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Increased plasma concentrations of sICAM-1, sVCAM-1 and sELAM-1 in patients with \textit{Plasmodium falciparum} or \textit{P. vivax} malaria and association with disease severity

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\textbf{SUMMARY}

Increased serum concentrations of soluble intercellular adhesion molecule-1 (sICAM-1), soluble endothelial leucocyte adhesion molecule-1 (sELAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) were detected in Danish malaria patients infected with sequestering \textit{Plasmodium falciparum} or non-sequestering \textit{P. vivax} parasites, as well as in patients with sepsis or meningitis. Levels of soluble adhesion molecules remained elevated in the \textit{P. falciparum} patients for several weeks after initiation of treatment. Plasma concentrations of sICAM-1, sVCAM-1 and sELAM-1 were higher in Gambian children with severe \textit{P. falciparum} malaria than in children with mild malaria. Plasma levels of sVCAM-1 and sELAM-1 were significantly correlated. Plasma levels of sELAM-1 and sVCAM-1 may reflect endothelial inflammatory reactions and these reactions may be harmful for humans infected with malaria parasites.

\textbf{INTRODUCTION}

Cerebral malaria is one of the major severe disease manifestations of \textit{Plasmodium falciparum} infections. Sequestration of \textit{P. falciparum}-infected erythrocytes in the brain almost certainly plays an important part in the pathogenesis of this condition. \(^1\) Adhesion of infected erythrocytes to both endothelial cells and non-parasitized erythrocytes, forming rosettes, is believed to contribute to the sequestration of parasites and the obstruction of blood flow in brain capillaries and post-capillary venules. \(^2,3\) \textit{Plasmodium falciparum} isolates can bind to several adhesion molecules \textit{in vitro} including CD36, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and endothelial leucocyte adhesion molecule-1 (ELAM-1). \(^4,5\) High plasma concentrations of cytokines such as tumour necrosis factor-\(\alpha\) (TNF-\(\alpha\)) are associated with cerebral malaria, especially cases with a fatal outcome. \(^6,7\) TNF may have direct toxicity on endothelial cells, induce harmful concentrations of nitric oxide, and also play an important role in the pathogenesis of cerebral malaria by up-regulating the expression of the adhesion molecules ICAM-1, ELAM-1 and VCAM-1. \(^8,9\) This up-regulation of adhesion molecules may further increase the number of sequestering parasites in the brain. \(^9\) A marker of lymphocyte activation, soluble interleukin-2 receptor (sIL-2R), is also associated with cerebral malaria, suggesting that lymphocyte activation may contribute to the pathogenesis of cerebral malaria. \(^10,11\)

In comparison with \textit{P. falciparum}, infections with \textit{P. vivax} are relatively benign and are without cerebral manifestations. \textit{Plasmodium vivax} parasites do not generally sequester in the human host and it is not known whether endothelial inflammatory reactions take place in \textit{P. vivax}-infected patients.

Meningococcal meningitis involves inflammation of the tissues lining the brain and meninges, producing diminished integrity of the blood–brain barrier, and allowing meningococci to accumulate in the cerebrospinal fluid. \(^12\)

We have measured sICAM-1, sVCAM-1 and sELAM-1 as markers of endothelial inflammation in the peripheral blood of Danish patients with malaria or meningococcal disease, to investigate whether such inflammatory reactions are a uniform characteristic of these diseases. In addition, we have related the levels of these adhesion molecules to disease severity in Gambian patients with malaria.

\textbf{MATERIALS AND METHODS}

\textit{Danish patients}

Serum samples were collected from patients admitted to the
Department of Infectious Diseases, University Hospital, Copenhagen, Denmark as described by I. C. Bygbjerg (manuscript in preparation). Fifteen patients had *P. falciparum* malaria, eight patients had *P. vivax* malaria and 11 patients had meningitis and/or sepsis caused by *Neisseria meningitidis*.

All malaria cases (age 9–61 years) were uncomplicated. The diagnosis of malaria was confirmed by examination of Giemsa-stained thick films, and the species of malaria was determined by microscopy of thin films.

All patients with meningitis and/or sepsis (age 19–72 years) caused by *N. meningitidis* had severe disease, as the patients were comatose and/or had hypotension (systolic arterial blood pressure below 90 mmHg) and/or required artificial respiration or pressor support (catecholamine infusion). One patient died from the infection. The diagnosis of meningitis was made on the basis of clinical symptoms and the findings of polymorphonuclear pleocytosis in the cerebrospinal fluid, with positive microscopy or positive culture. The diagnosis of septicaemia was based on the finding of fever, systolic blood pressure < 90 mmHg and a positive blood culture.

Informed consent for additional analysis of blood samples was obtained from the patient, or close relatives, if the patient was unable to communicate.

Plasma and serum samples were collected from healthy Gambian adults as controls.

**Gambian patients**

Plasma samples were collected during the rainy season from children with mild or severe *P. falciparum* malaria admitted to the Royal Victoria Hospital in Banjul or the Medical Research Council (MRC) Laboratories at Fajara, the Gambia. Clinical malaria was defined as fever with parasitaemia of 2500 parasites/microlitre or more, in the absence of any other cause for the fever. Severe malaria was defined according to the World Health Organization criteria. The group with severe malaria comprised 29 children with cerebral malaria (coma score of 2 or less on the modified Glasgow coma scale), four children with severe anaemia (haemoglobin of less than 5 g/dl) and one child with hyperparasitaemia (parasitaemia > 1 million per microlitre blood), and two children with combined causes of severe disease. Thirty-nine children with mild malaria were treated as out-patients with oral medication.

Informed consent was obtained from the parent or guardian of each child. The project was approved by the Gambia Government/MRC Ethical Committee.

Seventeen children attending the out-patient clinic at the MRC Laboratories with minor complaints, mainly infections other than malaria, were included as controls. These children had no detectable parasitaemia at the time of blood collection.

**Blood sampling**

In Copenhagen, blood samples were collected by venepuncture and serum was prepared by centrifugation of the clotted blood samples.

In the Gambia, blood samples were collected by venepuncture into heparinized vacutainers (Becton Dickinson, Rutherford, NJ) and plasma obtained.

**Determination of soluble adhesion molecules**

Plasma and serum concentrations of sICAM-1, sVCAM-1 and sELAM-1 were measured by ELISA kits according to the manufacturer's instructions (British Biotechnology Products, Abingdon, UK). The sensitivity of each of the assays was < 2.5 ng/ml.

**Statistical methods**

The Wilcoxon–Mann–Whitney test was used for intergroup comparisons. *P*-values lower than 0.05 were considered significant. Spearman rank-order correlation coefficient ($r^s$) was used for evaluation of parameter association. Two-tailed *P*-values lower than 0.05 were considered significant.

The levels of sICAM-1, sVCAM-1 and sELAM-1 in Gambian children with mild or severe malaria were compared after correction for levels of parasitaemia using analysis of covariance.

**RESULTS**

**Soluble adhesion molecules in Danish patients**

Danish patients with *P. falciparum* malaria, *P. vivax* malaria or...
with meningitis and/or sepsis had increased plasma concentrations of sICAM-1, sVCAM-1 and sELAM-1 compared with healthy individuals (Fig. 1). Serum concentrations of sVCAM-1 were significantly correlated with concentrations of sICAM-1 ($r = 0.37$, $P < 0.05$) but not with sELAM-1 ($r = 0.15$, $P > 0.1$).

Consecutive samples were collected over a period of 25 days to 1 month from five *P. falciparum* patients (Fig. 2). Elevated concentrations of sICAM-1, sVCAM-1 and sELAM-1 continued for the first 3 days after initiation of treatment, then showed a small decline over the subsequent 3–4 weeks, but had still not reached control values by this time.

**Parasitaemia in Gambian patients**

Levels of parasitaemia at the time of plasma collection ranged from 0 to more than 750,000 parasites/µl in 37 children with mild malaria and 17 children with severe malaria. Levels of parasitaemia in the remaining children with clinical malaria were unknown. Differences in parasitaemia between children with mild or severe malaria were not statistically significant. Levels of parasitaemia in all the children with malaria correlated with plasma concentrations of sVCAM-1 ($r = 0.71$, $P < 0.005$) and with plasma concentrations of sELAM-1 ($r = 0.61$, $P < 0.01$), but not with plasma concentrations of sICAM-1 ($r = 0.27$, $P > 0.05$).

**Associations between soluble adhesion proteins and severity of malaria in Gambian children**

Significantly higher plasma concentrations of sICAM-1 were detected in children with severe malaria compared with children with mild malaria ($P < 0.05$) and with children with other mild non-malarial diseases ($P < 0.05$) (Fig. 3a).

Plasma concentrations of sICAM-1 were significantly correlated with plasma concentrations of sIL-2R previously reported in these children ($r = 0.63$, $P < 0.05$).

In comparison with children with mild malaria or mild non-malarial diseases, children with severe malaria had significantly higher plasma concentrations of sVCAM-1 and sELAM-1 ($P < 0.05$ for each, respectively) (Fig. 3b, c).

Plasma concentrations of sVCAM-1 were significantly correlated with plasma concentrations of sELAM-1 ($r = 0.85$, $P < 0.0005$). Associations of plasma concentrations of sELAM-1 and sVCAM-1 with sICAM-1 only just reached significance ($r = 0.38$, $P = 0.04$ and $r = 0.45$, $P = 0.05$, respectively).

Figure 4 shows the levels of adhesion molecules in children with mild or severe malaria in relation to the levels of parasitaemia. Children with mild malaria could be divided into two groups, those with levels of sELAM-1 and sVCAM-1 similar to children with severe malaria and those with much lower levels. The association between plasma levels of...
sELAM-1 or sVCAM-1 and disease severity was maintained after correction for levels of parasitaemia (analysis of covariance, \( P < 0.01 \)).

**DISCUSSION**

ICAM-1 (CD54) is a 95 000 MW cell-surface glycoprotein. Both mononuclear cells and endothelial cells secrete ICAM-1, and ICAM-1 expression on endothelial cells is up-regulated by cytokines such as TNF-\( \alpha \), IL-1 and interferon-\( \gamma \) (IFN-\( \gamma \)).\(^{15,16}\)

ICAM-1 is a ligand for lymphocyte function-associated antigen-1 (LFA-1) and is one of the receptors responsible for adhesion of T lymphocytes, monocytes and granulocytes to endothelium. Circulating ICAM-1 has previously been detected in human sera.\(^ {17}\)

VCAM-1 is a 90 000 MW cell-surface glycoprotein. It is expressed on dendritic cells and vascular endothelium activated by TNF, IL-1 and IL-4.\(^ {18}\) It plays a major role in the adhesion of leucocytes to the endothelium by interaction with its ligand very late activation antigen-4 (VLA-4), which is expressed by lymphocytes and monocytes. Soluble VCAM-1 is detectable in serum from healthy individuals and is found in increased concentrations in patients with diabetes.\(^ {15}\)

ELAM-1 (E-selectin) is a 115 000 MW cell-surface glycoprotein. It is transiently expressed on vascular endothelial cells in response to either TNF-\( \alpha \) or IL-1 and represents a specific marker for endothelial damage or activation. The ELAM-1 ligand is a sialylated, fucosylated molecule that is expressed on neutrophils, monocytes and a subset of memory T cells. Low levels of ELAM-1 are detectable in sera of healthy individuals.\(^ {15,19}\)

Sudanese adults with clinical episodes of uncomplicated *P. falciparum* malaria have increased plasma levels of sICAM-1, sELAM-1 and sVCAM-1.\(^ {20,21}\) We detected raised plasma concentrations of sVCAM-1 and sELAM-1 in Gambian malaria patients. Increased concentrations of sELAM-1 in these patients indicate that endothelial inflammatory reactions take place.

In addition, we found significantly higher concentrations of sELAM-1 and sVCAM-1 in Gambian children with severe malaria compared with children with mild malaria. Children with mild malaria could be subdivided into those children with levels of adhesion molecules similar to children with severe malaria and those with much lower levels. The location of the endothelial inflammatory reactions may be critical to the clinical manifestations of infection, children with severe malaria having endothelial inflammatory reactions in the brain, while children with mild malaria but high levels of adhesion molecules may have endothelial inflammatory reactions in other parts of the body. It is also possible that the children with mild malaria and high sELAM-1 and sVCAM-1 levels without treatment would have progressed to severe disease.

ICAM-1, ELAM-1 and VCAM-1 bind malaria parasite-infected erythrocytes\(^ {22,23}\) and their up-regulation by inflammatory cytokines may increase sequestration of parasites to endothelium in the brain, leading to cerebral malaria.

We also detected raised plasma concentrations of sICAM-1 in Danish and Gambian malaria patients. It is possible that both activated lymphocytes and vascular endothelium are sources of the sICAM-1 found in plasma. We found significantly higher levels of sICAM-1 in Gambian children with severe malaria compared with children with mild malaria. Concentrations of sICAM-1 correlated with concentrations of sIL-2R and we have previously found an association between severity of *P. falciparum* malaria and plasma levels of sIL-2R.\(^ {11}\) Activated T cells may contribute to endothelial inflammation. Both type 1 and type 2 T-helper responses may increase parasite sequestration in the brain, since IFN-\( \gamma \) up-regulates endothelial expression of CD36, ICAM-1 and ELAM-1, while IL-4 increases that of VCAM-1 on endothelial cells.\(^ {22,23}\)

*Plasmodium vivax* malaria patients also had raised plasma levels of sICAM-1, sVCAM-1 and sELAM-1. *Plasmodium vivax* parasites do not sequester in brain capillaries, unlike *P. falciparum* parasites. Our results therefore indicate that endothelial inflammatory reactions may be induced in the absence of sequestering parasites. However, *P. vivax* parasites induce the secretion of inflammatory cytokines,\(^ {24,25}\) which may be responsible for the induction of the adhesion molecules. It is likely that sequestration of parasite-infected erythrocytes to sites with activated endothelial cells is more important in the development of severe disease than endothelial inflammatory reactions alone, as non-sequestering *P. vivax* parasites rarely cause cerebral malaria compared to sequestering *P. falciparum* parasites. It has previously been reported that patients with septic shock have elevated concentrations of sELAM-1 in their
serum. These studies indicated that elevated concentrations of sELAM-1 developed in conjunction with the haemodynamic manifestations of advanced septicemia. We detected increased concentrations of sICAM-1, sELAM-1 and sVCAM-1 in patients with meningitis and sepsis. Levels of the soluble adhesion molecules in these patients were similar to the levels observed in \textit{P. falciparum} and \textit{P. vivax} patients. Meningococcal bacteria do not sequester in capillaries like \textit{P. falciparum} parasites, but induce the secretion of TNF-\textalpha, which is linked with disease severity\textsuperscript{6,7} as in \textit{P. falciparum} malaria.\textsuperscript{6,7}

In conclusion, \textit{P. falciparum} and \textit{P. vivax} malaria parasites, as well as meningococcal bacteria, induce the secretion of the adhesion molecules sELAM-1, sVCAM-1 and sICAM-1. Inflammatory cytokines are likely to be responsible for the induction of secretion of adhesion molecules. The association of high levels of adhesion molecules with disease severity is compatible with the view that the endothelial inflammatory reactions induced by infections such as malaria are harmful to the human host.

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**REFERENCES**


