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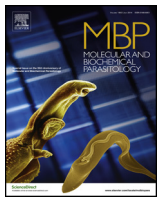

Review

The role of PfEMP1 adhesion domain classification in *Plasmodium falciparum* pathogenesis research

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ABSTRACT

The *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) family has a key role in parasite survival, transmission, and virulence. PfEMP1 are exported to the erythrocyte membrane and mediate binding of infected erythrocytes to the endothelial lining of blood vessels. This process aids parasite survival by avoiding spleen-dependent killing mechanisms, but it is associated with adhesion-based disease complications. Switching between PfEMP1 proteins enables parasites to evade host immunity and modifies parasite tropism for different microvascular beds. The PfEMP1 protein family is one of the most diverse adhesion modules in nature. This review covers PfEMP1 adhesion domain classification and the significant role it is playing in deciphering and deconvoluting *P. falciparum* cytoadhesion and disease.

Contents

1. Introduction

Cytoadhesion of *Plasmodium falciparum* infected erythrocytes (IE) is a major virulence determinant associated with pathological complications from IE binding to the endothelial lining of blood vessels [1]. Although this deadly parasite adhesion trait has been recognized for over a century [2], the molecular interactions involved in parasite binding in brain and other microvasculature are only partially understood. This deficiency exists in part because of the complexity of the *var* gene/*P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family that mediates endothelial binding [3]. Each parasite genotype encodes approximately 60 *var* gene copies and there is limited overlap of *var* repertoires between parasite genotypes [4–6]. Switching between *var* genes modifies the antigenic and binding properties of IEs, and orchestrates parasite binding tropism for placenta [7] and possibly other microvascular sites [8].

PfEMP1 proteins evolve under opposing binding and antibody selection pressures. This has resulted in extensive diversification of PfEMP1 adhesion domains. Within the protein family, some binding properties are common to many PfEMP1 [9], while others are rare or may have evolved to exploit specialized microvascular niches (e.g. placental binding) [7,10]. A major issue for pathogenesis research is whether specific PfEMP1–host receptor interactions are involved in severe malaria and, if so, whether there are common pathogenic mechanisms that could be targeted for intervention. This review covers the introduction of a system of PfEMP1 adhesion domain classification [11] and its application to malaria disease research.

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2. PfEMP1 adhesion domain classification

At the time of their discovery [12–14], a significant clue into PfEMP1 binding function was that they encode a recognizable binding module from *Plasmodium* erythrocyte invasion ligands, called the Duffy binding-like (DBL) domain [15,16]. This homology showed the PfEMP1 ectodomain contained multiple DBL domains and a new domain termed the cysteine-rich interdomain region (CIDR) [14]. Early sequence comparisons indicated that individual PfEMP1 domains maintained less than 50% amino acid identity and were much more divergent than DBL domains in erythrocyte invasion ligands [12–14]. The variability in PfEMP1 size and sequence suggested a potential explanation for parasite binding differences [17], but given the massive sequence diversity in the PfEMP1 family it was unknown if PfEMP1 binding was predictable or if there would be any disease binding patterns.

To investigate PfEMP1 structure and function, we used phylogenetic criteria to classify adhesion domains into different sequence types [11]. This analysis was performed on the first 20 *var* gene sequences in Genbank. It showed that DBL domains could be classified into four major types (α, β, γ, and δ) and CIDR domains into three major types (α, β, and γ). It also revealed higher domain organization in PfEMP1 proteins. Small PfEMP1 contained four extracellular domains; a DBLα-CIDRα tandem followed by a DBLδ-CIDRβ/γ tandem (Fig. 1). Large PfEMP1 proteins contained the same DBL-CIDR tandems, but had additional DBL domain types (β or γ) domains inserted before or after the C-terminal tandem (Fig. 1). The N-terminal DBL-CIDR tandem is the most conserved extracellular region and is referred to as the semi-conserved protein head structure [14]. Within a given *var* repertoire head structures maintain less than 50% amino acid identity highlighting the extensive diversification within the family [5].

At the time of this adhesion domain classification, the CIDR domain in the semi-conserved head structure had already been shown to bind CD36 [18,19] and ICAM1 binding had been mapped to a DBLβ domain [20] (Fig. 2). However, it was not known what proportion of PfEMP1 variants encoded CD36 or ICAM1 binding activity or if binding was predictable. Notably, one of first 20 PfEMP1 proteins had a distinct protein head structure; a DBLα-CIDRγ tandem instead of the more characteristic DBLα-CIDRα tandem. This unusual DBLα-CIDRγ head structure was known to mediate "rosetting", or the binding of IEs with uninfected red blood cells [21], but it was not known if it bound CD36. In addition, DBLβ domains were restricted to large PfEMP1 (Fig. 1). Together, these findings raised the questions whether small and large PfEMP1 encoded distinct binding properties and if adhesion domain classification could help predict PfEMP1 binding properties [11].

The initial observations on PfEMP1 architecture have largely held up [4–6]. While the number of DBL (α, β, γ, δ, ε and ζ) and CIDR (α, β, γ, δ, and PAM) sequence classes have slightly increased as more proteins have been analyzed, the same higher order domain organizations have been identified in geographically diverse parasites [6]. Although *var* repertoires are highly divergent, the majority of PfEMP1 are classified into three main groups on the basis of upstream sequence (ups) and chromosome location (Fig. 2) [22]. Group A (upsA) and group B (upsB) are present in subtelomeric regions and transcribed in opposite orientations. Group C (either upsC or upsB) are found in central chromosome regions. The *var* repertoire also contains three unusual strain transcendent variants (var1CSA, var2CSA, and type 3 *var*). Subsequent sequence comparisons have also led to further sub-classification of domains (e.g. CIDRα1.1) and the identification of PfEMP1 domain cassettes (DC), or tandem arrangements of two or more domains of particular subclasses (e.g. DC8) (Fig. 3) [6]. The fact that DCs are discernable despite extensive gene recombination in the *var* gene family

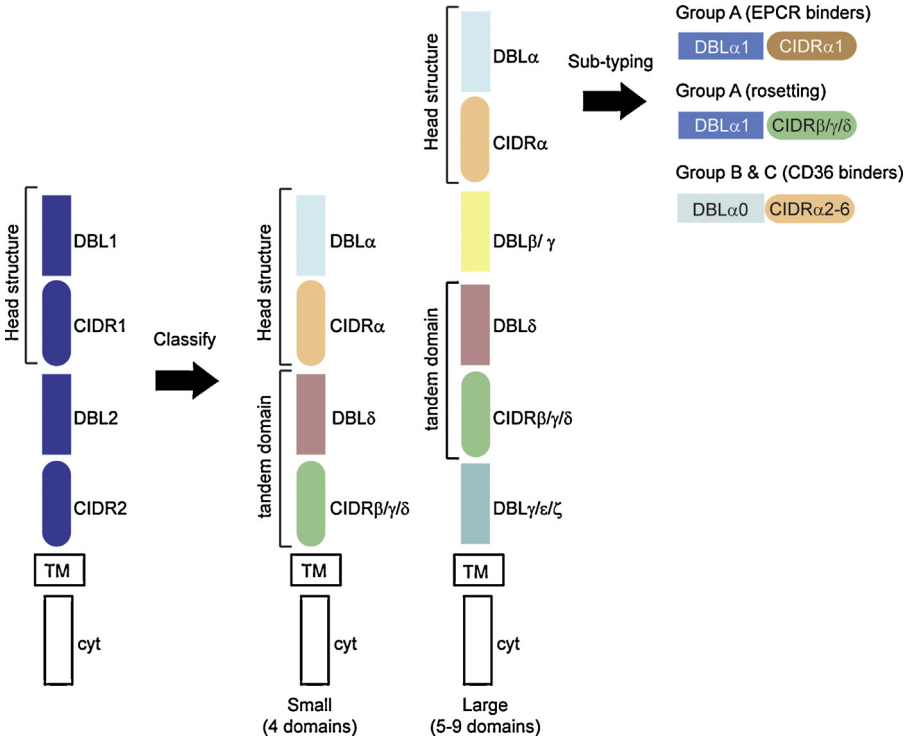

Fig. 1. Adhesion domain classification of PfEMP1 proteins. The blue PfEMP1 shows a typical arrangement of PfEMP1 domains. The first arrow indicates how adhesion domain classification reveals higher domain organization in PfEMP1. Specific DBL and CIDR domain types form preferential tandem domain arrangements (DBLα-CIDRα and DBLδ-CIDRβ/γ/δ). The same tandem arrangements are present in small (4-domain) and large (5 or more domain) PfEMP1, but large proteins contain unique DBL subtypes (β or γ) that are not present in small proteins. The second arrow indicates that further sub-classification of adhesion domains (e.g. CIDRα1) distinguishes three different PfEMP1 head structure types and functional differences between group A, B, and C proteins. TM is transmembrane, cyt is cytoplasmic tail.