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Review

Disguising itself—insights into *Plasmodium falciparum* binding and immune evasion from the DBL crystal structure

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Abstract

Duffy-binding like (DBL) domains are common to two different families of malaria proteins that are involved in parasite invasion of erythrocytes or cytoadhesion of infected erythrocytes. DBL domain crystal structures have recently been solved for two different erythrocyte binding ligands, EBA-175 and the *Plasmodium knowlesi* α Duffy binding protein. These structures reveal different mechanisms for DBL binding and erythrocyte invasion. This review summarizes recent work on DBL domain binding and immune evasion and proposes a new structural model for how these domains adapted to intense antibody surveillance at the infected erythrocyte surface.
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Keywords: Malaria; Antigenic variation; Cytoadherence; PfEMP1; *var*; DBL; EBL

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1. Introduction

During the blood stage of infection, *Plasmodium falciparum* merozoites invade erythrocytes and infected erythrocytes sequester from blood circulation by binding host endothelium [1]. Erythrocyte invasion and infected erythrocyte cytoadhesion require the binding function of a common adhesion module found in two distinct families of parasite ligands, the Erythrocyte Binding Ligand (EBL) family of erythrocyte invasion ligands and the *P. falciparum* Erythrocyte Membrane Protein 1 (PfEMP1) family of cytoadherence ligands found on the surface of infected erythrocytes. This adhesive domain, called the Duffy-binding like (DBL) domain, was first described as part of the Duffy Binding Protein (DBP), an important invasion ligand of *P. vivax* and *P. knowlesi* for erythrocytes [2]. As both invasion and cytoadherence are mechanisms contributing to parasite virulence, it is important to understand how this shared domain functions in the context of two different protein families. Moreover, as one of the most versatile and polymorphic adhesive modules in nature, knowledge about how DBL domains function as immunoevasive binding ligands can aid the development of vaccine or receptor blockade interventions for malaria. This

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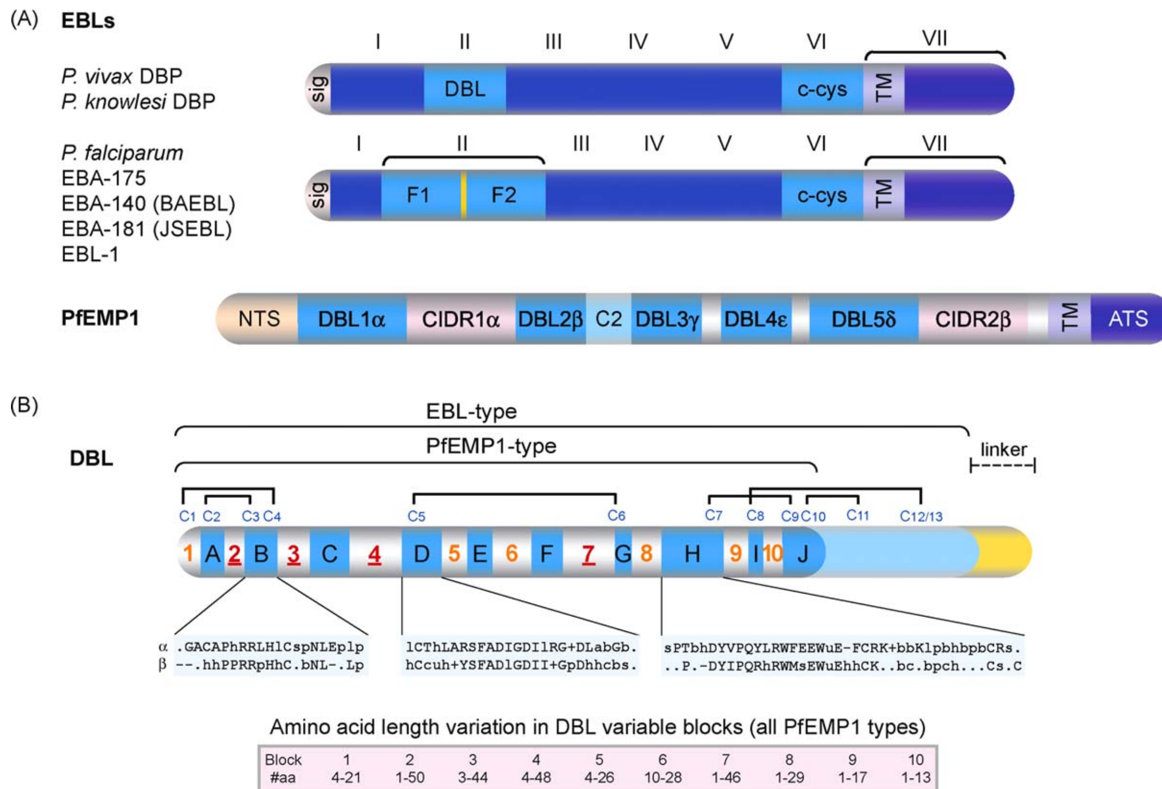
review outlines studies of the EBL family of invasion ligands and the PfEMP1 family of cytoadhesion ligands, as well as discusses how the recently published crystal structures of the DBL domains from two well studied EBL ligands, *P. knowlesi* DBP (PkDBP) and *P. falciparum* EBA-175 [3,4], can help address these questions.

2. DBL domains in EBL invasion ligands

Merozoite invasion into red blood cells is a complicated process, but can be described as four steps: attachment, reorientation, junction formation, and entry [2,5]. While the initial attachment involves merozoite surface proteins, the subsequent events of reorientation, junction formation, and invasion require the contents release of three intracellular organelles: micronemes, rhoptries, and dense granules [2]. During these invasion processes, parasite proteins are proteolyzed and released from the merozoite surface, a property that has been exploited to identify or discover new invasion ligands [6].

Among the shed proteins are members of the EBL protein family, which localize to micronemes, but are believed to be exported to the merozoite surface during invasion [7]. *P. vivax* has a single known EBL protein, PvDBP, which binds the Duffy blood group antigen/receptor for chemokines (DARC) on the surface of erythrocytes [8,9]. Because this interaction is required for invasion by *P. vivax* merozoites [10], malaria infection due to this species is notably low in West Africa due to the corresponding infrequency of DARC in the population [2]. The *P. knowlesi* DBP has been shown to be essential for junction formation [15,16], and it is assumed that EBLs in *P. falciparum* and other species have analogous functions, although this has not yet been formally demonstrated. *P. falciparum* has multiple pathways of erythrocyte invasion and a repertoire of several EBLs, including EBA-175, BAEBL (EBA-140), JSEBL (EBA-181), and EBL-1 [11–13]. A fifth EBL, PEBL, is a pseudogene in the 3D7 strain [14]. Individual *P. falciparum* parasite isolates are capable of expressing these four *ebl* genes simultaneously [17]. Of these, EBA-175 has been argued to have the most important role in invasion, although genetic disruptions of individual genes (EBA-175, BAEBL or JSEBL) indicate that none is absolutely required for parasite invasion, suggesting these proteins operate through parallel and redundant pathways of invasion [2,18–22].



Amino acid length variation in DBL variable blocks (all PfEMP1 types)

Block	1	2	3	4	5	6	7	8	9	10
#aa	4-21	1-50	3-44	4-48	4-26	10-28	1-46	1-29	1-17	1-13

Fig. 1. Domain architectures of EBL and PfEMP1 proteins. (A) The EBL family of proteins has a related protein structure that contains one or two DBL domains, termed F1 and F2. The multi-domain structure of PfEMP1 proteins is composed of DBL, CIDR, and C2 domains, as well as an N-terminal segment (NTS), transmembrane domain, and acidic terminal segment (ATS). c-cys, C-terminal cysteine-rich domain; sig, signal sequence; TM, transmembrane domain. (B) DBL domains contain 10 semi-conserved (A–J) and variable blocks (1–10). Variable blocks, labeled with red, underlined numbers possess greater length variation. The table shows the extent of amino acid length variation at variable loops between all PfEMP1-type DBL sequences. Variability differs between different types, with DBLδ sequences being especially variable in length [43]. The 80% consensus sequences of the most conserved DBL blocks (B, D, and H) are shown for the DBL α and β sequence types [43]. Conserved residues are identified with capital letters representing the amino acid. A period (.) represents sequence and/or length polymorphism among members of a type, while a dash (—) indicates polymorphism between different types. Residues with similar character are coded as follows: c, charge (D, E, H, K, R); +, positive (H, K, R); h, hydrophobic (A, C, F, I, L, M, V, W, Y); p, polar (C, D, E, H, K, N, Q, R, S, T); s, small (A, C, D, G, N, P, S, T, V); u, tiny (A, G, S); b, big (E, K, R, I, L, N, S, Y, W). DBL domains have conserved cysteine residues numbered C1 to C13. The disulfide bond structure of EBL-type DBL domains is shown [3,4]. The homology between EBL and PfEMP1-type DBL domains ends shortly after the tenth cysteine.

The EBL protein architecture is divided into six regions that include two cysteine-rich motifs called region II (RII) and region VI (Fig. 1) [9]. RII in PvDBP and PkDBP is responsible for binding directly to DARC on erythrocytes, thus it is called the Duffy binding domain [23]. In *P. falciparum* EBLs, RII consists of two cysteine-rich regions, F1 and F2, which are homologous in sequence to the Duffy binding domain of PvDBP/PkDBP, and therefore called Duffy binding-like (DBL) domains [11]. The tandem DBL domains in RII of EBA-175, BAEBL, and JSEBL have also been shown to encode the direct erythrocyte binding activity of the proteins, but bind different sialoglycoproteins on the erythrocyte surface [24–26].

The only receptor known for EBA-175 is glycophorin A [24], but the receptors for BAEBL and JSEBL are only partly characterized. Some reports suggest that BAEBL solely binds glycophorin C [21,29], but others conclude it can bind to other unknown receptors as well [26,30]. For JSEBL, the various erythrocyte receptors have not been identified [2]. An additional level of complexity in the binding model is that single amino acid variation in BAEBL (mapping primarily to the F1 DBL domain) and JSEBL (mapping either to the F1 or F2 DBL domains) impacts their ability to bind [25,26]. In contrast, allelic differences in the minimal binding region of EBA-175, which is localized to the F2 domain, do not change its in vitro binding characteristics [11,31]. It is not clear whether amino acid polymorphism in JSEBL and BAEBL changes only their affinity for sialic acid in certain sialoglycoproteins or whether it completely changes their binding specificity to new erythrocyte receptors, including sialic acid independent pathways [25,26]. The clinical significance of the BAEBL and JSEBL sequence polymorphism is unknown, but may be used by the parasite to circumvent mutations in host receptors.

The evidence that amino acid differences in the F1 DBL domain of BAEBL impact its binding [25] suggests either that BAEBL and EBA-175 have different binding regions (F1 versus F2), or alternatively that changes in one DBL domain (F1) impact the entire RII or binding region. Because EBA-175, JSEBL, and BAEBL all have a sialic acid-dependent component to their binding, it is conceivable that they could have a related binding pocket. Therefore, knowledge about the EBL binding pocket may suggest drug intervention strategies that block one or potentially multiple pathways of EBL-mediated invasion at a time. Antibodies to specific EBLs have been able to reduce parasite invasion, but they do not abolish it [32,33]. Together, these results suggest that multiple antigens from *ebl* and/or other invasion ligand families will need to be combined within a vaccine to provide broadly neutralizing protection against *P. falciparum* infection.

3. DBL domains in infected erythrocyte cytoadhesion ligands

After invading erythrocytes, all *Plasmodium* species appear to export clonally variant antigens to the infected erythrocyte surface, but the gene families responsible for antigenic variation differ between species [34–36]. *P. falciparum* and its closest relative, the chimpanzee malaria *P. reichenowi* [37], are the only known *Plasmodium* species to encode variant antigen families that have DBL domains (*P. reichenowi* Genome Project, The Wellcome Trust Sanger Center) [38–40]. In *P. falciparum*, this family consists of *var* genes that encode PfEMP1 antigens. *P. falciparum* and *P. reichenowi* diverged approximately 6–7 million years ago, suggesting that *var* genes/PfEMP1 proteins originated in this branch of the lineage and evolved from *ebl* genes, which are common to all *Plasmodium* species.

From the perspective of human disease, the emergence of *var* genes was an important development for *P. falciparum* virulence. *Var* genes are encoded in approximately 60 different copies per haploid parasite genome, and are extremely diverse within and between parasite isolates [40,41]. By encoding adhesive functions into a variant antigen family, *P. falciparum* parasites are able to change binding and antigenic properties as they switch expression of different *var* genes. This, in turn, enables infected erythrocytes to bind microvasculature endothelium and sequester from blood circulation [1]. Cytoadhesion and sequestration may provide a selective advantage to the parasite from spleen-dependent killing mechanisms and may also have immunomodulatory properties on host dendritic cells [42]. Further, cytoadhesion is a primary determinant of organ-specific pathology during *P. falciparum* infections, as in the cases of cerebral and pregnancy-associated malaria [1]. Current research efforts seek to determine whether specific PfEMP1 variants predispose parasites to cause disease and have a related protein structure or adhesion domain(s) that can be therapeutically targeted across parasite isolates.

The binding region of PfEMP1 proteins is composed of a variable number of two types of adhesive domains, DBL domains and Cysteine-rich Interdomain Regions (CIDR) [43]. As in EBLs, the DBL domains from PfEMP1 proteins bind a variety of different receptors, both carbohydrate and protein. DBL sequences in this family average less than 50% amino acid identity [44], but can still be classified into six different types (α, β, γ, δ, ε, and X) based on sequence similarity [41,43]. Further in-depth sequence analysis revealed that each DBL domain can be divided into 10 semi-conserved blocks (A–J) and 10 variable blocks (1–10) (Fig. 1) [43]. The semi-conserved blocks contain a higher number of invariant or biochemically-conserved residues that are presumably important for domain folding. The question arises whether DBL sequence classification may provide insight into binding. Although there are examples of adhesive properties, such as binding to ICAM-1 [45–47], that have been mapped to particular domain types, high throughput methodologies and a larger binding dataset will be required to refine and validate the relationship between sequence classification and adhesion.

To improve current binding algorithms, it would be helpful to know if DBL domains share a single binding site or have separate and distinct binding sites to accommodate different interactions. Whereas the DBL domains of EBLs contain 12–13 invariant cysteine residues [9], the absolute number of cysteine residues differs more significantly between the DBL domains of PfEMP1 proteins [43]. Although the homology between PfEMP1-type and EBL-type DBL domains ends shortly after the tenth invariant cysteine (Fig. 1), the conserved cysteines of EBL-type DBL domains can be used as a frame of reference to compare binding

properties of analogous regions from all DBL sequences. Using these criteria, a minimal binding region has been mapped to a central region in the DBL domain between conserved cysteines 5 and 8 that is involved in EBL binding (in PvDBP, PkDBP and the EBA-175 F2 DBL domain) to erythrocyte receptors and PfEMP1 binding to complement receptor 1 and chondroitin sulfate A [27,28]. However, the evidence suggests that while this minimal binding region is necessary and sufficient for binding, it exhibits lower binding affinity than the entire domain [28]. Furthermore, ICAM-1 binding requires both central DBLβ residues and residues from the flanking C2 domain [28,47]. Additionally, another study has shown that a 67 amino acid fragment from FCR3-CSA DBL3γ, located C-terminal to the C5-C8 core binding region, has the capacity to bind CSA [48]. Therefore, while core binding residues may map between C5 and C8 for several DBL binding interactions, flanking residues seem to impact the affinity and/or specificity of adhesion.

4. DBL three-dimensional structure and receptor binding

Recently, the three-dimensional structures were solved of the tandem DBL domains (RII) from *P. falciparum* EBA-175 [3], and the single DBL domain from PkDBP, referred to as Pkα-DBL [4]. The structure of EBA-175 RII was solved alone, as well as in a co-complex with a sialic acid derivative, α-2,3,sialyllactose to identify the glycan binding site [3]. These structures represent important milestones in the understanding of erythrocyte invasion and suggest that EBA-175 and PkαDBP use different mechanisms for binding erythrocyte receptors.

Significantly, despite limited sequence conservation, the two DBL domains in EBA-175 and the single DBL domain in PkDBP have highly similar overall structures (Fig. 2C) [3,4]. The DBL fold was shown to be entirely novel and primarily composed of α-helices. Each DBL domain was further subdivided into two or

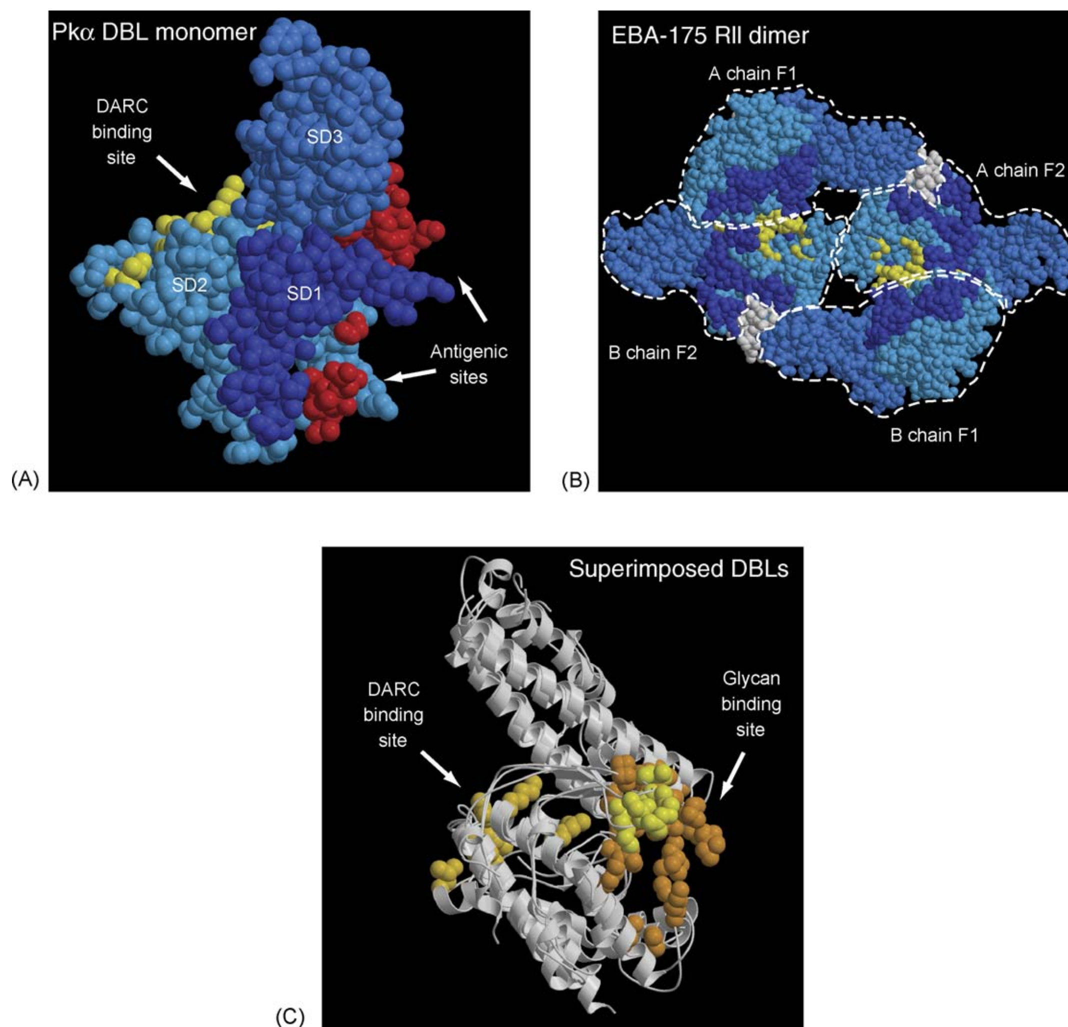


Fig. 2. Structural comparisons of DBL domains that bind as a monomer versus a dimer. (A) The PkαDBL domain is believed to engage the DARC receptor on erythrocytes as a monomer. The DARC binding site residues (yellow) and polymorphic residues (red) cluster on opposite faces of the DBL fold. Subdomains 1–3 [4] are labeled. (B) The EBA175 tandem DBL domain (RII) interacts with glycophorin A as a dimer that resembles a pretzel with two channels in the middle [3]. The two RII molecules in the EBA-175 dimer are labeled as A or B chains, including their respective F1 and F2 DBL domains. Subdomains retain the same shades of blue as panel A. The glycan contact residues are highlighted in yellow, and the linker region between F1 and F2 is depicted in white. (C) The three solved DBL domains – PkαDBL, F1 and F2 – have nearly identical structures, as shown here by superimposing them. The binding sites are shown in gold (PkαDBL), yellow (F1), and orange (F2). Images in this figure and Fig. 3 were produced using MOLSCRIPT [58] and Raster3D [59] software.

three subdomains, depending on the authors' definitions [3,4]. For this review, we are adopting the three subdomain nomenclature of Singh et al. [4] in order to facilitate comparisons between DBL sequences, but note that the boundary for the third subdomain was very similar in both publications. The approximate boundaries for subdomains 1–3 encompass invariant cysteines 1–4, 5–6, and 7–12, respectively (Fig. 1) [4]. Subdomain 1 is smaller (~37 residues) in Pkα-DBL and lies juxtaposed to subdomain 2 (Fig. 2A). Subdomain 2 is primarily α-helical and contains one of the six disulfide linkages occurring in EBL-type DBL domains [4]. Subdomain 3 consists of two long α-helical towers, approximately 40 Å in length. The towers are connected by a surface exposed loop, which itself may be anchored through a complex series of three disulfide bonds (Fig. 3) [4]. These towers are among the most conserved regions in all DBL sequences (H and J blocks) [43]. Overall, the three DBL structures are nearly identical, as defined by structural superimposition, being within ~2 Å of each other (Fig. 2C) [4].

An important feature revealed by the crystal structures is that part of the explanation for how DBL domains are able to bind different substrates is that they have different binding sites for different interactions and sometimes binding involves dimerization. In EBA-175, RII dimerization was shown to be critical for binding to glycophorin A on erythrocytes [3]. The sialic acid binding site was mapped to a dimer interface formed by the interaction of two different RII monomers that assembled

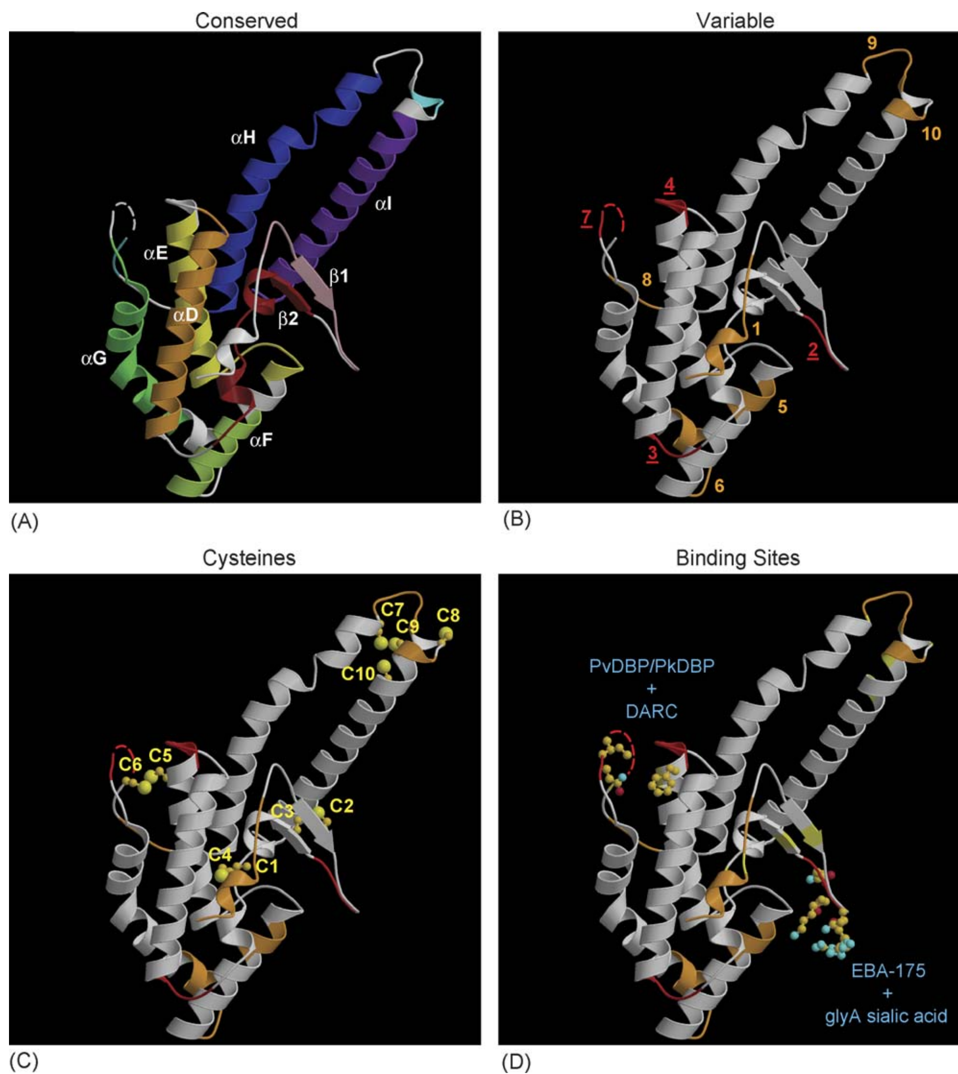


Fig. 3. A proposed model for DBL conservation and diversity mapped onto the EBA-175 F1 DBL domain structure. Conserved and variable blocks in PfEMP1-type DBL domains were defined from a multiple alignment of 58 different DBL sequences aligned to EBA-175 [43]. These regions are mapped here on the F1 DBL domain crystal structure [3]. (A) Conserved blocks A–J are: A, pink; B, red; C, orange; D, yellow; E, yellow-green; F, green; G, light blue; H, blue; I, cyan; J, purple. The β sheets and α helices are labeled according to Tolia et al. [3]. (B) Variable blocks 1–10. As in Fig. 1, regions displaying greater sequence length polymorphism are red; regions displaying less sequence length polymorphism are orange [43]. (C) Conserved cysteine residues, C1–C10, are depicted in ball and stick form, where C atoms are gold and S atoms are yellow. The variable blocks remain red and orange on the ribbon structure. (D) Receptor binding sites. The *P. knowlesi*/*P. vivax* DBP residues that interact with DARC are located on an opposite face of the DBL structure than the *P. falciparum* EBA-175 glycan binding site. Residues involved in the binding interactions are shown in ball and stick form with the following atom colors: C, gold; N, cyan; O, crimson; S, yellow. The locations of the conserved cysteine residues are yellow on the ribbon structure.

in an anti-parallel fashion, termed a handshake interaction. The RII dimer resembles a pretzel with two long central channels (Fig. 2). Four of the six glycan binding sites are buried in the channel and two others are exposed at its surface. Consistent with the truncation analysis [28], the binding site includes the minimal binding region between C5 and C8. However, the entire binding site encompasses binding residues from all three DBL subdomains, albeit these are contributed by the F1 DBL subdomain 1 and the F2 DBL subdomains 2 and 3 (Fig. 2). Tolia et al. [3] further speculate that during merozoite invasion, EBA-175 dimerizes around glycophorin A, which is itself a dimer, and this generates signals through the cytoplasmic tail required for the invasion process.

In contrast, the Pkα-DBL interaction with DARC does not appear to require dimerization, and in fact, the dimerization elements defined in EBA-175 RII are missing in DBL domains from both PkDBP and the orthologous PvDBP [4]. Instead, site-directed mutagenesis suggests that the DARC binding site maps to an exposed region on subdomain 2 on an opposite side of the DBL fold from the sialic acid binding site (Fig. 2C) [4,49,50]. Overall, these comparisons suggest that a single binding site will not be sufficient to explain all DBL interactions with their varied receptors.

If DBL domains have more than one binding site, how might this new information be applied towards interventions that prevent malaria disease? Clearly, we need to learn more about how EBLs bind their varied substrates in order to rationally design drugs that simultaneously interfere with multiple pathways of *P. falciparum* invasion. The available evidence suggests that DBL domains have at least two distinct (and possibly more) binding sites, and can act alone or in dimeric association depending on the particular receptor interaction. Thus far, there is no indication that the PkDBP interaction with DARC requires more than the proposed single DBL binding site [4]. However, for EBA-175, a sialic acid binding site has been mapped [3], but the DBL surface contacted by the peptide backbone of glycophorin A has not been defined. Since both the glycophorin A peptide backbone and sialic acid contribute to RII binding [24], it will be interesting to test whether the DARC binding site is involved in glycophorin A binding. Furthermore, although dimerization does not appear to be a general requirement for DBL binding, it will be useful to test whether dimerization is involved in the sialic acid-dependent binding of BAEBL and JSEBL, and also whether these two EBL proteins have a common glycan binding site with EBA-175. Thus, there are still significant gaps in our understanding of DBL binding, but investigation into this topic will be greatly aided by the EBA-175 RII and Pkα-DBL crystal structures.

The new DBL structures also provide insight into parasite immune evasion that may be valuable for vaccine design. Using the Pkα-DBL scaffold, Singh et al. showed that polymorphic residue clusters in the PvDBP are located on the opposite face of the DBL fold from the DARC binding site and could contribute to immune evasion (Fig. 2) [4,51]. This observation supports the "just in time release" model proposed by Adams et al. [7] in which the PvDBP is sequestered in micronemes until needed for invasion, when the DBL domain quickly engages the DARC receptor on the erythrocyte membrane, thereby preventing antibody access to the DARC binding site. One prediction of this model is that immunization with *P. vivax* DBL antigens will be capable of eliciting antibodies to the binding pocket that will not be affected by polymorphism selected by natural immunity [4].

5. Implications from the DBL crystal structure for PfEMP1 binding and immune evasion

Like the gp120/gp41 envelope glycoprotein of human immunodeficiency virus (HIV) [52], PfEMP1 proteins have adhesive properties, but are among the most polymorphic antigens in nature. Just as the three-dimensional structure for a partial gp120 fragment has been valuable for investigating the structure and function of this protein [53], the DBL structures provide a new perspective to understand malaria pathogenesis. Whereas DBL domains in EBLs display relatively limited allelic variation resulting from point mutations and gene recombination [31,54], PfEMP1-type DBL domains are extremely polymorphic [43]. This difference probably relates to the intensity of immune pressure on PfEMP1 proteins at the infected erythrocyte surface. From a structural and evolutionary perspective, it is interesting to consider how the DBL domain adapted to constant antibody surveillance.

In terms of infected erythrocyte cytoadherence, it may be significant that DBL domains were pre-adapted for binding. Therefore, binding sites that have been defined in EBA-175 RII and Pkα-DBL may be informative for identifying analogous binding regions in PfEMP1 proteins. Although the minimal binding requirements for some PfEMP1 interactions differ from EBLs, this is not necessarily incompatible with this interpretation. For instance, the DBLβ interaction with ICAM-1 requires the flanking C2 domain (Fig. 1A) [45–47]. Interestingly, for both EBA-175 and DBLβ-C2, critical binding residues map beyond cysteine 10, in what may be an equivalent binding pocket. This would imply that the glycan binding site in EBA-175 has been adapted to bind ICAM-1, but this has yet to be experimentally confirmed.

Understanding the structural basis for DBL binding and immune evasion will also provide insight into malaria pathogenesis. To investigate how PfEMP1-type DBL domains diversify under immune pressure, we have taken the variable and semi-conserved blocks defined from multiple DBL alignments [43] and mapped them onto the EBA-175 F1 domain structure (Fig. 3A and B). This analysis supports the hypothesis that the semi-conserved blocks comprise a DBL framework, primarily composed of α-helices. This framework is mostly buried, as the amount of flexibility allowed for the scaffold might be expected to be under structural constraints and hidden, by necessity, from antibody. In contrast, the variable blocks are all surface exposed as parts of loops or turns that can protrude from the scaffold (Fig. 3B).

In addition, our analysis suggests that the disulfide bonds have a critical role for how the DBL domain adapted to constant immune pressure. Namely, each of the 10 invariant cysteines can form disulfide-bonding pairs in the F1 domain that seem

to support a variable region (Fig. 3C). Because some of the variable loops tolerate greater length variation [43], it is likely that the role of the cysteines is to keep the protein scaffold stable and allow the parasite to explore more surface space at these variable loops (Fig. 2C). Both the total number of cysteine residues in DBL sequences and the absolute amount of amino acid length variation observed at specific loops differs between different DBL sequence types [43]. It will be interesting to investigate how these differences are accommodated. Notably, many of the variable loops in the HIV surface envelope glycoprotein are also braced by disulfide bonds at the base of the loop [53] suggesting this may be a more general structural solution that different pathogens have adapted for immune evasion. A final intriguing point made apparent by the polymorphism analysis is that both the EBA-175 sialic acid binding site and the PvDBP DARC binding site are extremely variable (in sequence and length) when compared against all types of DBL sequences (Fig. 3D). This suggests that some of the diversity may be under binding selection, and/or that variation around the binding site may be important for immune evasion. Thus, it is clear that the parasite has devised an ingenious solution for binding and immune evasion that uses cysteine scaffolding to hold together largely buried, semi-conserved framework elements and place variable protuberances at the surface of the DBL fold (Fig. 3).

Similar structural and sequence diversity considerations will likely have important application for guiding pregnancy malaria vaccine development focused on an unusually conserved PfEMP1 protein called *var2csa*, which is involved in infected erythrocyte sequestration in the placenta [55]. Unlike most *var* genes which are not conserved between strains, all parasite isolates appear to have a *var2csa* homolog [55]. To put the known *var2csa* sequence diversity into broader context, *var2csa* DBL domains can vary by as much as 25–30% amino acid identity as compared to ∼30% variation between HIV envelope glycoproteins [52]. While HIV uses glycosylation “shields” and rapidly mutates its glycoprotein adhesive domains due to an error-prone DNA polymerase and can tolerate mutations that abolish function because of the high amplification potential of the virus, we speculate that evolution of *var* genes involves mutation and gene conversion/recombination that maximizes polymorphism at discrete positions in the DBL fold (variable turns and loops in Fig. 3). These positions may have been engineered to tolerate extensive change in order to provide the parasite greater flexibility to evade the immune response.

DBL polymorphism is not limited to the variable blocks, as the framework regions also vary (Fig. 1) [43]. Depending on their level of surface exposure, variant framework residues may be diversifying under either antibody or cellular immune selection. Although specific epitopes recognized by helper T cells have not yet been mapped in DBL sequences, it will be interesting to determine if buried polymorphic residues are presented to helper T cells. One speculative possibility is that PfEMP1 polymorphism may also be under selection from cellular immunity and that sequence variation or “altered peptide ligands” may subvert the development of broadly reactive T helper cells to the variant antigens.

6. Conclusion

Clonal antigenic variation is a common strategy employed by numerous pathogens to evade immunity. *P. falciparum* and *P. reichenowi* employ an interesting variation on this theme, as the parasite has adapted a binding domain from an invasion ligand to incorporate adhesive properties into variable erythrocyte surface antigens. Other *Plasmodium* species and *Babesia bovis* sequester infected erythrocytes, but have come up with distinct gene and protein structure solutions for binding [56,57] which are not yet characterized. The new DBL crystal structures provide a framework on which to model DBL diversity in three-dimensional space. Based upon comparative structural and sequence polymorphic considerations, we propose a model for DBL binding and immune evasion which emphasizes the importance of cysteine disulfide bonds for stabilizing surface-located variable loops and protuberances. Insights from the new DBL structures will promote a more complete, mechanistic understanding of erythrocyte invasion and *P. falciparum* pathogenesis, and facilitate the design of vaccine and ligand-blocking therapeutics.

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