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Complement activation in primiparous women from a malaria endemic area is associated with reduced birthweight

A. Khattab [a,*], P.G. Kremsner [b,c], S. Meri [a,d]

[a] Infection Biology Program, Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Haartmaninkatu 3, P.O. Box 21, 00014 Helsinki, Finland
[b] Institute for Tropical Medicine, University of Tübingen, Tübingen, Germany
[c] Medical Research Unit, Albert Schweitzer Hospital, Lambaréné, Gabon
[d] Helsinki University Central Hospital, Helsinki, Finland

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ABSTRACT

The hallmark of placental malaria (PM) due to *Plasmodium falciparum* infection is the accumulation of mature-stage parasites, monocytes and macrophages in the maternal vascular bed of the placenta. The mechanisms leading to morbidity and mortality in PM are incompletely understood. However, an inflammatory response in the placenta has been related to both severe anemia in the mother and low birthweight (<2500 g) in the newborn. In this study we analyzed whether complement activation as a mediator of inflammation could contribute to poor pregnancy outcome in PM. The concentrations of the soluble terminal complement complex (TCC) were measured as an indicator of complement activation in placental, cord and peripheral blood samples from 146 women from a malaria endemic area. Placental and cord plasma samples of primiparous women, a group vulnerable to PM, showed significantly higher levels of TCC than multiparous women. Additionally, in women with malaria history during pregnancy or placental infection by *P. falciparum* at delivery, the TCC levels in the corresponding placental and cord plasma samples were significantly higher than in the malaria negative group. In multiple regression analysis parity was shown to be the main determinant of TCC levels. Placental plasma samples corresponding to babies weighing less than 2700 g had significantly higher levels of TCC than babies carrying more weight. In conclusion, both primiparity and *P. falciparum* infection were related to a local increase of complement activation in the placentas. Association between reduced birthweight and higher levels of TCC in placental blood suggests a role for complement activation in influencing the pregnancy outcome in malaria exposed women.

1. Introduction

Adults living in malaria endemic areas have normally been exposed to repeated infections with *Plasmodium falciparum* and are, therefore, clinically immune to the disease [1]. However, this is not true in the case of pregnant women who can suffer from serious disease despite apparent immunity. The disease, known as placental malaria (PM), is more pronounced in primiparous women than in multiparous women [2]. The severity of placental malaria is attributed mainly to the lack of antibodies against a particular protein (var2CSA) from the multicopy variant antigens, PfEMP1s, encoded by the *var* gene family [3]. These variant antigens are expressed on the surface of *P. falciparum*-infected erythrocytes (IEs) and mediate cytoadherence to endothelial cell receptors such as CD36 and ICAM-1 [4]. Cytoadherence of IEs in the microvasculature can lead to tissue damage and organ dysfunction thereby contributing to the pathology of malaria [5]. In pregnant women, the placenta provides a new niche for the selective cytoadhesion of IEs. The IEs bind via var2CSA to chondroitin sulfate A, a glycosaminoglycan, expressed on the surface of the syncytiotrophoblast layer lining the placental chorionic villi [3,6]. Since var2CSA is an unusually conserved protein among other PfEMP1s [7], immunity to PM is acquired relatively soon and one or two pregnancies in malaria endemic areas are generally enough to confer protection [8]. Cytoadhesion properties of IEs displaying var2CSA explain the accumulation of IEs in the maternal vascular area of *P. falciparum*-infected placentas in primiparous women [9]. The infected erythrocytes and consequent inflammation could lead to the recruitment of monocytes and macrophages into the placenta during infection [10]. The resulting possible outcomes of PM are maternal anemia,

* Corresponding author. Tel.: +358 468834423.
E-mail address: ayman.khattab@helsinki.fi (A. Khattab).

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abortion, stillbirth, prematurity, intrauterine growth retardation and low birthweight (LBW). LBW is the greatest risk factor for death during the first month of life [11]. With up to 125 million pregnancies at risk of malaria infection every year [12] there is an urgent need for a better understanding of the pathophysiological mechanisms leading to poor outcomes associated with PM.

Increased complement activation has been identified in non-infectious pathological pregnancies [13], as well as in malaria in general [14]. The complement system is a specific effector arm of the humoral immunity. It plays a key role in destroying foreign invading microbes and in the clean-up of endogenous waste products. The complement system can be activated by antibodies binding C1q in the classical pathway or by mannan binding lectin or ficolins to sugar residues on the target surface in the lectin pathway. The alternative pathway is spontaneously activated by binding of C3b to foreign surfaces. Activation results in the assembly of C3-cleaving enzymes (C3 convertases) on target surfaces. Amplification via the alternative pathway leads to more C3b deposition on the target. The terminal pathway is activated when C3b forms a complex with the C3-convertases and generates C5-convertases that can cleave C5, thereby yielding one anaphylatoxin C5a and one C5b molecule. Association of C5b with C6, C7, C8, and multiple C9 molecules ultimately leads to the generation of C5b-9, also known as the membrane attack complex (MAC). However, much of the C5b generated does not result in the formation of MAC, but is diverted by control proteins (e.g. clusterin and S protein) to form a soluble, lytically inert complex called soluble C5b-9. C5b-9 is referred to commonly as Terminal Complement Complex or TCC [15]. Despite the strong cytolytic activity of complement many microbes including the malaria parasite, are able to escape complement attack. Complement can attack microbes or human cells that are marked with antibodies or have an activating altered cell surface. Paternal alloantigens in the fetoplacental unit can trigger antibody production by the maternal immune system [16]. Antibodies could thus activate complement in the placental compartment through immune complex formation. However, the placental cells are usually protected by a panel of complement regulators expressed by the fetal cytotrophoblast cells and by the syncytiotrophoblast. In healthy pregnancies, these regulators such as CR1 (CD35), DAF (CD55), MCP (CD46) and protectin (CD59) down regulate complement activation and protect the placenta from complement-mediated damage [17]. In addition, the soluble complement inhibitors, factor H (FH) and C4b binding protein (C4bp) can keep complement activation under control in the fluid phase and to some extent on surfaces, as well. In certain pathological situations, pregnancy complications might occur. Particularly in preeclampsia and in antiphospholipid syndrome, complement activation is believed to contribute to the pathogenetic processes [13,18]. In PM, the IEs bind to the syncytiotrophoblasts of the placenta [6]. The bound IEs display parasite antigens on their surfaces. These could thus be targets for maternal antibodies and activate the complement system [19]. Merozoite egress from an infected erythrocyte is accompanied by the release of *Plasmodium* proteins (e.g. GPI-anchored proteins) and toxins (e.g. hemozoin – a *Plasmodium*-generated hemoglobin degradation product) into the placental intervillous space. These could either directly or via immune complex formation also lead to complement activation. Collectively, the above considerations have led us to suspect that complement activation in the placenta in connection with *P. falciparum* infection could lead to a poor pregnancy outcome in PM.

The complement C3a and C5a anaphylatoxins and the soluble TCC are widely used markers for complement activation. Their use singly or in combination with each other depends largely on the questions and conditions of the measurements. It is important to note that C3a and C5a, in particular, bind actively to specific cell receptors upon their generation. Therefore, the concentration of C3a rapidly decreases over time and C5a cannot be measured from clinical samples [20]. On the other hand, the soluble TCC generated from complement activation remains in its measurable free form at a steady concentration for a longer period of time [20]. Thus, in the present study the level of complement activation was estimated by measuring the concentration of the soluble TCC in placental, peripheral and cord plasma samples collected from pregnant women at delivery from a malaria endemic area. TCC levels were then analyzed with respect to PM manifestations and outcomes.

2. Materials and methods

2.1. Study participants

The study cohort has been described elsewhere in an earlier study [21]. Briefly, one hundred and fifty women, who presented at the maternity ward of the Albert Schweitzer Hospital in Lambaréné, Gabon for delivery between May and October 2002, were recruited in the study. Malaria in the region is hyperendemic and perennially transmitted, and the disease is predominantly due to *P. falciparum* [22]. Whenever possible, demographic data (e.g. ethnic group, date of birth, weight) were collected from the study participants upon admission. At delivery, data regarding characteristics of the newborn baby (vital status at birth, birthweight, sex and the presence of twins) were collected. For those attending prenatal care, information regarding malaria infections during the course of pregnancy was obtained from the maternity record book, which documented peripheral parasitemia by thick blood smears (TBS). Written informed consents were obtained from all of the women and the study was approved by the ethics committee of the International Foundation of the hospital. A more detailed summary of the study cohort characteristics, that was not shown in the earlier study describing the cohort [21], is shown in Table 1.

2.2. Blood samples

Peripheral blood was collected from the study participants within 1 h after delivery in an EDTA-containing monovette. Blood was also extracted directly from the placenta. This was done by making several 1-cm deep incisions into the maternal side of the placenta and the flowing blood was drawn into an EDTA syringe (monovette without needle). Cord blood was collected immediately after placental expulsion following delivery. This was done by drawing venous blood into an EDTA-containing monovette via puncture of the ethanol-sterilized umbilical vein at a site distal to the placenta, to minimize the possibility of cross-contamination of maternal and cord blood. Blood samples collected from the three different compartments were used for preparing TBS to detect the presence of malaria parasites and to determine parasite densities using a previously described method [23]. EDTA-plasma samples were obtained from the withdrawn blood by centrifugation and preserved at −70 °C until further use.

2.3. Sandwich ELISA for the determination of the fluid-phase TCC in peripheral, placental and cord plasma

For the TCC ELISA, 96-well microtiter plates (Maxisorp NUNC, Thermo Scientific) were coated with the anti-C9 neoepitope Ab WU13-15 (5 μg/ml in 0.2 M Na_2CO_3, pH 10.6; Hycult Biotech, Uden, The Netherlands) and incubated overnight at 4 °C. After

Table 1
Characteristics of the study group.

	All women ($n = 150$)	Primiparous women ($n = 45$)	Multiparous women ($n = 105$)	*P* value
Age mean years, (SD)[a]	25.65 (7.32)	18.91 (3.32)	28.57 (6.6)	<0.001*,[b]
P. falciparum positive:				
Placental TBS at delivery, *n* (%)	21 (14)	9 (20)	12 (11.4)	0.16[d]
Reported[c], *n* (%)	39 (26)	18 (40)	21 (20)	0.01*,[d]
Total, *n* (%)	55 (37)	23 (51)	32 (30.4)	0.016*,[d]
Hemoglobin:				
Mean g/dl, (SD)[a]	11.03 (1.99)	11.23 (2.37)	10.94 (1.80)	0.46[d]
Anemic women, *n* (%)	71 (47)	19 (42.2)	52 (49.5)	0.37[d]
Birthweight:				
≤2500 g, *n* (%)	16 (10.6)	8 (17.7)	8 (7.6)	0.065[d]
≤2700 g, *n* (%)	32	14 (31.1)	18 (17.1)	0.056[d]

*Indicates significant value.
[a] Standard deviation.
[b] Wilcoxon rank-sum test.
[c] extracted from maternity record book.
[d] Pearson χ^2 test.