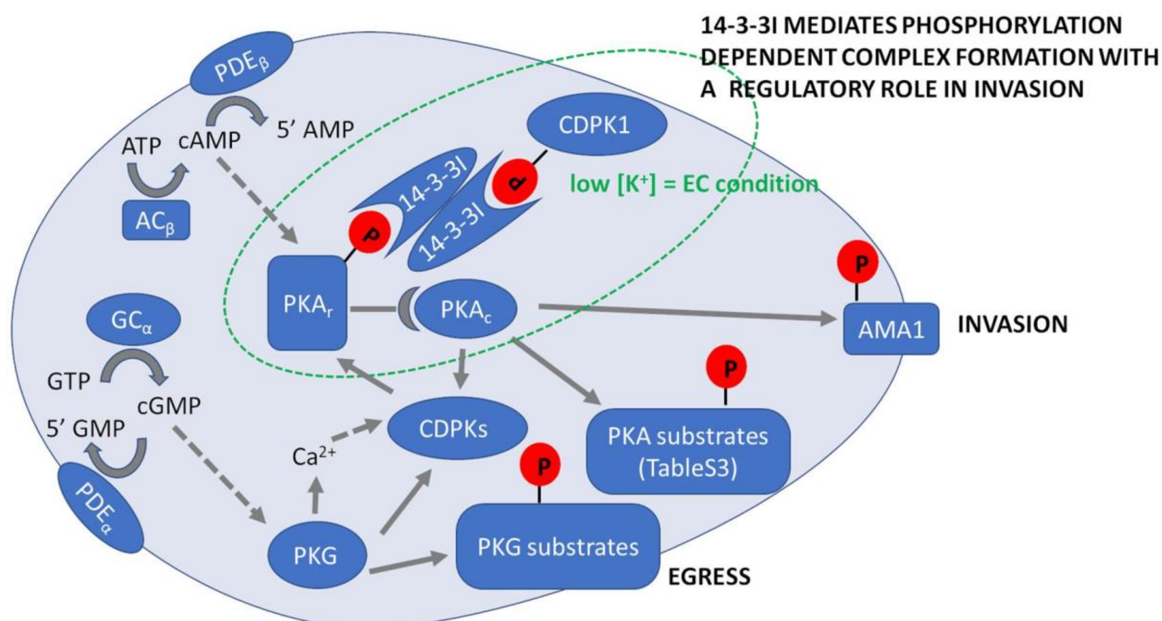

Fig. 3. Key components of cAMP signalling in the plasmodium

7. CURRENT ANTIMALARIAL DRUGS AND THEIR MODES OF ACTION

Nowadays, most antimalarial agents target the asexual phase of malaria infection, which is responsible for causing symptomatic illness. The pre-erythrocytic stage, on the other hand, is not as appealing as it does not produce clinical symptoms. Malarial treatment has been based on the development of natural products, semi-synthetic, and synthetic compounds since the 1940s [94]. Antimalarial agents are classifying into three main groups: quinoline derivatives, antifolates, and artemisinin derivatives. Currently, no single drug has been identified or manufactured that can effectively eliminate all strains of the Plasmodium species. Quinolines, which are alkaloids extracted from the bark of Cinchona trees, are the most commonly used antimalarial agents for malaria treatment. Quinine, in particular, became the standard therapy for malaria from the mid-19th century to the 1940s [95]. The therapeutic use of Quinine has been limited due to the emergence of resistant strains of P. falciparum, as well as its toxicity. However, Quinine is still used to treat severe malaria, usually in combination with another agent. This helps to reduce the duration of treatment and minimize side effects [96].

Atovaquone is the first antimalarial agent approved for targeting the Plasmodium mitochondria. It works by inhibiting electron transport through blocking cytochrome b parts of the cytochrome bc1 complex, acting as a ubiquinone analog. When combined with proguanil, atovaquone is considered safe and effective for pregnant women and children. It is effective against the sexual stages of the parasite in both the host and the mosquito, thus preventing malaria transmission from mosquito to human. This fixed combination is marketed under the trade name Malarone [97,98].

Chloroquine is the preferred drug for treating malaria because it is effective, safe, and affordable. However, the misuse of chloroquine quickly resulted in the emergence of P. falciparum species that are resistant to it [99]. Antifolates are a also type of antimalarial agents that work by inhibiting the synthesis of folic acid, which is necessary for the production of nucleotides and amino acids. These agents specifically target the Plasmodium species during the schizont stage within both erythrocytes and hepatocytes. One example of an antifolate is sulfadoxine, which has a similar structure to p-aminobenzoic acid (PABA), a key component of folic acid. By inhibiting the enzyme dihydropteroate synthase, which is crucial for the biosynthesis of nucleic acids, sulfadoxine effectively impedes the synthesis of dihydrofolic acid [97].

In 1972, scientists discovered Artemisinin in Artemisia annua. Artemisinin and its derivatives, such as artemether, dihydroartemisinin, arteether, and artesunate, have a wide range of effectiveness. Artemisinin is effective in inhibiting all stages of parasites within red blood cells, especially during the early phase of their growth. Additionally, it helps to prevent the transmission of gametocytes from humans to mosquitoes [100]. Artemisinin and its derivatives are highly effective against strains of malaria that are resistant to chloroquine and mefloquine. They are safe, powerful, and act quickly to kill the parasite in the bloodstream, regardless of the Plasmodium species involved. However, it is important to note that Artemisinin alone cannot eliminate the dormant liver stages of the parasite. Additionally, these drugs have a short half-life and are poorly absorbed by the body, which can contribute to the development of drug resistance. Therefore, it is recommended to use Artemisinin derivatives in combination with other antimalarial agents for more effective treatment [101].

The current strategy for combating antimalarial drug resistance involves the use of drug combinations. The World Health Organization (WHO) recommends the use of artemisinin-based combination therapy (ACT) as the first-line treatment for managing uncomplicated P. falciparum. This is because combining different drugs in the treatment decreases the development of drug resistance and minimizes side effects [102].

8. MALARIA VACCINES

The process of administering a vaccine to enhance the development of disease resistance in the immune system is known as vaccination. Vaccines typically contain proteins or toxins from the organism, along with a weakened, live, or killed version of a virus or microbe. By stimulating the body's immune response, vaccines help prevent illness caused by infectious agents. Getting vaccinated is the most effective way to prevent the spread of infectious diseases. The RTS,S vaccine is the most clinically advanced vaccine against P. falciparum. It is a subunit vaccine. It consists of a

single recombinant protein called the P. falciparum circumsporozoite protein (PfCSP), and it is administered with the adjuvant AS01. In a phase 3 clinical trial involving infants aged 5-17 months, it was found that three vaccinations with RTS,S/AS01 provided 51.3% vaccine efficacy (VE) against all episodes of P. falciparum clinical disease within one year [103]. Furthermore, when four vaccinations were given over a period of 21 months, the vaccine efficacy over four years was 36.3% [104]. It is believed that the protection provided by RTS,S is primarily due to antibodies [105].

The PfSPZ Vaccine takes a unique approach using live, nonreplicating, radiation-attenuated, aseptic, purified, cryopreserved P. falciparum sporozoites (SPZ). Initial studies have shown that it provides approximately 60-100% vaccine efficacy (VE) for up to 14 months against controlled human malaria infection [101,106] in malaria-naïve US adults [107]. An important aspect of the PfSPZ Vaccine's effectiveness is that it needs to be administered through direct venous inoculation [60,108]. This method stimulates the production of circulating PfCSP-specific antibodies and both circulating and liver-resident T cell responses [109 (Mordmüller, 2017 #12]).

While PfCSP-specific antibodies have been found to offer short-term VE [110], preclinical animal models have shown that durable sterilizing protection requires PfSPZ-specific T cells [108]. In malaria-naïve humans, the presence of multifunctional CD4+ T cells producing cytokines in the blood has also been associated with protection from PfSPZ Vaccines [111]. It is possible that priming a protective CD4 and CD8 T cell response after receiving the PfSPZ Vaccine is partially facilitated by γδ T cells [112]. The frequency of Vδ2+Vγ9+ T cells at the time of initial PfSPZ immunization has been correlated with the induction of PfSPZ-specific T cells and a positive outcome in adults [107]. In mice, γδ T cells are also necessary for the induction of protective immunity when immunized with rodent malaria sporozoites [112].

9. DRUG RESISTANCE MECHANISMS AND EMERGING CHALLENGES

The main factors that facilitate the emergence of resistance to existing antimalarial drugs such as, parasite mutation rate, overall parasite load, strength of drug selected, treatment compliance, and poor adherence to malaria treatment guidelines [113]. Improper dosing, poor pharmacokinetic properties, fake drugs lead to inadequate drug exposure on parasites [114]. Poor-quality antimalarial may aid and abet resistance by increasing the risk of hyperparasitaemia, recrudescence, and hypergametocyopaenia, wrong APIs such as the use of halofantrine instead of artemisinin which without chemical analysis will be invisible to investigators but not to parasites [115,116].

Mutations that cause resistance to antimalarial drugs primarily occur naturally and are independent of the drug's effects. These mutations are often referred to as spontaneous mutations. The development of drug resistance in malaria happens in two stages. In the first stage, an initial genetic event occurs, resulting in a mutant parasite that possesses a new genetic trait providing a survival advantage against the drug. In the second stage, these resistant parasites are selected and begin to multiply, ultimately leading to a parasite population that is no longer susceptible to treatment. In some cases, resistance can be attributed to a single point mutation, while for other drugs, multiple mutations at different sites are necessary. These acquired mutations enable the survival and reproduction of the resistant parasite, while susceptible parasites are eliminated under the pressure of the drug [117].

The importance of pharmacokinetics in determining the effectiveness of antimalarial drugs and in contributing to the development and spread of drug resistance has received increased attention [118]. In the past, drug plasma levels were seldom measured, leading to the assumption that all cases of clinical treatment failure were caused by inherent parasite resistance. Typically, the chosen dosage is the lowest amount that achieves a positive response in order to minimize potential side effects. However, as resistance has expanded, it has been discovered that even relatively small amounts of drugs can enable the significant spread of resistant parasites. This is because the therapeutic level necessary to clear partially resistant parasites is often higher than what is needed to eliminate fully susceptible parasites [119].

The spread of drug-resistant malaria parasites is made easier by using medications that have longer elimination phases. During the period after treatment, the remaining antimalarial activity acts as a "selective filter" that can prevent infection by

sensitive parasites but allows infection by resistant parasites. Medications such as chloroquine, mefloquine, and piperaquine, which stay in the bloodstream for longer periods of time, continue to act as a selective filter even after they have been stopped [120]. The length of the terminal elimination half-life is an important factor in determining whether an antimalarial drug will lose its effectiveness due to the development of resistance. Drugs like mefloquine, piperaquine, and chloroquine remain in the host's bloodstream for extended periods, allowing resistant parasites to be selected over time [121].

The parasite genome undergoes spontaneous changes, such as single nucleotide variations and multiple mutations in different genes. These alterations enable the pathogen to develop resistance to drug action over time, resulting in unexpected outcomes. Known drug-resistance genes, such as pfcrt, pfmdr1, pfk13, pfmrp1, pfdhfr, and pfdhps, exhibit genetic polymorphisms that often counteract the effectiveness of drugs used to control the disease [122,123].

During the asexual blood stage of the Plasmodium life cycle, chloroquine targets the polymerization of free haem within the food vacuole of the parasite. In the food vacuole, the haemoglobin obtained from the host is broken down into amino acids. These amino acids are then used for protein synthesis by the parasite. Additionally, the haemoglobin also contains Fe2+, which is digested alongside the amino acids [124]. Haem, which contains Fe2+, undergoes oxidation to form protoporphyrin IX (FPIX) containing Fe3+. FPIX is toxic to the parasite, but it is converted to the polymer haemozoin. Haemozoin formation is disrupted by chloroquine, a process that is crucial for the parasite's survival. The primary mechanism of chloroquine resistance involves the efflux of the drug through the P. falciparum chloroquine-resistance transporter (pfcrt), which is located in the food vacuole. The SNP K76T in pfcrt is universally associated with chloroquine resistance in Africa [125] and globally the development of chloroquine resistance is associated with additional mutations in pfcrt, including K76T [126,127].

10. CONCLUSION

This review offers valuable insights into the molecular machinery of malaria infection, illuminating host-parasite interactions and potential therapeutic targets.

We have explored the intricate lifecycle of the malaria parasite, emphasizing the molecular mechanisms involved in invasion, erythrocyte, and immune attack. Understanding these processes at the molecular level is crucial for developing interventions that can disrupt the parasite's lifecycle and prevent infection. Host-parasite interactions play a critical role in malaria pathogenesis, and we have discussed the molecular factors involved in the host immune response, signaling pathways, and genetic factors that influence susceptibility to malaria. By unraveling these interactions, we can gain a deeper understanding of the disease and identify novel approaches for intervention. These insights pave the way for developing targeted therapies that can modulate the immune response and mitigate disease severity.

Furthermore, we have discussed the current antimalarial drugs and the challenges posed by drug resistance. Identifying new therapeutic targets is essential for overcoming drug resistance and developing more effective treatments. By targeting specific molecular mechanisms and host-parasite interactions, we can strive toward the goal of eradicating malaria and alleviating the burden it imposes on global health. Continued research in this field is essential to uncover new therapeutic strategies and ultimately achieve success in the fight against malaria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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