Severe malaria in Cameroonian children: correlation between plasma levels of three soluble inducible adhesion molecules and TNF-α

Viviane H.M. Tchinda a, b, *, Armand D. Tadem b, Ernest A. Tako c, Gilbert Tene d, Josephine Fogako b, Philoma Nyonglema b, Grace Sama b, Ainong Zhou e, Rose G.F. Leke b

a The Medical Research Centre, Institute of Medical Research and Medicinal Plant Studies (IMPM), Ministry of Scientific Research and Innovation, Yaoundé Cameroon, P.O. Box 3851, Messa – Yaoundé, Cameroon
b The Biotechnology Center, University of Yaoundé I, Yaoundé, Cameroon
c Department of Biology, Georgetown University, Washington, DC, USA
d The Pediatric Unit, Chantal Biya Foundation, Yaoundé Central Hospital, Yaoundé, Cameroon
e AZ Data Clinic, Inc., Rockville, MD, USA

Received 29 November 2006; received in revised form 20 February 2007; accepted 21 February 2007
Available online 27 February 2007

Abstract

Plasma levels of three soluble inducible adhesion molecules, namely: intercellular adhesion molecule-1 (sICAM-1), vascular cell adhesion molecule-1 (sVCAM-1) and endothelial leucocyte adhesion molecule-1 (sELAM-1) or sE-selectin and the pro-inflammatory cytokine, tumour necrosis factor-alpha (TNF-α) were measured in well-defined clinical groups of children with severe and uncomplicated malaria. The goal of the study was to investigate the role of these molecules in immunopathogenic processes associated with severe malaria in Cameroonian children. Results showed significantly increased plasma concentrations of sICAM-1, sVCAM-1 and sE-selectin in children with severe malaria compared to those with uncomplicated malaria and healthy children (P < 0.001). TNF-α levels increased significantly in children with severe malaria, approximately 2-folds compared to those with uncomplicated malaria and about 3-folds compared to healthy children (P < 0.001). More importantly, levels of TNF-α strongly correlated with those of the three adhesion molecules and were significantly associated with increased risk of death (P = 0.03). In addition, children who died from severe malaria showed higher mean levels of all measured factors compared to those who recovered, with significant differences observed with sICAM-1 (P < 0.001) and sE-selectin (P = 0.002). Furthermore, children with severe malarial anemia relative to those without, showed significantly elevated levels of the three soluble molecules; and sICAM-1 was significantly associated with increased risk of severe anemia. Taken together, these results confirm the role of TNF-α and the three adhesion molecules in pathogenic processes associated with severe malaria in children, and suggest an association between sICAM-1 and severe malarial anemia.

© 2007 Elsevier B.V. All rights reserved.

Keywords: Severe malaria; Adhesion molecules; Cytokine; Cerebral malaria; Severe malarial anemia; Immunopathogenic processes

1. Introduction

Malaria remains one of the main causes of childhood morbidity and mortality in sub-Saharan Africa where
more than a million children mostly under the age of 5 years die from the disease each year (World Health Organization, 2003). Many of these children die from severe complications such as cerebral malaria, severe anemia associated or not with respiratory distress. The development of severe malaria probably results from a combination of parasite-specific factors, such as adhesion and sequestration in the vasculature and the release of bioactive molecules, together with host inflammatory responses. These include cytokine and chemokine production and cellular infiltrates (Mackintosh et al., 2004). During the erythrocytic cycle, malarial toxins direct systemic release of pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), which act on many other cellular systems such as endothelium to upregulate the expression of adhesion molecules (Pober, 1988; Gearing and Newman, 1993; Miller et al., 1994). At least three inducible adhesion molecules, intercellular adhesion molecule-1 (ICAM-1), E-selectin or endothelial leucocyte adhesion molecule-1 (ELAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are involved in the cytoadherence of malarial erythrocytes to endothelial cells, leading to obstruction of blood vessels in the brain of patients with cerebral malaria (Ockenhouse et al., 1992; Turner et al., 1994; Dobbie et al., 1999). In addition to endothelial expression of adhesion molecules, soluble forms are released into the serum, either because of shedding from endothelial cell surface or differential mRNA splicing to form a truncated, soluble form with no cytoplasmic anchor sequence (Pigott et al., 1992). Several studies conducted either in malaria patients or in a primate model for human severe malaria have shown that serum levels of soluble forms of these adhesion molecules were elevated and correlated with disease severity (Hviid et al., 1993; Wenisch et al., 1994; Muanza et al., 1999; Kawai et al., 2003). It has also been shown that African children with severe malaria have increased levels of circulating TNF-α, and the highest concentrations are associated with the most severe form of the disease (Grau et al., 1989; Kwiatkowski et al., 1990). Several lines of experimental evidence have also shown the role of pro-inflammatory cytokines such as TNF-α in the pathogenic process of malarial anemia, including severe disruption of erythropoiesis, and suppression of proliferation of erythroid progenitor cells in human marrow culture (Roodman et al., 1987; Clark and Chaudhri, 1988; Shaffer et al., 1991). The immunopathogenic mechanisms underlying severe malaria are still poorly understood and remain a subject of debate; TNF-α appears to be central to these mechanisms, by up-regulating the expression of adhesion molecules at the surface of endothelium, amongst others. In this study, we investigated the correlation between plasma levels of TNF-α and circulating forms of ICAM-1, VCAM-1 and E-selectin in well-characterized clinical groups of Cameroonian children with severe and uncomplicated malaria.

2. Materials and methods

2.1. Study site

The study was conducted in Yaoundé, the capital city of Cameroon. Transmission of malaria occurs throughout the year, but the level of transmission is variable throughout the city, depending on the presence of marshes and ponds where mosquitoes breed. During the high transmission period, severe malaria anemia cases occur more frequently while cases of cerebral malaria tend to increase during the low transmission season (dry season).

2.2. Study subjects

Children who participated in this study were recruited at the emergency and external consultations’ sections of the Pediatric Unit of Yaoundé Central Hospital, known as The Chantal Biya Foundation. This is the main referral hospital in town where most severe malaria cases are referred to and managed accordingly. The 212 children investigated were of both sex and aged between 6 months and 10 years. 114 were severe malaria cases and 55 were uncomplicated malaria cases according to World Health Organization established criteria (World Health Organization, 2000). 43 age-matched healthy children without malaria parasites were recruited to serve as normal controls. Informed consent was obtained from the parents or guardians of all children before their inclusion into the study and the study was approved by the National Ethical Committee of Cameroon.

2.3. Clinical definition of malaria and study groups

Malaria infection was defined as presence of fever (axillary’s temperature $\geq 37.5^\circ C$ or rectal temperature $\geq 38^\circ C$) or previous episodes of fever before consultation (assessed by the mother or guardian at home) in a child presenting with symptoms of severe or uncomplicated malaria as described by The World Health Organization without any other clinical sign. Children were classified into five study groups according to their clinical picture and presence of malaria parasites...
in their peripheral blood smear.

1. Cerebral malaria (CM): defined as a Blantyre coma score of <3 (persisting for more than 30 min after effective treatment of hypoglycemia or seizures) in a child with *Plasmodium falciparum* parasitemia and no other apparent cause of coma.

2. Severe malaria anemia (SMA): according to WHO’s definition criteria, SMA in children is defined as hemoglobin level <5.0 g/dl (or hematocrit <15%) combined with a malaria parasitemia. But due to the fact that some children with respiratory distress and hemoglobin equal to 5 g/dl showed signs of decompenzated anemia, they were also classified in this study as severe anemia cases.

3. Others severe forms (OSF) of malaria: defined as complications other than the previous cited, mainly cases of seizures, prostration, hypoglycemia, etc., with a positive peripheral blood smear.

4. Uncomplicated malaria (UM): defined as clinical symptoms of malaria, a positive peripheral parasitemia but with none of the above complications.

5. Healthy malaria negative (HMN): defined as age-matched healthy children with a negative peripheral blood smear.

2.4. Case management

Children were given the best standards of treatment for severe and uncomplicated malaria authorized by the National Malaria Control Program, Ministry of Health of Cameroon during the recruitment period (2001–2003). All children with severe malaria were treated with either arthemether given intramuscularly or intravenous quinine. Those with uncomplicated malaria were treated with amodiaquine syrup. Admitted patients were followed-up on a daily base until discharge.

2.5. Laboratory analyses

Blood samples were collected from children on admission before administration of anti-malarial therapy and were immediately transported to the laboratory for processing and storage. Analyses on fresh blood included parasitological determination of malaria parasitemia by microscopy, blood glucose level (glucometer One-Touch Basic™), hematocrit and hemoglobin level to assess anemia, and full blood count (performed microscopically using a Neubauwer). Plasma was immediately isolated after centrifugation, shared into different aliquots and stored frozen at −70°C until used for immunological experiments.

2.6. Parasitological analysis and assessment of anemia

Both thick and thin smears were prepared on the same slide, then dried thin smear was first fixed in methanol, and the whole slide (both thin and thick films) was then stained with Diff-quick solutions A (Eosin red) and B (Thiazine blue) [Baxter International, Deerfield, IL, USA]. Stained slides were dried and examined by two different microscopists. A slide was considered to be positive if malaria parasites were detected in the blood smear and negative if parasites were not detected after examination of 200 oil-immersion fields of the thick smear. Thin films were mostly used to confirm malaria parasite species. Parasite density per micro-liter of blood was determined on thick blood smear by counting the number of parasites present per 200 leukocytes; this number was then multiplied by the average leukocyte count in individual child, and then divided by 200.

For assessment of anemia, heparinized micro capillary tubes were filled with fresh blood and spun at 15,000 rpm for 5 min using a microcapillary centrifuge (IEC Micro-MB centrifuge). The packed cell volume (PCV) was determined using a micro-hematocrit reader. Hemoglobin level (g/dl) was determined directly from PCV by dividing each value obtained by three.

2.7. Measurement of plasma levels of the three soluble inducible adhesion molecules and TNF-α

These were performed using commercially available ELISA kits. For sICAM-1 and TNF-α, a sandwich ELISA method using DuoSet® ELISA Development System kits (R&D Systems, Inc. Minneapolis, USA) were used. All the necessary reagents: capture antibody, detection antibody, standards and enzyme conjugate were provided with the kits and all experiments were carried out following the manufacturer’s instructions. For sVCAM-1 and sE-selectin, Parameter® kits (R&D Systems Inc. Minneapolis, USA) with pre-coated plates were used and all necessary reagents were also provided with the kits and experiments were carried out following the manufacturer’s instructions.

2.8. Calculation of results

A standard curve was performed for each assay by plotting on a graph paper, the mean absorbance for each standard dilution on the y-axis against the corresponding concentration on the x-axis, and then a
best-fit curve was drawn through the points on the graph. The concentrations of each tested molecule (sICAM-1, sVCAM-1, sE-selectin and TNF-α) in individual sample were determined by interpolation of corresponding mean absorbance on the standard curve.

2.9. Statistical analysis

Mean concentrations of different tested molecules were calculated and between-group comparisons were done using Kruskall–Wallis or Wilcoxon rank-sum tests. Chi-square test was used for between-group comparison of categorical measures. Correlations between adhesion molecules concentrations and cytokine levels were examined using Pearson correlation coefficients. The level of significance was set at 0.05. All the analyses were performed using SAS software (Version 9.1, SAS Institute, Cary, NC, USA).

3. Results

3.1. Clinical and biological characteristics of the study population

A total of 212 children both male and female participated in this study. They included: 114 children with severe malaria among whom, 27 (24%) had cerebral malaria, 42 (37%) had severe malarial anemia and 45 (39%) had other forms of malaria complications, mainly seizures, prostration, hemoglobinuria and hyperparasitemia. 55 children had uncomplicated malaria and 43 were healthy children without malaria parasites. A summary of clinical characteristics of the five study groups is given in the Table 1.

Children with clinical symptoms of severe malaria were significantly younger than those with uncomplicated malaria, with the lowest mean hemoglobin level. In fact, we found in these children that age was an important risk factor for severe malaria ($\chi^2 = 6.39$, $P < 0.01$) and severe anemia ($\chi^2 = 6.60$, $P < 0.01$). Moreover, children with severe malarial anemia were those of significantly younger age (mean age = 19.34 ± 16.56 months) than those having cerebral malaria (mean age = 39.15 ± 23.26 months) or other forms of malaria complications (mean age = 29.68 ± 22.33 months) [$P < 0.001$]. Higher mean levels of malaria parasitemia and total number of white blood cells (WBC) were also found in children with severe malaria compared to those with uncomplicated malaria (see Table 2).

3.2. Mortality among children with severe malaria

Overall 18 children (15.8%) with severe malaria died usually within 24 h of admission. These included 6 of the 27 children with cerebral malaria (22.22%), 8 (19 %) of the 42 with severe malarial anemia and 4 (8.88%) of the 45 with other forms of malaria associated complications. Demised children were of significantly younger age (mean age = 19.22 ± 14.57 months) compared to those who recovered from infection (mean age = 32.89 ± 25.15 months) [$P < 0.005$]. They also showed significantly higher mean levels of total WBC (19,284 ± 16,829 versus 11,338 ± 6722 WBC/µl of blood) [$P < 0.007$].

Table 1
Clinical characteristics of the five study groups

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>Study groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CMa</td>
</tr>
<tr>
<td>Number of subjects</td>
<td>27</td>
</tr>
<tr>
<td>Fatal cases (%)</td>
<td>6 (22.22%)</td>
</tr>
<tr>
<td>Pallor</td>
<td>11 (40%)</td>
</tr>
<tr>
<td>Prostration</td>
<td>21 (78%)</td>
</tr>
<tr>
<td>Impaired level of consciousness</td>
<td>27 (100%)</td>
</tr>
<tr>
<td>Coma (Blantyre coma scale &lt;3)</td>
<td>27 (100%)</td>
</tr>
<tr>
<td>Seizures</td>
<td>24 (89%)</td>
</tr>
<tr>
<td>Circulatory collapse</td>
<td>5 (19%)</td>
</tr>
<tr>
<td>Abnormal breathing</td>
<td>6 (22%)</td>
</tr>
<tr>
<td>Jaundice</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>Hemorrhagic signs</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Dehydration</td>
<td>7 (26%)</td>
</tr>
<tr>
<td>Hemoglobinuria</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

a CM: cerebral malaria; SMA: severe malaria anemia; OSF: other severe forms; UM: uncomplicated malaria; HMN: healthy malaria negative.
b The corresponding clinical sign was not observed in any subject.
3.3. Plasma levels of TNF-α and the three soluble inducible adhesion molecules in children with different clinical presentations

Mean plasma levels of TNF-α were significantly elevated, approximately 2-folds in children with severe malaria compared to those with uncomplicated malaria, and about 3-folds compared to healthy malaria negative controls ($P<0.001$). There was no statistically significant difference between children with uncomplicated malaria and healthy malaria negative children ($P=0.106$). Children with cerebral malaria showed the highest mean levels of TNF-α, but no significant difference was observed between the three severe malaria categories ($P>0.05$) [Table 3]. Higher mean plasma levels of TNF-α were found in children who died from severe malaria compared to those who recovered from infection, but the difference was not statistically significant (Table 4). TNF-α and severe anemia were significantly associated with an increased risk of death ($\chi^2 = 4.34$, $P=0.03$ and $\chi^2 = 3.11$, $P<0.07$, respectively).

Results of the three adhesion molecules showed a general trend towards higher mean levels of sICAM-1, sVCAM-1 and sE-selectin in children with severe malaria and moderately increased levels in those with uncomplicated malaria compared to low levels in normal children without malaria parasites (Table 3). Differences were statistically significant ($P<0.001$) between severe and uncomplicated malaria groups and between the latter and normal children ($P<0.001$). No significant differences were found between the three severe malaria groups. Children dying from severe malaria showed higher mean levels of the three soluble molecules compared to those who recovered, and significant differences were observed with sICAM-1 ($P<0.001$) and sE-selectin ($P=0.002$) [Table 4]. Moreover, levels of sICAM-1, sVCAM-1 and sE-selectin significantly correlated ($P=0.0006; 0.009$ and $<0.0001$, respectively) with those of TNF-α, a predictor of mor-

Table 3
Mean levels of sICAM-1, sVCAM-1, sELAM-1 and TNF-α in children with different clinical presentations of malaria

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Molecules tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sICAM-1 (ng/ml)</td>
</tr>
<tr>
<td>CM (n=27)</td>
<td>705.93 ± 248.18</td>
</tr>
<tr>
<td>SMA (n=42)</td>
<td>811.86 ± 306.34</td>
</tr>
<tr>
<td>OSF (n=45)</td>
<td>665.61 ± 269.15</td>
</tr>
<tr>
<td>UM (n=55)</td>
<td>524.16 ± 230.32</td>
</tr>
<tr>
<td>HMM (n=43)</td>
<td>286.92 ± 148.40</td>
</tr>
</tbody>
</table>

* CM: cerebral malaria; SMA: severe malaria anemia; OSF: other severe forms; UM: uncomplicated malaria; HMM: healthy malaria negative; sICAM-1: soluble intercellular adhesion molecule-1; sVCAM-1: soluble vascular cell adhesion molecule-1; sELAM-1: soluble endothelial leucocyte adhesion molecule –1; TNF-α: tumour necrosis factor-alpha.
ICAM-1: soluble intercellular adhesion molecule-1; VCAM-1: soluble vascular cell adhesion molecule-1; ELAM-1: soluble endothelial leucocyte adhesion molecule-1; TNF-α: tumour necrosis factor-alpha.

Table 4
Mean levels of sICAM-1, sVCAM-1, sELAM-1 and TNF-α in children who recovered from severe malaria compared to those who died

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Molecules tested</th>
<th>sICAM-1 (ng/ml)</th>
<th>sVCAM-1 (ng/ml)</th>
<th>sELAM-1 (ng/ml)</th>
<th>TNF-α (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall (n = 212)</td>
<td>sICAM-1 (ng/ml)</td>
<td>589.01 ± 307.62</td>
<td>847.99 ± 436.47</td>
<td>149.77 ± 87.19</td>
<td>83.66 ± 166.13</td>
</tr>
<tr>
<td>Recovered (n = 151)</td>
<td>sVCAM-1 (ng/ml)</td>
<td>566.07 ± 297.69</td>
<td>834.88 ± 428.53</td>
<td>143.51 ± 83.70</td>
<td>72.18 ± 137.47</td>
</tr>
<tr>
<td>Died (n = 18)</td>
<td>sELAM-1 (ng/ml)</td>
<td>833.66 ± 313.63</td>
<td>987.84 ± 506.11</td>
<td>216.55 ± 97.85a</td>
<td>206.15 ± 331.08</td>
</tr>
</tbody>
</table>

α Significant differences (P < 0.001 and P = 0.002, respectively, for sICAM-1 and sELAM-1 [sE-selectin]) between children who died from severe malaria and those who recovered.

4. Discussion

The overall objective of this study was to investigate the role of three soluble inducible adhesion molecules namely, sICAM-1, sVCAM-1 and sE-selectin, and the pro-inflammatory cytokine TNF-α in immunopathogenetic processes associated with severe malaria in Cameroonian children. In the present study, we used three well-characterized clinical groups of children with different presentations of severe malaria, while in many published studies, severe malaria in children has always been classified into two main forms namely; cerebral malaria and severe malarial anemia. Here we generated a third clinical group with malaria associated complications other than cerebral malaria or severe anemia, mainly cases of seizures, prostration, hypoglycemia, etc, combined with a positive peripheral blood smear.

Our data showed that severe malarial anemia and other forms of malaria complications were the predominant complications encountered in children consulting with severe malaria at the Pediatric unit of Yaoundé Central hospital; most cases occurring during the high transmission period while cases of cerebral malaria occur infrequently and tend to increase during the dry season when transmission is low. These observations are in agreement with those previously reported in areas with intense transmission to malaria (Marsh, 1992; Miller et al., 1994; Snow and Marsh, 2002).

Our current results on TNF-α showed evidence for increased levels of this cytokine during severe malaria in children (cerebral malaria and severe malarial anemia especially), its significant correlation with levels of the three soluble inducible adhesion molecules and the significant association of high levels of this cytokine with poor outcome. In fact, TNF-α is one of the most studied cytokine in malaria. Earlier studies on the role of TNF-α in the pathogenesis of malaria showed low serum levels at physiological concentrations to be beneficial while very high levels were harmful and associated with severe malaria especially cerebral malaria (Grau et al., 1989; Kwiatkowski et al., 1990; Kern et al., 1992; Marsh, 1992; Miller et al., 1994). In addition, TNF-α and other pro-inflammatory cytokines including IL-1 and IL-6 have also been implicated in the pathogenesis of severe malarial anemia, through suppression of erythropoiesis (Roodman et al., 1987; Clark and Chaudhri, 1988; Shaffer et al., 1991). As TNF-α is downregulated by IL-10, an anti-inflammatory cytokine that plays a role in T-helper type 2-like immune response and that appears to stimulate erythropoiesis (Wang et al., 1996); it has been proposed that P. falciparum-infected persons who produce balanced levels of IL-10 to regulate the excessive TNF-α activity could escape from developing severe or moderate malarial anemia (Othoro et al., 1999; May et al., 2000; Nussenblatt et al., 2001). Moreover, results of the study by Nussenblatt et al. (2001) have also suggested that younger children do not maintain IL-10 production in response to the inflammatory process, and this mechanism may contribute to the more severe anemia found in younger children. In line with this, it is possible that children with severe malarial anemia in the present study may as well develop an impaired Th2 type response (low IL-10) as the latter were significantly younger that those without severe anemia.
Our results also provide evidence for increased plasma concentrations of soluble forms of the three inducible adhesion molecules sICAM-1, sVCAM-1 and sE-selectin during malaria, with the highest levels being associated with severe forms of the disease as previously reported (Hviid et al., 1993; Wenisch et al., 1994; Muanza et al., 1999; Kawai et al., 2003). Moreover, levels of all the three molecules were significantly elevated in children with severe malaria compared to those with uncomplicated infection, while results of previous studies in adults did not show any significant difference for sVCAM-1 and sE-selectin in the two mentioned groups (Muanza et al., 1999). This may be due to differences in severe malaria in children and adults.

Correlation between high levels of soluble adhesion molecules and TNF-α is in agreement with the hypothesis that in response to malarial toxins, TNF-α is released by macrophages and together with other inflammatory cytokines may act on many cellular systems such as the vascular endothelium to upregulate the expression of adhesion molecules, especially ICAM-1 leading to cytoadherence of erythrocytes infected with late stages of malaria parasites. This phenomenon leads to the blockade of deep capillaries in the brain causing cerebral malaria (Pober, 1988; Gearing and Newman, 1993, Miller et al., 1994). Indeed, increased expression of adhesion receptors ICAM-1, E-selectin, and VCAM-1 has been shown in the brain of fatal malarial cases (Ockenhouse et al., 1992; Turner et al., 1994), thus associating these molecules to the pathogenesis of cerebral malaria. Additionally, it was suggested that amongst the implicated adhesion molecules, ICAM-1 may have the more important role as it is widely distributed on cerebral vessels and adhered parasitized erythrocytes in cerebral microvessels co-localize with endothelial expression of ICAM-1 (Turner et al., 1994). Then, the adhesion of infected erythrocytes to ICAM-1 was found to be greater in patients with cerebral malaria compared with other infected groups (Newbold et al., 1997).

Although, many studies in the literature have correlated endothelial expression of adhesion molecules with the pathogenesis of cerebral malaria, the relationship of increased plasma levels of soluble forms of these molecules to pathogenic processes of severe clinical signs such as severe anemia remains to be established. In fact, our data showed that plasma levels of sICAM-1 were significantly associated with increased risk of severe malaria, particularly severe malarial anemia and that children with severe malarial anemia showed significantly higher mean levels of the three soluble adhesion molecules and TNF-α, compared to those without. Thus, suggesting that adhesion molecules, particularly ICAM-1 may also be indirectly involved in the pathogenesis of severe malarial anemia.

In fact, the expression of ICAM-1 is known to be upregulated by TNF-α and we found a significant correlation between the levels of both molecules, thus suggesting that the mechanism underlying the possible role of sICAM-1 in the pathogenesis of severe malarial anemia is most probably related to the excessive production of TNF-α at the site of endothelial inflammation. Therefore, the high expression of ICAM-1 at the surface of activated endothelial cells and other cell types, together with the increased release of soluble forms; may contribute to the pathogenesis of severe malarial anemia by increasing the sequestration of infected erythrocytes and other cells such as monocytes and activated T cells, with known affinity for ICAM-1 (Hviid et al., 1993; Jenkins et al., 2006). Thus, interactions between infected erythrocytes sequestered through binding to ICAM-1 at the surface of activated endothelial cells or infected erythrocytes attached to soluble form of the molecule, and monocytes or activated T cells during their trafficking, will contribute to the amplification of the local endothelial inflammation with the release of more pro-inflammatory cytokines such as TNF-α, since monocytes are key source of many pro-inflammatory mediators. This will lead to the development of severe anemia. Although VCAM-1 and E-selectin can mediate adherence of infected erythrocytes and other cells to endothelium, their soluble forms do not seem to play a significant role in the pathogenesis of severe malarial anemia as we did not find any significant association between the levels of these two molecules and risk of severe anemia. This is probably due to the differences in the levels and control of expression of these two molecules compared to ICAM-1, as well as the differences in the kinetics of the disease-induced changes in circulating forms during the course of infection. ICAM-1 is a molecule with a wider cell distribution and it has been shown that enhanced ICAM-1 expression on endothelial cells can be sustained in the continuous presence of inflammatory cytokines, whereas ELAM-1 expression rapidly returns to basal levels (Hviid et al., 1993; Peyron et al., 1994; Wenisch et al., 1994). This may indicate that the intensity of endothelial inflammation together with cells sequestration can be prolonged with ICAM-1 receptor, leading to prolonged production of inflammatory mediators; thus sICAM-1 could have a prolonged effect on host-parasite interaction as previously stated (Wenisch et al., 1994).

Taken together these observations support the hypothesis that TNF-α upregulates the production of circulating
forms of inducible adhesion molecules, which are associated with disease severity in children. However, increased levels of TNF-α have also been observed during uncomplicated malaria, and high levels were also found in a large proportion of asymptomatic parasitemic children (Mshana et al., 1991). Other studies have shown that certain polymorphisms at the promoter region of TNF-α gene are associated with severe malaria in African populations (McGuire et al., 1999; May et al., 2000; Aidoo et al., 2001; Wassmer et al., 2003). Additionally, results of other studies on adhesion molecules suggest that genetic polymorphisms at host adhesion molecules loci are important variables in the susceptibility of African patients to severe malaria (Fernandez-Reyes et al., 1997; Amodu et al., 2005).

Acknowledgements

We wish to thank the children and their parents without whose cooperation; this study would not have been possible. We also thank the staff, the nurses and doctors of the hospital for their care of the patients. We are grateful to Professors Diane W. Taylor and Isabella A. Quakyi for their helpful contributions. This work received financial support from the UNICEF-UNDP-World Bank-WHO Special Programme for Research and Training in Tropical Diseases (Re-entry grant no. A00769). V.H.M.T. was supported in part by training grant 5D43 TWHO1264, Fogarty International Center.

References


