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VAR2CSA and protective immunity against pregnancy-associated *Plasmodium falciparum* malaria

Lars Hviid and Ali Salanti

Centre for Medical Parasitology at Institute for Medical Microbiology and Immunology, University of Copenhagen and Department of Infectious Diseases, Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark

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Address for correspondence: Lars Hviid, Centre for Medical Parasitology, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark. Fax +45 35 45 76 44, e-mail: lhcmp@rh.dk

SUMMARY

People living in areas with stable transmission of *P. falciparum* parasites acquire protective immunity to malaria over a number of years and following multiple disease episodes. Immunity acquired this way is mediated by IgG with specificity for parasite-encoded, clonally variant surface antigens (VSA) on the surface of infected erythrocytes (IEs). However, women in endemic areas become susceptible to *P. falciparum* infection when they become pregnant, particularly for the first time, regardless of previously acquired protective immunity. This conundrum was resolved when it was observed that the selective placental accumulation of IEs that characterizes pregnancy-associated malaria (PAM) is caused by an immunologically and functionally unique subset of VSA (VSA_{PAM}) that is only expressed by parasites infecting pregnant women, and that protective immunity to PAM is mediated by IgG with specificity for VSA_{PAM}. In this review we summarize the research leading to the identification of the distinctly structured PfEMP1 variant VAR2CSA as the dominant PAM-type VSA and as the clinically most important target of the protective immune response to placental *P. falciparum* infection.

Keywords: Antibodies, malaria, *Plasmodium falciparum*, pregnancy, immunity, VAR2CSA, variant surface antigens

INTRODUCTION

Plasmodium falciparum malaria is both the commonest and most serious form of malaria affecting humans, and is an infection of colossal medical and economic consequence (Sachs and Malaney 2002; Miller *et al.* 2002; Greenwood and Mutabingwa 2002). In areas of intense parasite transmission, severe *P. falciparum* malaria is concentrated among infants and young children, because acquisition of protective immunity causes malaria-related mortality and severe morbidity to decrease with increasing age. However, development of immunity is slow, and substantial protection is only achieved after a number of disease episodes. Even then, immunity is partial, and though severe morbidity is rare among adults, sterile protection is probably never achieved. Several studies have documented the efficacy of passive immunization of malaria patients with IgG from clinically immune adults, showing that antibodies targeting asexual blood-stage antigens are an important component of protective immunity acquired following natural exposure to *P. falciparum* parasites (Cohen, McGregor and Carrington 1961; McGregor, Carrington and Cohen 1963; Sabchareon *et al.* 1991). Trans-placental transfer of IgG also seems to be responsible for resistance to malaria for the first months of life in infants born to clinically immune mothers (Bruce-Chwatt 1952). Antibodies with specificity for a number of antigens expressed by asexual blood-stage parasites are likely to contribute to this protection. However, parasite-encoded, clonally variant surface antigens (VSA) expressed on the surface of the infected erythrocytes (IEs) appear to be of particular importance (Marsh *et al.* 1989), and the intra- and inter-clonal diversity of VSA go a long way towards explaining the sluggish acquisition of immunological protection (reviewed by Hviid 2005). The general rule of *P. falciparum* malaria as a childhood disease in areas of intense parasite

transmission has one important exception that has attracted substantial attention in recent years: pregnancy-associated malaria (PAM).

PREGNANCY-ASSOCIATED MALARIA

It is a long-recognized fact that pregnant women are at increased risk of malaria, with adverse maternal and foetal consequences (reviewed by Duffy and Desowitz 2001). This pregnancy-related susceptibility is apparent despite previously acquired protective immunity, and even in malaria-endemic areas, *P. falciparum* infection is therefore both more prevalent and more severe in pregnant women than in their non-pregnant peers (Walton 1949). It has often been speculated that PAM is an unavoidable consequence of maternal immuno-suppression or -modulation to protect the foetal allograft from rejection (Menendez 1995). However, pregnancy-induced immune modulation mainly affects the cellular arm of immunity, whereas humoral immunity (upon which protection from malaria hinges) is largely unaffected (reviewed by Guilbert, Abbasi and Mosmann 2001). Furthermore, susceptibility to PAM is concentrated among women of low parity, in particular primigravidae (McGregor 1984). This suggests that susceptibility to PAM is due to a specific absence of immunity to a particular form or subset of *P. falciparum* parasites that can only infect pregnant women, and that protection against PAM-specific parasites is acquired following exposure to them during pregnancy in a manner similar to acquisition of protection from malaria in general. The hypothesis of a pregnancy-specific subset of *P. falciparum* is further supported by the facts that PAM is characterized by a selective accumulation of IEs in the placenta (Blacklock and Gordon 1925) and that pregnancy-associated parasitaemia generally resolves spontaneously shortly after expulsion of the placenta at delivery (Nguyen-Dinh *et al.* 1988).

PAM is caused by functionally and immunologically unique parasites

Erythrocytes infected by mature (trophozoite and schizont) stages of *P. falciparum* parasites can bind to a range of different receptors in the host vasculature. The first adhesion receptor identified was CD36 (Barnwell, Ockenhouse and Knowles, II 1985; Ockenhouse *et al.* 1989), followed by a range of other molecules, including the proteoglycan chondroitin sulphate A (CSA) (Rogerson *et al.* 1995; Robert *et al.* 1995). The first direct piece of evidence in favour of the hypothesis of a discrete parasite subset being responsible for PAM was the finding that IEs isolated from the placenta of women with PAM exclusively bind to CSA, a receptor that is rarely – if ever – exploited as an adhesion receptor by *P. falciparum*-IEs in non-PAM infections (Fried and Duffy 1996). This key observation was followed by the similarly important observation that placental and CSA-adhering IEs are not only functionally but also immunologically distinct from all other IEs (Fried *et al.* 1998; Beeson *et al.* 1999; Ricke *et al.* 2000). As adhesion of IEs in general is mediated by VSA, these findings together pointed to a functionally and immunologically distinct subset of VSA that is exclusively expressed by placental and CSA-adhering parasites; a subset often referred to as VSA_{PAM}.

Protective immunity to PAM is mediated by IgG with specificity for PAM-specific VSA

It is generally suspected that protective immunity to malaria depends on VSA-specific IgG that can interfere with receptor-specific IE adhesion (David *et al.* 1983; Udeinya *et al.* 1983), and that the piecemeal acquisition of protective immunity to malaria reflects the need to acquire a broad repertoire of such antibodies with specificity for a multitude of antigenically distinct VSA (Marsh and Howard 1986; Bull *et al.* 1998). The

importance of VSA_{PAM} in protective immunity against PAM was therefore reinforced when it was shown that serum antibodies can inhibit adhesion of IEs to CSA in a parity-dependent manner (Fried *et al.* 1998; Ricke *et al.* 2000). In addition to inhibition of IE adhesion, opsonization and phagocytosis of IEs may also play an important role in protective immunity to PAM. The dominance of cytophilic subclasses among VSA_{PAM}-specific IgG (Megnekou *et al.* 2005; Elliott *et al.* 2005) and the finding of impaired opsonization of VSA_{PAM}-expressing IEs in HIV-infected women (Mount *et al.* 2004; Keen *et al.* 2006) support this possibility. In any case, levels of VSA_{PAM}-specific IgG at delivery correlate inversely with placental parasitaemia (Staalsoe *et al.* 2001), and direct and compelling evidence of the clinical importance of CSA adhesion-inhibitory as well as VSA_{PAM}-specific IgG have recently become available (Duffy and Fried 2003; Staalsoe *et al.* 2004).

Molecular identification of VSA_{PAM},

Most efforts to identify VSA_{PAM} in molecular terms have focused on the best-described family of VSA, *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) (Leech *et al.* 1984). These high-molecular weight antigens are encoded by the *var* multi-gene family (Baruch *et al.* 1995; Su *et al.* 1995; Smith *et al.* 1995). Scherf *et al.* (1998) observed transcription of a particular *var* gene, dubbed var^{CSA}, in *P. falciparum* FCR3 selected for IE adhesion to CSA, and the Duffy-binding-like (DBL) 3-γ domain of the encoded PfEMP1 variant was found to have affinity for CSA (Buffet *et al.* 1999). Most *P. falciparum* genomes contain a *var* gene very similar to var^{CSA}, and these related genes were grouped together in the *var1csa* sub-family of *var* genes (Salanti *et al.* 2002; Rowe *et al.* 2002). These findings, and the observation that *var1csa* is often highly transcribed by placental parasites (Fried and Duffy 2002), fitted the earlier prediction

(Fried *et al.* 1998) that the antigen mediating IE adhesion to CSA, and the suspected target of PAM-specific immunity, was conserved between parasite clones. However, a number of other findings do not support the hypothesis of VAR1CSA as a PAM-type VSA, or its involvement as a target of protective immunity against PAM. Thus, high transcription of *var1csa* is not restricted to placental and CSA-adhering parasites (Rowe *et al.* 2002; Kyes *et al.* 2003), and levels of VAR1CSA-specific IgG do not correlate with gender or parity, in contrast to expectation regarding VSA_{PAM}-specific IgG (Jensen *et al.* 2003). Finally, the ability of IEs to adhere to CSA is maintained after disruption of *var1csa* (Andrews *et al.* 2003).

An unusually structured gene,* var2csa, *is selectively transcribed by CSA-adhering and placental parasites

The availability of the complete *P. falciparum* genome (Gardner *et al.* 2002) opened the possibility of more precise analysis of *var* gene transcription than had previously been possible with degenerate primers (Taylor *et al.* 2000). Taking this approach, Salanti *et al.* (2003) analyzed changes in *var* gene transcription in response to selection of NF54-IEs for adhesion to CSA, which resulted in acquisition of the VSA_{PAM}-type gender-specific and parity-dependent IgG recognition pattern of selected IEs (Ricke *et al.* 2000). The striking result was that the selection resulted in markedly increased and dominant transcription of a single, distinctly structured, *var* gene, PFL0030c (Salanti *et al.* 2003). PFE1640w, which belongs to the *var1csa* sub-family previously implicated in adhesion of IE to CSA, is a pseudo-gene in NF54/3D7, but data from other parasite lines have shown that selection for IE adhesion to CSA does not increase the abundance of *var1csa* transcripts (Salanti *et al.* 2003). Genes with high similarity to PFL0030c, and now grouped in the *var2csa* sub-family, appear to be present in all *P. falciparum* clones

and to be selectively transcribed by CSA-selected and placental parasites alike (Salanti *et al.* 2003; Duffy *et al.* 2005; Tuikue Ndam *et al.* 2005). The findings that several VAR2CSA domains have affinity for CSA (Gamain *et al.* 2005) and that disruption of *var2csa* interferes with the ability to acquire the CSA-adhering phenotype further supports the PAM-relevance of this sub-family (Viebig *et al.* 2005; Duffy *et al.* 2006). It is noteworthy that the *var2csa* response to CSA-selection had gone unnoticed in earlier studies because they used degenerate primers targeting DBL1-α or DBL1-γ encoding sequences (Taylor *et al.* 2000; Fried and Duffy 2002); domains that are not present in VAR2CSA.

VAR2CSA has the characteristics expected of VSA$_{PAM}$

The distinct structure of *var2csa* compared to other *var* genes corresponded well with the expected functional and antigenic uniqueness of the PAM-type VSA it was assumed to encode (Lavstsen *et al.* 2003; Kraemer and Smith 2003). Indeed, analysis of levels of VAR2CSA-specific IgG in *P. falciparum*-exposed adults confirmed the female-restricted and parity-dependent pattern expected of VSA$_{PAM}$ (Salanti *et al.* 2004). Importantly, VAR2CSA is present on the surface of intact VSA$_{PAM}$-type IEs and absent from the surface of IEs that do not have this phenotype (Salanti *et al.* 2004; Barfod *et al.* 2006). Finally, we have shown that high plasma levels of VAR2CSA-specific IgG correlate with protection from adverse clinical consequences of PAM (Salanti *et al.* 2004).

VAR2CSA appears to be the dominant target of the protective immune response to PAM

The importance of VSA-specific IgG relative to IgG with other antigen specificities for clinical protection against *P. falciparum* malaria is unknown, but may be high (Marsh *et al.* 1989). A parallel issue is the importance of IgG with specificity for VAR2CSA relative to other VSA_{PAM} specificities in protection against PAM. To address this latter question, we cloned memory B cells from recently pregnant, *P. falciparum*-exposed multigravidae, using a recently developed and highly efficient immortalization method (Traggiai *et al.* 2004). The clones were subsequently screened for production of VSA_{PAM}-specific monoclonal IgG using a panel of IEs displaying the VSA_{PAM} phenotype. All except one of the eight VSA_{PAM}-specific monoclonals had specificity for either the DBL3-X or the DBL5-ε domain of VAR2CSA (Barfod *et al.* 2006). The characteristics of the remaining antibody suggest that it is also VAR2CSA-specific. Although *var2csa* is composed of alternating stretches of substantial and restricted interclonal diversity (Salanti *et al.* 2003; Trimnell *et al.* 2006; Dahlbäck *et al.* 2006), all the human monoclonal IgG antibodies targeted polymorphic rather than conserved epitopes (Barfod *et al.* 2006). This finding suggests that domains of IE surface-expressed VAR2CSA that are accessible to protective antibodies are under selection pressure that favours polymorphism, in agreement with conclusions drawn from *in silico* analysis of *var2csa* (Trimnell *et al.* 2006; Dahlbäck *et al.* 2006). This pressure is most likely due to protective host immunity, supporting the clinical significance of the VAR2CSA-specific IgG response to PAM.

CONCLUDING REMARKS

VAR1CSA (as well as several non-PfEMP1 antigens) continues to be implicated in the pathogenesis as well as in the protective immune response to pregnancy-specific *P. falciparum* infection (Chia *et al.* 2005; Badaut *et al.* 2006), and is being explored as a candidate for development of vaccines against PAM (Gamain *et al.* 2004; Chia *et al.* 2005; Bir *et al.* 2006). However, from our perspective the bulk of current evidence identifies VAR2CSA as the main antigen mediating placental sequestration of *P. falciparum*-IEs and as the dominant and clinically most relevant target of the human IgG response to placental *P. falciparum* infection.

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