Molecules in focus

**PfEMP1: An antigen that plays a key role in the pathogenicity and immune evasion of the malaria parasite *Plasmodium falciparum***

Noa D. Pasternak, Ron Dzikowski*

Department of Microbiology and Molecular Genetics, The Institute for Medical Research Israel - Canada, The Kuvin Center for the Study of Infectious and Tropical Diseases, The Hebrew University-Hadassah Medical School, Jerusalem 91200, Israel

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**ABSTRACT**

The deadliest form of human malaria is caused by the protozoan parasite *Plasmodium falciparum* affecting millions worldwide every year. *P. falciparum* virulence is attributed to its ability to evade the human immune system by modifying infected host red blood cells to adhere to the vascular endothelium and to undergo antigenic variation. The main antigenic ligands responsible for both cytoadherence and antigenic variation are members of the *P. falciparum* Erythrocyte Membrane Protein-1 (PfEMP1) family. These polymorphic proteins are encoded by a multi-copy gene family called var. Each individual parasite expresses a single *var* gene at a time, maintaining the remaining ~60 *var* genes found in its genome in a transcriptionally silent state. As the antibody response against the single expressed PfEMP1 develops, small sub-populations of parasites switch expression to alternative forms of PfEMP1 and re-establish the infection. Therefore, PfEMP1 is considered a key player in the pathogenicity of *P. falciparum*.

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1. **Introduction**

Malaria is one of the major infectious diseases influencing human kind today. Each year 300–600 million people worldwide are infected with malaria parasites. *Plasmodium falciparum* is the Apicomplexan parasite responsible for the deadliest form of human malaria causing 1–3 million deaths a year, primarily of young African children (Snow et al., 2005). *P. falciparum* is transmitted by Anopheles mosquitoes and replicates within the circulating red blood cells of an infected individual. The virulence of *P. falciparum* is attributed to the parasites’ ability to modify the erythrocyte surface to adhere to and evade the host immune attack. The major antigenic ligand found to be responsible for the cytaadhesive properties of the infected red blood cells (iRBC) are members of the *P. falciparum* Erythrocyte Membrane Protein-1 (PfEMP1) family. These antigenically variable proteins are placed on knob like structures on the surface of the iRBC and bind to different host vascular adhesins, including CD36, ICAM1, VCAM1, and CSA (Kraemer and Smith, 2006). Different binding phenotypes cause sequestration in different organs and contribute to life threatening manifestations of the disease. PfEMP1 is also a major surface antigen that mediates antibody-dependent immune response. This response often clears the majority of infected cells from the circulation. However, small sub-populations of parasites switch expression to a different PfEMP1 avoid the antibody response and re-establish infection (Smith et al., 1995). This process is referred to as antigenic variation and is responsible for the persistent nature of the disease, as well as the waves of parasitemia, typical for *P. falciparum* infections (Miller et al., 1994). The extreme antigenic variability and the great breadth in adhesive phenotypes within the PfEMP1 repertoire make it the principal virulence factor of malaria.

2. **PfEMP1 structure**

PfEMP1s are large proteins, ranging in size between 200 and 350 kDa. All PfEMP1s share some conservation in their main structural features: an N terminal segment (NTS); variable numbers of Duffy Binding Like domains (DBL; α–ε); one or two cysteine-rich interdomain regions (CIDR; α–γ); a trans-membrane (TM) domain; a C2 domain; and a conserved intra-cellular acidic terminal segment (ATS) (Kraemer and Smith, 2006). Despite these similarities in their basic architecture, a comparison of the amino acid sequences of PfEMP1 paralogs revealed extreme sequence diversity among different parasite isolates. These sequence diversities are mainly in the DBL extracellular adhesion domains that are exposed to constant selection pressure by the human immune response. This high level of sequence diversity is believed to be created through recombination that generates new combinations of the proteins’ structural domains. Thus, in natural parasite populations there is virtually...
unlimited potential for antigenic variation within their PfEMP1 repertoire (Kyes et al., 2007).

PfEMP1 is expressed by late erythrocytic stage parasites with an association to the presence of knobs on the surface of iRBCs (Horrocks et al., 2005). The cytoplasmic tail of PfEMP1 anchors to one of the proteins composing the cytoplasmic scaffold of knobs known as the Knob Associated Histidine Rich Proteins (KAHRPs) (Waller et al., 2002). Although the mechanisms by which PfEMP1 is trafficked and anchored to the surface of the iRBC is still elusive, major advances in our knowledge have been made recently (Charpian and Przyborski, 2008). The translocation of PfEMP1 through the parasitophorous membrane is thought to be mediated by a conserved motif that shares features with Plasmodium export element (PEXEL/ VTS), a pentameric motif found in Plasmodium exported proteins. Once in the RBCs' cytosol, PfEMP1 is directed to parasite derived protein sorting site referred as Maurer's cleft (MC) and then trafficked through the clefts to the iRBCs' surface (Haeggström et al., 2007). This process was shown to involve a novel Skeleton Binding Protein 1 (SBP1) (Cooke et al., 2006; Maier et al., 2007). Recently, an extensive knock out project of all the genes that were predicted to have the PEXEL motif has identified eight additional proteins participating in the export of PfEMP1 from the parasite to the cell membrane of the iRBC (Maier et al., 2008).

3. Biological function of PfEMP1

Following invasion into the RBC the parasite “takes over” the biology of the host cell, changing its morphology and modifying it to adhere. PfEMP1 was shown to be a key molecule in defining the cytoadhesive properties of the iRBC to neighboring uninfected RBCs, forming structures called rosettes, as well as binding to several endothelial receptors. The cytoadhesive properties induced by PfEMP1 allow the iRBC to sequester in deep micro-vessels by binding to blood vessel walls thus enabling the parasite to complete its cell cycle, multiply and re-invade without being removed by the spleen. The variable extracellular domains of PfEMP1 (CIDR and DBL) have the ability to bind a variety of host receptors including cluster determinant 36 (CD36), intercellular adhesion molecule 1 (ICAM1), thrombospondin (TSP), complement receptor 1 (CR1), and chondroitin sulfate A (CSA) (Kraemer and Smith, 2006). Specific subsets of PfEMP1 bind specific host receptors based on their unique DBLs and CIDR components. Different binding phenotypes have been associated with specific disease pathologies, thus, the cytoadhesive properties of PfEMP1 contribute to most severe acute clinical pathologies of the disease. Interestingly, hemoglobin mutations may confer resistance to the severe manifestations of the disease. Infected erythrocytes carrying hemoglobin C (HbC) were shown to display abnormal knob formation and reduced PfEMP1 on their surface and as a result exhibited a decrease in their ability to form rosettes and bind CD36 and ICAM1 (Fairhurst et al., 2005). In addition to its role in cytoadherence, PfEMP1 is also the major antigen exposed by the parasite on the surface of the iRBC. Its great antigenic diversity and unique regulation of switching between different antigenic variants of PfEMP1 enable the parasite to evade immune attack and to maintain long-term chronic infections (Fig. 1).

4. Regulation of PfEMP1 expression

The regulation of PfEMP1 was recently comprehensively reviewed by (Scherf et al., 2008). The completion of P. falciparum genome sequence revealed that different forms of PfEMP1 are encoded by different members of a multi-copy gene family named var. About 60 var genes are found in the P. falciparum genome located mostly in subtelomeric regions but also in central regions of the chromosomes. They can be divided into few subclasses based on their 5' UTR sequence alignment. All var genes share a similar structure—a long first exon encoding the variable extracellular domain of PfEMP1, a well-conserved intron and a short second exon coding for an acid terminal segment that anchors the protein into the cytoskeleton of the host erythrocyte membrane. Within each var locus there are two defined promoters: (1) the var promoter upstream of the open reading frame responsible for production of the mRNA and (2) a promoter within the intron driving production of non-coding “sterile” transcripts.

To avoid unnecessary exposure of its antigenic repertoire, each parasite expresses only a single var gene at a time, a phenomenon known from other eukaryotic organisms as “allelic exclusion”. Immune evasion through antigenic variation in malaria therefore depends on the ability of the parasite to express only a single var gene at a time, and then to switch expression at a very low rate to another gene that will also be expressed in a mutually exclusive manner. These changes appear to be tightly regulated at the transcription level in an epigenetically controlled process. var gene regulation involves Promoter–promoter interaction, chromatin remodeling, and sub-nuclear localization. Pairing between a var promoter and the promoter found within the intron of each var gene is required for var gene silencing and mutually exclusive expression. A var promoter that is unpaired with the adjacent intronic promoter is constitutively active and is not recognized by the mechanism that controls mutually exclusive expression. This may indicate that the sterile transcripts driven by the intron promoter possess a functional role in var gene regulation; however, this hypothesis still awaits proof. The production of a functional protein was shown to have a role in maintaining allelic exclusion in other eukaryotic systems by initiating a negative feedback cascade that blocks the transcription of the rest of the gene family. However, in P. falciparum the proper regulation of the var gene family depends only on the non-coding regions surrounding each gene and PfEMP1 production is not necessary for their silencing and mutually exclusive expression. Epigenetic regulation of var gene expression also involves alterations in chromatin structure, in particular the modulation of histone tail acetylation and methylation. P. falciparum possesses a homolog to the yeast histone deacetylase SIR2 protein (PSIR2) that is involved in specifying the assembly of a silent chromatin structure by binding to regions that are transcriptionally repressed and seems to affect silencing of a subset of subtelomeric var genes. In addition, “histone code” epigenetic marks similar to those defined in other eukaryotic systems are involved in chromatin memory in P. falciparum, thus ensuring the expression of the same PfEMP1 variant over many generations. Ultra-structural studies into the P. falciparum nucleus revealed a distinct euchromatic domain within the peripheral heterochromatin rich region. var genes move to a specific spot within the nucleus upon changes in their activation state suggesting a model of regulation through an exclusive nuclear expression site. Indeed, it has been recently demonstrated that such a var specific expression site might exist in the P. falciparum nucleus, however, it is not restricted to accommodation of a single active var promoter at a time. Activation of large numbers of episomal var promoters simultaneously silenced all endogenous var genes indicating that a limiting factor that is required for var gene activation exists within the parasite nucleus (Dzikowski and Deitsch, 2008).

5. Clinical significance

PfEMP1 mediated adherence of iRBCs to human endothelial receptors is associated with the most severe forms of the dis-
Fig. 1. The immune-dominant PfEMP1 mediate adhesion of the infected RBC. PfEMP1 is expressed by the malaria parasite *P. falciparum* on the knobs formed on the surface of infected erythrocytes. The variable extracellular regions DBLs and CIDR mediate adhesion through binding to several endothelial receptors such as CD36, ICAM1 and CSA. In addition, PfEMP1 mediate adhesion to uninfected erythrocytes forming rosettes. (PV, parasitophorous vacuole; MC, Maurer’s cleft).

ease such as cerebral and pregnancy-associated malaria (Kraemer and Smith, 2006). Severe malaria has been correlated with specific expression of a subset of PfEMP1 that was also correlated with tissue specific binding in the heart and brains of people dying from malaria (Montgomery et al., 2007). Sequestration of iRBCs in the brain post-capillary venules that leads to cerebral malaria is associated with specific binding to ICAM1 by a subset of PfEMP1 forms that contain a DBLβ domain. RBCs infected with most parasite isolates can bind CD36, which is abundant in the liver, lungs, kidney and muscle (Baruch et al., 2002). Binding to CD36 was also found to be synergistic to ICAM1 binding (Yipp et al., 2000) and therefore might enhance cerebral malaria. The other severe pathology of the disease, known as pregnancy-associated malaria (PAM), is caused by sequestration of iRBCs in the intervillous blood spaces of the placenta through binding the primary placental receptor chondroitin sulfate A (CSA). This often leads to premature delivery, mortality of the fetus and severe anemia in the mother. A single PfEMP1 encoded by a distinct var gene (var2csa) that is highly expressed by placental parasite isolates was found to bind specifically to CSA through a distinct DBLβ domain (Nunes and Scherf, 2007). PAM is a syndrome that affects only primigravid women and after the first exposure they develop an effective antibody response against placental binding parasites. This acquired immunity is attributed to the acquisition of antibodies that recognize the highly conserved regions of var2csa among parasite isolates (Rogerson et al., 2007). In view of high variability of the rest of the var gene family among parasite isolates, the PfEMP1 encoded by var2csa is an exceptionally promising candidate for syndrome specific vaccine development against PAM.

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