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REVIEW ARTICLE

Plasmodium falciparum RIFINS: Role in malaria pathogenesis

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Abstract

Malaria kills an estimated 600,000 people each year, especially children under five years who reside in sub-Saharan Africa. Malaria fatalities are associated with severe forms such as cerebral malaria, acute respiratory failure, severe anaemia, renal failure, hypoglycaemia, and pulmonary oedema. Although the underlying pathogenic mechanisms in immune responses and parasite immune evasion, cytoadherence of parasitized red blood cells, and rosetting are enumerated, the mechanisms are not fully understood. P. falciparum parasite-derived surface protein, repetitive interspersed family (RIFIN) genes are involved in rosetting, blocking microcirculation, and playing a role in malaria pathogenesis; it is unclear which RIFIN family genes are involved in the various pathogenic mechanisms in malaria. RIFINs are the extensive malaria family genes expressed throughout the malaria parasite stages, indicating their diverse roles. Malaria pathogenesis occurs in erythrocyte-stage infection, and the expression of RIFINs at this phase could play a diverse role in the various pathogenic mechanisms. They are involved in major phenomena such as cytoadherence, merozoite evasion, and immune evasion. RIFINs aid in the immune evasion of P. falciparum through various molecular interactions by binding to the inhibitory receptors LAIR1, LILRB1, and LILRB2. RIFINs in severe forms of malaria (such as cerebral malaria and severe anaemia) require a considerable understanding to target and control malaria severity and mortality. RIFINs are implicated in severe malaria and are discussed together with other variant surface antigens such as STEVORS or PfEMP1 in the specific pathophysiology of malaria. This review details the role of RIFINs in the various malaria pathophysiological mechanisms underlying severe malaria and mortality.

Keywords: *Plasmodium falciparum*, Severe malaria, cerebral malaria, rosette formation, cytoadherence, malaria immunity, variant surface antigens.

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Introduction

Malaria is one of the perilous infectious diseases that affect humans (World Health Organization, 2016). *Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, Plasmodium vivax*, and *Plasmodium knowlesi* are the causative organisms for human malaria. More than 200 million people are affected by malaria each year, with more than 600,000 deaths in 2021 (World Malaria Report, 2021). Most deaths are primarily caused by Plasmodium falciparum, largely affecting children below the age of five, pregnant women in Sub-Saharan Africa, and non-immune travellers as well (World Health Organization, 2017). The majority of fatalities are caused by severe malaria, which includes cerebral malaria and severe malarial anaemia (Lawton et al., 2022).

Severe malaria is linked to cytoadherence to cell surfaces and the sequestration of parasitized red blood cells (pRBCs). Although all human malaria species adhere to cell surfaces, P. falciparum is more likely to sequestrate. P. falciparum infection spans a wide spectrum of clinical manifestations, ranging from asymptomatic infection to severe disease (including cerebral malaria, acute respiratory failure, severe anaemia, renal failure, hypoglycaemia, and pulmonary oedema) (Marsh & Snow, 1999). Patients with cerebral malaria initially experience unconsciousness as a result of vascular constriction caused by rosettes and some fibrillose components (Ndam & Deloron, 2007). Malaria pathogens have developed techniques to live inside infected hosts, one

tactic is changing the surface antigen expression to evade the host immune system (Deitsch et al., 2009).

Plasmodium species express and export family genes called variable surface antigens (VSA genes), for which each individual parasite expresses only one protein product (Fernandez et al., 1999; Mkumbaye et al., 2017). P. falciparum parasite has ≥ 60 erythrocytes membrane protein 1 (PfEMP-1) different var genes whose binding domain cassettes DC8 or DC13 are implicated in severe disease (Almelli et al., 2014a; Ndam et al., 2017). The DC8 or DC13 has a superior binding phenotype for diverse human endothelium including intercelullar adhesion molecule 1 (ICAM-1) and Endothelial protein C receptor (EPCR), cluster of differentiation 36 receptor (CD36), and chondroitin sulfate A (CSA) and leads to sequestration of pRBCs within small vessels which obstruct organs (Angeletti, 2013).

The mechanical obstruction of microcirculation by pRBCs, or the formation of rosettes, contributes to microvascular disease. P. falciparum parasite-derived surface protein, repetitive interspersed family (RIFIN), has been implicated in rosetting and blocking microcirculation, leading to malaria pathogenesis (Goel et al., 2015). This review discusses the role and underlying mechanism of P. falciparum RIFINs in the pathogenesis of malaria.

The molecular characteristics of Plasmodium falciparum RIFINs

RIFINs form the largest family of surface proteins in Plasmodium falciparum, with 150–200 copies per haploid genome. They are two-exon genes with a conserved domain architecture that is tiny (1000 base pairs or less) (Cheng et al., 1998). The Plasmodium falciparum 3D7 strain RIFINs can be split into two main categories: A and B-RIFINs (Joannin et al., 2008). The difference is largely due to a 25-amino acid indel present only in the conserved N-terminal region of A-RIFINs (Joannin et al., 2008) (figure 1)

Protein domains are illustrated as signal peptide (SP), short hypervariable region (V1) and hypervariable domain (V2), transmembrane domains (TM), brown (pexel motif), purple (indel, a 25 amino acid sequence unique to RIFIN-A), conserved (C), and semi conserved (SC) domains. RIFIN proteins are carried by infected erythrocytes to the Maurer's clefts and then to the erythrocyte membrane. The RIFIN proteins' C-terminal and semi-conserved domains are inserted into the erythrocyte membrane. Consequently, RIFIN B proteins are inserted by two transmembrane domains and express only a few amino acids at the surface of pRBC. Immune cells are bound by the C-terminal of RIFIN B, which enhances immune evasion. RIFINs are exported across parasitophorous vacuoles into the host cell by Pexel and signal peptideS. The hypervariable region of RIFINs generates antigenic variability. RIFIN A proteins are inserted by one transmembrane domain. Surface-exposed loops of RIFINs bind to immune cells to trigger immune invasion by parasites.

Compared to A-RIFINs, B-RIFINs are more hydrophobic (Goel et al., 2015). RIFINs are trafficked to the pRBC membrane via the parasite Maurer's clefts (MCs).

Members of the two sub-families have two transmembrane domains: a Plasmodium export element (PEXEL) or vacuolar transport signal (VTS) motif with an N-terminal signal peptide which is required to export target proteins outside the parasite (Horrocks & Muhia, 2005). A signal peptide and just one transmembrane (TM) region are present in the majority of A-RIFINs, whereas two TM regions and a signal peptide are present in the majority of B-RIFINs. After multiple sequence alignments with known STEVOR and RIFIN proteins, the similarities between the RIFIN and STEVOR proteins are minimal, making it easy to spot differences between them (Joannin & Kallberg, 2011). RIFINs are comparatively more resistant to trypsin treatment and require a high dosage of trypsin to remove them from the surface of the pRBCs (Bachmann et al., 2015). The TM domain at the N-terminus of RIFIN

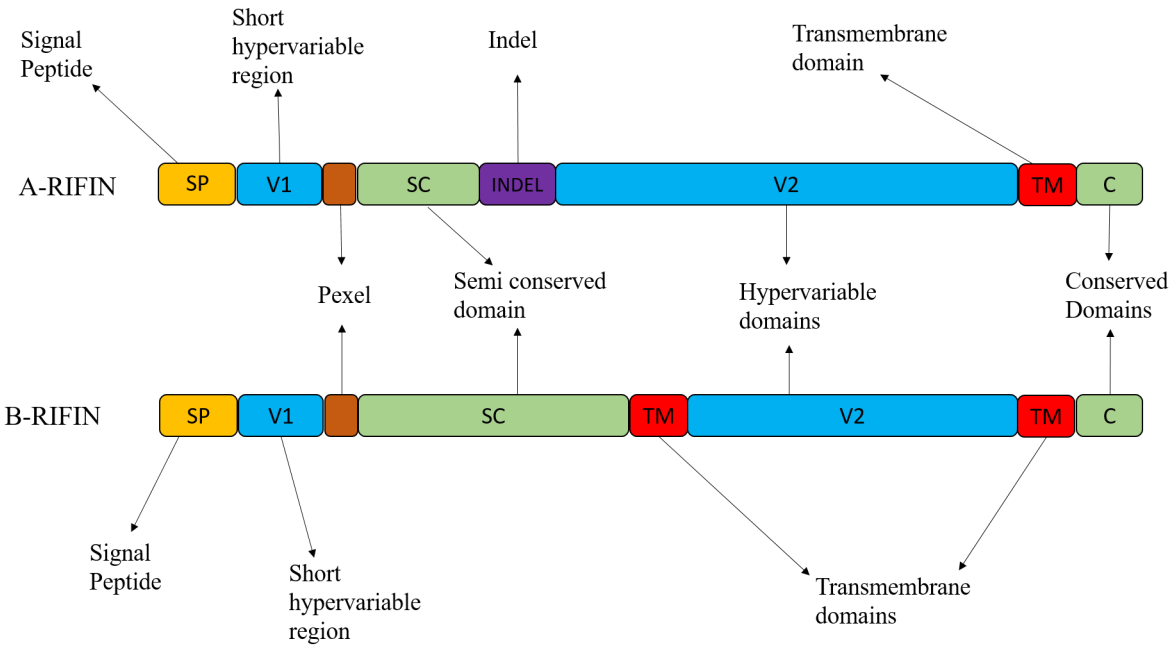

Figure 1 | General Structure of RIFIN A and B

contains a high concentration of alanine and glycine residues and conserved central proline residue, suggesting a connection with ion channel function (Schneider & Mercereau-Puijalon, 2005).

Interactions Between RIFINs and Other Virulence Factors

The expression of VSAs on the surface of pRBCs is a significant virulence factor of P. falciparum. These surface-derived antigens aid the adherence of parasites to the vasculature. They include PfeMP1 (Leech et al., 1984), STEVOR (Blythe et al., 2008; Kaviratne et al., 2002), RIFIN (Cheng et al., 1998; Fernandez et al., 1999; Kyes et al., 1999), and SURFIN (Winter et al., 2005). Several of these VSAs are exported by parasite-induced membrane structures called "Maurer's clefts" (MCs) that are found in the cytoplasm of pRBCs. MCs are distinct from the tubulovesicular network (TVN) that extends from the parasitophorous vacuole membrane (PVM) in that they serve as a sorting substrate for protein transport (Lanzer et al., 2006; Wickert & Krohne, 2007). Clonal diversity in the expression of PfEMP1 and RIFINS is thought to have evolved as a strategy for immune evasion, increasing the likelihood of extended parasite life, gametocyte maturation, and transmission to the subsequent host. PfEMP1 and RIFIN polypeptides initially emerge at the ring stage, 6–10 hours post-invasion (Haeggström et al., 2004). RIFIN proteins are thought to be concurrently expressed with PfeMP1 on the surface of pRBCs (Kyes et al., 2000). Haeggström and associates showed that RIFINs and PfEMP1 are transported across the cytoplasm of pRBCs via a shared trafficking route (Haeggström et al., 2004), in that would use lateral diffusion to traffic most or all of the gap between the erythrocyte plasma membrane and the parasite, once inserted into the cell membrane network. (Haeggström et al., 2004). PfEMP1, RIFIN, and STEVOR are believed to exhibit a mutually exclusive pattern of expression where one member at a time is expressed and others remain silent (Subudhi et al., 2016). The expression of RIFINs peaks at 12–27 hours (ring and trophozoite stages) after invasion, whereas STEVORs peak at 22–32 hours (trophozoite and schizont stages) after invasion of merozoites (Scherf et al., 2008).

STEVOR is expressed on the surface of pRBCs after the expression of both PfEMP1 and RIFIN, demonstrating its essential role in the development of late-stage parasites (Ferreira et al., 2004). It has been suggested that the enhanced rigidity of pRBCs due to STEVOR amplifies the sequestration mediated by PfeMP1 (Sanyal et al., 2012).

Expression of RIFINs at various stages of the parasite life cycle

Plasmodium has a complicated life cycle that necessitates the expression of certain proteins in both invertebrate and vertebrate hosts for both intracellular and extracellular survival, for invading various cell types, and for evading host immune responses. Targeting specific parasite life stages and/or specific proteins expressed at these phases will result in the most effective treatment methods, including antimalarial vaccines and medications. Proteomic and transcriptomic studies have shown that RIFINs are significantly expressed in the gametocytes, merozoites, and sporozoites stages of Plasmodium species (Bozdech et al., 2003; Florens et al., 2002; Le Roch et al., 2003).

Mosquitoes inject sporozoites after consuming a blood meal. Even though sporozoites spend only a few minutes in the bloodstream, sporozoites are the invasive stage that has the apical specialized machinery needed to invade host cells. RIFINs are mostly up-regulated in sporozoites (Florens et al., 2002; Le Roch et al., 2003), indicating that they play a major role in ensuring the survival of sporozoites against hostile environments in the blood and a successful hepatocyte invasion by sporozoites. The rif gene PF3D7_1300600 has been reported to dominate in the sporozoite stage (Mwakalinga et al., 2012; Wang et al., 2010).

Merozoites are released from pRBCs, and after a brief period in the plasma, they infiltrate new red blood cells (RBCs). RIFINs are highly co-expressed with other genes and are involved in merozoite invasion and remodelling of RBCs in the merozoite and the ring stage (Subudhi et al., 2016). A-type RIFINs are found at the apical tip of merozoites, whereas B-type RIFINs are found in the cytoplasm (Petter et al., 2007). Several RIFINs are co-expressed between 11 and 12 hours post-RBC invasion (Painter et al., 2018). P. falciparum 3D7 rif transcript PF3D7_1300600 has been discovered to be exported to the surface of invading merozoites, intimationtheir possible role in sensing or attachment to new RBCs (Mwakalinga et al., 2012). The rif gene PF3D7_1400400 has been implicated in merozoites and late ring stage in vitro, and PF3D7_0900600 has been implicated in merozoite, early ring, and late ring stage in vitro (Daily et al., 2005).

Transcriptome studies on developing gametocytes show persistent transcription of RIFINs with a distinct expression of variants (Wang et al., 2010). The expression of several RIFINs peaks at stages III and IV of the gametocytes and significantly decreases at stage V (Mwakalinga et al., 2012). The stage V gametocytes (mature) express high quantities of the type B rif gene, PF3D7_1300600, which likewise the predominates sporozoite rif transcript profile (Mwakalinga et al., 2012; Wang et al., 2010). The P. falciparum 3D7 rif transcript PF3D7_0900500 is most abundant during gametocyte stages II and III.

RIFINs are more expressed in vivo than in vitro throughout all stages of the parasite's life cycle (Daily et al., 2005). Types A and B RIFINs exhibit varying patterns of expression throughout the life cycle, and their localization to numerous subcellular sites indicates that they are involved in a variety of biological processes (Petter et al., 2008). In contrast to their cellular localization in asexual stages, the A-type RIFINs are expressed in gametocytes, whereas the B-type RIFINs do not appear to be exported to the erythrocyte in intra-erythrocytic sexual stage parasites (Petter et al., 2007).

Immunity against RIFINs

Exposure or repeated exposure to Plasmodium falciparum results in the gradual development of protective and

acquired clinical immunity against malaria (Doolan et al., 2009). For instance, the immunoglobulin G (IgG) antibody isotype repertoires against the cytophilic region of VSA have been shown to be involved in the opsonization of pRBCs (Yone et al., 2005). Similarly, antibodies against variant surface antigens such as RIFINs induce immune responses, which play a role in modulating the pathophysiology of malaria (Holder, 1999).

Anti-RIFIN antibodies are associated with protection against severe malaria through parasite clearance (Abdel-latif et al., 2003). Immune evasion techniques, including cytoadherence and rosetting of the parasite, may be blocked by antibodies against RIFINs (Gonzales et al., 2020). These antibodies may help natural killer (NK) cells kill pRBCs directly by inducing opsonic phagocytosis or antibody-dependent cellular cytotoxicity (Chan et al., 2019). Malaria patients develop receptor-containing antibodies, including a piece of the LAIR1 and LILRB1 exons (Chen et al., 2021; Tan et al., 2016). These antibodies are a class of widely neutralizing proteins that can simultaneously detect several LAIR1 and LILRB1-binding RIFINs (Tan et al., 2016), as well as block the interaction of LAIR1-binding RIFINs and LAIR1 on immune cells (Xie et al., 2021). Specific RIFIN family members have been identified as the target antigens of the LAIR1-containing antibodies (Tan et al., 2020). These antibodies are able to efficiently agglutinate and opsonize pRBCs for phagocytosis, suggesting that they might facilitate parasite clearance.

Additionally, IgGs with broad anti-RIFIN activity have been associated with protection against malaria and inhibit RIFIN's ability to bind to host leucocytes (Saito et al., 2017) to facilitate opsonization of P. falciparum-infected RBCs (Sakoguchi et al., 2021). In a particular study, children who had high levels of antibodies against four RIFIN variants had a significantly lower chance of contracting febrile malaria, indicating that RIFINs could be a significant target for protective immunity (Kanoi et al., 2020). Mwakalinga and colleagues also showed that there is a high prevalence of IgG antibodies to some specific RIFIN variants (Mwakalinga et al., 2012). Taken together, these findings illustrate a biologically relevant mechanism that may be suitable for vaccine development.

Immune evasion/immune suppression in humans

The survival, evasion, and development of malaria parasites require the ability of the merozoites to escape the host immune system and successfully invade the RBCs. Malaria-exposed individuals develop anti-parasitic immunity, such as antibodies and cellular immunity (T-cells), to clear invading malaria parasites. The anti-parasitic antibodies bind to pRBCs or mediate opsonization followed by phagocytosis by circulating macrophages (Gomes et al., 2016). However, Plasmodium parasites utilize multiple mechanisms, including the expression of variable surface, antigens to evade the human immune response (Kyes et al., 2001).

The presence of a hypervariable region and a huge repertoire of rif genes in the genome of a single parasite provides strong evidence that they are involved in antigenic variation during the pathogenic mechanisms of malaria parasites (Kaur & Hora, 2018). Some subsets of RIFINs are utilized by Plasmodium falciparum to evade the immune system of the host through the activation of inhibitory immune receptors, including leukocyte-associated immunoglobulin-like receptor 1 (LAIR1) and leukocyte immunoglobulin-like receptor B1 (LILRB1) (Harrison et al., 2020; Saito et al., 2017; Tan et al., 2016). The leukocyte immunoglobulin-like receptor (LILR) family is expressed on the surface of myeloid cells, B cells, certain NK cells, certain T-cell subsets, and major histocompatibility complex molecules (MHC) class 1 (Colonna et al., 1997). RIFIN variants PF3D7_1254800 and PF3D7_0223100 bind to LILRB1, while PF3D7_1101100 and PF3D7_1040300 bind to LAIR1 (Harrison et al., 2020; Saito et al., 2017; Xu et al., 2021) and prevents activation of immune cells

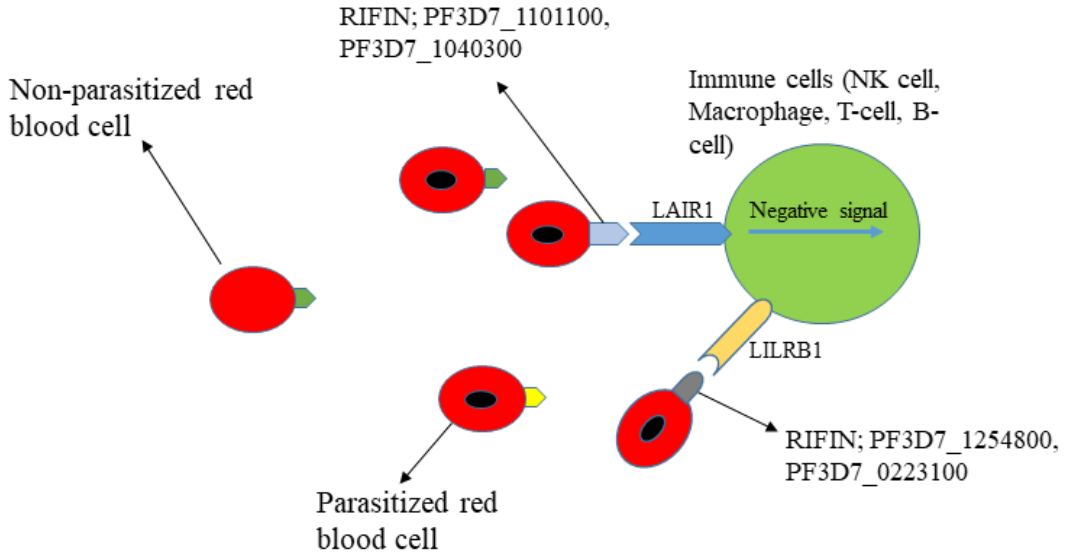

Figure 2 | Immune evasion mechanisms by P. falciparum RIFINs

expressing LILRB1 and LILR1(Saito et al., 2017) (Figure 2).

P. falciparum drives the expression of RIFINs on the exterior surface of pRBCs. Distinct RIFINs attack host inhibitory receptors LILRB1 and LAIR1 on the surface of immune cells (NK cells, Macrophages, T-cells and B-cells) to downregulate the innate and adaptive immune responses, enhancing parasites ability to evade the host immune system, which results in suboptimal immunity formation, which leads to severe malaria.

These interactions enable malaria parasites to escape immune clearance and offer survival advantages to the parasites. For example, the LILRB1-binding RIFIN, PF3D7_1254800, stimulates the reduction of NK cell cytotoxicity and the inhibition of B cells' generation of immunoglobulin M (IgM), which provides parasites with protection from macrophage phagocytosis and natural killer cell-mediated killing (Chew et al., 2020; Saito et al., 2017). Interactions between RIFINs and the leukocyte-associated immunoglobulin-like receptors are reported to be observed in more severe malaria cases than in mild instances, indicating that these interactions may be responsible for the disease's severity (Saito et al., 2017). Furthermore, some subsets of RIFINs bind to the inhibitory receptor LILRB2 to evade the host immune system during P. falciparum infection (Sakoguchi et al., 2021; Sakoguchi & Arase, 2022). Sakoguchi and associates demonstrated that the RIFIN PfKH02_070016000 binds to LILRB2, and domain 3 of LILRB2 is involved in RIFIN binding while domains 1 and 2 of LILRB2 are associated with binding to HLA class I molecules, indicating that, like LILRB1 and LAIR1, the inhibitory receptor LILRB2 is a target of RIFIN for P. falciparum immune evasion (Sakoguchi et al., 2021).

The importance of RIFIN proteins in immune defence is demonstrated by the association between the level of anti-RIFIN antibodies in infected patients' plasma and the removal of parasites from circulation (Abdel-latif et al., 2003). Therefore, the significance of RIFINs as immunological targets significantly promotes their prospects for vaccine development (Chan et al., 2014).

Rosette formation

Rosetting occurs when pRBCs attach to non-pRBCs, resulting in the activation of parasite ligands and RBC surface receptors (Miller et al., 2002; Udomsangpetch et al., 1989). It is a phenomenon of spontaneous binding between pRBCs and non-pRBCs (Barragan et al., 2000; Deans & Rowe, 2006; Udomsangpetch et al., 1989). Rosetting plays a part in the sequestration of RBCs and the development of severe malaria (Kaul et al., 1991). Rosettes are resistant to pulling forces but less so to shearing forces (Nash et al., 1992).

The pathophysiological effects of malaria parasites on hosts are aided through rosetting by: 1. circumventing the host immune system by protecting the freshly released merozoites and pRBCs from host invasion inhibitory antibodies; 2. ensuring parasite survival by creating an environment that will allow newly released merozoites to quickly infiltrate non-pRBCs (Deans & Rowe, 2006; Wahlgren et al., 2017). Receptor molecules such as blood group B and A sugars, glycophorin A (GYPA), complement receptor 1 (CR1), heparan sulfate (HS)-like molecules, and glycosaminoglycans play a part in rosetting (Barragan et al., 2000; Goel et al., 2015; Goerdeler et al., 2021).

The pathogenesis of severe malaria is aided by RIFINs, which facilitate RBC vascular binding and mediate aggregation and rosette formation (Goel et al., 2015; Sanyal et al., 2012; Tibúrcio et al., 2012). Small rosettes are formed when RIFINs bind glycophorin A on blood group O, while large rosettes are formed when RIFINs bind N-acetylgalactosamine on blood group A (Carlson & Wahlgren, 1992; Goel et al., 2015). Most rif genes have been associated with parasite-mediated rosetting (Rowe & Kyes, 2004). In a study to determine the functions of RIFINs, a semi-quantitative examination revealed that the percentage of PfEMP1 surface-positive pRBCs and RIFIN

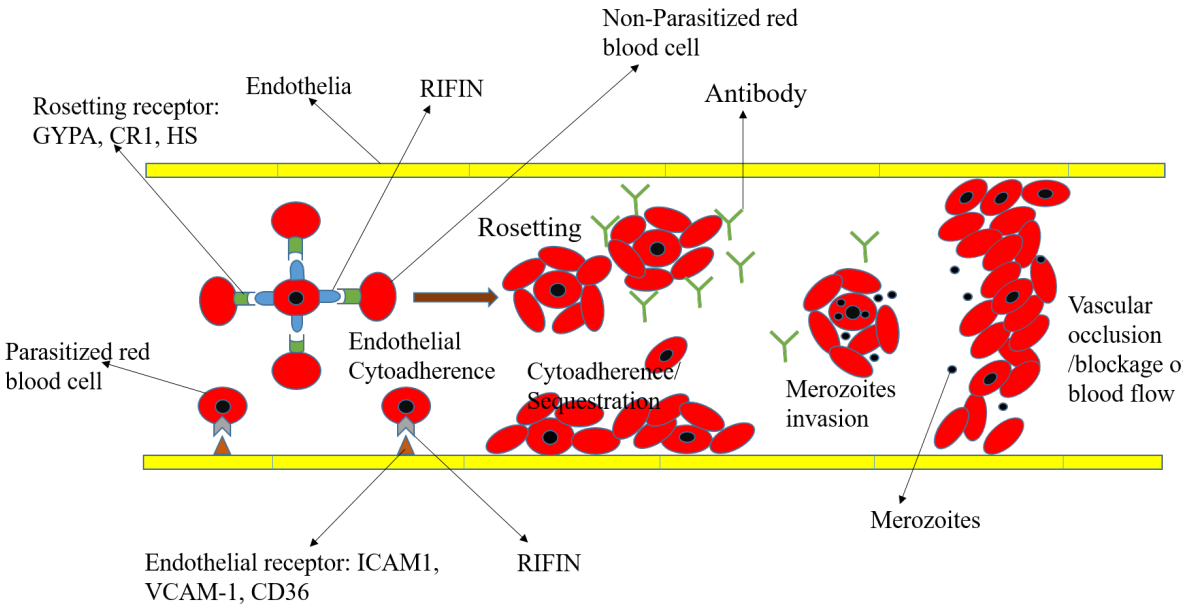

Figure 3 | Cytoadherence and rosetting phenomenon by P. falciparum RIFINs

correlated with the rates of rosetting, and upon selection for rosetting, both RIFINs and PfEMP1 were upregulated (Goel et al., 2015). These findings suggest that, likewise, PfEMP1 and RIFINs have a fundamental function in the development of severe malaria and therefore could be a potential target for therapy. Rosetting leads to the obstruction of the microvasculature and blood flow in various organs and tissues, such as in cerebral malaria (Kaul et al., 1991; Miller et al., 2002). This leads to an oxygen shortage in the tissues, excessive lactate generation, and a drop in blood and tissue pH, all of which can result in respiratory distress, coma, and severe anaemia (Pain et al., 2001). Immunoglobulin M, macroglobulin, albumin, and fibrinogen, which are host serum proteins, also aid in rosetting by either binding directly to the parasite adhesion molecules or by having non-specific effects on erythrocyte aggregation (Semblat et al., 2015).

Severe malaria

Severe malaria is characterized by pathophysiological abnormalities caused by inflammation, vascular endothelial failure, and sequestration of parasites due to high parasitaemia (Cunnington et al., 2013a; Cunnington et al., 2013b). RIFINs have been implicated in severe malaria due to their ability to mediate parasite sequestration through rosetting and cytoadherence (Cunnington et al., 2013b) (Figure 3).

The consecutive expressions of RIFINs found on the surface of pRBCs suggest a crucial role in the survival of the parasite. P. falciparum-pRBCs express RIFINs, which adhere to non-pRBCs and capillary endothelium. pRBCs employ specific RIFINs, which are expressed later in the parasite's lifecycle to adhere to receptors (ICAM1, VCAM-1, CD36) on endothelial cells to facilitate cytoadherence and sequestration to avoid splenic clearance. Rosettes are formed when pRBCs express distinct RIFINs to attach to surface receptors (GYPA, CR1, HS) on non-pRBCs. Rosetting helps with sequestration in vivo by utilizing RIFIN to adhere pRBCs to the endothelial and cause a vascular blockage, worsening of the condition, and pathogenesis of malaria. Cytoadherence and rosetting protect parasites from the host immune response and provide a convenient environment for the freshly released merozoites to enhance efficient and effective evasion.

Most malaria deaths in children are caused by three major syndromes; cerebral malaria, hyperlactatemia or acidosis, and severe anaemia (Cunnington al., 2013b; Phillips et al., 2017; Wassmer & Grau, 2017). RIFINs could interact with the host's immune cells and may cause the blood-brain barrier to break and activate endothelial cells (Pereira et al., 2020). Various rif genes are up-regulated in cerebral malaria, therefore, suggesting that they could play a major in the pathogenesis of cerebral malaria through promoting host cell invasion pathways and mediating immune system evasion in the host (Almelli, 2015; Almelli et al., 2014b).

Cytoadherence

Cytoadherence involves interactions between parasite ligands exported to the surface of pRBCs and the host endothelium receptors (Oquendo et al., 1989). The pRBCs cling to vascular endothelium and sequester in deep vasculature of different organs during the pathophysiology of severe malaria (Heddini et al., 2001; Ho et al., 1990).

The cytoadherence of Plasmodium falciparum pRBCs to host receptors is a major phenomenon in the pathological process of malaria which is linked with millions of deaths each year, especially among children in sub-Saharan Africa (Chen et al., 2000). Cytoadherence offers a survival benefit to the malaria parasites by evading host antibodies and the complement system to avoid the splenic clearance process (Barnwell et al., 1983; Cooke et al., 1995; Ho & White, 1999). The spleen filter and eliminates parasites that are unable to bind to vascular endothelium from the bloodstream (Rasti et al., 2004). Trophozoites and schizonts cytoadhere and sequestrate in deep organs' microvasculature to avoid passage through the spleen (Luse & Miller, 1971). RIFINs play a major role in the cytoadherence/microvascular binding of P. falciparum (Goel et al., 2015; Kyes et al., 1999). RIFINs enhance adherence to the capillary endothelial lining by binding to receptors such as ICAM-1, VCAM-1, CD36 (Fernandez et al., 1999; Rowe et al., 2009). RIFINs embedded in the RBCs' surface membrane mediate the adhesion of pRBCs in several microvascular beds, including the brain leading to cerebral malaria (Wahlgren et al., 2017). Malaria parasites achieve immunological protection by expressing variant RIFINs at different times to cytoadhere or form rosettes leading to microcirculatory blockage, localized hypoxia, metabolic problems and multiple organ failure (Yam et al., 2017a) (Figure 3, table 1).

Evolutionary dynamics and selection pressures

Significant variations in transcription occur during the adaptation of malaria parasites. These modifications are specific to genes implicated in the pathogenicity of malaria parasites. In particular, RIFINs demonstrate variable expression throughout the adaptation process (Chew et al., 2022). Unlike other genes, which exhibit a distinct maximum and minimum apex of expression, no such pattern is observed for RIFINs (Chew et al., 2022), which is consistent with earlier findings (Llinás et al., 2006). The variable region of RIFINs has been shown to be the main target of selection for positive diversification, and most mutation sites are found in the variable region due to high frequencies of amino acid changes in this region, and about 20 haplotypes have been identified in about 53 variable region sequences in RIFINs (Xu et al., 2023). This evolutionary adaptation could be a result of host immune pressures. RIFIN binds to immune receptors to evade the immune system by acting as a ligand for LILRB1, LILRB1, and LAIR1 or as a target for antibodies harboring anomalies. As a result, there are a large number of mutations in the variable region that evade host immune responses. McInerney and associates (2003) demonstrated that some subgroups of the RIFIN family are subject to a variety of selective pressures. Malaria-infected individuals usually develop an antibody response to RIFINs (Abdel-latif et al., 2003), and it is presumed that because they are detected by the host immune system, they are subjected to intense selective pressures. The logic for positive selection in some subgroups of RIFINs may be due to their location on the surface of the cell, their degree of expression, or possibly their marginally distinct role (McInerney et al.,

Table 1. Mechanisms and pathogenesis of *Plasmodium falciparum* RIFINs

RIFIN	Mechanisms	Pathogenesis	Reference
PF3D7_0401400	Immune evasion	Severe malaria	(Saito et al., 2017)
PF3D7_0937500	Immune evasion		(Saito et al., 2017)
PF3D7_0732200	Immune evasion		(Saito et al., 2017)
PF3D7_1479700	Immune evasion		(Saito et al., 2017)
PF3D7_1254400	Immune evasion		(Saito et al., 2017)
PF3D7_0632200	Immune evasion		(Saito et al., 2017)
PF3D7_1480000	Immune evasion		(Saito et al., 2017)
PF3D7_0700200	Immune evasion		(Saito et al., 2017)
PF3D7_0632700	Immune evasion		(Saito et al., 2017)
PF3D7_1100400	Immune evasion		(Saito et al., 2017)
PF3D7_1040700	Immune evasion		(Saito et al., 2017)
PfIT_060035900	Rosette formation		(Ch et al., 2016)
PF3D7_1254800	Immune evasion/rosetting	Severe malaria	(Harrison et al., 2020; Kassegne et al., 2020; Kaur & Hora, 2018; Saito et al., 2017)
PF3D7_0632400	Immunosuppression/evasion	Severe malaria	(Chen et al., 2021; Saito et al., 2017)
PF3D7_0324800	Immune evasion/cytoadherance	Severe malaria	(Almelli, 2015; Chen et al., 2021)
PF3D7_1041000	Immune evasion	Severe malaria	(Chen et al., 2021)
PF3D7_1300700	Immune evasion	Severe malaria	(Chen et al., 2021)
PF3D7_1300600	Transcript in gametocyte stage		(Florens et al., 2002; Mwakalinga et al., 2012; Wang et al., 2010)
PF3D7_0900500	Cytoadherence/transcript in gametocyte stage		(Florens et al., 2002)
PF3D7_0100400	Immune evasion/rosetting		(Goel et al., 2015; Saito et al., 2017)
PF3D7_0110100	Cytoadherence		(Goel et al., 2015)
PF3D7_0223100	Immune evasion	Severe malaria	(Kassegne et al., 2020; Saito et al., 2017)
PF3D7_1400600	Cytoadherence	Cerebral malaria	(Hebert et al., 2007; Kassegne et al., 2020; Xu et al., 2021)
PF3D7_1040300	Immunosuppression		(Xu et al., 2021)
PF3D7_0401200	Immunosuppression		(Xu et al., 2021)
PF3D7_1000600	Cytoadherence/rosetting		(Hebert et al., 2007; Kassegne et al., 2020)
PF3D7_0713000	Rosetting/cytoadherence		(Kassegne et al., 2020)
PF3D7_0632100	Rosetting/cytoadherence		(Kassegne et al., 2020)
PF3D7_1040900	Rosetting/cytoadherence		(Kassegne et al., 2020)
PF3D7_1300400	Immune evasion/rosetting/cytoadherence	Cerebral malaria, Severe malaria anaemia	(Almelli et al., 2014b; Kassegne et al., 2020; Lawton et al., 2022; Saito et al., 2017)
PF3D7_1254700	Rosetting/cytoadherence		(Kassegne et al., 2020)
PF3D7_1150300	Rosetting/cytoadherence	Cerebral malaria, severe malaria anaemia	(Almelli et al., 2014b; Kassegne et al., 2020; Lawton et al., 2022)
PF3D7_0808800	Rosetting/cytoadherence		(Kassegne et al., 2020)
PF3D7_0114700	Rosetting/cytoadherence	Severe malaria anaemia	(Kassegne et al., 2020; Lawton et al., 2022)
PF3D7_1101300	Rosetting/cytoadherence		(Kassegne et al., 2020)
PF3D7_1100300	Rosetting/cytoadherence	Cerebral malaria, severe malaria anaemia	(Almelli et al., 2014b; Kassegne et al., 2020)
PF3D7_0401300	Rosetting/cytoadherence		(Kassegne et al., 2020)
PF3D7_1101100	Immune evasion/cytoadherence		(Hebert et al., 2007; Saito et al., 2017)
PF3D7_0300200	Cytoadherence		(Hebert et al., 2007)
PF3D7_0631800	Cytoadherence		(Hebert et al., 2007)
PF3D7_1200500	Cytoadherence		(Hebert et al., 2007)
PF3D7_0500500	Cytoadherence		(Hebert et al., 2007)
PF3D7_0324800	Cytoadherence		(Hebert et al., 2007)
PF3D7_0114700	Cytoadherence		(Hebert et al., 2007)
PF3D7_0425700		Cerebral malaria	(Almelli et al., 2014b)
PF3D7_0401600		Cerebral malaria	(Almelli, 2015)
PF3D7_0101000	Immune evasion/Cytoadherence		(Hebert et al., 2007; Saito et al., 2017)
PF3D7_0100900		Cerebral malaria	(Almelli, 2015)
PF3D7_0324500		Cerebral malaria	(Almelli, 2015)
PF3D7_0900200	Immune evasion/Cytoadherence		(Hebert et al., 2007; Saito et al., 2017)
PF3D7_1400600	Cytoadherence		(Hebert et al., 2007)
PF3D7_0400300	Cytoadherence		(Hebert et al., 2007)
PF3D7_0400700	Cytoadherence		(Hebert et al., 2007)
PF3D7_0600300	Immune evasion		(Almelli, 2015; Saito et al., 2017)
PF3D7_0600800		Cerebral malaria	(Almelli, 2015)
PF3D7_0100200	Immune evasion		(Kassegne et al., 2020; Saito et al., 2017)
PF3D7_1000200	Immune evasion		(Saito et al., 2017)

2003). Positive selection will be evident only if the adaptive advantage offered by the mutation is adequate to overcome the random drift of genes (McInerney et al., 2003). Therefore, a protein will not be under the same selective pressure as one that is produced more frequently and/or has the ability to trigger a robust adaptive immunological response if it is only infrequently expressed or if it does not stimulate an intense antibody reaction. In a study to determine the natural selection and genetic diversity in the global populations of Plasmodium falciparum, Xu and colleagues showed that RIFINs from Ghana-imported cases had the highest level of genetic diversity among cases from Thailand, Cambodia, Myanmar, Vietnam, Mali, and Senegal (Xu et al., 2023), which indicates that climate affects RIFINs as well as the parasite life cycle and transmission. This identifies significant differences in RIFIN patterns that may have significant effects on the development of RIFIN-based vaccines.

Therapeutic Implications

RIFINs are a family of surface-exposed, antigenically varied proteins that play a role in rosetting and immune evasion. Immune evasion is a key survival mechanism for pRBCs, and it offers surface-exposed molecules involved in this process the potential to be targets for drugs or vaccines. Rosetting is a mechanism that plays a role in the pathophysiology of severe malaria (Carlson et al., 1990; Kaul et al., 1991; Rowe et al., 1995; Wahlgren et al., 1992), and molecules that prevent the occurrence of rosetting could be produced primarily for use in treating the disease. The ability of RIFINs to form rosettes independently of PfEMP1 (Yam et al., 2017b) makes them potential candidates for therapy. There may be a protective role for anti-RIFIN antibodies due to their correlation with both long-term persistence and quick parasite clearance in the sera of malaria-affected patients (Abdel-latif et al., 2003). These results collectively demonstrate the potential of RIFIN proteins as therapeutic targets and vaccination candidates.

Future Perspectives

A thorough understanding of the roles played by RIFINs is required to fully grasp the relationship between antigenic variation on the surface of pRBCs and parasite-induced diseases. Comprehensive transcriptome and proteomic analysis would be necessary for target identification since RIFINs exhibit variable expression, clonal variation, and antigenic switching. Furthermore, there is a dearth of structural and functional information on individual members of the RIFIN family. Members of the RIFIN family, along with PfEMP1, appear to be desirable additions to multistage and multisubunit vaccines due to their multistage transcription and roles in immune escape. To produce widely reactive antibodies against these RIFINs, it is critical to either find a highly immunogenic component expressed at all stages of parasite development or develop a common sequence that utilizes surface-exposed conserved epitomic sections of RIFINs.

Abbreviations

PfEMP1	Plasmodium falciparum erythrocyte membrane protein 1
RIFIN	Repetitive interspersed family proteins
STEVOR	Subtelomeric variable open reading frame proteins
PRBCs	Parasitized red blood cells
DC8	Domain cassette 8
DC13	Domain Cassette 13
CD36	Cluster of differentiation 36 receptor
ICAM-1	Intercelullar adhesion molecule 1
EPCR	Endothelial protein C receptor
CSA	Chondroitin sulfate A
MCs	Maurer's clefts
PEXEL	Plasmodium export element
VTS	Vacuolar transport signal
TM	Transmembrane
VSA	Variable surface antigen
GYPA	Glycophorin A
VCAM-1	Vascular cell adhesion molecule 1
CR1	Complement receptor 1
HS	Heparan sulfate
RBC	Red blood cell
SURFIN	Surface-associated interspersed gene family

Author contributions

AKK developed the idea. PED, JA, YKO, and AKK wrote and reviewed the manuscript. AKK and PED designed the figures. All authors read and approved the final version of the manuscript.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflict of interest.

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