Cerebrospinal Fluid Cytokine Levels and Cognitive Impairment in Cerebral Malaria

Chandy C. John,* Angela Panoskaltsis-Mortari, Robert O. Opora, Gregory S. Park, Paul J. Orchard, Anne M. Jurek, Richard Idro, Justus Byarugaba, and Michael J. Boivin

Department of Pediatrics, University of Minnesota Medical School, Minneapolis, Minnesota; Department of Paediatrics and Child Health, Makerere University Faculty of Medicine, Kampala, Uganda; International Neurologic and Psychiatric Epidemiology Program (INPEP), College of Osteopathic Medicine, Michigan State University, East Lansing, Michigan

Abstract. Cerebrospinal fluid (CSF) and serum levels of 12 cytokines or chemokines important in central nervous system (CNS) infections were measured in 76 Ugandan children with cerebral malaria (CM) and 8 control children. As compared with control children, children with cerebral malaria had higher cerebrospinal fluid levels of interleukin (IL)-6, CXCL-8/IL-8, granulocyte-colony stimulating factor (G-CSF), tumor necrosis factor-α (TNF-α), and IL-1 receptor antagonist. There was no correlation between cerebrospinal and serum cytokine levels for any cytokine except G-CSF. Elevated cerebrospinal fluid but not serum TNF-α levels on admission were associated with an increased risk of neurologic deficits 3 months later (odds ratio 1.55, 95% CI: 1.10, 2.18, P = 0.01) and correlated negatively with age-adjusted scores for attention (Spearman rho, −0.34, P = 0.04) and working memory (Spearman rho, −0.32, P = 0.06) 6 months later. In children with cerebral malaria, central nervous system TNF-α production is associated with subsequent neurologic and cognitive morbidity.

INTRODUCTION

Cerebral malaria (CM) is a deadly disease that affects more than 500,000 children in sub-Saharan Africa every year and kills ~110,000 of these children. The pathogenesis of CM is thought to involve both parasite sequestration in the cerebral microvasculature, with tissue hypoxia and ischemic damage, and immunologic responses to P. falciparum, including cytokine responses. Cytokines and chemokines may protect from disease by direct and indirect effects on the parasite, but they may also contribute to disease, through recruitment of inflammatory cells, augmented production and activity of other cytokines, and direct toxicity to cells and tissue. Murine models of CM have clearly demonstrated involvement of brain parenchymal cells, with activation of microglial cells, damage to astrocytes, and increased mRNA expression of genes regulating tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ). Most human studies of central nervous system (CNS) cytokine responses in CM to date have relied on post-mortem analysis of cytokine expression in the brain tissue of children who died of CM. Studies of cytokine levels in children with CM have generally assessed serum levels of these cytokines, but these levels, although potentially important in the systemic disease caused by CM, may not correspond to cytokine levels and activity in the CNS.

Recent studies providing evidence for a degree of local breakdown of the blood–brain barrier (BBB) in CM provide a plausible mechanism for intrathecal cytokine production by brain parenchymal cells. Cytokines and chemokines could cross the BBB in an area of breakdown and affect brain tissue directly, or P. falciparum soluble exoantigens may cross the BBB and stimulate cytokine and chemokine production by microglial cells and astrocytes (as reviewed by Medana and Hunt). Microglial cell production of TNF-α occurs in murine CM and is thought to be part of the pathogenesis of this disease. Thus, CNS cytokines and chemokines may play a major role in CM pathogenesis, which, depending on the cytokine or chemokine and the timing of production, may be protective or injurious.

Cerebrospinal fluid levels of cytokines and chemokines have been assessed in other infectious and non-infectious diseases as an indicator of CNS cytokine levels, and have correlated with disease severity. With the recent advances in suspension array technology assays, levels of multiple cytokines and chemokines can be assessed from a single CSF sample, allowing a more complete profile of cytokine and chemokine activity in a disease process. In the present study, CSF and serum levels of 12 cytokines or chemokines considered important in the pathogenesis of CM and in other CNS infections were assessed in Ugandan children with CM and control children without evidence of neurologic disease. CSF cytokine levels on admission were then compared with neurologic outcomes 3 months after discharge and cognitive outcomes 6 months after discharge.

MATERIALS AND METHODS

Study population and recruitment. The study was conducted at Mulago Hospital, Kampala, Uganda. Children 4–12 years of age were recruited as part of two studies assessing the complications of CM. A total of 86 children with CM, 76 children with uncomplicated malaria, and 99 community children without evidence of acute illness were recruited. CSF samples were obtained from 76 of the 86 children with CM. Control samples for CSF testing consisted of stored samples from 8 children 8–15 years of age with inherited metabolic disorders who were seen at the University of Minnesota Children’s Hospital, Fairview. These children underwent lumbar punctures as a part of routine testing for bone marrow transplant evaluation. None were acutely ill or had evidence of infectious disease at the time of testing. Cerebrospinal fluid was kept frozen at −70°C until testing was performed.

A complete description of the Ugandan study cohorts has been previously published. Briefly, children with CM were enrolled if they were admitted to Mulago Hospital and met the WHO criteria for CM: coma (Blantyre coma scale ≤ 2 or Glasgow coma scale ≤ 8), P. falciparum on blood smear, and no other cause for coma. Lumbar punctures were performed to rule out meningitis and encephalitis unless the child had...
clinical contraindications to lumbar puncture. A CSF leukocyte count of >5 leukocytes/mm³ or the presence of bacteria on CSF Gram stain or culture were exclusion criteria. Ugan
dan Ministry of Health national guidelines for drug treatment of CM (including quinine for 7 days) were followed.

Blood samples of 5 mL were obtained by venipuncture on admission from 80 children with CM. Blood samples for serum testing were collected in a Vacutainer serum separator tube (BD Diagnostics, Franklin Lakes, NJ), gently inverted 4–5 times, allowed to clot in a horizontal position for 30 minu
tes, and then centrifuged at 1,000 × g for 10 minutes. The separated serum was pipetted into aliquots and frozen at −70°C until testing was performed; 72 of the 76 children who had CSF samples had matched serum samples for cytokine testing.

Written informed consent was obtained from the parents or guardians of study participants. Ethical approval for the study was granted by the Institutional Review Boards for Human Studies at Makerere University Faculty of Medicine, University Hospitals of Cleveland, Case Western Reserve University and Indiana Wesleyan University. Ethical approval for testing of CSF samples from the children with metabolic disorders was granted by the Institutional Review Board for Human Studies of the University of Minnesota.

Neurologic and cognitive testing. A complete neurologic exam was done on children with CM at discharge and 3 and 6 months after discharge. Cognitive testing was also done in children with CM who were 5 years of age or older at discharge and 3 and 6 months after discharge. Cognitive testing was performed in the areas of attention, working memory and tactile-based learning, and age-adjusted z-scores calculated using scores from age-matched healthy Ugandan children, as previously described. Six of 71 children (8.5%) still had deficits at 3 months, and only 1 of 68 (1.5%) had deficits at 6 months. CSF cytokine testing was performed on 8 paired CSF and serum samples on a single plate. Cytokine values on repeat testing correlated highly with those on previous testing (e.g., Spearman’s rho 0.53, P < 0.001). Repeat testing was independently associated with increased risk of neurologic deficits at 3 months.

Cerebrospinal fluid cytokine levels in children with cerebral malaria and control children. Children with CM had significantly higher CSF levels of G-CSF, IL-1α, IL-6, CXCL8/IL-8, and TNF-α than control children (Figure 1). Levels of IFN-γ, IL-1β, IL-10, CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, and CCL5/RANTES did not differ significantly between children with CM and control children, although for each of these cytokines, there were individual children with CM who had elevated levels (Figure 1).

Repeat testing was performed on 8 paired CSF and serum samples on a single plate. Cytokine values on repeat testing correlated highly with those on previous testing (e.g., Spearman’s rho for CSF CXCL8/IL-8, 0.97, P < 0.001, and for serum IL-8, 0.89, P = 0.003).

Cerebrospinal fluid cytokines and neurologic and cognitive deficits. Neurologic sequelae were assessed by complete neurologic exam in 71, 71, and 68 of the children with CM at the time of discharge, 3 months and 6 months later, respectively. As reported previously, 19 of 71 children (26.8%) had neurologic deficits at discharge, consisting primarily of hyper
eflexia and hypertonia, but also including spastic quadriplegia, vision and hearing impairments, ataxia, lack of coordination, and attention deficit with inability to follow instruc
tions. Six of 71 children (8.5%) still had deficits at 3 months, and only 1 of 68 (1.5%) had deficits at 6 months. CSF cytokine levels of the 5 cytokines that differed between children with CM and control children (G-CSF, IL-1α, IL-6, CXCL8/IL-8, TNF-α) were compared in children with and without neurologic deficits at 3 months. Children with neurologic deficits at 3-month follow-up had higher admission CSF TNF-α levels (P = 0.02) and lower G-CSF (P = 0.01) and CXCL8/IL-8 (P = 0.05) levels than children without deficits (Table 2). In a logistic regression model including all 3 cytokines, increased TNF-α levels (odds ratio [OR] 1.55, 95% confidence interval [CI]: 1.10, 2.18, P = 0.01) and lower G-CSF levels (OR 0.98, 95% CI: 0.96, 0.99, P = 0.01) were independently associated with increased risk of neurologic deficits at 3 months.

Cognitive testing was limited to children over 5 years of
FIGURE 1. CSF levels (pg/mL) of 12 cytokines and chemokines in 76 Ugandan children with cerebral malaria (CM) and 8 North American children without neurologic disease. Lines depict median values in each group. For all values depicted on a log scale, undetectable cytokine levels were given a value of 0.1 pg/mL, or $10^{-1}$ pg/mL. The following outlier values (pg/mL) are not depicted: G-CSF, 14268.2; IL-1β, 2,951.7; IL-1ra, 21960.4; IL-6, 2,337.1; IL-10, 1,036.7; TNF-α, 688.8; CCL3/MIP-1α, 2,302.6; CCL4/MIP-1β, 20,909.4.
age. Cognitive testing was performed in the areas of attention, working memory, and tactile-based learning as previously described.15 Forty-four children completed cognitive assessment at discharge; 42 of the 44 children were available for cognitive testing at 6-month follow-up, and 37 of the 42 had serum and CSF samples available for cytokine testing. In the 37 children, CSF levels of TNF-α correlated negatively with age-adjusted z-scores for tests of attention (Spearman’s rho, −0.34, P = 0.04) and working memory (Spearman’s rho, −0.32, P = 0.06). In contrast, CSF levels of the 4 other cytokines that differed between children with CM and control children did not correlate with cognitive outcome scores. Serum cytokine levels also did not correlate with cognitive outcome scores (data not shown).

DISCUSSION

The pathogenesis of CM is becoming better defined through the complementary study of murine models and disease in human populations, but much remains to be learned. Currently, it is thought that CM results from a number of events, including parasite sequestration that leads to local ischemia and hypoxia, accumulation of CD4+ and CD8+ T cells, monocytes and platelets, local cytokine release, and stimulation of other pathways, including the kynurenine pathway.2 Animal models strongly suggest a role for microglial activation and cytokine production in the pathogenesis of CM, but evidence for this in human studies has until now been largely limited to assessment of mRNA expression in the brain tissue of small numbers of individuals who died of CM.13,14 In the present study of Ugandan children with CM, we document elevated CSF levels of pro- and anti-inflammatory cytokines, a lack of correlation between CSF and serum cytokine levels, an association between elevated CSF TNF-α levels on admission, and subsequent neurologic and cognitive impairment. Taken together, our findings provide evidence of CNS cytokine production in children with CM, and suggest that levels of these cytokines in the CNS but not peripherally may be associated with subsequent CNS injury. The study findings support the concept that the cells in brain parenchyma are not “innocent bystanders” in CM,19 but rather play an active role in the process.

Unlike other central nervous system infections, such as bacterial meningitis or viral encephalitis, in which the organism crosses the BBB and directly infects the brain, in CM, P. falciparum is confined to the endovascular space of the brain, and CM is not accompanied by a CSF leukocytosis, elevated protein, or other signs of CNS inflammation.37 For this reason, the main focus of research in CM has been on the vascular side of the BBB, and there has been an emphasis on the importance of parasite sequestration leading to local tissue hypoxia and damage. However, the coma produced by CM, the lack of stroke-like findings in most children with CM, and the dramatic and sometimes rapid recovery that is seen in CM are not completely consistent with this mechanism. Studies demonstrating that there is local impairment of the BBB in individuals with CM18,38 opened the possibility that leukocytes, serum cytokines, or P. falciparum exoantigens might cross the BBB, activate microglial cells and astrocytes and thus involve them in the pathogenesis of CM. Work by Medina, Hunt, and others demonstrated convincingly that in murine CM models a number of these processes were occur-

### TABLE 1

Cerebrospinal fluid (CSF) and serum levels of cytokines and chemokines in 72 Ugandan children with cerebral malaria*

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>CSF level</th>
<th>Serum level</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median, pg/mL (min, max)</td>
<td>Median, pg/mL (min, max)</td>
<td></td>
</tr>
<tr>
<td>G-CSF</td>
<td>114.3 (1,3, 14268.2)</td>
<td>117.0 (8.4, 3070.9)</td>
<td>0.70</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>0 (0.0, 688.8)</td>
<td>87.3 (0.0, 3369.6)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>IL-1β</td>
<td>0 (0.0, 2951.7)</td>
<td>0 (0.0, 1063.1)</td>
<td>0.003</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>83.2 (1,8, 21960.4)</td>
<td>1142.6 (160.1, 120668.8)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>IL-6</td>
<td>18.1 (1,5, 23371.4)</td>
<td>507.2 (0.0, 14074.0)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.2 (0.0, 1036.7)</td>
<td>690 (0.0, 12393.0)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1.3 (0.0, 688.8)</td>
<td>73.7 (0.0, 559.4)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Chemokine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CXCL8/IL-8</td>
<td>595.2 (38.4, 16918.0)</td>
<td>61.7 (0.7, 844.4)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>CCL2/MCP-1</td>
<td>471.4 (75.0, 4033.8)</td>
<td>211.6 (0.5, 14253.4)</td>
<td>0.0003</td>
</tr>
<tr>
<td>CCL3/MIP-1α</td>
<td>5.5 (0.0, 2302.6)</td>
<td>329.0 (0.0, 11116.0)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>CCL4/MIP-1β</td>
<td>14.8 (3.6, 20909.4)</td>
<td>936.0 (0.0, 5670.0)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>CCL5/RANTES</td>
<td>4.3 (0.0, 715.2)</td>
<td>24508.4 (62.0, 46400)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

* N = 71 for G-CSF, IL-1ra, CXCL8/IL-8, and CCL2/MCP-1.
† Serum levels of all cytokines but G-CSF, IL-1ra, CXCL8/IL-8, and CCL2/MCP-1 previously published.16
‡ Wilcoxon matched-pairs signed rank test.

### TABLE 2

Cerebrospinal fluid cytokine levels in 71 Ugandan children with cerebral malaria, according to presence of neurologic deficits at 3-month follow-up

<table>
<thead>
<tr>
<th>Cytokine/chemokine</th>
<th>Children without deficits, N = 64</th>
<th>Children with deficits N = 7</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median, pg/mL (min, max)</td>
<td>Median, pg/mL (min, max)</td>
<td></td>
</tr>
<tr>
<td>G-CSF</td>
<td>121.7 (5.7, 14286.2)</td>
<td>29.4 (1.3, 183.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>86.0 (1.8, 21960.4)</td>
<td>16.9 (2.6, 446.7)</td>
<td>0.18</td>
</tr>
<tr>
<td>IL-6</td>
<td>18.6 (3.4, 23371.4)</td>
<td>14.3 (1.9, 58.6)</td>
<td>0.30</td>
</tr>
<tr>
<td>CXCL8/IL-8</td>
<td>658.0 (79.6, 16918.0)</td>
<td>208.5 (38.4, 2004.7)</td>
<td>0.05</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1.3 (0.0, 688.8)</td>
<td>4.3 (0.9, 12.6)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* Wilcoxon rank-sum test.
ring in the brain, including stimulation of microglial cells at the BBB by *P. berghei* antigens, microglial activation\(^{46}\) and TNF-\(\alpha\) production\(^{11}\) and astrocyte injury.\(^{10}\) Other studies showing increased mRNA levels of TNF-\(\alpha\) and IFN-\(\gamma\) in the brain but not other organs of *P. berghei*-infected mice with CM\(^{46}\) also support the concept that murine CM is at least in part an encephalitis. However, this activity has been difficult to test in human studies. Studies done to date assessing CNS cytokine production in humans have been limited to two studies of CSF cytokines (TGF-\(\beta\) and TNF-\(\alpha\)), both of which reported normal CSF levels of these cytokines.\(^{39,40}\) The assays used in these earlier CSF cytokine studies may have had higher limits of detection than the assay used in the present study, which detects as little as 1.28 pg/mL of TNF-\(\alpha\). In support of our findings, autopsy studies of children who died of CM have revealed areas of the brain with increased expression of TNF-\(\alpha\), IL-1\(\beta\),\(^{13,41}\) TGF-\(\beta\),\(^{13}\) and CCL5/RANTES.\(^{14}\) However, these studies had small sample sizes and, being post-mortem studies, could not assess the associations of these cytokines with long-term sequelae in children with CM. In the present study, larger sample size, ability to test multiple cytokines from a single CSF sample, and a prospective cohort study design allowed us to demonstrate for the first time that CNS levels of specific pro- and anti-inflammatory cytokines are elevated in children with CM and are associated with subsequent neurologic and cognitive morbidity in these children. The lack of correlation between CSF and serum cytokine levels for all cytokines except G-CSF suggests that these cytokines are produced within the CNS. The strongest evidence for CNS cytokine/chemokine production was for CXCL8/IL-8, for which CSF levels were significantly higher than serum levels. It has been hypothesized that attachment of the *P. falciparum* PfEMP-1 antigen to endothelial cell ICAM-1 alters signaling pathways and leads to increased tight junction permeability, allowing soluble antigens, leukocytes, cytokines, and other factors to cross the BBB and activate and/or damage microglial cells, astrocytes, and pericytes.\(^{42}\) Microglial cells, the resident macrophages of the brain, have numerous membrane receptors, including MHC class II molecules, toll-like receptors (TLR), and numerous cytokine and chemokine receptors,\(^{43}\) and produce a number of cytokines and chemokines in response to infection or stimulation, including TNF-\(\alpha\), IL-1\(\beta\), RANTES,\(^{44}\) IL-6,\(^{45}\) CXCL8/IL-8,\(^{46}\) CCL2/MCP-1,\(^{47}\) CCL3/MIP-1\(\alpha\), and CCL4/MIP-1\(\beta\).\(^{48}\) Microglial cells may be able to respond to antigen presented in areas where the BBB is impaired due to damage to endothelial cell tight junctions. Astrocytes can also produce cytokines, including IL-6,\(^{45}\) CCL2/MCP-1,\(^{49}\) RANTES,\(^{45}\) CXCL8/IL-8,\(^{49}\) and G-CSF\(^{50}\) in response to viral infection or other stimulation. If CD4+/CD8+ T cells or monocytes cross the impaired BBB, they could also be a potential source of CNS cytokines. However, there are few of these cells within the CSF, so the production, in particular, of larger amounts of CXCL8/IL-8 in the CSF than in serum argues for microglial cell/astrocyte origin as opposed to migrating T cell or monocyte origin. However, the possibility of monocyteic cellular infiltrates that remain in brain tissue rather than in CSF cannot be completely excluded. Genetic differences in innate or adaptive host immune responses to *P. falciparum* exoantigens or other stimuli, diversity of parasite genotype and virulence, or a combination of these factors may affect BBB impairment and CNS cytokine production in children with CM, and may explain the elevation of specific cytokine levels in some but not all children with CM. Variations in the time course of CNS cytokine production and/or highly localized CNS cytokine production may also have affected the ability to detect CSF cytokine levels in some children.

Central nervous system cytokine production may lead to protection or damage of neural cells, depending on the specific cytokine, timing of its production, and the amount produced. Production of pro-inflammatory cytokines by microglia can induce neuronal damage or death,\(^{51}\) but the effects of pro-inflammatory cytokines may be modulated by production of anti-inflammatory cytokines\(^{52}\) or by other pro-inflammatory cytokines.\(^{53}\) CXCL8/IL-8, the cytokine with the highest CSF levels in the present study, is a potent neutrophil chemotactrant,\(^{34,54,55}\) and G-CSF, which was also elevated in the CSF, decreases neutrophil apoptosis.\(^{56}\) Together, the effects of these cytokines/chemokines on neutrophils could lead to damage from increased neutrophil activity in the CNS. Neutrophil activity has been postulated to be important in murine CM pathogenesis in one study,\(^{57}\) but not in other murine or human studies. Elevated CSF concentrations of CXCL8/IL-8 have been documented in individuals with traumatic brain injury\(^{58,59}\) and Alzheimer’s disease,\(^{60}\) but it remains unclear whether CXCL8/IL-8 is involved in the pathogenesis of cognitive impairment in these diseases. In the present study, elevated levels of CXCL8/IL-8 and G-CSF were seen in the children without neurologic deficits. Thus, the role of CXCL8/IL-8 and G-CSF in human CM pathogenesis and morbidity is unclear, and it is possible that these cytokines are associated with neuroprotection rather than neurotoxicity in human CM.

Interestingly, TNF-\(\alpha\), the only CSF cytokine associated with neurologic and cognitive impairment in the present study, is the primary cytokine implicated in fatal murine CM.\(^{11}\) In the present study, serum levels of TNF-\(\alpha\) or other cytokines did not correlate with neurologic or cognitive outcomes, corresponding to murine model observations that systemic administration of TNF-\(\alpha\) does not lead to brain changes consistent with CM in *P. vinckeii*-infected mice.\(^{64}\) TNF-\(\alpha\) released in the CNS may cause neurotoxicity by inducing the release of other cytokines or nitric oxide, enhancing superoxide production, or potentiating glutamate receptor-mediated neurotoxicity.\(^{62–64}\) The evidence that CNS TNF-\(\alpha\) is critical to murine CM pathogenesis gives biologic plausibility to the association of elevated CNS TNF-\(\alpha\) levels with subsequent neurologic and cognitive impairment in children with CM. However, the numbers of children assessed for neurologic or cognitive sequelae in the present study (71 and 37 children, respectively) did not allow for detection of strong associations, and further studies are required to confirm these findings.

The evidence in the present study for CNS production of cytokines in CM, and the suggestion of a relationship between neurologic and cognitive sequelae, lend credence to the idea that interventions designed to dampen specific CNS cytokine responses, such as CNS TNF-\(\alpha\) production, could decrease long-term morbidity in CM. An earlier clinical trial of antibodies to TNF-\(\alpha\) in children with CM showed an association of increased neurologic deficits with this antibody treatment, possibly because these antibodies retain TNF-\(\alpha\) within the circulation.\(^{65}\) The present study findings suggest that CNS-specific TNF-\(\alpha\) inhibition might be required to reduce CNS morbidity. In light of the high frequency of cognitive morbidity pre-
vously documented by our group in children with CM,\textsuperscript{15} CNS-specific interventions are urgently needed.

Study limitations include the lack of CSF samples from children with uncomplicated malaria or severe malaria without CSF symptoms, and the relatively small number of control samples. These limitations were unavoidable. Lumbar puncture in children with malaria but without CSF symptoms would be unethical, so it is impossible to determine if CNS cytokine release is seen in other clinical presentations of malaria. Samples could potentially be obtained from African children with other diseases such as meningitis or encephalitis. However, it is well established that numerous cytokines are elevated in these disease processes,\textsuperscript{26,32,66} so documentation of elevation of cytokine levels in children with CM as compared to children in a baseline non-inflamed state could not be accomplished by comparison with these samples. Because there are few situations in which a lumbar puncture can ethically be performed in a child without CSF symptoms, the availability of the CSF samples from children with metabolic disorders but no CNS symptoms allowed us to compare the CSF levels to baseline values in children; even with the small number of controls, we were able to establish that there were highly significant differences in CSF cytokine levels for several cytokines. Furthermore, elevated levels of TNF-$\alpha$ in children with CM correlated with neurologic and cognitive impairment in these children, supporting a potential pathophysiological role for TNF-$\alpha$ in neurologic sequelae of CM.

In conclusion, we provide the strongest evidence to date that African children respond to CM with CNS cytokine production. The possible association of specific cytokines with neuroprotection or neurotoxicity in children with CM, particularly the association of TNF-$\alpha$ with neurologic and cognitive sequelae, requires further evaluation. If production of specific cytokines in the CNS is associated with increased morbidity in children with CM, interventions to decrease production of these cytokines in the brain may lead to improved outcomes.

Received October 6, 2007. Accepted for publication November 16, 2007.

Acknowledgments: The authors thank the study participants and their families for their participation in this study, and the collaborative study team that performed clinical care and testing, cognitive testing, laboratory investigations, and data entry. This study was supported by an NIH Fogarty Institute grant (R21 TW-006794) to Chandy C. John and a Fulbright African Regional Research Award to Michael J. Boivin. The authors have no financial or other conflicts of interest.

Authors’ addresses: Chandy C. John, Angela Panoskaltsis-Mortari, Paul J. Orchard, Gregory S. Park, and Anne M. Jurek, University of Minnesota Department of Pediatrics, 420 Delaware St SE, 850 Mayo, MMC-296, Minneapolis, MN 55455, Telephone: (612) 624-1966, Fax: (612) 624-8927, E-mails: cc@umn.edu, panos001@umn.edu, orchaa001@umn.edu, parkx479@umn.edu, and jureck@epi.umn.edu. Robert O. Opoka, Richard Idro, and Justus Byarugaba, Department of Paediatrics and Child Health, Makerere University, Mulago Hospital, Box 7051, Kampala, Uganda, E-mails: opokabob@yahoo.com, and byarugaba@yahoo.com, Michael J. Boivin, International Neurologic and Psychiatric Epidemiology Program (INPEP), College of Osteopathic Medicine, Michigan State University, 324 West Fee Hall, East Lansing, MI 48824, E-mail: Michael.Boivin@hc.msu.edu.

REFERENCES

21. Winter PM, Dung NM, Loan HT, Kneen R, Wills B, Thu le T,


