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Original article

Plasmodium falciparum isolates from patients with uncomplicated malaria promote endothelial inflammation

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Abstract

The ability of *Plasmodium falciparum* infected erythrocytes (Pf-IEs) to activate endothelial cells has been described; however, the interaction of the endothelium with Pf-IEs field isolates from patients has been less characterized. Previous reports have shown that isolates alter the endothelial permeability and apoptosis. In this study, the adhesion of 19 uncomplicated malaria isolates to Human Dermal Microvascular Endothelial Cells (*HDMEC*), and their effect on the expression of ICAM-1 and proinflammatory molecules (sICAM-1, IL-6, IL-8, and MCP-1) was evaluated.

P. falciparum isolates adhered to resting and TNFα-activated HDEMC cells at different levels. All isolates increased the ICAM-1 expression on the membrane (mICAM-1) of HDMEC and increased the release of its soluble form (sICAM-1), as well the production of IL-6, IL-8 and MCP-1 by HDMEC with no signs of cell apoptosis. No correlation between parasite adhesion and production of cytokines was observed.

In conclusion, isolates from uncomplicated malaria can induce a proinflammatory response in endothelial cells that may play a role during the initial inflammatory response to parasite infection; however, a continuous activation of the endothelium can contribute to pathogenesis.

Keywords: *Plasmodium falciparum*; Clinical isolates; Endothelial cells; Endothelial activation; Inflammation; Uncomplicated malaria

Q1 1. Introduction

The sequestration of *Plasmodium falciparum*-infected erythrocytes (Pf-IEs) in the microvasculature and the inflammatory response of the host are the main pathogenic mechanisms of malaria [1,2]. Parasite sequestration results from the cytoadherence of the Pf-IEs to the endothelial cells, mediated by the parasite PfEMP-1 proteins and endothelial receptors such EPCR, CD36 and ICAM-1, among others [3–9]. The mass of sequestered Pf-IEs leads to microvascular obstruction [10–13], alteration of tissue perfusion, hypoxia, acidosis [10,12], local inflammation and endothelial activation [14].

The systemic endothelial activation in malaria results from the sequestration of Pf-IEs, besides others factors such as presence of parasite material released by the schizont rupture and the induction of proinflammatory cytokines. Endothelial activation is characterized by the widespread release of molecules such as von Willebrand factor (vWF) [15,16], soluble ICAM-1 [15–17] angiopoietin-2 (ang-2) [15,16,18,19], and soluble Tie-2 receptor [15,16]. Although there is a relationship between severity and endothelial activation, the increase of these molecules has also been found in uncomplicated malaria and asymptomatic infections [18,20–22].

In vitro models have shown that *P. falciparum* laboratory strains (FCR3 and 3D7) activate endothelial cells resulting in expression of proinflammatory cytokines, adhesion molecules,

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signal transduction and apoptosis and/or survival [23−30]. Pf-IEs increase the expression of ICAM-1 on the endothelial cell membrane [23,28,31] and activate CD36 dephosphorylation, leading to an increased affinity for this receptor [30]. A proinflammatory state in endothelial cells has also been observed, Pf-IEs increase the secretion of cytokines such IL-6 [23,26], IL-1 and TNFα [26], and chemokines such IL-8 (CXCL8) [23,24,26], MCP-1 [23], MIP3A, Gro-alpha and RANTES [26], which are important in the recruitment of leukocytes to the endothelium during inflammation.

Sequestration of *P. falciparum* to endothelia occurs in infected patients; however, its association with the variable symptoms and complications of the disease is not well established, thus a better understanding of the endothelial response to the parasite infection, may help to further understand the pathogenic mechanisms in malaria and to the possible design of novel therapeutic strategies. Endothelial cell activation in *P. falciparum* malaria has been mostly studied using laboratory strains [23−31], but there are few reports with field isolates [32,33]. The aim of this study was to explore whether uncomplicated malaria isolates induce inflammatory activation of endothelial cells and if these changes are associated with cytoadherence.

2. Materials and methods

2.1. Endothelial cultures

Primary HDMEC were obtained from Clonetics®-Lonza (CC-2543) and cultured according to the distributor's recommendations. The cells were incubated at 37 °C, 5% CO_2, in EBM-2 (Lonza, CC-3156) medium with growth supplements (Lonza, CC-4147) in T25 tissue culture flask (Falcon). The cells were passaged 3 to 5 times before use.

2.2. P. falciparum *strains cultures*

The strains FCB1 and FCB2 (Colombia) and FCR3 and 3D7 (Africa) were cultured in A+ erythrocytes in RPMI-1640 medium (Sigma) supplemented with 25 mM HEPES (Sigma), 0.2 mM hypoxanthine (Sigma), 16 μg/ml gentamicin (Sigma), 21.6 mM of $NaHCO_3$ (incomplete medium); plus 10% A+ human serum (complete medium) [34]. The cultures were adjusted to 1−2.5% haematocrit and 1−15% parasitaemia and incubated in 5% CO_2 at 37 °C. Mycoplasma spp. contamination was evaluated by PCR [35]. Maintenance of the Knob-positive phenotype was carried out using gelatine (Sigma) [36].

2.3. P. falciparum *isolates cultures*

P. falciparum isolates from Turbo-Antioquia (Colombia) and Tumaco-Nariño (Colombia) were obtained from patients with uncomplicated malaria during 2005−2006 and 2011−2012. All patients were informed about the study and asked to participate voluntarily by signing a written Informed Consent, approved by the Ethics Committee of the Facultad de Medicina of Universidad de Antioquia. Uncomplicated malaria was defined as parasitaemia with symptoms associated with malaria (chills, fever and sweating) or other unspecific symptoms (headache, muscle pain, weakness, nausea, diarrhoea, others), without symptoms and signs of severity or evidence of a vital organ dysfunction [37].

Blood samples in EDTA were centrifuged three times in RPMI-1640 at 600 × g, for 5 min at room temperature and erythrocytes were cultured at 2% haematocrit. Parasite stabilates stored in liquid nitrogen were thawed as follows: A 12% saline solution (0.4 ml/0.5 ml of blood) was added for 5 min, followed by a 1.5% saline solution (9 ml/0.5 ml of blood). The samples were centrifuged at 600 × g for 5 min at room temperature and the supernatants were removed. Next, a 0.9% saline solution plus 0.2% dextrose (9 ml/0.5 ml of blood) was added, thereafter samples were centrifuged at 600 × g for 5 min at room temperature and the pellet of erythrocytes cultured at 2% haematocrit.

2.4. Concentration of mature stages of P. falciparum

The mature stages (trophozoites and schizonts) were obtained by floatation on gelatine [36]. Ten volumes of 1% type A gelatine (Sigma G2625) were added to one volume of Pf-IEs in incomplete RPMI-1640 and incubated at 37 °C for 40−50 min. The supernatant with the mature stages was collected, centrifuged at 600 × g for 5 min and the parasitaemia was calculated in Giemsa stained smears.

2.5. Cytoadherence tests

The HDMEC cells were cultured in lab-tek II chambers (Nunc) at 60−80% of confluence and incubated with mature stage Pf-IEs (300 ul, 10% parasitaemia, 1% haematocrit) in adhesion medium (RPMI-1640, BSA at 0.5%, pH 6.7) for 1 h at room temperature under agitation (100 rpm). The non-adhered erythrocytes were removed by washing 4 times with adhesion medium. The HDMEC cells with adherent erythrocytes were fixed overnight in 2% glutaraldehyde and stained with 1% Giemsa for 45 min. The number of adhered Pf-IEs was quantified under microscope in 300 HDMEC cells [38].

2.5.1. Adhesion blocking by anti-CD36 and anti-ICAM1

Endothelial surface receptors were blocked with 10 ug/ml of monoclonal antibodies anti-CD36 (clone 185-1G2, abcam ab76521) and anti-ICAM-1 (clone 15.2, AbDserotec MCA532) for 40 min at room temperature in the adhesion medium, the cells were washed and the cytoadhesion assay was performed.

2.6. Co-culture of HDMEC cells with Pf-IEs

The HDMEC cells at 90−100% confluence in 6 wells dishes (Nunc) were incubated with Pf-IEs suspension (1 ml, 10% parasitaemia, 3% haematocrit) in culture medium EGM-

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