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Lars Hviid

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EXPERT REVIEWS

The case for PfEMP1-based vaccines to protect pregnant women against *Plasmodium falciparum* malaria

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Lars Hviid

Centre for Medical Parasitology, University of Copenhagen and Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark
and
Department of International Health, Immunology, and Microbiology, CSS Building 22, Øster Farimagsgade 5, PO Box 2099, 1014 Copenhagen K, Denmark
lhviid@sund.ku.dk

Vaccines are very cost-effective tools in combating infectious disease mortality and morbidity. Unfortunately, vaccines efficiently protecting against infection with malaria parasites are not available and are not likely to appear in the near future. An alternative strategy would be vaccines protecting against the disease and its consequences rather than against infection *per se*, by accelerating the development of the protective immunity that is normally acquired after years of exposure to malaria parasites in areas of stable transmission. This latter strategy is being energetically pursued to develop a vaccine protecting pregnant women and their offspring against mortality and morbidity caused by the accumulation of *Plasmodium falciparum*-infected erythrocytes in the placenta. It is based on a detailed understanding of the parasite antigen and the host receptor involved in this accumulation, as well as knowledge regarding the protective immune response that is acquired in response to placental *P. falciparum* infection. Nevertheless, it remains controversial in some quarters whether such a vaccine would have the desired impact, or indeed whether the strategy is meaningful. This article critically examines the relevance of several perceived obstacles to development of a vaccine against placental malaria.

Keywords: malaria • PfEMP1 • placenta • *Plasmodium falciparum* • pregnancy • vaccine • VAR2CSA

Malaria in pregnancy & VAR2CSA-specific vaccination to prevent it

Each year approximately 55 million women living in areas with stable transmission of *Plasmodium falciparum* parasites become pregnant [1]. Most of these women live in Africa, and it is among them that the large majority of episodes of malaria in pregnancy occur – episodes that every year cost aproximately 100,000 infants their lives in Africa alone [2]. Although an additional 70 million pregnancies occur in areas with unstable transmission of *P. falciparum* parasites or with transmission of *Plasmodium vivax* only, these pregnancies are only at very limited risk of malaria-related complications [1]. Not very much is known about the clinical impact of *P. vivax* infection during pregnancy, but it appears to be less striking [3] and due to infection-related fever and anemia rather than to parasitemia *per se* [4]. Thus, in terms of numbers and severity, by far the major part of the problem of malaria in pregnancy is caused by *P. falciparum* infections in women living in areas of stable transmission in Sub-Saharan Africa. It is these women a VAR2CSA-based vaccine is aimed at protecting.

The particular virulence of *P. falciparum* is closely related to the ability of *P. falciparum*-infected erythrocytes (IEs) to adhere to various vascular beds. This tissue-specific IE adhesion, or sequestration, is mediated by parasite-encoded proteins expressed on the IE surface, primarily members of the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family (reviewed in [5]). The other malaria parasites regularly infecting humans – *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale* – do not possess antigens corresponding to PfEMP1, and erythrocytes infected by these species do not or only to a limited extent sequester. The schizont-infected cell agglutination (SICA) antigen family of *Plasmodium knowlesi*, which was recently shown to also naturally infect humans [6,7],

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appears orthologous to PfEMP1 [8], but little is known about the role of SICA proteins in the pathogenesis of human *P. knowlesi* infections.

The basis for development of VAR2CSA-based vaccines against placental malaria

Adults living in areas of stable *P. falciparum* transmission usually benefit from substantial clinical protection from malaria because of immunity acquired in response to *P. falciparum* infections during childhood (for a review see [9]). Pregnant women, and in particular primigravidae, constitute an important exception to this general rule (for a review see [10]). The infections in these pregnant women are often inconspicuous with limited overt symptoms and scant peripheral parasitemia. Nevertheless, placental parasitemia can be high, because IEs selectively accumulate in this organ [11]. This has been known for a very long time, but it was only 15 years ago that it was discovered that the IEs accumulating in the placenta specifically adhere to the glycosaminoglycan chondroitin-4-sulfate (usually referred to as chondroitin sulfate A [CSA]) in the intervillous space [12]. This pivotal finding was matched 7 years later, when the parasite ligand of the CSA receptor was identified as a particular PfEMP1 variant called VAR2CSA [13,14].

With this knowledge, scientists have since been working to develop a VAR2CSA-based vaccine specifically aimed at protecting women against placental *P. falciparum* infection [15]. By immunization, the goal is to achieve before the first pregnancy a level of immunological protection corresponding to the level otherwise achieved only over several pregnancies. The aim is to induce VAR2CSA-specific antibodies that can inhibit adhesion of IEs to CSA in the placenta and opsonize VAR2CSA-positive IEs for phagocytosis. To the author's knowledge, there is currently no credible alternative vaccination strategy to specifically reduce the problem of malaria in pregnancy.

Reasons why VAR2CSA-based vaccination against malaria in pregnancy might not work

It is generally accepted that CSA-specific IE adhesion and VAR2CSA expression are centrally involved in the pathogenesis of placental *P. falciparum* infection, and that VAR2CSA is an important target of acquired immunity to the syndrome. Nevertheless, the utility of the PfEMP1-based intervention against malaria in pregnancy is regularly challenged with reference to a series of obstacles (**Box 1**). To the author's knowledge, the significance of these hindrances and knowledge gaps has not previously been systematically reviewed and will be attempted here.

CSA might not be the only host receptor for placenta-sequestering IEs

The identification of CSA as the placental adhesion receptor for IEs [12] was a momentous follow-up on reports that *P. falciparum* IEs can adhere to CSA *in vitro* [16,17]. IEs from nonpregnant hosts do not have this phenotype. The discovery made it possible to develop a coherent theory accounting for previously unexplained epidemiological observations. Thus, the accumulation of IEs in the placenta might be explained by the abundance of CSA on the syncytiotrophoblast surface and in particular in the intervillous space. The high susceptibility of pregnant women to *P. falciparum* infection despite having acquired substantial protective immunity during childhood might be explained by the unavailability of CSA for IE sequestration in other tissues, and the consequent lack of VAR2CSA-specific immunity to CSA-adhering parasites in primigravidae. This supposition would also explain the marked concentration of susceptibility to malaria in pregnancy among primigravidae (because susceptibility to placental infection decreases as protection is acquired when the immune system is exposed to the parasite ligand binding to CSA). The alternative theory favored by most at the time (and still by some) was that malaria in pregnancy is an unfortunate but unavoidable consequence of immunosuppression required to protect the fetus from rejection. However, this general immunosuppression hypothesis cannot adequately explain the parity-dependent susceptibility to malaria in pregnancy.

On the other hand, the observation by Fried and Duffy did not rule out the existence of additional placental IE receptors, although those authors did not find any among the many they tested [12]. For this reason, it has been repeatedly proposed since that CSA is just one among several receptors [18]. The non-sulfated glycosaminoglycan hyaluronic acid and the neonatal Fcγ receptor have received particular attention in this respect due to high-profile reports of their involvement [18,19], but present evidence weighs heavily against significant roles for either of these receptors in placental IE sequestration [20–22]. Although adhesion of placental IEs to CD36 (a common phenotype among isolates from nonpregnant donors) was tested and excluded (significant adhesion in zero out of 11 isolates) as a placental receptor in the original report on CSA adhesion [12], it has been reported in some studies as a minor phenotype (three out of 17 isolates [23] and one out of seven islotates [24]) among placental isolates. However, all the placental isolates with CD36-adhering IEs also contained CSA-adhering IEs, and the most parsimonious explanation is that these isolates included both IEs adhering to CSA in the placenta and IEs adhering to CD36 elsewhere [23–26]. Even if some placenta-sequestering IEs were able to bind to CD36 *in vitro*, such adhesion is probably irrelevant in terms of placental sequestration, as CD36 does not appear to be expressed by the syncytiotrophoblast [27].

The theory that acquired protective immunity to malaria in pregnancy is mediated by antibodies interfering with CSA-specific IE adhesion was first substantiated shortly after the identification of CSA as a placental receptor for adhesion of *P. falciparum* IEs [28]. Primigravidae were found not to possess IgG inhibiting CSA-specific IE adhesion, whereas IgG from multigravidae had high levels of such antibodies. Follow-up studies have confirmed and extended the theory that the parasite ligand mediating IE adhesion to CSA in the placenta is antigenically distinct from parasite molecules responsible for IE sequestration in other tissues. Thus, levels of IgG with specificity for CSA-adhering IEs were found to be much lower in *P. falciparum*-exposed men and children than in sympatric women [23,29], and not significantly different from levels in nonexposed control donors [29,30]. Furthermore, levels of CSA-adhering IE-specific IgG among

P. falciparum-exposed women were found to correlate with the parity of the donors [23,28–30]. The clinical importance of these antibodies is supported by studies demonstrating correlations between their levels and protection from maternal anemia, low infant birth weight and premature delivery [31,32]. The absence of similar associations for IgG with specificity for IE surface antigens expressed by clonally identical *P. falciparum* not adhering to CSA strengthens the likelihood of a causal relationship between levels of CSA-adhering IE-specific IgG and clinical protection from adverse pregnancy outcome [32].

Taken together, these observations strongly suggest that CSA is the major, and very likely the only, placental receptor for clinically significant adhesion of IEs in the placenta. In any case, no other receptor has been identified to which only placental IEs adhere, and that has a tissue distribution making its involvement in the pathogenesis of placental malaria plausible.

VAR2CSA might not be the only parasite ligand involved in placental IE sequestration

Obviously, the identification of CSA as the placental IE adhesion receptor spurred a hunt for the corresponding parasite ligand. The focus was on the PfEMP1 proteins expressed on the IE surface [33]. These proteins had just been shown to be encoded by a multigene family involved in antigenic variation and switches in IE adhesion phenotype [34–36]. A clonally variant – and immunologically distinct – antigen selectively expressed during infection of pregnant women seemed probable, as it would explain why protective immunity does not develop prior to the first pregnancy. After some initial false leads, the search resulted in the identification of a PfEMP1-encoding *var* gene (*var2csa*) that is selectively transcribed by all placental parasites as well as by parasites selected *in vitro* for IE adhesion to CSA [13,14,37–39]. Like all other *var* genes, *var2csa* encodes a modular protein composed of a series of so-called Duffy-binding-like (DBL) and cysteine-rich inter-domain region (CIDR) domains [36]. However, the domains encoded by *var2csa* are structurally distinct from those of other PfEMP1 proteins [40], as would be expected from the functional specialization of VAR2CSA [13]. Several studies have documented that transcription of *var2csa* by placental and CSA-adhering parasites results in IE surface expression of the corresponding approximately 350-kDa PfEMP1 protein (VAR2CSA) [14,25,41].

A number of DBL and CIDR domains from PfEMP1 proteins other than VAR2CSA have been reported to have some affinity for CSA [42–45]. However, these are probably irrelevant in terms of placental malaria [46], and the central importance of VAR2CSA was further strengthened when it was recently shown that its affinity for CSA is several orders of magnitude higher than described for any other PfEMP1 domain [47,48]. Furthermore, selective knockout of *var2csa* abrogates or greatly diminishes the ability to select for IE adhesion to CSA *in vitro* [49–51]. Finally, clinical protection from placental malaria is associated with VAR2CSA-specific IgG levels [14], IE adhesion to CSA is efficiently inhibited by recombinant VAR2CSA and by VAR2CSA-specific antibodies [25,47,48], and the naturally acquired IgG response to CSA-adhering IEs is completely focused on VAR2CSA [52].

Box 1. Potential obstacles to VAR2CSA-based vaccination against malaria in pregnancy.

VAR2CSA-based vaccine development will be compromised if:

- CSA is not the only clinically significant host receptor for placental sequestration of *Plasmodium falciparum* IEs
- VAR2CSA is not the only clinically significant *P. falciparum* ligand mediating CSA-specific placental sequestration of IEs
- The pathogenesis of malaria in pregnancy depends critically and directly on parasite populations sequestering outside the placenta
- Antibodies to CSA-adhering IEs cannot adequately control placental parasitemia
- Interclonal diversity of VAR2CSA prevents its use in vaccination
- Sufficient funding for preclinical research and clinical trials is not available

CSA: Chondroitin sulfate A; IE: Infected erythrocytes.

Several recent studies have mapped the CSA-binding region of VAR2CSA to the DBL2X-CIDR$_{PAM}$ region [53–55], with only minimal involvement of the previously implicated DBL3-X domain [56–58]. The involvement of a low molecular weight (22 kDa) unidentified protein in binding of VAR2CSA-positive IEs to CSA [59] has not been substantiated.

Direct identification by mass spectrometry of PfEMP1 on the surface of placental and CSA-adhering IEs has proved difficult [60,61], probably due to the low abundance of PfEMP1 in the IE membrane, complicated by technological difficulties [62]. This approach nevertheless yielded data pointing to a non-PfEMP1 hypothetical conserved protein (PFI1785w), as peptides from this protein were exclusively identified in placental IE samples [61]. Selective transcription of this and four additional conserved proteins (including PFD1140w) among placental parasites has since been reported in whole-genome transcriptional profiling studies [39,63]. Since surface expression of VAR2CSA is restricted to CSA-adhering IEs, serum IgG with specificity for CSA-adhering IEs and VAR2CSA is generally absent from men (sex specificity), whereas the levels of these antibodies among women increase with the number of pregnancies experienced (parity dependency) [14,29,30,64]. The higher serum levels of IgG with specificity for PFD1140w [39] and PFI1785w [63] among *P. falciparum*-exposed women than men were therefore taken as evidence that these antigens are also of relevance to malaria in pregnancy. However, truly sex-specific antibody recognition of either PFD1140w or PFI1785w was not apparent (but difficult to evaluate because of the lack of control samples from nonexposed donors), and parity dependency was not demonstrated in either study [39,63]. Furthermore, it is not yet known whether one or both these proteins are present on the IE surface, let alone involved in IE sequestration in the placenta.

Gysin *et al.* have described adhesion of ring-stage-infected erythrocytes (8 h postinvasion) to placenta tissue *in vitro* [65]. Ring-stage IE adhesion would exclude a role for PfEMP1, as these proteins (including VAR2CSA) are not present on the IE surface until about 16 h after parasite invasion, and the responsible parasite ligand was identified as RAP2 in a subsequent study by the

same authors [66]. However, these findings are difficult to reconcile with the heavily documented stage-specific accumulation of mature IEs in the placenta *in vivo* [11], and to the author's knowledge, evidence for the involvement of RAP2 in the pathogenesis of placental malaria has not been forthcoming.

Taken together, these observations strongly point to VAR2CSA as the major, and very likely only, *P. falciparum* ligand mediating clinically important adhesion of IEs in the placenta. In any case, substantial evidence of significant alternative candidates is not yet available.

P. falciparum *parasites sequestering in tissues other than the placenta might be involved in the pathogenesis of malaria in pregnancy*

Pregnant women are unquestionably at increased risk of malaria compared with their nonpregnant peers, and the consequences of infection tend to be more severe [67,68]. Pregnant women appear to be particularly attractive to the mosquitoes that transmit the disease [69,70], and perhaps pregnancy-induced immune modulation temporarily reduces their ability to control a malaria parasite infection – whether acquired during pregnancy or before conception [71]. Unfortunately, there is very little data available regarding the role in the pathogenesis of malaria in pregnancy of parasites not binding to CSA, not expressing VAR2CSA and sequestering in tissues other than the placenta. Such parasites, if present in a pregnant woman before the placenta has developed sufficiently to support VAR2CSA-mediated intervillous IE adhesion to CSA, could potentially be a Trojan horse source of placental infection due to their potential for switching to expression of VAR2CSA. That way, placental parasitemia might not necessarily require infection during the last two thirds of pregnancy when the placenta is fully formed, but could possibly originate from a clinically inconspicuous or completely asymptomatic infection acquired earlier in pregnancy – or even long before conception. This might explain the borderline association between first-trimester infections and low infant birth weight [72,73], and the less than expected seasonality of malaria in pregnancy observed in several studies (reviewed in [74]). Finally, it is the most reasonable explanation for cases of placental malaria in women without exposure to parasite transmission for several years prior to conception [75,76]. But it is speculation, and as far as the author is aware, studies specifically designed to study the Trojan horse hypothesis have not been done, and such studies may not in fact be practical.

Apart from this putative indirect role, it is repeatedly suggested that parasites not expressing VAR2CSA and sequestering in organs other than the placenta also play a direct and important role in malaria in pregnancy [26,77]. Although peripheral blood *P. falciparum* parasitemia in pregnant women living in stable parasite transmission areas often seems to be derived from a parasite population with a placental sequestration focus [23,78–80], such a population may also be just one of several populations present in the peripheral blood [12,23,79]. These populations can be clonally different from each other [81] or genotypically identical subpopulations expressing different PfEMP1 proteins and with different tissue tropisms.

In summary, there is currently no substantial evidence that nonplacental (sub-)populations play a significant direct role in the pathogenesis of malaria in pregnancy. By contrast, there is a very substantial body of evidence that placenta-sequestering IEs adhering to CSA through VAR2CSA are an unequivocal and substantial cause of adverse pregnancy outcome. Determination of the relative importance of placental and nonplacental infections for adverse pregnancy outcome may have to await results of clinical trials of vaccination against placental parasitemia.

Antibodies with specificity for CSA-adhering IEs might not be sufficient to control placental parasitemia

There is substantial evidence linking IgG specific for CSA-adhering and VAR2CSA-positive IEs to protection from adverse pregnancy outcome [14,28,30–32]. However, there is also evidence that placental parasitemia can persist in the presence of this type of immunity [28,82–85], and this is often cited to question the importance of IgG-mediated immunity to CSA-adhering IEs. It is usually overlooked that the statistical power to detect a relationship between IgG to CSA-adhering IEs and pregnancy outcome was compromised in several studies by the overall low levels of potentially inhibitory antibodies due to concomitant use of insecticide-treated bed nets [85], inclusion of primigravidae only [84,86], or by the small sample size [82]. Nevertheless, these studies raise the important issue of the quantity (levels) and quality (function) of IgG to IE surface antigens needed to substantially impact placental parasitemia.

Let us look at the role of quantity first. In one study, where both IgG levels to CSA-adhering IEs and placental parasite loads were quantified, a clear inverse correlation between the two was observed in multigravidae but not among primigravidae [30]. In another study, placental parasite density was inversely correlated with infant birth weight [82]. In combination, these data suggest that it is high placental parasite densities that really matter in terms of adverse pregnancy outcome, and that high IgG levels to CSA-adhering IEs are required to (and can) reduce them sufficiently. If this indeed is the case, it follows that simple recording of the absence or presence of specific IgG and placental parasitemia is insufficient when relating acquired immunity to protection. Further quantitative data are clearly needed.

The aforementioned data also indicate that qualitative differences in CSA-adhering IE-specific IgG may be important, a notion supported by the finding that levels of IgG to CSA-adhering IEs and levels of IgG capable of inhibiting IE adhesion to CSA do not always correlate well [82]. Recent data clearly support that IgG recognizing epitopes near the CSA-binding region of VAR2CSA can be potent inhibitors of IE adhesion to CSA, whereas antibodies binding to more distant epitopes are ineffective [25,53–55,87–89]. Additional qualitative data are therefore needed.

Finally, the timing and rate of acquisition of the relevant antibodies are also likely to play a role. The primary IgG response to CSA-adhering IEs among primigravidae occurs later and develops slower than the secondary response typically seen among multigravidae [90]. In fact, these differences, rather than qualitative differences in the IgG acquired, were suspected to

be contributing to the absent correlation between levels of IgG to CSA-adhering IEs and placental parasite load among primigravidae [30]. More longitudinal studies are clearly needed.

The interclonal diversity of critical VAR2CSA epitopes might prohibit development of an effective vaccine

VAR2CSA is a member of the PfEMP1 family of proteins. Each *P. falciparum* genome contains approximately 60 genes called *var* that encode different PfEMP1 variants with high sequence diversity and affinity for many different host receptors [34–36]. The intraclonal variability among PfEMP1 proteins in general is replayed at a more limited scale within VAR2CSA itself, since many *P. falciparum* clones harbor more than one *var2csa* gene in a way that appears to be biologically significant [91–93]. This intraclonal diversity is further compounded by substantial interclonal polymorphism, yielding a potentially very large number of antigenically distinct variants.

VAR2CSA is not only antigenically distinct from all other PfEMP1 variants [14]; it is also characterized by a higher degree of interclonal conservation [13,94]. Nevertheless, the extracellular domains of VAR2CSA are composed of highly conserved segments interspersed by stretches with very substantial amino acid diversity [94,95]. There has therefore been concern that antibody epitopes of importance to acquired protective immunity might be preferentially located in variable regions of VAR2CSA as a consequence of diversifying selection pressure [52,96], which would constitute a serious obstacle to the development of a VAR2CSA-based vaccine.

Immuno-epidemiological evidence uniformly shows a high concordance of serum antibody reactivity to genotypically distinct CSA-adhering and VAR2CSA-expressing IEs [28,82,97], suggesting either the presence of interclonally conserved antibody epitopes (Figure 1A) or the presence of a broad repertoire of antibody specificities (Figure 1B). Fortunately for vaccine development, several independent lines of research support the former possibility [52,53,98–103]. Although one study has cast doubt on the functional importance of such interclonally conserved epitopes [104], it is offset by several reports providing evidence of substantial interclonal conservation of epitopes recognized by IgG that can block CSA-specific adhesion [25,47,87,88].

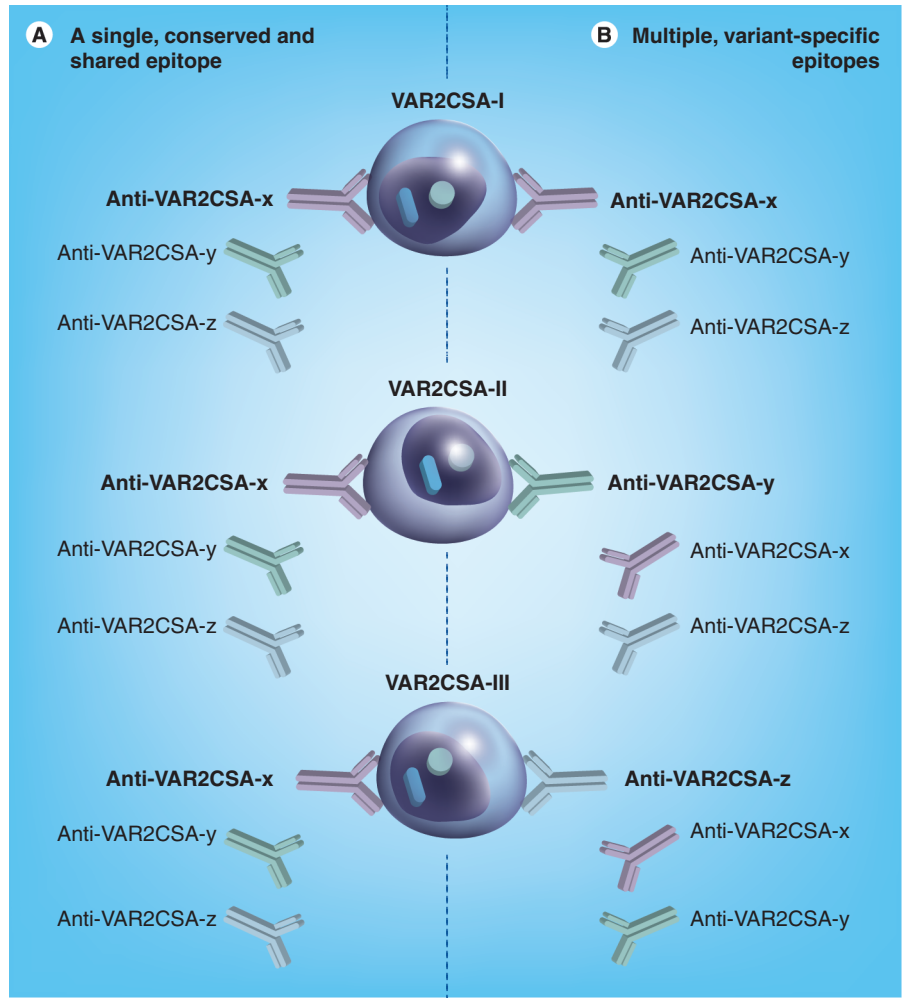

Figure 1. IgG recognition of VAR2CSA$^+$ infected erythrocytes. Assume a scenario, where three different clones of *P. falciparum*, each expressing a distinct variant of VAR2CSA (VAR2CSA-I, VAR2CSA-II, VAR2CSA-III) on the infected erythrocyte surface are exposed to a polyclonal anti-serum containing IgG with specificity for the three different epitopes in VAR2CSA (Anti-VAR2CSA-x, Anti-VAR2CSA-y, Anti-VAR2CSA-z). In the scenario represented in **(A)**, the three clones are all recognized by one of the antibodies (anti-VAR2CSA-x that recognizes a single, conserved epitope shared by all three variants) but not by the two others. In the scenario shown in **(B)** the three clones are also recognized by only one antibody each, but a different one in each case (anti-VAR2CSA-x, anti-VAR2CSA-y and anti-VAR2CSA-z, respectively, each recognizing a distinct, variant-specific epitope). In both scenarios, each clone is recognized by one of the three VAR2CSA-specific antibodies present in the antiserum, leading to highly concordant antibody recognition of the three clones. However, only **(A)** involves an interclonally conserved epitope that is present in all three VAR2CSA variants (and recognized by cross-reactive antibody Anti-VAR2CSA-x).

Clearly, much work remains, and studies of less heterogeneous antigens, such as AMA-1, indicate that unanticipated problems could lie ahead. Despite this caveat, the bulk of presently available evidence points to the presence of interclonally conserved and functionally important antibody epitopes in VAR2CSA that can be exploited in vaccine development.

Expert commentary

A vaccine specifically against placenta-sequestering IEs is not the only – or even the best – solution to malaria in pregnancy.

Obviously, a *P. falciparum* vaccine inducing efficient (preferably sterile), long-lasting (life- or at least decades long) immunity to all parasites regardless of their tissue tropism would be a superior solution to the problem. Such a vaccine would be expected to protect against all *P. falciparum* malaria syndromes, including placental malaria. The problem, however, is that such a vaccine does not exist – despite many attempts to create it, encouraging results and high hopes. It remains an open question whether it ever will. By contrast, a vaccine based on conserved parasite antigens and inducing relatively short-lasting, partial immunity may well be around the corner. Given to small children, such a vaccine is likely to have a substantial effect on malaria-related morbidity and mortality early in life, but unlikely to prevent placental malaria – and therefore unlikely to have a major impact on malaria in pregnancy. A vaccine specifically aimed at protecting pregnant women – and particularly their unborn babies – from the adverse consequences of placental *P. falciparum* infection would be an extremely useful supplement to a partially efficacious pediatric vaccine against malaria. Admittedly, such a vaccine does not exist either, but if the resources for its development can be found, it is a goal that is potentially reachable in the not too distant future. It is a goal that appears much more realistic than an ideal vaccine inducing life-long, sterile immunity to all *P. falciparum* parasites. The available evidence indicates that a vaccine based on VAR2CSA that can inhibit IE adhesion to CSA in the placental intervillous space and opsonize VAR2CSA-positive IEs for phagocytosis would markedly reduce mortality and morbidity from malaria in pregnancy. It would not prevent all forms or types of malaria in pregnant women, but it would probably remove a very substantial proportion of the problems suffered by pregnant women living in areas of stable *P. falciparum* transmission. It follows that even a highly efficacious VAR2CSA-based vaccine should not be relied upon as the only measure to protect pregnant women in low-endemicity areas against malaria, because they are likely to be susceptible to *P. falciparum* parasites other than those sequestering in the placenta. What transmission patterns and intensities would render vaccination with a VAR2CSA-based vaccine cost–beneficial is beyond the scope of this article, but clearly something that must be considered carefully.

Overall, there are still lacunae in our knowledge, and we should strive to fill these. Some knowledge gaps have been mentioned earlier; others have been described elsewhere [2,105]. Our recent finding that VAR2CSA-positive IEs appear to be able to shield themselves from immune recognition illustrates that further complications might well lie ahead [89]. However, none of them cast serious doubt that a vaccine capable of markedly reducing placental *P. falciparum* parasite loads would be highly likely to have a major impact on maternal and infant morbidity and mortality caused by malaria in pregnancy [2,74]. It is the author's opinion that using weakly supported obstacles such as those discussed earlier and listed in **Box 1** (except for the last bullet point) as arguments that may delay or jeopardize the development of such a vaccine could be irresponsible [77].

Five-year view

Progress in the next 5 years regarding development of a vaccine to protect pregnant women against placental *P. falciparum* infection will depend mainly on the availability of funding. In the last decade, there has been tremendous progress in the understanding of the pathogenesis and immunology of placental malaria, which constitutes an exceptionally solid evidence-based foundation for clinical vaccine development. Despite this, the recent decline in funding for the preclinical research required to define optimal vaccine constructs and formulations constitutes a very serious and immediate threat to continued progress. Similar difficulties can be envisioned regarding funding for the clinical trials themselves. The reasons for this precarious situation are many; the international economic setback, lack of political awareness, and the change in the malaria research agenda from control to elimination and eradication are important examples. Finally, a solution must be found to the practical problem of being able to formally document in a clinical trial the protective effect of VAR2CSA-based vaccination. Pregnant trial participants are likely to receive standard preventive treatment against placental malaria and to be issued with insecticide-treated bed nets. These interventions are known to markedly reduce the incidence of placental malaria, and on top of this, transmission of *P. falciparum* parasites at many potential trial sites has been declining in recent years. Under such circumstances it may well prove to be very difficult to demonstrate convincing vaccine efficacy, even when testing an efficacious vaccine.

If these obstacles can be overcome, it is reasonable to speculate that an experimental, VAR2CSA-based vaccine could be in clinical trials within the next 5 years. Hopefully, consensus among sponsors, policy-makers and researchers regarding the appropriate target product profiles and clinical development plans for such a vaccine can be achieved. This will require broad discussions involving all stakeholders and considering all available evidence.

In all likelihood, the eventual target population of a placental malaria vaccine is girls at puberty. A vaccine inducing sufficiently long-lasting immunological memory to allow vaccination against placental malaria as part of routine childhood vaccination programs might not be achievable, and a vaccine with duration of protection so short that it must be given during pregnancy is probably not worth pursuing. On this premise, a vaccine against placental malaria combined with the already available vaccine against cervical carcinoma appears an attractive option.

Financial & competing interests disclosure

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Key issues

- The major part of the problem of maternal and infant morbidity and mortality related to malaria in pregnancy is the result of placental *Plasmodium falciparum* infections in women living in areas of stable *P. falciparum* transmission.
- The particular virulence of *P. falciparum* malaria is related to the expression of proteins (in particular PfEMP1) on the infected erythrocyte surface, where they mediate adhesion to a range of vascular host receptors.
- Placental sequestration of infected erythrocytes is mediated by the PfEMP1 protein VAR2CSA. This parasite ligand has nanomolar affinity for the placental host receptor chondroitin sulfate A (CSA).
- There is no firm evidence supporting a direct role of *P. falciparum* parasites not expressing VAR2CSA in the pathogenesis of malaria in pregnancy.
- Clinical protection against adverse pregnancy outcome due to placental malaria is mediated by IgG with specificity for CSA-adhering, VAR2CSA-expressing infected erythrocytes.
- Interclonally conserved and functionally important epitopes that could be exploited in the development of a vaccine against placental malaria are present in VAR2CSA.
- Inadequate funding appears to be the most serious obstacle to the successful development of a VAR2CSA-based vaccine to protect against maternal and neonatal morbidity and mortality in areas of stable transmission of *P. falciparum* parasites.

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