Commentary

Adhesion Specificities of *Plasmodium falciparum*-Infected Erythrocytes Involved in the Pathogenesis of Pregnancy-Associated Malaria

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Plasmodium falciparum malaria remains a major cause of mortality and severe morbidity in large parts of the world. Most of those who die or become seriously ill from the disease are young children or pregnant women. The adhesion of infected erythrocytes (IEs) in various tissues—a process called sequestration—is thought to be key to the pathogenesis of *P. falciparum* malaria and is therefore a topic of high research priority. Sequestration is thought to be the result of interaction between parasite-encoded variant surface antigens (VSA) on the outer membrane of IEs and a range of host receptors. In this issue of *The American Journal of Pathology*, Muthusamy et al¹ demonstrate that IEs that can accumulate in the placenta and thereby cause a severe form of malaria known as placental or pregnancy-associated malaria (PAM) bind to chondroitin sulfate proteoglycans (CSPG) but not to another host receptor, hyaluronic acid (HA), which has also been implicated in the pathogenesis of PAM.

The Pathogenesis of *P. falciparum* Malaria

In areas with stable parasite transmission, *P. falciparum* malaria is concentrated among young children because protective immunity to the disease is acquired over a period of years of repeated infections. Pregnant women constitute a notable exception to this rule, since they are highly vulnerable to infection, particularly during a first pregnancy. It is becoming increasingly clear that the reasons for the slow acquisition of immunity and the sudden reappearance of susceptibility during pregnancy are related. The immune system detects and destroys IEs in the spleen, and the parasites try to avoid this by putting sequestration molecules on the surface of IEs that prevent their passage to the spleen. The host immune system counters this by producing antibodies that interfere with the adhesion of IEs and increase their detectability.

The parasites in turn can switch among a large array of different adhesion ligands. This immunological “arms race” is almost certainly the reason why it takes children several years, and many disease episodes, to acquire substantial protective immunity to *P. falciparum* malaria. But what is the relationship to PAM?

Chondroitin Sulfate A and Hyaluronic Acid As Infected Erythrocyte Receptors

P. falciparum IEs can bind to the sulfated glycosaminoglycan chondroitin sulfate A (CSA),² which constitutes the polysaccharide part of CSPG. CSA is composed of a chain of alternating sugars (*n*-galactosamine and glucuronic acid) in which sulfation of the *n*-galactosamine moieties mainly occurs at carbon 4 (CSA is therefore also known as chondroitin-4-sulfate). The profound significance of this adhesion specificity became clear when it was demonstrated³ that the IEs isolated from the placenta of pregnant women use CSA rather than host receptors exploited by IEs isolated from nonpregnant hosts, and vice versa. Placental IEs are thus functionally distinct from IEs causing malaria in nonpregnant individuals, and placenta-sequestering parasites appear to be unable to survive in nonpregnant hosts.⁴ Furthermore, the VSA expressed by placental and CSA-adhering IEs (VSA_{PAM}) are antigenically completely distinct from other VSA.^{5,6} These findings suggest that the reason why pregnant women become susceptible to *P. falciparum* malaria despite previously acquired protective immunity to the disease is not due to pregnancy-related immunosuppression as previously thought. Rather, the parasites causing PAM express VSA (VSA_{PAM}), which is not expressed by

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parasites infecting nonpregnant individuals and to which primigravidae consequently have no pre-existing immunity. The observations that susceptibility to PAM falls with increasing parity and that levels of VSA_{PAM}-specific antibodies correlate with plasma inhibition of IE adhesion to CSA⁷ and with protective immunity to PAM^{8,9} all support this scenario. Furthermore, they suggest that vaccination against PAM may be feasible, which is a highly attractive possibility both in its own right and as an important proof of concept for efforts to develop VSA-based vaccines against malaria in general.

Matters were complicated when it was reported that another glycosaminoglycan, HA, was an additional placental IE adhesion receptor.¹⁰ IE binding to HA seemed to be weaker than to CSA, less common among placental IEs, and less PAM-specific than adhesion to CSA, and the relative importance of CSA and HA as placental adhesion receptors for IEs has been the subject of some controversy. Since the original report, various studies have reported that IEs can bind to HA independently of their affinity for CSA^{10,11} or have dismissed binding to HA as the result of contaminating CSA in the HA preparations used.^{12,13}

Adhesion Abilities and Opportunities

Even if some IEs can bind to HA independent of CSA, is such adhesion relevant to PAM? Several CSPG types are present in the placenta, but not all are equally important for IE adhesion. IEs previously selected *in vitro* for adhesion to bovine CSA (a convenient standard protocol to obtain PAM-type IEs^{2,6}) adhere well to the CSPG dominating in the intervillous space but stick poorly to cell-associated CSPG.¹⁴ This observation corresponds well to the mainly intervillous location of placental IE sequestration.¹⁵ Low-sulfated CSA is the dominant glycosaminoglycan in the intervillous CSPG type, although small amounts of HA can also be detected.^{12,14} In their new study, however, Gowda's group¹ presents evidence that HA is in fact not present in the intervillous space or on the syncytiotrophoblast. Rather, the HA detected previously seems to have been due to contamination from the umbilical cord (where HA is abundant), as predicted by Fried et al.¹³ This is in contrast to CSA, which is present both in the intervillous space and on the syncytiotrophoblast. Thus, HA is absent and CSA present at the sites where placental IE sequestration occurs. Therefore, even if IEs can bind to HA independently of CSA binding, it is unlikely to be relevant to the pathogenesis of PAM.

Concluding Remarks

The new study by Muthusamy et al.¹ in combination with several previous studies concentrates the scientific spotlight on CSA as the key host receptor relevant to PAM. Selection of parasites for adhesion to CSA results in IEs expressing VSA_{PAM}, which seem more or less synonymous with a particular interclonally conserved member of the PfEMP1 protein family called VAR2CSA.^{16,17} As

PfEMP1 is thought to be responsible for IE binding to host receptors in general, VAR2CSA is now the prime candidate for IE adhesion to CSA. Reassuringly, VAR2CSA contains CSA-binding domains, and parasites lacking the gene encoding it due to knockout can no longer be selected for adhesion to CSA.^{18,19} Does this mean that the solution to the puzzle of PAM lies in careful dissection of the interaction between CSA and VAR2CSA and finding ways of blocking it? Perhaps, but several unresolved issues remain. Although VAR2CSA appears to be the only PfEMP1 associated with the VSA_{PAM} phenotype,^{16,17} many other PfEMP1 variants contain domains with affinity for CSA.^{20,21} Furthermore, CSA is present in nonplacental tissues such as brain endothelium²² (which has even been used by some to select for pregnancy-type parasites), and yet there is no pre-existing immunity to VSA_{PAM} at first pregnancy. In addition, very recently, Gowda's group²³ has published evidence to suggest that the parasite molecule directly responsible for IE adhesion to CSA is a low-molecular weight protein rather than high-molecular weight molecules such as VAR2CSA. Thus, it is probably safe to say that the puzzle of PAM pathogenesis and immunity has not quite been solved. Important new pieces, such as the one reported in this issue of the *AJP*, crop up at regular intervals. Determining how to make the old and new pieces fit—and to decide which of the pieces may not in fact belong to the PAM puzzle at all—will continue to be a major challenge and an exciting topic to follow.

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