Plasmodium falciparum during pregnancy: a puzzling parasite tissue adhesion tropism

M. C. NUNES[1] *and* A. SCHERF[1]*

[1] *Unité de Biologie des Interactions Hôte-Parasite, Institut Pasteur, CNRS, 75724 Paris, France*

SUMMARY

P. falciparum malaria severely affects pregnant women and children. Despite immunity through lifelong exposure to malaria, pregnant women become susceptible to infections causing anaemia, abortions and low birth weight. They experience massive accumulation of infected erythrocytes (IEs) in the placenta. Adhesion of IEs to host endothelial receptors is mediated by members of a large diverse protein family called *P. falciparum* erythrocyte membrane protein 1 (PfEMP1). Pregnancy malaria is generally associated with the emergence of a distinct subset of parasites expressing a unique PfEMP1 that binds to the host-receptor chondroitin sulfate A (CSA). Resistance to pregnancy malaria is associated with the acquisition of antibodies that block IEs binding to placental CSA. The absence (or rare occurrence) of CSA-binding parasites in malaria patients (children, men and non-pregnant women) suggests that these parasites become virulent only during pregnancy. The molecular mechanisms used by *P. falciparum* to achieve the timely expression of the Pf-CSA ligand in pregnant women remain puzzling. In this review we will discuss two hypothetical mechanisms by which CSA-binding parasites may arise during pregnancy. The first, a selection process by the placenta of a distinct sub-population of *P. falciparum* expressing a particular PfEMP1. The second, an induction mechanism that facilitates the expression of a particular PfEMP1 protein by specific host factor(s) present only during pregnancy.

Key words: Malaria, placenta, *Plasmodium falciparum*, var2CSA.

INTRODUCTION

Malaria is one of the major causes of morbidity and mortality in developing countries. Human malaria may be caused by any one of four *Plasmodium* species: *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*. *P. falciparum* is the parasite that causes the most severe form of malaria.

In natural infections, *P. falciparum* infected erythrocytes (IEs) circulate in the peripheral blood for a period corresponding to roughly 18 hours after invasion (ring stage) (Silamut *et al*. 1999). Later, in blood-stage development, parasite-encoded adhesion molecules are inserted into the erythrocyte membrane and adhesion of IEs to host endothelium receptors is observed. The adhesion to the host endothelium prevents the circulation of IEs and may assist the parasite in evading clearance of mature forms in the spleen (Pasloske and Howard, 1994). Severe pathology is observed when IEs sequester themselves deep within the vascular beds of tissues or in high numbers in different vital organs. The accumulation results in capillary blockage and the shortage of oxygen and nutrients. In addition, the production of toxic metabolites and other factors by the parasite induces a host pro-inflammatory response that can lead to vascular damage and organ dysfunction (Miller, Good and Milon, 1994; Warrell, 1987). The adhesion to host cell endothelium receptors is essential for the survival of the parasite.

The principal molecule mediating IE adhesion is *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) – a large, highly variant parasite antigen protein, encoded by the polymorphic *var* multigene family (Baruch *et al*. 1995; Smith *et al*. 1995; Su *et al*. 1995). PfEMP1 determines the host adhesion receptor preference of the IEs. Examples of these are CD36, intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), E-selectin and chondroitin sulfate A (CSA) (Craig and Scherf, 2001). Exposure of PfEMP1 on the IE surface stimulates a variant specific antibody response by the host (Miller *et al*. 2002). There are approximately 60 *var* genes per haploid genome, which are predominantly situated in the subtelomeric regions of the parasite genome (Gardner *et al*. 2002). Only a single variant of the *var* gene family is expressed at the surface of an IE at any given time. *P. falciparum* switches expression from one *var* gene to another, successively (Scherf *et al*. 1998). The switching in expression between the different *var* genes allows the parasite to undergo clonal antigenic variation, thus eluding the host's immune

* Corresponding author: Artur Scherf, Unité de Biologie des Interactions Hôte-Parasite, CNRS URA 2581, Institut Pasteur, 25, rue du Dr. Roux, 75724 Paris Cedex 15, France. Tel: 33-1-45688616. Fax: 33-1-45688348. E-mail: ascherf@pasteur.fr

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response and maintaining a persistent infection. In addition to its role in immune evasion, switching in PfEMP1 expression is associated with fundamental changes in parasite adhesion phenotype leading to sequestration in various tissues and ultimately results in variable disease outcomes (for review see Kyes, Horrocks and Newbold, 2001).

EMERGENCE OF UNIQUE PARASITE VARIANTS IN PREGNANT WOMEN

In malaria-holoendemic areas, clinically protective immunity is acquired during the first 5–10 years of life. In these areas adults had experienced several malaria episodes from the time they were born throughout their lives. Consequently, many have developed a repertoire of anti-PfEMP1 antibodies against the most common variants of the membrane protein. This form of adaptive immunity enables them to curb *P. falciparum* infection, thus rarely experiencing severe malaria. Pregnant women in such endemic areas, especially during their first pregnancy, are an exception. *P. falciparum* infection during first pregnancy poses serious health risks for the mother and her baby, and can lead to maternal anaemia, foetal growth retardation, low birth weight, premature delivery and even death of both mother and child (Menendez, 1995; Rogerson and Brown, 1997). This foeto-maternal syndrome is best described by the term 'pregnancy associated malaria' (PAM). Principally during first pregnancy, IEs massively sequester to the maternal side of the syncytiotrophoblast (a multinucleated cell layer derived from the foetus that lines the intervillous space that is in contact with maternal blood) that lines the placenta. IEs mainly accumulate in the intervillous space and only a sub-population appears to be directly attached to the syncytiotrophoblast surface (Muthusamy *et al*. 2004). Placental IE sequestration is a virulence phenomenon exhibited by *P. falciparum* and is apparently not shared by other malarial parasites.

PAM is associated with the emergence of a sub-population of IEs that are expressing PfEMP1 proteins that are structurally, antigenically and functionally distinct from those expressed by parasites that infect children and non-pregnant women. The placenta supports the clonal expansion of a unique subset of parasites that do not adhere to the usual endothelial receptors used by IEs like CD36 and ICAM-1, but instead bind to CSA and possibly other receptors (see below) (Fried and Duffy, 1996). Since the occurrence of a PfEMP1 variant with CSA-binding ability is low in non-pregnant patients, during first pregnancy women are deficient in antibodies against this PfEMP1 form. However, after multiple (more than one or two) pregnancies women (in malaria endemic areas) become immune to CSA-binding infected erythrocytes (IE^{CSA}) by producing specific antibodies associated with resistance to infection and to improved pregnancy outcomes (Duffy and Fried, 2003; Fried *et al*. 1998*b*). Sera of multigravida women possess antibodies that recognize IE^{CSA} and inhibit their sequestration, whereas such antibodies are detected at very low levels in the sera of children, men or women before the third trimester of their first pregnancy (Fried *et al*. 1998*b*; Ricke *et al*. 2000). Primigravida women, who do not yet have effective titers of antibodies against the IE^{CSA}, offer a new niche for the sequestration and proliferation of these parasites.

A switch from CD36 binding to CSA binding may change the tissue tropism of IEs leading to extensive sequestration of IEs in placental intervillous space. This is probably a key event in malaria pathogenesis during pregnancy. Nonetheless, despite all the accomplishments achieved in PAM research, the adhesive tropism of placental parasites is still enigmatic. Available evidence from different laboratories suggests that a particular *var* gene, *var2CSA*, is selectively expressed during placental infection and in IEs selected to adhere to CSA *in vitro* (Gamain *et al*. 2005; Salanti *et al*. 2003; Viebig *et al*. 2005). It is believed that var2CSA PfEMP1 plays a major role in PAM (Salanti *et al*. 2004). In IEs with non-CSA binding abilities, *var2CSA* is silent and different members of the *var* gene family are transcribed during chronic infection. Switching in transcription from one *var* gene to another is associated at the molecular level with epigenetic changes in the gene locus (Duraisingh *et al*. 2005; Freitas-Junior *et al*. 2005; Ralph and Scherf, 2005). The rate at which parasites switch expression from one *var* gene to another is difficult to measure in patients (Peters *et al*. 2002) and only some studies from *in vitro* cultured parasites are available (Horrocks *et al*. 2004). Therefore, an intriguing question is how a *var* gene that is apparently not expressed (or only rarely) in patients comes to be the predominant *var* gene expressed during pregnancy. We discuss in this review how the IE^{CSA} phenotype may arise *in vivo* by two different pathways which may act independently or in concert.

SELECTION MECHANISM

During pregnancy a new highly vascularized organ, the placenta, emerges in the woman's body. The unusual vascular bed of the placenta could, in theory, select for IEs expressing rare variant binding phenotypes (var2CSA) that circulate in very small numbers in the peripheral circulation of the primigravida. This would be possible if the placental structure was able to support the binding of IEs that do not bind to host receptors which are expressed in men and women. These parasites, when in contact with placental receptors such as glycosaminoglycans,

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could adhere to them and expand as a clonal population.

The placental intervillous space contains an extracellular proteoglycan matrix, with the main glycosaminoglycan being CSA (Achur *et al*. 2000; Alkhalil *et al*. 2000). As previously mentioned, epidemiological and molecular data strongly suggest that CSA is the principal receptor for IE adhesion in the placenta. However, it is important to note that the glycosaminoglycan CSA is expressed in non-placental tissues. Therefore, it is a striking observation that IE^{CSA} are only present at high levels during pregnancy and that anti-IE^{CSA} antibodies are largely restricted to pregnant women. Indeed, CSA is expressed throughout the surface of endothelial cells in the microvasculature of other target organs, such as the brain and the lung (Dittman and Majerus, 1990; Wong *et al*. 1991). Furthermore, it has been demonstrated that IE^{CSA} cytoadhere to monkey brain microvascular endothelial cells and human lung epithelial cells, apparently expressing chondroitin sulfate (Robert *et al*. 1995; Traore *et al*. 2000).

One explanation for placental tropism could be that the placental receptors are somehow different from the vascular receptors in other organs. CSA is a glycosaminoglycan composed of repeating disaccharide units of D-glucuronic acid linked to N-acetyl-D-galactosamine with a sulfate group at position C4 of the galactosamine. CSA molecules are very heterogeneous in their sulfate content, and this property influences their ability to support IE binding (Achur *et al*. 2003). An unusually low-sulfated form of CSA is expressed in the placenta, and efficiently supports placental IE binding (Achur *et al*. 2000). These low-sulfated glycosaminoglycans are most likely the main receptor for the opportunistic cytoadhesion of IEs in the placenta (Fried *et al*. 2006).

IE^{CSA}-specific sequestration has also been observed in male *Saimiri sciureus* monkeys (Pouvelle *et al*. 1997) and *in vitro* studies mimicking physiological conditions have also shown that IEs^{CSA} cytoadhere strongly enough to resist the wall shear stresses observed in the microvasculature (Pouvelle *et al*. 2003). Therefore, it is perplexing that a well-adapted phenotype for microvessel sequestration is limited to placental cytoadhesion.

The presence of other co-receptors in the placenta that bind IEs efficiently or that favour CSA adhesion could help to explain the exclusivity of placental binding. Placental and laboratory isolates with differences in adhesion patterns to CSA receptors and with the ability to bind to several receptors *in vivo* have been reported (Avril *et al*. 2004; Beeson *et al*. 2000; Rasti *et al*. 2006; Tuikue Ndam *et al*. 2004). In addition to CSA, another member of the glycosaminoglycan family, hyaluronic acid (HA), and nonimmune immunoglobulins (IgG and IgM) have been proposed to mediate IE adhesion and sequestration in the placenta (Beeson *et al*. 2000; Creasey *et al*. 2003; Flick *et al*. 2001; Rasti *et al*. 2006), but evidence for their roles is still unclear (Fried *et al*. 2006; Rasti *et al*. 2006). Placental isolates were shown to bind HA *in vitro* (Beeson *et al*. 2000), however, this result might have been influenced by a CSA contamination in the HA preparations used (Fried *et al*. 2006; Rasti *et al*. 2006). A recent study (Rasti *et al*. 2006) suggested that nonimmune Igs bind to the surface of IE^{CSA} allowing IE to bind to Ig receptors (Fc receptors) in the placenta. Nevertheless, the presence of HA and Fc receptors either in the intervillous space or in the syncytiotrophoblast lining of placentas is still obscure (Fried *et al*. 2006; Lyden *et al*. 2001; Valiyaveettil *et al*. 2001). Some unidentified co-receptors exclusively from placental tissue could be responsible for this particular tropism.

INDUCTION MECHANISM

An alternative way by which var2CSA expressing parasites may emerge during pregnancy is through its *var2CSA* activation by locally-released placental factors or serum-specific factors present only during pregnancy. These may act directly or indirectly to promote switching to the expression of *var2CSA*. The modulation of antigenic expression by host factors would require that the parasite be able to 'sense' extracellular changes in its surroundings and could activate specifically one particular *var* gene. The fact that the promoter region of the *var2CSA* gene has a unique promoter sequence not shared with other *var* genes – which have common 5′UTR sequence types, either upsA-, upsB- or upsC-type (Gardner *et al*. 2002; Lavstsen *et al*. 2003) – provides circumstantial evidence for a possible alternative epigenetic control which may result from the parasite sensing a new extracellular environment.

Active signaling between the host and parasite has been suggested in the *P. knowlesi*/rhesus monkey model. *In vivo* experiments have suggested that antigenic variation occurs only in the presence of the host spleen, and that it may be induced by antibodies reactive with the surface variant antigens of the parasite (Barnwell *et al*. 1983; Brown, 1973; David *et al*. 1983). Furthermore, experimental evidence supported the belief that the new parasite population may not appear due to the selection of variant subpopulations, but as a result of an induced phenotypic switch (Barnwell *et al*. 1983; Galinski and Corredor, 2004). These observations reinforce the hypothesis that the host environment may provide regulatory cues that influence the expression and switching of surface variant antigens.

Several physiological changes occur in pregnant women and these include dramatic increases in the levels of several hormones (Roberts, Walker and

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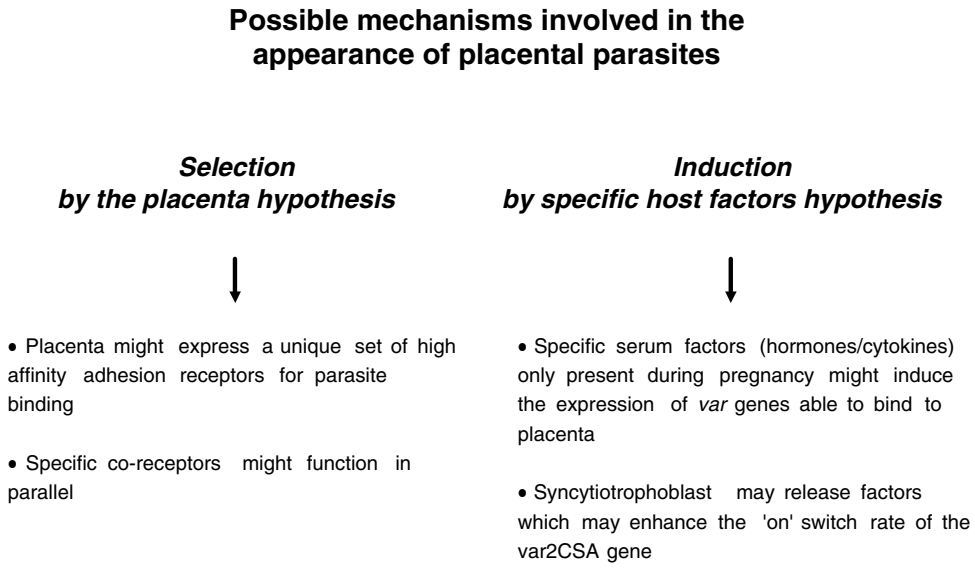


Fig. 1. Proposed mechanisms involved in placental adhesion tropism. Factors that may control the occurrence of a *P. falciparum* population expressing *var2CSA* during pregnancy.

Alexander, 2001). Furthermore, the mother's immune system needs to alter its normal composition to prevent rejection of the foetal allograft. This immunomodulation often increases the susceptibility to certain parasitic infections (Luft and Remington, 1982; Shirahata *et al*. 1992). It is possible that hormones and/or cytokines or other factors released into the blood during pregnancy facilitate the activation of *var2CSA*.

The influence of different hormones during malaria infections has been documented. Two prominent examples are: host circadian changes in the levels of the hormone melatonin have been shown to control synchronous maturation of the parasite *in vivo* (Hotta *et al*. 2000) and sex hormones have been shown to influence the length and severity of the infection in *P. chabaudi* (Benten *et al*. 1992*a*, *b*) most likely by modifying the immune response through specific interactions (Roberts, Satoskar and Alexander, 1996).

The role of pregnancy hormones in malaria has not been studied in great depth. A few studies have shown that the hormones cortisol and prolactin, whose levels are elevated during pregnancy, were detected at different levels among primigravida and multigravida women. Cortisol concentrations were significantly higher in primigravida as opposed to multigravida women and prolactin concentrations were significantly lower (Bouyou-Akotet *et al*. 2004, 2005). A relationship between these hormonal changes and malaria was drawn, due to the antagonist effect these two hormones have on the immune system. This effect could explain the incidence of infection in primigravidae since the highest cortisol levels were in *P. falciparum* infected primigravida women (Vleugels *et al*. 1989; Bouyou-Akotet *et al*. 2004). Several other hormones have altered levels during pregnancy and it will be interesting to study their role in the parasite's asexual blood-stage gene expression.

Cross-talk between the endocrine and the immune system is high during pregnancy. Cytokines play an important role in placental development. Different cytokines have been localized at the placenta-uterine interface where they probably play an important role in regulation of placental development. The human placenta secretes different cytokines and inflammatory molecules both locally and to the maternal systemic circulation (Bowen *et al*. 2002). In a successful pregnancy there is a predominance of T helper type 2 cytokines at the maternal-foetal interface (Wegmann *et al*. 1993). During malaria infection, the production of pro-inflammatory factors increases and the type 2 helper T cells response weakens (Fried *et al*. 1998*a*). We speculate that the changes in cytokines levels not only alter the immune balance of the pregnant woman but might be able to induce gene switching in circulating parasites. Local increases in cytokine concentration at the placenta or in the peripheral circulation may be sensed by the parasite leading to *var2CSA* activation.

The production of hormones and other pregnancy regulated factors in pregnant women may directly influence the parasite or modify host cellular functions enabling parasitic proliferation.

CONCLUSION

In this review we have explored two possible models that could lead to *var2CSA* activation during PAM (Fig. 1). The selection mechanism is generally used in the literature to explain the appearance of this new phenotype. However, it does not make clear why IEs expressing *var2CSA* are not detected in

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non-pregnancy malaria infections. The fact that the parasite adhesion receptors described so far in the placenta exist in other organs (Pouvelle *et al*. 2003) and that ICAM1 is present on the surface of syncytiotrophoblasts but is not involved in placental parasite sequestration is puzzling (Beeson *et al*. 1999) and does not convincingly explain the placental tissue tropism by 'selection' alone.

There are examples of unknown host factors able to alter parasitic gene expression, like the *P. knowlesi*/rhesus monkey model described above. We favour an alternative mechanism that would imply the sensing by the parasite of specific maternal factors.

The 'induced' switch model is an attractive model to explain *var2CSA* activation given that this *var* gene has a 5′ region unique among the *var*-gene 5′ sequences. The work of several groups regarding the intrinsic switching of *var* variants in the absence of external immune response suggests that the immune response may not directly cause *var* gene switching (Horrocks *et al*. 2004; Roberts *et al*. 1992). The immune responses and cytoadhesion/selection *in vivo* may provide a higher level of control over pre-programmed/stochastic switching events. In addition, several host factors are released into the blood circulation only during pregnancy. During the intraerythrocytic life cycle, *P. falciparum* exports a number of proteins to the surface of the IEs and it is possible that some of these proteins can function as receptors for hormones or cytokines. The identification of possible *P. falciparum* hormone or cytokine receptors may open new avenues in our understanding of the underlying molecular processes that lead to placental malaria.

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