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Increased Concentrations of Interleukin-6 and Interleukin-1 Receptor Antagonist and Decreased Concentrations of Beta-2-Glycoprotein I in Gambian Children with Cerebral Malaria

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To investigate the pathogenic versus the protective role of cytokines and toxin-binding factors in *Plasmodium falciparum* infections, we measured the concentrations of tumor necrosis factor alpha, interleukin-1α (IL-1α), IL-1β, IL-1 receptor antagonist, and IL-6, as well as soluble receptors of tumor necrosis factor and IL-6 (sIL-6R) in serum of Gambian children with cerebral malaria, mild or asymptomatic malaria, or other illnesses unrelated to malaria. Because cytokine secretion may be triggered by toxic structures containing phosphatidylinositol (PI), we also measured concentrations of anti-PI antibodies and the PI-binding serum protein beta-2-glycoprotein I. We found increased concentrations of IL-6, sIL-6R, IL-1ra, and some immunoglobulin M antibodies against PI in children with cerebral malaria, but those who died had decreased concentrations of beta-2-glycoprotein I. We conclude that increased concentrations of cytokines and soluble cytokine receptors represent a normal host response to *P. falciparum* infections but that excessive secretion of cytokines like IL-6 may predispose to cerebral malaria and a fatal outcome while beta-2-glycoprotein I may protect against a fatal outcome of cerebral malaria.

Cerebral malaria is the most severe complication of *Plasmodium falciparum* infections. Only a minority of infections progress to cerebral malaria (11). The reasons why some children but not others develop cerebral malaria are not fully understood. Cytokines, including tumor necrosis factor alpha (TNF-α), may protect against malaria by stimulating host immune responses to the parasite. However, cytokines may also contribute to the immunopathological complications of malaria. Elevated concentrations of TNF-α and interleukin-1α (IL-1α) have been found in African children with severe malaria (9, 21), and studies on a murine malaria model have indicated that a variety of cytokines, including TNF-α, play a role in the development of cerebral malaria (7). In addition, activated T lymphocytes may play a pathogenic role in the development of clinical malaria in humans (24) and of cerebral malaria in mice (8). TNF-α secretion is induced by phosphatidylinositol (PI)-containing agents secreted by malaria parasites (2). Protection against pathogenic concentrations of cytokines may be achieved by production of cytokine antagonists which block the activity of the cytokine or by production of factors such as antibodies capable of blocking the activity of cytokine-inducing agents. Antibodies against PI block TNF-α secretion induced by malaria parasites (1). The serum protein beta-2-glycoprotein I has been reported to bind to negatively charged phospholipids like PI (26, 28).

We have prospectively studied 140 Gambian children with clinical malaria, 50 children with asymptomatic malaria, and 100 children with clinical illnesses unrelated to malaria. To look for potential markers of disease severity or mediators of protection, we measured concentrations of cytokines, cytokine receptors, and phospholipid-binding factors in serum at the time of hospital admission and related the results to clinical outcome. We found that IL-6 and IL-1 receptor antagonist (IL-1ra) levels were raised in children with cerebral malaria and found decreased concentrations of serum beta-2-glycoprotein I in children who died of malaria.

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MATERIALS AND METHODS

Donors and blood sampling. Our study was carried out with permission of The Gambia Government/Medical Research Council Ethical Committee between September and November 1992 at the Royal Victoria Hospital, Banjul, The Gambia, and at the Medical Research Council Laboratories, Fajara, The Gambia. Parents or guardians gave informed consent for the participation of their children in the study. Five clinical groups were defined, as follows.

(i) CM. The CM group included children with cerebral malaria, defined as a coma score of 2 or less (22), persisting for more than 30 min after any convulsions had ceased and hypoglycemia had been corrected, in children with *P. falciparum* parasitemia and with no evidence of meningitis or any other cause of coma. This group of children was subdivided into CM(s) (survivors) and CM(f) (fatalities). Children who died within 1 h of presentation were excluded from the study.

(ii) MM. The MM group included children with mild malaria, defined as a febrile illness in children with asexual *P. falciparum* parasites on blood film, without any other satisfactory explanation for the fever, and with none of the defined features of severe malaria.

(iii) AM. The AM group included children with asymptomatic malaria, defined as children with asexual *P. falciparum*

parasites but with an axillary temperature of less than 37.5°C and without any clinical symptoms of malaria.

(iv) SC. The SC group included children with severe illnesses requiring in-patient management and with no malaria parasites seen on blood film. The majority of these children had severe malnutrition, severe lower respiratory tract infections, meningitis, or sepsis. There were no deaths in this group of children.

(v) MC. The MC group included children with relatively mild illnesses requiring outpatient treatment and with no malaria parasites seen on blood film. The majority of these children had gastroenteritis or upper or lower respiratory tract infections.

Patients were treated in accordance with The Gambia government guidelines, and blood was taken immediately before or simultaneously with administration of treatment, which was not delayed as a result of the sampling. Thick blood smears were stained with Giemsa, and parasite density was calculated as previously described (10). Serum samples were frozen within 2 h of collection and kept frozen at −20°C for 1 to 4 months in The Gambia. The samples were then transported on dry ice to Denmark and stored at −70°C until they were analyzed. The concentrations of all cytokines in individual samples were measured on the same day without prior thawing.

Lumbar punctures were performed in children with altered consciousness at the time of admission unless clinically contraindicated. The cerebrospinal fluid (CSF) was inspected immediately. Concentrations of cytokines, antibodies, and beta-2-glycoprotein I in CSF were measured as described below for the serum samples.

Determination of cytokines and cytokine receptors. Enzyme-linked immunosorbent assay (ELISA) kits were used as specified by the manufacturers to measure levels of TNF-α (T-Cell Diagnostics, Cambridge, Mass.), IL-1β (Immunotech, Marseilles, France), soluble TNF receptor type I (sTNF-RI; Bender MedSystems, Vienna, Austria), and soluble IL-6 receptor (sIL-6R; Research and Development Systems, Minneapolis, Minn.) in serum and CSF. Assay sensitivities were 10, 5, 80, and 140 pg/ml, respectively.

IL-6, IL-1α, and IL-1ra levels in serum and CSF were measured by double-sandwich ELISA with monospecific polyclonal rabbit antibodies to purified recombinant cytokines as described previously (12, 13, 15). Briefly, Immuno-Maxisorb plates (Nunc, Roskilde, Denmark) were coated with protein A affinity-purified immunoglobulin G (IgG). Nonattached sites were blocked with 5% human serum albumin in phosphate-buffered saline (PBS). Samples were diluted in PBS supplemented with 2% normal rabbit serum (DAKO, Glostrup, Denmark), 10 mM EDTA, 2,000 KIE (Kallikrein inactivating units) of aprotinin per ml, and 5 mM DL-dithiothreitol. Biotinylated rabbit antibodies against recombinant cytokines were used as detecting antibodies together with streptavidin-peroxidase. Color development was carried out with orthophenyl diamine and H_2O_2 and measured at 492 nm. The assays of IL-1α and IL-6 were calibrated with international standards of the respective cytokines (National Institute for Biological Standards and Controls, Potters Bar, United Kingdom). The inter- and intra-assay coefficients of variation for the concentration range between 8 pg/ml and 1 ng/ml were less than 15%. The limit of sensitivity of these ELISAs was 8 to 30 pg/ml. There was no cross-reactivity and no reaction with human IL-1β, IL-2, IL-4, IL-8, IL-10, TNF-α, lymphotoxin, alpha interferon, or gamma interferon.

Determination of anti-PI IgM antibodies. The levels of anti-PI IgM antibodies in serum were measured by an indirect ELISA as described previously (14, 16). In brief, polyvinyl chloride microtiter plates (Flow Laboratories, Rockville, Md.) were coated with PI overnight. Two different procedures were used. Fetal calf serum used in procedure A contains beta-2-glycoprotein I, and antibodies may react with phospholipids complexed with beta-2-glycoprotein I in this system while antibodies reactive in ELISA with procedure B are more likely to react with free phospholipids (14).

TABLE 1. Donor characteristics[a]

Group	No. per group	Age (mo)	Sex (F/M)	Parasitemia (10^4/μl)	Hemoglobin level (g/dl)	Body temp (°C)
CM	74	36	42/32	7.5	8.1	39.1
CM(f)	11	40	7/4	6.0	8.6	39.1
CM(s)	63	36	35/28	9.0	8.1	39.0
MM	66	36	36/30	9.0	8.0	39.0
AM	50	40	27/23	1.5	ND[b]	36.4
SC	54	22	29/25	0	8.5	38.0
MC	46	32	26/20	0	8.0	38.4

[a] Median values at admission are shown.
[b] ND, not done.

(i) ELISA procedure A. After being washed with washing solution (2.0 M NaCl, 0.04 M Mg_2SO_4), the plates were blocked for 30 min with washing solution containing 2% human serum albumin. The plates were then washed again. A 100-μl volume of serum diluted 1:100 in washing solution containing 10% fetal calf serum was added to each well for 90 min. The washing procedure was repeated, 100 μl of anti-IgM peroxidase-conjugated antibodies (DAKO, Copenhagen, Denmark) diluted in washing solution containing 10% fetal calf serum was added, and the plates were incubated for 1 h. After a final wash, peroxidase binding was measured with orthophenyl diamine and H_2O_2 as substrates. Optical densities (ODs) were read at 492 nm, and all measurements were performed in triplicate.

(ii) ELISA procedure B. After being washed with washing solution (2.0 M NaCl, 0.04 M Mg_2SO_4), the plates were blocked for 30 min with PBS (pH 7.2) containing 1% casein and 0.3% gelatin. The plates were then washed again. A 100-μl volume of serum diluted 1:100 in PBS containing 0.3% gelatin was added to each well for 90 min. The washing procedure was repeated, 100 μl of anti-IgM peroxidase-conjugated antibodies (DAKO) diluted in PBS containing 1% human serum albumin was added, and the plates were incubated for 1 h. After a final wash, peroxidase activity was measured with orthophenyl diamine and H_2O_2 as substrates. ODs were read at 492 nm, and all measurements were performed in triplicate.

On each ELISA microtiter plate, laboratory standards including sera with high antibody titers against PI were assayed and control wells without serum (background value) were included. To account for day-to-day variations, results were expressed in ELISA units (EU) calculated as 100 × [OD(sample) − OD(background)]/[OD(positive control) − OD(background)].

Determination of beta-2-glycoprotein I. The concentrations of beta-2-glycoprotein I were determined by rocket immunoelectrophoresis as described previously (27).

Statistical methods. Because of skewed data distributions, the nonparametric Mann-Whitney U test was used for intergroup comparisons on logarithmically transformed data. *P* values lower than 0.05 were considered significant. The Spearman rank order correlation coefficient (*r*) was used for evaluation of parameter association. *P* values lower than 0.01 were considered significant. All calculations, including determinations of correlation coefficients, were performed by use of the

TABLE 2. Cytokine concentrations in various subsets of Gambian children

Group	Concn (pg/ml) of [a]:			
	TNF-α	IL-1α	IL-1β	IL-6
CM	24 (<10–53), $n = 73$	<8 (<8–<8), $n = 64$	43 (9–71), $n = 54$	48 (<10–171), $n = 67$
CM(f)	27 (<10–108), $n = 10$	<8 (<8–<8), $n = 8$	40 (11–51), $n = 5$	114 (<10–574), $n = 9$
CM(s)	24 (<10–53), $n = 63$	<8 (<8–<8), $n = 56$	43 (9–71), $n = 49$	39 (<10–152), $n = 58$
MM	20 (<10–47), $n = 58$	<8 (<8–<8), $n = 62$	40 (<5–75), $n = 28$	22 (<10–95), $n = 62$
AM	21 (<10–47), $n = 27$	<8 (<8–<8), $n = 10$	43 (36–78), $n = 15$	<10 (<10–62), $n = 26$
SC	17 (<10–53), $n = 52$	<8 (<8–<8), $n = 50$	38 (<5–49), $n = 29$	<10 (<10–81), $n = 49$
MC	<10 (<10–35), $n = 44$	<8 (<8–87), $n = 35$	31 (<5–153), $n = 19$	<10 (<10–10), $n = 38$

[a] Median values at admission and 25th to 75th percentiles (in parentheses) are shown; n is the number of samples tested.

Epi Info computer program (Centers for Disease Control and Prevention, Atlanta, Ga.).

RESULTS

Donor characteristics. A total of 290 children were enrolled in the study; some of their characteristics are summarized in Table 1. Nine children with cerebral malaria also had severe anaemia. Eleven children with cerebral malaria (15%) died; one of these children had severe anaemia as well. The median age of the children with severe nonmalarial illnesses was significantly lower than that of the children from the other groups ($P < 0.005$).

Concentrations of cytokines and cytokine receptors. Concentrations of TNF-α (Table 2) were significantly higher in children with cerebral malaria than in children with severe nonmalarial illnesses ($P < 0.05$). Children who died of cerebral malaria had higher concentrations of TNF-α than did children who survived, although this difference did not reach statistical significance. Concentrations of sTNF-RI (Table 3) were significantly higher in children with clinical malaria (mild or cerebral malaria) than in children with nonmalarial diseases and children with asymptomatic malaria ($P < 0.0005$). The association between levels of sTNF-RI and the outcome of cerebral malaria was not tested because of the small number of samples ($n = 4$) tested from children who had died of malaria. Within the group of children with cerebral malaria, paired measurement of TNF-α and TNF-RI was possible for 39 children who survived and 4 who died. The TNF-α/TNF-R ratio varied from 0 to 16.9×10^{-3} with a median of 1.57×10^{-3} for the survivors and from 1.77×10^{-3} to 8.52×10^{-3} with a median of 5.71×10^{-3} for the children who died. Concentrations of IL-1α (Table 2) were generally lower than 8 pg/ml in serum of children with malaria. These concentrations were statistically lower in children with cerebral or mild malaria than in children with mild nonmalarial illnesses ($P < 0.0001$). Recovery experiments were performed to investigate whether the low levels of IL-1α in children with clinical malaria were caused by IL-1α binding factors. Recovery of measured IL-1α ranged from 83 to 139% when 500 pg of recombinant IL-1α per ml was added to five serum samples from children with clinical malaria, indicating that there were no interfering factors in the samples.

Concentrations of IL-1β (Table 2) were not statistically different between the five groups of Gambian children.

Concentrations of IL-1ra (Table 3) were significantly higher in children with cerebral malaria than in children with severe nonmalarial illnesses or children with mild malaria ($P < 0.005$). Children with clinical malaria had significantly higher concentrations of IL-1ra than did children with asymptomatic malaria ($P < 0.0001$). Within the group of children with cerebral malaria, there was no significant difference in IL-1ra levels between children who died and children who survived. IL-1ra concentrations correlated with concentrations of sTNF-RI ($r = 0.44$, $P < 0.005$), with rectal temperatures ($r = 0.54$, $P < 0.005$), and, finally, with a low coma score (data not shown) ($r = -0.26$, $P < 0.01$).

Concentrations of IL-6 (Table 2) in children with cerebral malaria were higher than in the other groups of children. The difference approached significance in comparison with children with mild malaria ($P = 0.17$) and was statistically significant for comparison with children with severe nonmalarial illnesses ($P < 0.05$), children with mild nonmalarial illnesses ($P < 0.0001$), and children with asymptomatic malaria ($P < 0.05$). Children who died of cerebral malaria had higher concentrations of IL-6 than did children with mild malaria ($P < 0.05$). IL-6 concentrations correlated with TNF-α concentrations ($r = 0.23$, $P < 0.005$) and with IL-1ra concentrations ($r = 0.18$, $P < 0.01$).

Concentrations of sIL-6R (Table 3) in children with cerebral malaria were higher than in children with mild malaria ($P < 0.05$).

Concentrations of beta-2-glycoprotein I. Concentrations of beta-2-glycoprotein I (Table 3) were significantly lower in children with cerebral malaria than in children with severe

TABLE 3. Concentrations of sTNF-RI, IL-1ra, sIL-6R, and beta-2-glycoprotein I in various subsets of Gambian children

Group	Concn of[a]:			
	sTNF-RI (ng/ml)	IL-1ra (pg/ml)	sIL-6R (pg/ml)	Glycoprotein (μg/ml)
CM	15.6 (10.5–23.0), $n = 42$	2,400 (1,944–3,240), $n = 71$	369 (303–437), $n = 26$	83 (68–107), $n = 52$
CM(f)	14.0 (8.3–37.6), $n = 4$	2,733 (2,145–3,606), $n = 11$	ND	64 (51–83), $n = 5$
CM(s)	15.7 (10.5–23.0), $n = 38$	2,350 (1928–3150), $n = 60$	369 (303–437), $n = 26$	92 (73–111), $n = 47$
MM	15.2 (11.6–20.0), $n = 35$	2,009 (1,331–2688), $n = 59$	305 (275–366), $n = 29$	94 (88–115), $n = 48$
AM	7.8 (5.9–10.7), $n = 16$	860 (200–1,120), $n = 33$	330 (168–343), $n = 3$	103 (58–118), $n = 7$
SC	7.1 (4.6–9.8), $n = 39$	1,660 (731–2,766), $n = 49$	343 (277–370), $n = 18$	116 (94–143), $n = 43$
MC	5.4 (3.4–7.0), $n = 20$	2,022 (1,655–2,640), $n = 40$	ND	83 (69–111), $n = 17$

[a] Median values at admission and 25th to 75th percentiles (in parentheses) are shown; n refers to the number of samples tested.

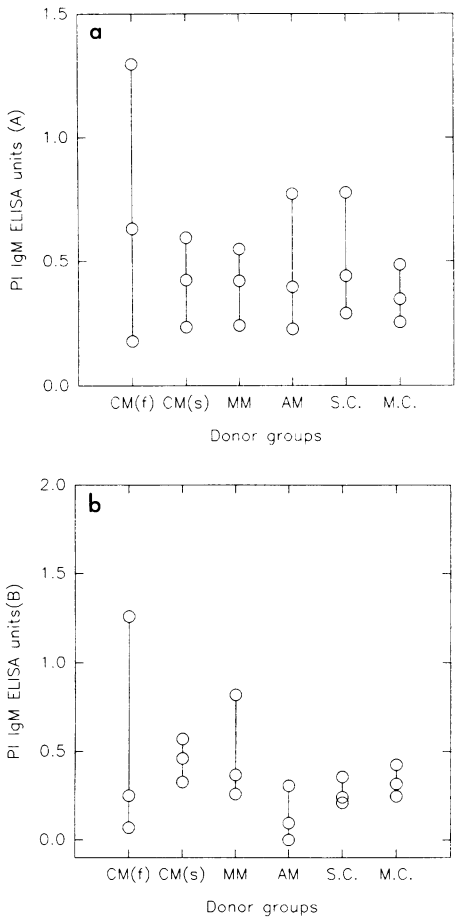

FIG. 1. Serum anti-PI IgM antibody reactivities in various subsets of Gambian children. Distribution of admission IgM reactivities in Gambian children who had cerebral malaria and died [CM(f)], cerebral malaria and survived [CM(s)], mild malaria (MM), asymptomatic malaria (AM), severe illnesses unrelated to malaria (S.C.) and mild illnesses unrelated to malaria (M.C.). Antibody reactivities obtained by using procedures A (a) and B (b) are shown. The figure shows medians and 95% confidence intervals.

nonmalarial illnesses ($P < 0.05$). Children who died of cerebral malaria had significantly lower concentrations of beta-2-glycoprotein I in serum than did children with mild malaria ($P < 0.05$) or children who survived cerebral malaria ($P = 0.069$). Concentrations of beta-2-glycoprotein I decreased with decreasing coma score ($r = 0.24$, $P < 0.01$).

Anti-PI IgM antibody reactivities. Anti-PI IgM reactivities measured by procedure A (Fig. 1a) were not statistically different between the five groups of Gambian children. However, within the group of children with cerebral malaria, those who died had significantly higher IgM reactivities than the survivors did ($P < 0.05$). IgM reactivities increased with age ($r = 0.20$, $P < 0.01$). Anti-PI IgM reactivities measured by procedure B (Fig. 1b) were significantly higher in children who survived cerebral malaria than in children with severe nonmalarial illnesses ($P < 0.05$). The group of children who survived cerebral malaria had also a higher median level of IgM reactivity than did the children who died of cerebral malaria, but because of the highly variable IgM reactivities in the latter group, the difference was not significant. Children with clinical malaria had significantly higher IgM reactivities than did children with asymptomatic malaria ($P < 0.0005$). The IgM reactivities also correlated with age ($r = 0.18$, $P < 0.01$), with serum levels of IL-1α ($r = 0.25$, $P < 0.01$), and with rectal temperature ($r = 0.17$, $P < 0.01$).

TABLE 4. Concentrations of cytokines, cytokine receptor, and beta-2-glycoprotein I in paired samples of sera and CSF collected from children with cerebral malaria

Factor (no. of children tested)	Median concn in:	
	Serum	CSF
TNF-α (pg/ml) (18)	21	<10
IL-1α (pg/ml) (15)	<8	<8
IL-1β (pg/ml) (18)	49	28
IL-1ra (pg/ml) (15)	1,778	<10
IL-6 (pg/ml) (16)	148	72
sTNF-RI (ng/ml) (7)	12.1	5.1
Beta-2-glycoprotein I (μg/ml) (5)	104	0

Comparison of concentrations of cytokines, receptors, and beta-2-glycoprotein I in CSF and serum. Concentrations of TNF-α, TNF-RI, IL-1α, IL-1β, IL-1ra, IL-6, and beta-2-glycoprotein were negative or much lower in CSF samples than in the corresponding serum samples (Table 4). The CSF/serum ratio was highest for IL-6. The mean concentration of TNF-α detected in CSF (11 pg/ml) was not appreciably different from values reported previously (9). Anti-PI IgM antibody reactivities were low in CSF samples (data not shown).

DISCUSSION

Several cytokines, i.e., TNF-α, lymphotoxin, IL-1α, IL-1β, and IL-6, show a range of overlapping biological activities including pyrogenicity. These cytokines may play a crucial role in both protection against malaria parasites and malaria immunopathology. The cytokines induce pyrexia, which may retard the development of *P. falciparum* (20) and enhance the efficiency of cellular immune responses against the malaria parasite (19). However, other effects of these cytokines, such as induction of hypoglycemia and modulation of the adhesive properties of infected erythrocytes and endothelial cells, may be detrimental to the host (4).

We have measured simultaneously the levels of several different endogenous pyrogens in serum of Gambian children with malaria and unrelated diseases of different severity with the purpose of evaluating each cytokine as a marker of disease severity. There have been several previous reports on cytokine levels in serum and plasma and their relation to the outcome of different diseases. These studies, as well as our own, have several limitations. Most cytokines have short half-lives in serum (5). Thus, measured levels of cytokines reflect secretion levels only immediately before blood collection and not activity during the previous days. In The Gambia, it usually takes several hours to reach a clinic, and cytokine concentrations at the onset of cerebral symptoms, which may be a more important clinical determinant than concentrations on admission, cannot be measured. Other limitations to these studies are localized secretion of cytokines, which may not be reflected by the levels in serum. Cytokines may show synergistic interactions, which may affect the biological activity of each cytokine and may not be reflected by individual cytokine measurements. Nevertheless, we believe that it is important to try to define the relative importance of different cytokines in the pathogenesis of cerebral malaria.

In this study, we found that children who died of cerebral malaria had the highest mean concentrations of TNF-α of all

donor groups, confirming previous findings (9, 21, 29), although the mean concentration of TNF-α in children with fatal cerebral malaria, 87 pg/ml, was lower than the previously reported concentrations of 709 pg/ml (9) and 269 to 284 pg/ml (21). These data indicate that TNF-α may be less important in the initiation of cerebral malaria than in the clinical progression from coma to death. However, the highest concentration of TNF-α in serum, exceeding 1,000 pg/ml, was found in a child with mild malaria, indicating that the concentration of TNF-α in serum alone is not a specific marker of disease severity.

We found very low levels of IL-1α in children with clinical malaria. This is in contrast to a previous study which showed that IL-1α concentrations increased with severity of malaria (21). In the study reported by Kwiatkowski, children who died of malaria had concentrations of 29 to 44 pg of IL-1α per ml, which were much higher than the IL-1α concentrations we detected in our study. On the other hand, we measured higher levels of IL-1α in children with unrelated diseases than the levels (mean, <10 pg/ml) reported by Kwiatkowski for similar children. The reasons for these differences remain unexplained. We added dithiothreitol to the serum samples to avoid false-positive results caused by rheumatoid factors (6), but other serum factors may affect the results of different ELISAs measuring IL-1α. Levels of IL-1β were not associated with the severity of malaria.

We found that high levels of IL-6 were associated with a fatal outcome of malaria, in agreement with a previous finding in a study of European malaria patients (17). This finding resembles the previously reported association between TNF-α levels and fatal malaria (21). The association between TNF-α and disease severity may be related to the capacity of TNF to induce IL-6 secretion.

In summary, our cytokine measurements indicate that with the possible exception of IL-1α, all the endogenous pyrogens are produced during cerebral malaria and that IL-6 appears to be the most sensitive marker of cerebral malaria.

Soluble cytokine receptors usually circulate in serum for longer than the corresponding cytokines and may therefore be more reliable markers of cytokine activation than measurement of the cytokines themselves. At least two different receptors for TNF exist. One of the receptors, TNF-RI, mediates the biological activities of TNF-α associated with endotoxic shock (23). High sTNF-RI levels have previously been reported in European malaria patients (18). We found high levels of sTNF-RI in the blood of children with clinical malaria, suggesting activation of a feedback mechanism to neutralize the harmful effects of excessive amounts of TNF-α. Interestingly, although the number of patients is small, we observed that four children who died of cerebral malaria had higher TNF-α/TNF-RI ratios than did children who survived cerebral malaria. Soluble IL-6R, like sTNF-RI, appears to be a marker of cerebral malaria.

IL-1ra is an antagonist protein which binds to both the two known IL-1 receptors without triggering the cells. IL-1ra therefore blocks the biological activity of both IL-1α and IL-1β. We found high levels of IL-1ra associated with cases of cerebral malaria, but there were no differences in IL-1ra levels in children who survived and children who died. The secretion of IL-1ra is apparently triggered by inflammatory processes. We do not know whether the measured IL-1ra is biologically active, because IL-1ra, like IL-1β but unlike TNF-α, IL-1α, and IL-6, is thermally denaturated (3). Further studies on the importance of IL-1ra in pathology and protection against malaria are needed.

Antibodies against cytokine-inducing antigens of the malaria parasite may protect against the formation of harmful levels of cytokines. The TNF-inducing component of both human and rodent malaria parasites appears to include a PI derivative (2, 25). Mouse antibodies against PI inhibit the production of TNF from macrophages induced by malaria parasites (1). We have previously reported that IgM antibodies against PI are produced during malaria episodes caused by *P. falciparum* in humans (16). In this study, we measured levels of anti-PI antibodies by two different procedures. In the procedure involving fetal calf serum, containing beta-2-glycoprotein I, we found that within the group of children with cerebral malaria, those who died had higher antibody reactivities against PI than did those who survived, indicating that this subpopulation of antibodies against phospholipids is associated with disease severity, in accordance with our previous findings (16). No association between IgM reactivities and disease severity was found when performing ELISA with gelatin and casein instead of fetal calf serum, but all groups of children with clinical disease had higher IgM reactivities than did children with asymptomatic malaria, suggesting that this population of antibodies against phospholipids is produced as a component of the inflammatory response. In summary, our ELISA measuring phospholipid antibodies provided no evidence to indicate a protective role for such antibodies in human malaria. It is possible that the type of phospholipid antibodies produced in mice after vaccination is different from the type of antibodies produced in humans during infection. Alternatively, protective antibodies may constitute only a fraction of the phospholipid antibodies measured in our ELISAs.

Finally, we found that levels of the phospholipid-binding serum protein beta-2-glycoprotein I were depressed in children who died of cerebral malaria. This finding suggests that this protein may be involved in protection against the severe clinical manifestations of malaria, possibly by the binding and neutralization of cytokine-inducing malaria antigens.

The concentrations of TNF-α, IL-1α, IL-1β, IL-1ra, and phospholipid antibodies in CSF were not elevated, even in patients with very high concentrations of the cytokines or antibodies in serum. This finding is in accordance with previous findings (9) but in contrast to the elevated levels of TNF-α in CSF described in patients with bacterial sepsis. It appears that the blood-brain barrier remains intact during severe malarial attacks.

In conclusion, we found that high concentrations of IL-6, IL-6R, and IL-1ra are associated with cerebral malaria in Gambian children. Our data indicate that several different endogenous pyrogens may be involved in the pathogenesis of cerebral malaria. Finally, we found that levels of the serum protein beta-2-glycoprotein I were decreased in children who died of cerebral malaria, indicating a possible protective role of this serum protein.

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