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The effect of *Plasmodium falciparum* infection on expression of monocyte surface molecules

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Summary *Plasmodium falciparum* infection may result in severe malaria in susceptible individuals. The pathogenesis of severe disease is probably a combination of the sequestration of infected erythrocytes and overstimulation of the immune response. Monocytes are a key source of many of the pro-inflammatory agents implicated but also are found sequestered in blood vessels. However, little is known about the monocyte phenotype in malaria disease. Flow cytometry was performed on fresh whole blood to determine surface expression of four receptors during acute severe and non-severe malaria and again during convalescence when uninfected. Three hundred and fifty-six children with *P. falciparum* infection were studied and were found to show increased expression of intercellular adhesion molecule-1 (ICAM-1), urokinase plasminogen activator receptor (uPAR), CD23 and chemokine receptor 5 (CCR5) ($P < 0.001$) during acute disease compared with convalescent levels. Using multivariate analysis, it was found that large increases in expression of ICAM-1 (odds ratio (OR) 2.44, 95% CI 1.80–3.32) and uPAR (OR 3.14, 95% CI 1.93–5.09) but small increases in expression of CD23 (OR 0.82, 95% CI 0.68–0.96) were independently associated with severe malaria. These results give an insight into the cellular processes occurring in severe malaria and suggest that pathology is based on a complex repertoire of pro- and anti-inflammatory processes.

1. Introduction

In endemic areas, *Plasmodium falciparum* malaria shows a wide clinical spectrum, ranging from asymptomatic infection to severe disease and death. The pathogenesis of cerebral and severe malaria is not well understood. Organ-specific pathogenesis probably requires sequestration of infected erythrocytes (IE) on endothelial cells. The

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cytoadhesion process of IEs is mediated by the variable and diverse *var* gene products, encoding *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1), that are displayed on the surface of IEs and can bind to many host cellular proteins. In addition, several immune and inflammatory mediators are heavily implicated in the pathophysiology of severe malaria (for review, see Clark and Cowden, 2003).

There is increasing awareness of the role of monocytes in malaria pathogenesis. It is well known that these cells are an important reservoir of many relevant pro- and anti-inflammatory mediators. Monocytes have also been found associated with sequestered IEs in cerebral post-mortem specimens (Oo et al., 1987; Patnaik et al., 1994; Porta et al., 1993). In a post-mortem survey of Malawian children who had died from cerebral malaria, 75% of the brain sections containing sequestered IEs had additional microvascular pathology often involving monocytes (Taylor et al., 2004). In a similar study of Malawian children, in 14 of 32 cases that had been clinically diagnosed with cerebral malaria, sequestered IEs were associated with intravascular monocyte aggregations (Clark et al., 2003). By contrast, in a post-mortem survey of Southeast Asian adults with cerebral malaria, no such inflammatory lesions were seen (Pongponratn et al., 2003), suggesting that accumulation of monocytes at the site of IE sequestration may be a distinct feature of pathological processes occurring in children. These monocytes potentially have a great effect on local cytokine concentrations.

Little is known about the effect of *P. falciparum* infection on circulating monocytes or whether monocyte activation might influence whether an individual develops severe disease. Accordingly, we studied the surface expression of ICAM-1 (intercellular adhesion molecule-1; CD54), CD23 (low affinity IgE receptor), uPAR (urokinase plasminogen activator receptor; CD87) and CCR5 (chemokine receptor 5; CD195). Each of these receptors has previously been implicated in the pathogenesis of severe malaria.

ICAM-1 is an adhesion molecule expressed on endothelial cells and leukocytes that is involved in leukocyte trafficking through endothelial layers via binding to β_2-integrins. It is also implicated in the pathophysiological process leading to cerebral malaria (Turner, 1997). Upregulation of ICAM-1 on the endothelium is widespread and co-localised with sequestered IEs in cerebral venules in post-mortem specimens from patients who died from cerebral malaria (Turner et al., 1994). ICAM-1 is cleaved from cellular surfaces to produce a soluble form (sICAM-1) and some investigators have found increased levels to be associated with severe malaria (Jakobsen et al., 1994; McGuire et al., 1996). However, these results must be treated with some caution as circulating sICAM-1 and endothelial cell surface ICAM-1 expression are not well correlated (Turner et al., 1998). In a case—control study in Kenya, adhesion of IEs to ICAM-1 was highest in those with cerebral malaria compared with asymptomatic controls (Newbold et al., 1997). An ICAM-1 polymorphism (ICAM-1^{Kilifi}), which results in functional differences in ICAM-1 binding to IEs (Adams et al., 2000), is common in sub-Saharan Africa and may predispose to cerebral malaria (Fernandez-Reyes et al., 1997).

Levels of IgE increase in *P. falciparum* infection, which crosslinks CD23 resulting in increased inducible nitric oxide synthase (iNOS) expression and TNF production by monocytes in vitro (Perlmann et al., 1999). uPAR is upregulated with the acute inflammatory response, increasing in response both to in vivo injection of endotoxin and TNF (Dekkers et al., 2000). uPAR is believed to associate with surface β_2-integrin molecules to augment monocyte adhesion to endothelial cells. In vitro, β_2-integrin-mediated adhesion of monocytes to the endothelium was enhanced in the presence of uPAR expression on the cell surface and inhibited by the blockade of uPAR using anti-uPAR monoclonal antibody, R3 (May et al., 1998). In post-mortem brain specimens from European adults, there was increased monocyte uPAR staining in those who had died from malaria compared with those who died from meningitis (Fauser et al., 2000). In the mouse severe malaria model, mice deficient in uPAR had a significantly delayed mortality (Piguet et al., 2000) and mice deficient in the CCR5 receptor showed less coma and death (Belnoue et al., 2003) compared with wild-type mice. CCR5 is expressed by monocytes and lymphocytes, binds to multiple chemokines and is thought to be involved in leukocyte recruitment. The level of CCR5 mRNA was higher in brain tissues from Ghanaian paediatric cerebral malaria cases than non-malaria controls (Sarfo et al., 2004).

Here we present our findings that the expression of all four molecules is upregulated during *P. falciparum* infection. In multivariate analysis, higher levels of ICAM-1 and uPAR, but lower levels of CD23, are associated with severe malaria.

2. Methods and materials

2.1. Study site and enrolment

The study was conducted at Kilifi District Hospital (KDH) on the Kenyan coast. Malaria transmission occurs throughout the year, although clinical disease increases dramatically after the rainfalls that usually occur biannually during November/December and April—June.

Between August 2003 and June 2004, all children admitted to KDH with a fever >37.5 °C and *P. falciparum* infection (at any density for children <1 year or >2500/μl in children ≥1 year old) were considered (Mwangi et al., 2005). Severe malaria was defined as blood film-positive *P. falciparum* infection plus one or more of the following symptoms: prostration (inability to sit or breast feed), coma, respiratory distress (deep breathing or intercostal recession) or severe anaemia (haemoglobin <5 g/dl). Those with clinical or microbiological evidence of an additional bacterial infection were excluded. Children were recruited into the study when the attending parent or guardian gave informed consent. Blood taken at admission and at a clinic visit 6 weeks later if the child was well and had a negative malaria blood slide was used.

The study was approved by the Research Ethics Committee, Liverpool School of Tropical Medicine and the Kenya Medical Research Institute National Ethical Review Committee.

2.2. Determining pigment-containing monocytes

Malaria pigment was detected on Giemsa-stained thick films by counting 200 monocytes and determining the percentage