Correspondence

Polymorphic and Conserved Targets of Antibodies against Plasmodium falciparum during Pregnancy

Defining the specificity of naturally acquired protective antibodies that target surface epitopes of placental-binding Plasmodium falciparum–infected erythrocytes (IEs) will facilitate the development of a vaccine for malaria during pregnancy. It is important to determine whether immunity is mediated by antibodies directed against conserved epitopes or by a repertoire of antibodies targeting polymorphic epitopes. VAR2CSA, a specific variant of the P. falciparum erythrocyte membrane protein 1 (PFEMP1) family, is the ligand for the adhesion of IEs to chondroitin sulfate A (CSA), the major receptor in the placenta, and appears to be the dominant target of protective antibodies that are reactive with placental IEs [1]. There is considerable interest in VAR2CSA as a vaccine antigen for the prevention of malaria during pregnancy; however, significant diversity in the VAR2CSA sequence poses challenges in vaccine development. In a recent article in the Journal, Oleinikov et al. [2] raised the question of whether functional antibodies that inhibit IE adhesion to CSA in the placenta are variant specific.

We found that adhesion-inhibitory antibodies demonstrated substantial variant specificity, which correlated with polymorphisms in VAR2CSA [3]. Acquired antibodies among pregnant women showed differential inhibition of different CSA-binding isolates expressing polymorphic variants of VAR2CSA. Variant-specific adhesion inhibition was also observed with rabbit antibodies generated against a CSA-binding isolate expressing VAR2CSA. Furthermore, our findings indicated that, although serum antibodies may be broadly reactive with surface antigens of different CSA-binding isolates, they are not necessarily broadly inhibitory. This may have important implications for understanding immunity and vaccine design. Other findings also point to adhesion-inhibitory antibodies being variant specific, as demonstrated by there being little correlation between different isolates with respect to the level of adhesion-inhibitory antibody in serum from pregnant women [4].

Results from mixed agglutination assays used to measure cross-reactive antibodies (antibodies that recognize 2 or more different isolates) suggested that naturally acquired antibodies predominantly target polymorphic rather than conserved epitopes [3]. However, mixed agglutination assays may lack sufficient sensitivity to detect cross-reactive antibodies in all instances, particularly if these antibodies are at lower concentrations than variant-specific antibodies. We found that a greater proportion of secundigravidae and multigravidae had antibodies to >1 CSA-binding or placental isolate than did primigravidae, suggesting that successive exposure to malaria during pregnancy leads to an expansion of the antibody repertoire and/or the acquisition of cross-reactive antibodies [3, 5].

On the basis of these observations, we believe it is unlikely that CSA-binding sites of VAR2CSA are highly conserved in sequence. Polymorphisms in VAR2CSA may have functional relevance because they appear to influence the fine specificity of binding interactions between IEs and structural motifs of CSA [6]. However, the presence of antibodies against different placental isolates among pregnant women from geographically diverse populations suggests that there is some limitation to global diversity [7]. It is possible that receptor-binding regions have a greater degree of antigenic restriction than do other regions of VAR2CSA because of constraints imposed by the requirement of receptor binding. However, this is not suggested by the available data. The ability of serum antibodies to inhibit the adhesion of different CSA-binding lines did not appear to be broader than that of total antibodies against surface epitopes of CSA-binding isolates [3], and the correlation between different isolates with respect to the level of adhesion-inhibitory antibody among pregnant women was weaker than that for total antibody against IE surface antigens [4]. There is also evidence from studies of other antigens that there can be substantial antigenic diversity of receptor-binding regions. For example, functional epitopes of apical membrane antigen 1 appear to be highly polymorphic [8], and an analysis of the binding region of Streptococcus pyogenes M protein revealed no evidence of antigenic restriction [9].

Cross-reactive antibodies have been generated in experimental animals by vaccination [1, 10]. However, antibodies generated by vaccination may differ greatly, in specificity and functional activity, from antibodies acquired through natural exposure. Our findings suggest that cross-reactive antibodies can be acquired by pregnant women via natural exposure to malaria during pregnancy but are not prominent [3]. The molecular basis for cross-reactivity is presently unclear. It is possible that cross-reactive antibodies recognize highly conserved regions of the VAR2CSA sequence; alternatively, cross-reactivity may instead reflect sharing of polymorphic epitopes between different VAR2CSA variants.

These issues require further investigation, given that the identification of common targets of protective antibodies would contribute enormously to our un-
understanding of immunity to placental malaria and to the development of effective interventions.

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References


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Reply to Beeson et al.

Beeson et al. [1] ask whether protective antibodies acquired by pregnant women target conserved or polymorphic epitopes of Plasmodium falciparum. They highlight our recent finding that seroreactivity to recombinant VAR2CSA domains is variant-dependent and cite other work showing that different chondroitin sulfate A (CSA)–binding parasite lines are variably inhibited by individual antisera. We agree with their conclusion that “the identification of common targets of protective antibodies would contribute enormously . . . to the development of effective interventions.” Such an advance must await the development of more specific reagents in order to adequately characterize protective epitopes.

Our group first showed that parasite-infected erythrocytes (IEs) bind to CSA in the placenta and that women develop resistance to placental malaria as they acquire antibodies that block IE binding to CSA. We also observed that naturally acquired anti-CSA-binding antibodies are globally cross-reactive, suggesting a finite number of conserved epitopes. Others have found that placental isolates vary in the degree to which they are inhibited by individual serum samples, but it is difficult to compare results between groups because the assays are not standardized. Furthermore, as Beeson et al. point out, assays may vary in their ability to detect antibodies to conserved versus polymorphic epitopes, and these different antibodies may be acquired at different rates in exposed individuals. For example, conserved regions might have relatively low immunogenicity and elicit antibodies more slowly, as was recently suggested for protective opsonizing antibody to streptococcal M protein [2].

Studies cited by Beeson et al. of polymorphic ligands in Plasmodium falciparum and Streptococcus pyogenes [3, 4] are instructive. Apical merozoite antigen (AMA)–1 of P. falciparum is essential for erythrocyte invasion. Although variant-specific invasion-inhibitory antibody targets a polymorphic loop of AMA–1 [3], engineered shark antibody to an adjacent conserved region (presumed to be the ligand-binding domain) has cross-strain invasion-inhibitory activity [5]. Thus, vaccine strategies could target conserved binding domains or a sufficient number of polymorphic loops to achieve broadly effective anti-adhesion immunity.

In contrast, the hypervariable region of the S. pyogenes M protein has been implicated as a ligand for C4b-binding protein (C4BP), despite undergoing complete sequence variation among isolates [4]. However, existing data do not exclude the possibility that M protein variants might bind at different or overlapping sites in C4BP, meaning that specific ligand-receptor interactions could be mediated by distinct sequences with conserved features.

These studies may help us to understand the interactions between VAR2CSA and CSA in the placenta. Different Duffy-binding like (DBL) domains of VAR2CSA bind to CSA in vitro. Although these domains are thought to be structurally similar to EBA175 [6] and Plasmodium knowlesi DARC (Duffy antigen receptor for chemokines)–binding [7] DBL domains, their sequences differ substantially. Conversely, the domain order for VAR2CSA is completely conserved among multiple isolates, and the sequences of individual DBL domains are relatively conserved among isolates. This may indicate that each domain is important for in vivo binding to CSA and that any single domain is insufficient. Of note, sulfation of CSA in the intervillous spaces (where IEs sequester) is low and heterogeneous [8]; distinct VAR2CSA DBL domains might bind different CSA entities, with the combination of domains providing sufficient avidity for IE adhe-
sition. It will be critical to know whether inhibition of a single domain may be sufficient to block IE binding to CSA.

Furthermore, as observed for AMA-1, anti-adhesion antibodies do not necessarily target binding residues. DBL4 and DBL5 do not bind to CSA in vitro but have the highest sequence conservation. This may indicate an important role in protein structure and function. Considering the surface accessibility of DBL5 in native VAR2CSA [9–11] and its conserved sequence, this domain should not be discarded as a potential target for vaccine development [11].

Our recent experiments suggest that seroreactivity to native VAR2CSA is significantly reduced by single amino acid substitutions in flexible loops but that Escherichia coli–expressed VAR2CSA domains can elicit broadly cross-reactive antibody at low titer [11]. No one has described VAR2CSA immunogens that elicit highly active functional antibody. Until we obtain specific reagents with a high degree of functional activity, we will be limited in our ability to decipher whether the critical epitopes in the CSA ligand are conserved or polymorphic. This knowledge will determine whether vaccines might target a single key epitope or a limited number of relatively conserved variants. As Beeson et al. stress, understanding the nature of the epitopes targeted by protective antibodies will greatly advance the development of a vaccine against placental malaria.

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Persistent Borrelia burgdorferi infection after Treatment with Antibiotics and Anti–Tumor Necrosis Factor-α

To the Editor—Yrjänäinen et al. [1] have demonstrated that mice infected with Borrelia burgdorferi, the spirochetal agent of Lyme disease, may have persistent culture-proven infection despite appropriate antibiotic therapy when treated with the immune modulator anti–tumor necrosis factor–α [1]. Their study adds to the list of animal experiments that have demonstrated persistent infection despite “appropriate” short-course antibiotic therapy in hamsters [2], mice [3–5], dogs [6, 7], and horses [8]. Furthermore, immune modulation can induce persistent B. burgdorferi infection and tissue pathology over a period of months to years in a rhesus macaque model of chronic Lyme disease [9, 10]. Thus, the findings of Yrjänäinen et al. are consistent with the findings of these previous animal studies.

The observation that short-course antibiotic therapy may fail to eradicate B. burgdorferi infection under experimental conditions in animals suggests that longer antibiotic treatment may be appropriate in humans who display evidence of persistent tickborne infection [11, 12].

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