



Doesn't It All Come Down to Function? How To Correlate VAR2CSA Antibodies with Protection

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We wish to respond to the article “Going native, or the risk of overreliance on recombinant antigens” (1) by Dr. Lopez-Perez, which questions our findings that antibodies to VAR2CSA can arise outside pregnancy. She asserts that relying on antibody recognition of recombinant proteins can yield misleading data on VAR2CSA antibody responses. We agree; it is not surprising that VAR2CSA may be folded or modified differently when expressed in a variety of cell systems, and this is expected to alter the recognition of the recombinant protein by antibodies. We contend that the most important way to characterize VAR2CSA antibodies is not based exclusively on antigen recognition but on whether those antibodies block the adhesion of infected erythrocytes (IE) to the placental receptor chondroitin sulfate A (CSA). Antibodies with inhibitory activity strongly correlate with protection against pregnancy-associated malaria (PAM) (2–6). The importance of function was further emphasized by Khattab et al. in 2004 (5): “The identification of functional antibodies which appear to inhibit the binding of parasites to CSA in the placenta is crucial to developing a successful vaccination strategy against PAM.”

The most established assay to measure functional antibodies is the inhibition-of-binding assay (IBA). We employed the IBA in all of our studies to show that the VAR2CSA antibodies observed in men and children from Colombia and Brazil were functional (7–9). By virtue of their inhibitory activity in the IBA, these antibodies presumably recognized native VAR2CSA expressed at the surfaces of IE. These findings prompted us to investigate the source of these VAR2CSA antibodies acquired outside pregnancy, which we discovered could be exposure to *Plasmodium vivax* (8). Sera from men and children with past exposure to *P. vivax* blocked parasite adhesion. We dissected the underlying mechanism of immune recognition and found that antibodies to *P. vivax* Duffy binding protein (PvDBP) cross-reacted with VAR2CSA and that both a mouse monoclonal antibody raised against PvDBP and PvDBP-affinity-purified human sera from Colombian men and children had strong blocking activity in the IBA (8). We further mapped the cross-reactive epitope to subdomain 1 (SD1) of PvDBP. Antibodies from Colombian men and children affinity-purified on a synthetic SD1 peptide significantly reduced parasite adhesion to CSA (9).

Collectively, our findings suggest that antibodies to an epitope in PvDBP are functional and inhibit the adhesion of *Plasmodium falciparum*-infected erythrocytes to CSA. While surprising, heterologous immunity between *Plasmodium* species is not without precedent (10). The Duffy binding-like (DBL) domains of these PvDBP and VAR2CSA proteins are structurally related and likely share epitopes that are evolutionarily conserved. These results point to new epitopes that can be exploited in vaccine design to elicit antibodies to VAR2CSA.

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We recognize that our findings challenge the dogma of parity-dependent immunity to PAM. Yet given that current vaccines based on recombinant VAR2CSA domains are hampered by extensive polymorphisms in native alleles and fail to elicit strain-transcending immune responses (11, 12), we hope to stimulate an open scientific dialogue about other, complementary vaccine approaches. Finding a way to protect pregnant women from malaria should be paramount.

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