Available online at www.sciencedirect.com
ScienceDirect
Infection, Genetics and Evolution 6 (2006) 459–463
Infection, Genetics and Evolution
www.elsevier.com/locate/meegid

Acquisition of antibodies to variant antigens on the surface of *Plasmodium falciparum*-infected erythrocytes during pregnancy

Nadine Fievet [a], Jean Yves Le Hesran [a], Gilles Cottrell [b], Souleymane Doucoure [a], Ibrahima Diouf [a], Jean Louis Ndiaye [a], Gwladys Bertin [b], Oumar Gaye [c], Sokhna Sow [d], Philippe Deloron [b,*]

[a] *Institut de Recherche pour le Développement (IRD), UR 010, Mother and Child Health in the Tropics, BP1386 CP 18524 Dakar, Senegal*
[b] *Institut de Recherche pour le Développement (IRD), UR 010, Mother and Child Health in the Tropics, Faculté de Pharmacie, 4 Avenue de l'Observatoire, 75006 Paris, France*
[c] *Laboratoire de Parasitologie, Faculté de Médecine, Université Cheick Anta Diop, Dakar, Senegal*
[d] *Centre de Santé de Thiadiaye, Senegal*

Received 6 December 2005; received in revised form 23 February 2006; accepted 26 February 2006
Available online 18 April 2006

Abstract

Pregnancy-associated malaria is characterized by *Plasmodium falciparum* adherence to chondroitin sulfate A (CSA) in placenta, through a particular variant surface antigen (VSA). VSA_{CSA}-specific IgG are involved in protection against placental malaria. In order to assess the relationship between VSA_{CSA}-specific antibody responses and parity as well as protection against placental malaria, the occurrence of *P. falciparum* infection was assessed in 306 pregnant women from a low malaria transmission area of Senegal. Anti-VSA_{CSA} antibodies against three placental parasite isolates were measured by flow cytometry, at enrollment and delivery. Placental infection prevalence rates were highest in primigravidae, but no clear decreasing trend was observed from the second pregnancy onwards. Anti-VSA_{CSA} antibody prevalence rates increased with parity. Both anti-VSA_{CSA} antibody prevalence rates and levels increased during pregnancy only in women infected with *P. falciparum*. Although a single or a very limited number of *P. falciparum* infections were able to induce an anti-VSA_{CSA} antibody response, the level or the quality of this response did not appear to confer protection against placental malaria infection.

Keywords: *Plasmodium falciparum*; Pregnancy; Malaria; Placenta; Variant surface antigen; PfEMP1

1. Introduction

In *Plasmodium falciparum* endemic areas, previously immune women become more susceptible to malaria during their first pregnancy. The prevalence rates of peripheral and placental parasitemias, as well as their densities, are highest in primigravidae (Brabin, 1983; Steketee et al., 1988), decreasing after the second pregnancy. Pregnancy-associated malaria (PAM) is characterized by the accumulation of infected red blood cells (IRBC) in the placenta (Fried and Duffy, 1996). PAM is responsible for low birth weight, abortion and maternal mortality (Menendez et al., 2000). Chondroitin sulfate A (CSA) expressed on syncytiotrophoblast mediates the adherence of IRBC to placenta (Fried and Duffy, 1996) in association with the expression of a distinct variant surface antigen (VSA) on the membrane of IRBC. The major *falciparum* antigen involved is the variant *P. falciparum* erythrocyte membrane protein-1 (PfEMP1), each variant mediating adhesion of IRBC to a specific host molecule (Buffet et al., 1999). PAM is caused by *P. falciparum* which express peculiar VSAs (VSA_{CSA}) that allow the parasite to bind CSA and sequester in the placenta (Rogerson et al., 1995). Conversely to the VSA expressed by parasites infecting non-pregnant hosts, these VSA_{CSA} are reasonably conserved antigens (Khattab et al., 2003). In areas of intense malaria transmission, antibody levels to VSA_{CSA} increase with gravidity, block the adhesion of IRBC to CSA (Fried et al., 1998; Gysin et al., 1999; Maubert et al., 1999; O'Neil-Dunne et al., 2001), and protect against low birth weight and maternal anemia (Staalsoe et al., 2004). A placental infection during first pregnancy was suggested to induce a

* Corresponding author. Tel.: +33 1 53 73 96 22; fax: +33 1 53 73 96 17.
E-mail address: Philippe.Deloron@ird.fr (P. Deloron).

1567-1348/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.meegid.2006.02.006

substantial antibody response to CSA-binding IRBC (Beeson et al., 2004), raising hope for a potential VSA-based vaccine against PAM. Studies reported that anti-VSA_{CSA}-specific antibodies are not acquired earlier than halfway through the first pregnancy (O'Neil-Dunne et al., 2001; Staalsoe et al., 2001). In the present work, we aimed to investigate the relationships between parity, placental infection and presence of anti-VSA_{CSA} antibody. In addition, we assessed the relation between the occurrence of *P. falciparum* infections during pregnancy and the development of an anti-VSA_{CSA} antibody response against three different placental isolates.

2. Materials and methods

2.1. Study population

We studied 306 pregnant women in Thiadiaye, Senegal, where the rainy season lasts from July to October (500 mm of annual rains). Malaria transmission is stable and seasonal, occurring following the first rains from mid-September to mid-November. The estimated average number of infective bites is 10 per person and per year (Robert et al., 1998). The Ethical Committee, Ministry of Health, Senegal provided ethical approval for this research. All participants gave informed consent.

Pregnant women presenting, from July 30 to October 15, 2001, to the Thiadiaye hospital for an antenatal care visit were enrolled in a cohort study if they were less than 6 months pregnant, they did not report a history of fever since the beginning of pregnancy, and they were determined to be free of detectable malaria based on negative rapid immuno-chromatographic tests, no detection of malaria parasites in thick blood smears and negative *P. falciparum*-specific PCR. Thus, all 306 women were, at enrollment, unlikely to have been infected by malaria parasites since being pregnant, but will be likely to be exposed to infective mosquito bites, as part of their pregnancy will occur during the transmission season.

Women were examined during each monthly antenatal consultation, and through weekly home visits, to assess the occurrence of a *P. falciparum* infection. At each home visit, axillary temperature was recorded, and in case of fever (axillary temperature $> 37.5\ ^{\circ}C$), a capillary blood sample was obtained for thick blood smear and *P. falciparum* PCR. At each antenatal care visit, a blood sample was obtained from all women, whether they presented or not with fever. At parturition, malaria infection was assessed by examination of both peripheral and placental blood smear by microscopy. In addition, 5 ml of peripheral blood were collected both at enrollment and at delivery. Plasma were isolated and stored at $-20\ ^{\circ}C$ until use. Blood collected on Whatman 3MM filter papers was dried and conserved at room temperature for genomic DNA extraction.

2.2. Malaria infection diagnosis

Peripheral and placental thick blood smears were Giemsa-stained and the number of asexual parasites against 200 leucocytes was counted, assuming a mean count of 8000 leucocytes per milliliter of blood. Genomic DNA was extracted using chelex from peripheral blood filter papers blots, and a *P. falciparum* species-specific PCR was performed (Singh et al., 1999). A one-round PCR was optimized, using the internal *P. falciparum* species-specific primers FAL1 (5′-TTAAACTGG-TTTGGGAAAACCAAATATATT-3′) and FAL2 (5′-ACA-CAATGAACTCAATCATGACTACCCGTC-3′). Each 50-μl amplification mixture contained 4 μl of DNA template, 0.25 μM of each primer (FAL1 and FAL2), 4 mM $MgCl_2$, Perkin-Elmer PCR buffer 10×, 200 μM of each deoxynucleoside triphosphate and 0.25 U of AmpliTaq Gold DNA polymerase (Perkin-Elmer Applied Biosystems). The thermal cycling conditions were as follows: step 1, 94 °C for 7 min; step 2, denaturation at 94 °C for 40 s; step 3, annealing at 58 °C for 2 min; step 4, extension at 72 °C for 2 min; repeat steps 2–4, 35 times and step 4 for 5 min. The PCR products were then analyzed by agarose gel electrophoresis stained by ethidium bromide.

2.3. Malaria parasites collection

Three *P. falciparum* isolates (P29, P48 and P53) were collected in November 2003 from infected placentas of O-type red blood cells in the Guediawaye Hospital maternity, in the suburbs of Dakar. After delivery the placenta was placed into a sterile dish and was carried to the laboratory within 2 h. It was rinsed once with 2% of penicillin–streptomycin in PBS and placed on a lid with the maternal side up. Placentas were perfused using a syringe inserted into the maternal side with 400 ml of PBS with 0.1% of sodium heparin and 2% penicillin–streptomycin. Collected IRBC were used directly for flow cytometry analysis, without being subjected to in vitro culture. In parallel, these parasite isolates were shown to bind CSA (Tuikue Ndam et al., 2004).

2.4. Flow cytometry

Antibodies (IgG) to VSAs were measured in the plasma samples collected at enrollment and delivery, by flow cytometry, as described previously (Staalsoe et al., 2001). In short, IRBC containing late stages from placental isolates were ethidium bromide-labeled, and sequentially incubated in 96-well round bottom plates with test plasmas (diluted 1:20), goat anti-human IgG (1:250) and FITC-conjugated rabbit anti-goat IgG (1:100). Samples were analyzed using a Facscalibur (Becton Dickinson) cytometer. Samples were coded and tested blindly. The level of IgG recognizing VSAs was expressed as mean fluorescence intensity (MFI) in the channel for ethidium bromide-labeled IRBC. For each isolate, the threshold for positivity was defined as two standard deviations above the mean MFI from 30 nulligravid women from Thiadiaye (P29: 39.5, P48: 98.4, P53: 19.6 MFI).

2.5. Statistical analysis

To study the evolution of anti-VSA_{CSA} antibodies according to the occurrence of a *P. falciparum* infection, we defined this