

Impaired Systemic Production of Prostaglandin E₂ in Children with Cerebral Malaria

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Prostaglandins (PGs) are important mediators of macrophage activity, vascular permeability, fever, erythropoiesis, and proinflammatory responses to infection. Our recent studies have shown that plasma levels of bicyclo-PGE₂ (a stable end product of PGE₂ metabolism) and leukocyte cyclooxygenase (COX)–2 gene expression are suppressed in children with malarial anemia. Since the role of PGs as immunomodulators of human cerebral malaria (CM) has not been examined, we investigated urinary levels of bicyclo-PGE₂/creatinine in children with varying clinical outcomes of CM. Among parasitemic children, those with asymptomatic parasitemia had the highest levels of bicyclo-PGE₂/creatinine, whereas those with CM had significantly lower levels of bicyclo-PGE₂. Systemic levels of bicyclo-PGE₂/creatinine were not significantly associated with parasitemia, hemoglobin levels, or levels of the PG-regulatory cytokine tumor necrosis factor- α but were positively correlated with levels of interleukin-10. The results presented here show that impaired systemic production of PGE₂ is associated with adverse outcomes of CM, whereas elevated levels of PGE₂ in asymptomatic parasitemia suggest a potential role for PGs in protective immunity.

Malaria is estimated to cause >2 million deaths annually [1]. Although there are diverse clinical presentations of malaria, cerebral malaria (CM) is a common feature of severe malaria in nonimmune and semi-immune individuals residing in areas with low to moderate rates of *Plasmodium falciparum* transmission. CM is a neurological syndrome characterized by encephalopathy, coma, and seizures. Although the underlying molecular mechanisms of CM have been only partially defined, at least 4 distinct groups of children whose conditions fulfill the World Health Organization definition of CM have been

described [2]. The central features in most children with CM include sequestration of parasitized red blood cells (pRBCs) within the cerebral microvasculature [3] and excessive systemic production of proinflammatory cytokines, such as tumor necrosis factor (TNF)- α , and of anti-inflammatory cytokines, such as interleukin (IL)–10 [4–8]. In addition, our previous studies have shown that, in Tanzanian children with malaria, systemic levels of nitric oxide (NO) and leukocyte expression of nitric oxide synthase type 2 (NOS2) protein are inversely associated with disease severity [7].

The relative balance of pro- and anti-inflammatory cytokines in the inflammatory environment regulates the expression of inducible genes, such as NOS2 and cyclooxygenase (COX)–2, which, in turn, mediate the immune response through generation of high levels of NO and prostaglandins (PGs), respectively [9]. PGs are generated in response to cellular activation that triggers the release of arachidonic acid (AA) from membrane glycerophospholipids by the actions of phospholipase A₂ [10]. Free AA is then converted into prostanoids (e.g., PGE₂, PGF_{2 α} , PGD₂, prostacyclin [PGI₂], and thromboxane-A₂) by the enzymatic activity of the COXs

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(COX-1 and COX-2 [also termed PGH synthase-1 and -2]) [11]. COX-1-generated PGs are typically important in maintenance of physiological homeostasis, whereas COX-2-generated PGs are important in inflammation and host defense [9]. The most extensively characterized prostanoid, PGE₂, regulates a number of key immunological processes relevant to malaria pathophysiology, including expression of cell adhesion molecules, release of proinflammatory mediators (such as TNF- α), vascular permeability, and tissue fluid dynamics [11]. In addition, inhibition of production of PGE₂ by nonsteroidal anti-inflammatory drugs, can suppress certain leukocyte functions, such as chemotaxis, phagocytosis, reactive oxygen species generation, and microbial killing (for review, see [12]).

Our recent studies of Gabonese children with severe malaria (characterized by severe anemia and high-density parasitemia) have shown that plasma levels of bicyclo-PGE₂ (a stable end product of PGE₂ metabolism) and leukocyte COX-2 gene expression are significantly reduced during acute malaria [13]. Although measurement of PGE₂ and COX-2 gene expression in those studies was performed before treatment with antimalarials and/or antipyretics, previous studies of Gabonese children within the same geographic region have shown that treatment of malaria-infected children with acetaminophen (paracetamol) is associated with adverse outcomes, such as prolonged time to clearance of parasites and decreased production of TNF- α and oxygen radicals [14]. Studies using murine models of CM have shown that blockade of formation of PGs, by aspirin, is associated with increased mortality [15] and that more-selective inhibition of COX-2 by celecoxib is associated with an earlier onset of CM [16]. Taken together, the findings of these studies suggest that enhanced production of PGE₂ is associated with protective effects and that reduced levels of PGE₂ may promote increased pathogenesis.

Since the role of PGE₂ in mediating protection against human CM is not known, we sought to determine the relationship between systemic production of PGE₂ and disease severity in children with and children without CM. We hypothesized that urinary levels of bicyclo-PGE₂ in Tanzanian children would be inversely associated with malaria disease severity. We also determined the relationship between production of PGE₂ and levels of the COX-2-regulatory cytokines TNF- α and IL-10, peripheral parasitemia, and anemia status (hemoglobin levels).

PATIENTS, MATERIALS, AND METHODS

Study participants. The present study was performed using cryopreserved samples collected from subjects enrolled at Muhimbili Medical Centre (MMC), Dar es Salaam, Tanzania, from May 1994 to January 1995, as described elsewhere [7]. The original study was approved by the College Research and Publications Committee of MMC and the Duke University Medical Center Investigational Review Board, and informed consent was

obtained from the parents of participating children in Kiswahili. Five groups of children 6 months to 9 years of age were prospectively recruited from the pediatric and surgical wards: (1) healthy control subjects (CONs) were characterized by absence of fever within the past 2 weeks, absence of parasites on thick blood film examination, a normal white blood cell (WBC) count, and a lack of any other detectable acute illnesses (a fracture >1 week old was permitted); (2) patients with asymptomatic parasitemia (AP) were characterized as were CONs, except that they had the presence of *P. falciparum* parasitemia on thick blood film examination; (3) patients with noncerebral malaria (NCM) were characterized by a febrile illness, with *P. falciparum* parasitemia >10,000 trophozoites/ μ L, no history of convulsions, and no other evident cause of fever, and were fully alert, normoglycemic, and without severe respiratory distress; (4) patients with CM with complete recovery (CMCR) were characterized by unarousable coma with a Blantyre coma score ≤ 2 that persisted for ≥ 30 min after the last convulsion, no other cause of coma evident from clinical analysis or analysis of cerebrospinal fluid, any level of *P. falciparum* parasitemia on thick blood film examination, and recovery without neurological sequelae; and (5) patients with CM complicated by death or neurological sequelae (CMDS) were characterized as were patients with CMCR, except that they had coma accompanied by death or neurological sequelae on discharge. Patients with malaria were managed according to standard MMC guidelines.

Collection of samples. Venous blood was drawn into sterile EDTA-containing vacutainers. Hemoglobin levels and WBC counts were determined by use of a Coulter counter. Thick and thin blood films were prepared; thick blood films were stained with Field's stains A and B, and thin blood films were stained with a Giemsa stain. The number of parasites per 200 WBCs was determined from thick blood films, and parasitemia (per microliter of whole blood) was calculated from the automated WBC count. An experienced microscopist examined 50 oil-immersion fields before a film was classified as negative. Urine was collected on admission and immediately stored at -70°C . Plasma (1–3 mL) was isolated from whole blood within 30 min of collection and was immediately frozen at -70°C , for later determination of cytokines.

Measurement of bicyclo-PGE₂ and cytokines. Since PGE₂ is rapidly converted to 13,14-dihydro-15-keto-PGE₂ in an in vivo environment, levels of bicyclo-PGE₂ were measured. Urine (500 μ L) was precipitated in 2 mL of ethanol for 15 min at 4°C . Samples were purified by solid-phase extraction, by use of 500 mg/3 mL DSC-18 (octadecyl bonded silica; Suppelco) tubes. PGs were eluted with ethyl acetate containing 1% methanol and were evaporated to dryness by vacuum centrifugation, followed by resuspension in commercially available EIA buffer (500 μ L; Cayman Chemical). To convert PGE₂ and the intermediary metabolites to bicyclo-PGE₂, samples were derivatized

Table 1. Clinical and laboratory measures of study participants.

Variable	CONs	AP	NCM	CMCR	CMDS	P
Group, no. of patients	18	8	31	14	19	
Age, years	3.9 ± 0.5	4.7 ± 0.9	2.9 ± 0.4	3.4 ± 0.5	3.3 ± 0.4	NS ^a
Geometric mean parasitemia, trophozoites/ μ L	0	396 ± 162	47,478 ± 8824	12,750 ± 3541	39,111 ± 10,457	<.0001 ^b
Hemoglobin level, g/dL	11.6 ± 0.3	9.6 ± 0.9	7.56 ± 0.4	6.58 ± 0.5	5.9 ± 0.5	<.0001 ^a
Urinary level of bicyclo-PGE ₂ , pg/mL	448.1 ± 81.3	821.3 ± 262.0	780.8 ± 97.6	572.2 ± 123.9	406.8 ± 98.4	<.01 ^b
Urinary level of creatinine, mg/mL	0.49 ± .07	0.40 ± 0.14	0.49 ± 0.04	0.42 ± 0.04	0.35 ± 0.04	NS ^a
Plasma level of TNF- α , pg/mL	ND	ND	76.3 ± 15.9	92.5 ± 26.3	154.9 ± 43.4	NS ^b
Plasma level of IL-10, pg/mL	ND	ND	285.6 ± 65.4	582.6 ± 150.1	727.2 ± 262.6	NS ^b

NOTE. Data are mean $\pm$ SE. AP, patients with asymptomatic parasitemia; CONs, healthy control subjects; CMCR, patients with cerebral malaria (CM) with complete recovery; CMDS, patients with CM with death or associated neurological sequelae; IL-10, interleukin-10; NCM, patients with noncerebral malaria; ND, not determined; NS, not significant; PGE₂, prostaglandin E₂; TNF- α , tumor necrosis factor- α .

^a Analysis of variance (ANOVA) performed on raw (untransformed) data.

^b ANOVA performed on log-transformed data.

overnight at 37°C in carbonate buffer (150 μ L), followed by addition of PBS (200 μ L) and EIA buffer (150 μ L). Levels of bicyclo-PGE₂ were determined by use of a quantitative sandwich EIA (Cayman Chemical). Urinary levels of creatinine were determined by use of an Ektachem autoanalyzer (Eastman Kodak). Plasma levels of human TNF- α and IL-10 were determined by use of a quantitative sandwich EIA, as reported elsewhere [7]. The sensitivities of detection of the cytokine assays were 15.6 pg/mL (TNF- α) and 4.7 pg/mL (IL-10).

Statistical analyses. Urinary levels of bicyclo-PGE₂ (picograms per milliliter) were measured in triplicate and at several different dilutions. Urinary levels of creatinine (milligrams per milliliter) were measured in triplicate, and plasma levels of cytokines (picograms per milliliter) were measured in duplicate. To account for potential differences in renal filtration rates between the various groups, urinary levels of creatinine were measured, and data were expressed as urinary levels of bicyclo-PGE₂/creatinine (picograms per milligram per milliliter). To examine potential differences between groups in parasitemia and levels of bicyclo-PGE₂/creatinine, TNF- α , IL-10, and hemoglobin, a 1-way analysis of covariance (ANCOVA) was performed on each variable, with age serving as a covariate. After each significant *F* test, post hoc pairwise mean comparisons were conducted by use of Fisher's least significant difference (LSD) multiple range test. The Fisher's LSD procedure effectively controls the family-wise type I error rate and accommodates unequal sample sizes across groups. To examine the strength of linear association between pairs of variables, bivariate zero-order and partial correlation analyses were conducted. Before performance of inferential statistical analyses, the following outcome variables were logarithmically transformed toward normality: levels of bicyclo-PGE₂/creatinine, TNF- α , and IL-10 and parasitemia. Hemoglobin levels did not require transformation, since they did not evidence significant skewness or kurtosis. Unless otherwise noted in Results, all ANCOVA and correlational analyses used the transformed versions of the afore-

mentioned variables. The only exception to this rule concerned hemoglobin levels, which, as noted above, did not require transformation. For all of the analyses presented, there were no missing data for any of the subjects.

RESULTS

Patient characteristics. The untransformed variables for the patient characteristics—including age (years), peripheral parasitemia (trophozoites per microliter), hemoglobin level (grams per deciliter), and levels of bicyclo-PGE₂ (picograms per milliliter), creatinine (milligrams per milliliter), TNF- α (picograms per milliliter), and IL-10 (picograms per milliliter)—are presented in table 1 for the 5 groups participating in the study: CON (*n* = 18), AP (*n* = 8), NCM (*n* = 31), CMCR (*n* = 14), and CMDS (*n* = 19).

Impaired systemic production of PGE₂ in CM. To determine whether systemic production of PGE₂ differed between children with varying degrees of disease severity, urinary levels of bicyclo-PGE₂ were measured and expressed relative to urinary levels of creatinine, to account for potential differences in renal filtration rates between the groups. The raw untransformed mean ($\pm$ SE) levels of bicyclo-PGE₂/creatinine for the groups is presented in figure 1. Since the data were not normally distributed, log transformation toward normality was performed. The log-transformed mean levels of bicyclo-PGE₂/creatinine for the 5 groups, after adjustment for age, were as follows: CON, 2.98; AP, 3.40; NCM, 3.12; CMCR, 2.86; and CMDS, 2.76. There was a significant difference in the age-adjusted mean levels of bicyclo-PGE₂/creatinine between the groups (*F*[4,74] = 2.73; *P* = .035) (figure 1). Relative to the AP group, the 2 CM groups had significantly lower levels of bicyclo-PGE₂/creatinine, as determined by use of Fisher's LSD multiple range tests (figure 1). In addition, relative to the NCM group, the CMDS group had significantly lower levels of bicyclo-PGE₂/creatinine (figure 1).

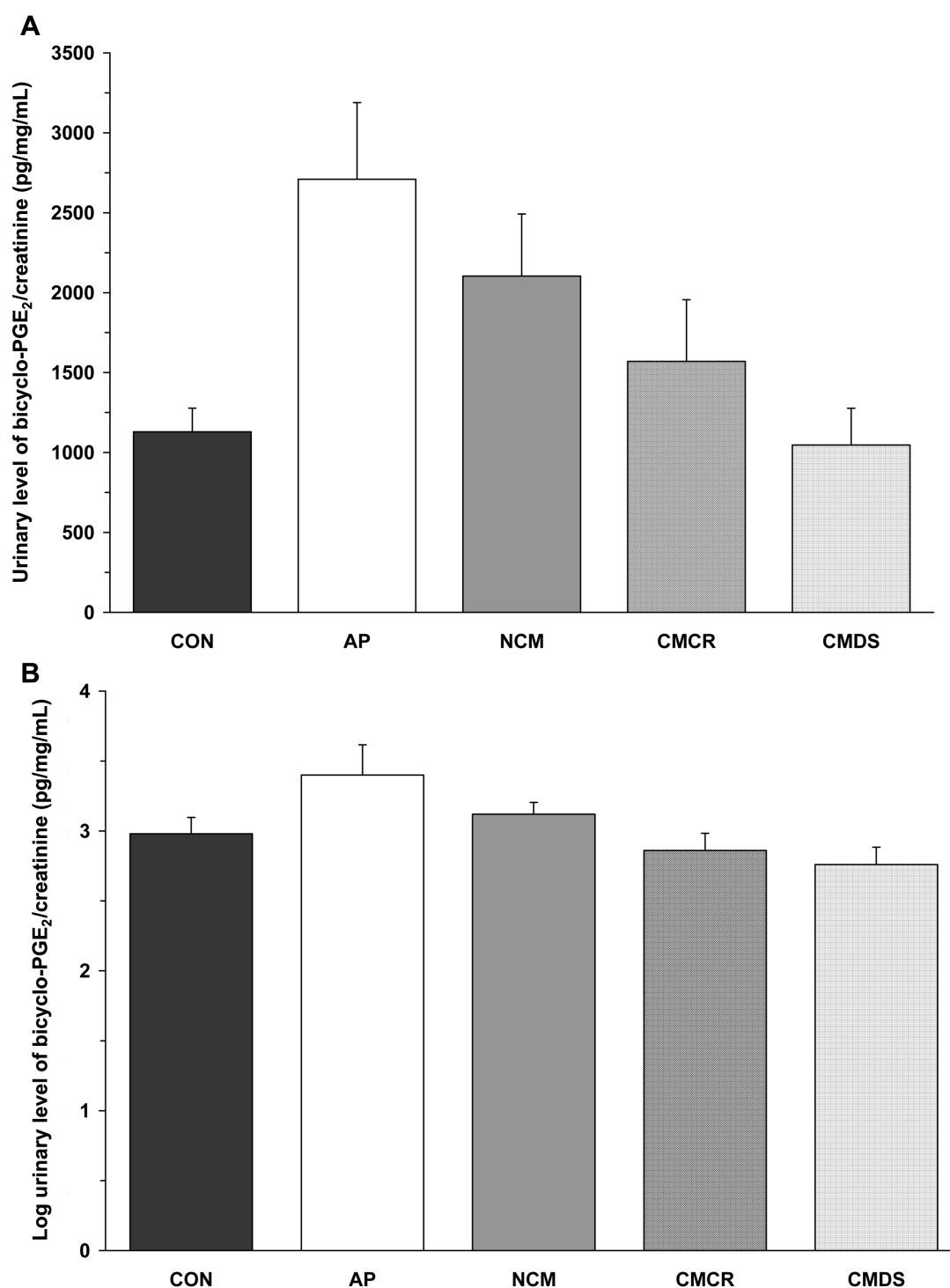


Figure 1. Systemic levels of bicyclo-prostaglandin E₂ (PGE₂) in different disease severity groups. Urine was collected aseptically from multiple groups of Tanzanian children: healthy control subjects (CONs; $n = 18$), patients with asymptomatic parasitemia (AP; $n = 8$), patients with noncerebral malaria (NCM; $n = 31$), patients with cerebral malaria (CM) with complete recovery (CMCR; $n = 14$), and patients with CM with death or associated neurological sequelae (CMDS; $n = 19$). Urinary levels of bicyclo-PGE₂ were measured by EIA and expressed per unit of urinary creatinine, to account for potential differences in hydration status. *A*, Untransformed mean ($\pm$ SE) levels of bicyclo-PGE₂/creatinine for all 5 groups; *B*, log-transformed mean ($\pm$ SE) levels of bicyclo-PGE₂/creatinine for all 5 groups. An analysis of covariance (with adjustment for age) indicated a significant difference in mean levels of bicyclo-PGE₂/creatinine between the groups ($F[4,74] = 2.73$; $P = .035$). Further analyses using Fisher's least significant difference multiple range tests indicated that (1) the 2 CM groups had significantly lower levels of bicyclo-PGE₂/creatinine than did the AP group, and (2) the CMDS group had a significantly lower level of bicyclo-PGE₂/creatinine than did the NCM group.

Relationship between systemic levels of PGE₂ and those of cytokines. Since TNF- α and IL-10 are important for regulation of production of PGs, through induction and suppression of COX-2 gene expression, respectively [17], plasma levels of TNF- α and IL-10 were examined in children with malaria, to determine whether changes in these COX-2-regulatory cytokines could account for the altered production of PGs between the groups. Although levels of TNF- α and IL-10 increased with disease severity, ANCOVA (with adjustment for age) failed to indicate significant between-group differences in levels of either cytokine (TNF- α : $F[2,44] = 1.07$, $P = .353$; IL-10: $F[2,44] = 0.36$, $P = .700$) (table 1). The log-transformed, covariate-adjusted mean levels of TNF- α (i.e., adjusted for age) for the NCM, CMCR, and CMDS groups were 1.50, 1.59, and 1.82, respectively. Similarly, the log-transformed, covariate-adjusted mean levels of IL-10 for the same 3 groups were 2.10, 2.28, and 2.30, respectively. No association was found between plasma levels of bicyclo-PGE₂/creatinine and those of TNF- α ($r = 0.11$; $P = .461$) (table 2). There was, however, a significant association between plasma levels of bicyclo-PGE₂/creatinine and those of IL-10 ($r = 0.34$; $P = .019$) (table 2).

Partial correlations were also calculated, to determine the influence of age and disease severity on the relationship between levels of bicyclo-PGE₂/creatinine and those of the cytokines. The association between levels of bicyclo-PGE₂/creatinine and those of TNF- α remained nonsignificant ($r_p = 0.08$; $P = .607$), whereas the association between levels of bicyclo-PGE₂/creatinine and those of IL-10 remained significant ($r_p = 0.31$; $P = .035$).

Relationship between systemic levels of PGE₂ and peripheral parasitemia. After adjustment for age, the log-transformed mean peripheral parasitemia values for the 5 groups were as follows: CON, 0; AP, 2.68; NCM, 4.64; CMCR, 4.10; and CMDS, 4.59 (figure 2). An ANCOVA (with adjustment for age) indicated a significant between-group difference in peripheral parasitemia ($F[4,72] = 124.35$; $P < .0001$) (table 1). Results of further analyses using Fisher's LSD multiple range tests indicated

that the AP group had significantly lower parasitemia than did the NCM and CM groups, whereas the NCM group had significantly higher parasitemia than did the CMCR group (figure 2). Although the zero-order correlation between the systemic level of bicyclo-PGE₂/creatinine and peripheral parasitemia was not significant ($r = 0.11$; $P = .345$), when age and disease severity were taken into account via partial correlation, the 2 variables became significantly correlated ($r_p = 0.31$; $P = .007$) (table 2).

Relationship between levels of PGE₂ and hemoglobin levels. Since anemia is frequently an important complicating feature in CM, hemoglobin levels were determined for the groups. There was a significant difference in hemoglobin levels between the groups ($F[4,74] = 23.95$; $P < .0001$) (table 1 and figure 3). Results of further analyses using Fisher's LSD multiple range tests demonstrated the following: (1) the CON group had significantly higher hemoglobin levels than did the other groups, (2) the AP group had significantly higher hemoglobin levels than did the 2 CM groups, and (3) the NCM group had significantly higher hemoglobin levels than did the 2 CM groups.

The association between levels of bicyclo-PGE₂/creatinine and hemoglobin levels was also examined, since PGE₂ is an important soluble factor for promoting efficient erythropoiesis [18, 19]. However, no significant association was found between levels of bicyclo-PGE₂/creatinine and hemoglobin levels ($r = -0.03$; $P = .86$) (table 2). When age, parasitemia, and disease severity were controlled by partial correlation, the association between levels of bicyclo-PGE₂/creatinine and hemoglobin levels remained nonsignificant ($r_p = 0.03$; $P = .80$) (table 2).

Our previous studies of Gabonese children with severe malarial anemia have shown a significant inverse association between plasma levels of bicyclo-PGE₂/creatinine and hemoglobin levels [20]. However, all of the children representing the control group in those studies had previous clinical episodes of malaria that were recorded during their participation in a longitudinal cohort study. Since we did not know the previous exposure

Table 2. Association between clinical and laboratory measures and levels of bicyclo-prostaglandin E₂/creatinine.

Variable	Pearson zero-order correlation		Partial correlation	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
TNF- α , pg/mL	0.11	NS	0.08 ^a	NS
IL-10, pg/mL	0.34	.019	0.31 ^a	.035
Geometric mean parasitemia, trophozoites/ μ L	0.11	NS	0.31 ^a	.007
Hemoglobin, g/dL				
All groups	-0.03	NS	0.03 ^b	NS
Malaria-infected groups	0.06	NS	0.06 ^b	NS

NOTE. IL-10, interleukin-10; NS, not significant; TNF- α , tumor necrosis factor- α .

^a With adjustment for age and disease severity.

^b With adjustment for age, disease severity, and parasitemia.

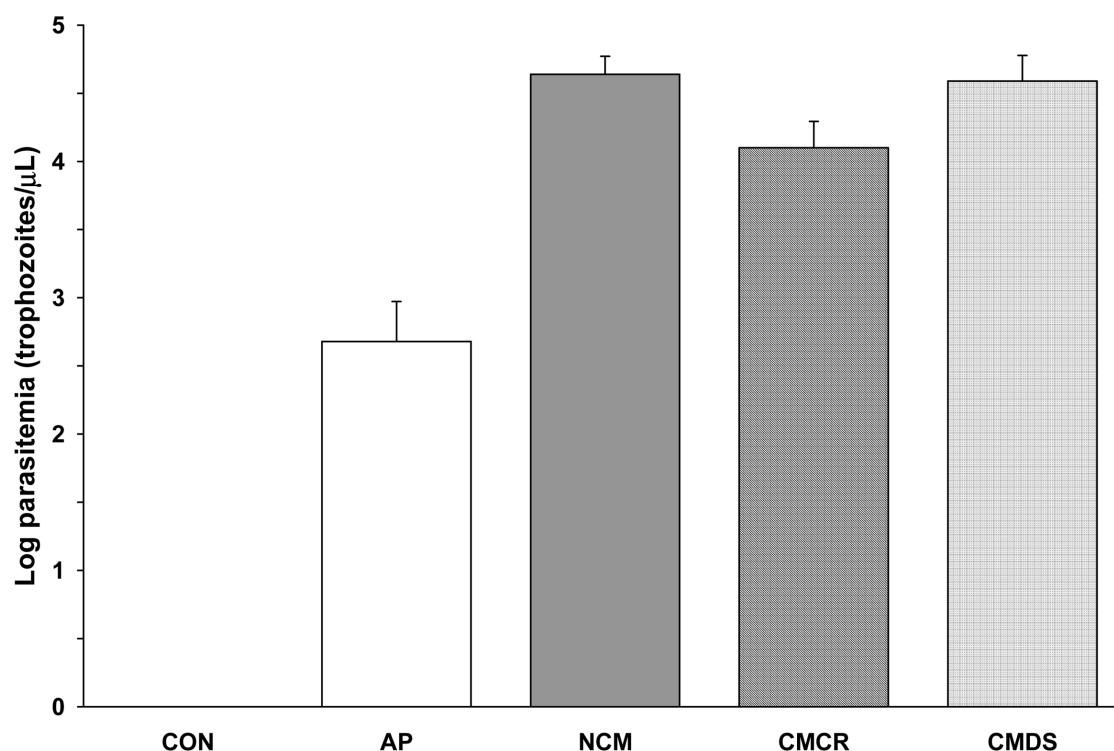


Figure 2. Peripheral parasitemia values in different disease severity groups. Peripheral parasitemia values were determined in children with differing disease severities: healthy control subjects (CONs; $n = 18$), patients with asymptomatic parasitemia (AP; $n = 8$), patients with noncerebral malaria (NCM; $n = 31$), patients with cerebral malaria (CM) with complete recovery (CMCR; $n = 14$), and patients with CM with death or associated neurological sequelae (CMDS; $n = 19$). Data are presented as mean ($\pm$ SE) log-transformed parasitemia values. An analysis of covariance (with adjustment for age) indicated a significant difference in peripheral parasitemia between the 5 groups ($F[4,72] = 124.35$; $P < .0001$). Further analyses using Fisher's least significant difference multiple range tests indicated that (1) the AP group had significantly lower parasitemia than did the 2 CM groups, and (2) the NCM group had significantly higher parasitemia than did the CMCR group.

rate of the control group in the present study, data on parasitemic children were reanalyzed with the control group excluded from the analysis. Upon reanalysis, there was still no significant association between levels of bicyclo-PGE₂/creatinine and hemoglobin levels ($r = 0.06$; $P = .634$) (table 2). The partial correlation (with adjustment for age, parasitemia, and disease severity) showed a nonsignificant positive association between levels of bicyclo-PGE₂/creatinine and hemoglobin levels ($r_p = 0.06$; $P = .66$) (table 2).

DISCUSSION

The lack of elevation of urinary levels of bicyclo-PGE₂ in Tanzanian children with CM, along with the findings of our previous studies (which showed an inverse correlation of peripheral blood mononuclear cell [PBMC] COX-2 gene expression and plasma levels of bicyclo-PGE₂ with disease severity in Gabonese children with malarial anemia) [13], illustrates that decreased biosynthesis of PGE₂ during falciparum malaria is associated with enhanced disease severity. The findings of these 2 studies are also consistent with those of our recent study that demonstrated that production of PGE₂ was suppressed in cir-

culating placental monocytes isolated from Kenyan women with malaria during pregnancy [21]. The inverse association between systemic levels of PGE₂ and malaria disease severity in all of our previous studies performed in participants from regions with differing malaria endemicity who had varied genetic backgrounds and different clinical manifestations of severe disease (i.e., malarial anemia vs. CM) suggests that suppression of systemic production of PGE₂ is a universal pathophysiological marker and/or mediator of malaria pathogenesis.

Previous studies of healthy adults (non-malaria exposed) have illustrated that salicylates and acetaminophen (paracetamol) can suppress urinary production of PGE₂ [22, 23]. Since some of the children in the present study received antimalarials and/or antipyretics before enrollment, systemic levels of PGs could have been altered by common treatment interventions for malaria. Although the effects of antimalarials on systemic production of PGE₂ are unknown, it is unlikely that treatment interventions alone account for the impaired production of PGE₂ in children with CM. For example, urinary levels of bicyclo-PGE₂ were lower in children with the most severe forms of CM, even though the number of individuals who reported

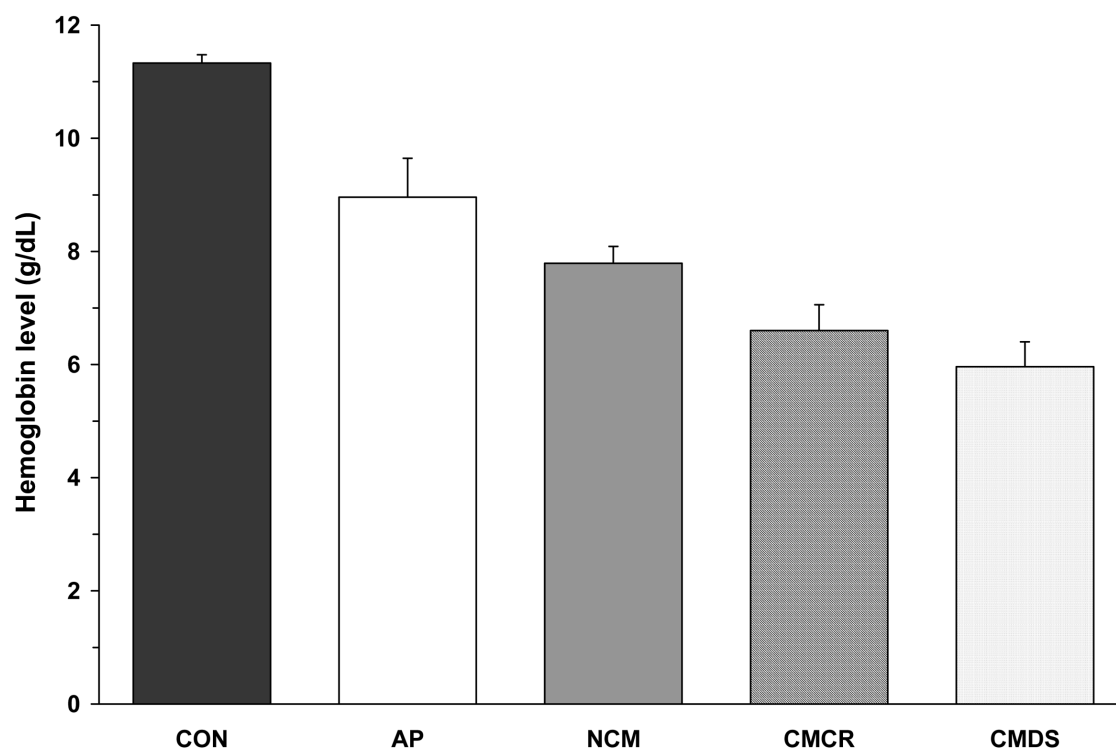


Figure 3. Hemoglobin levels in different disease severity groups. Hemoglobin levels were determined in children with differing disease severities: healthy control subjects (CONs; $n = 18$), patients with asymptomatic parasitemia (AP; $n = 8$), patients with noncerebral malaria (NCM; $n = 31$), patients with cerebral malaria (CM) with complete recovery (CMCR; $n = 14$), and patients with CM with death or associated neurological sequelae (CMDS; $n = 19$). The data are presented as raw, untransformed mean ($\pm$ SE) hemoglobin levels for all 5 groups. An analysis of covariance (with adjustment for age) indicated a significant difference in hemoglobin levels between the groups ($F[4,74] = 23.95$; $P < .0001$). Further analyses using Fisher's least significant difference multiple range tests indicated that (1) the CON group had significantly higher hemoglobin levels than did the other groups, (2) the AP group had significantly higher hemoglobin levels than did the 2 CM groups, and (3) the NCM group had significantly higher hemoglobin levels than did the 2 CM groups.

having received antimalarials and/or antipyretics was slightly lower in the CMDS group (86% medication usage) than in the CMCR group (92% medication usage). Although it is possible that treatment interventions may have some effect on urinary levels of PGE_2 , our previous results, which showed that plasma levels of bicyclo- PGE_2 are significantly suppressed in medication-naïve individuals with severe malarial anemia [13], illustrate that infection with *P. falciparum* can impair systemic production of PGE_2 . Our previous studies have also demonstrated that de novo levels of PBMC COX-2 mRNA and protein were significantly lower in children with severe malaria than in children with mild disease. This finding is important since suppression of production of PGE_2 , by salicylates and acetaminophen, likely occurs through blockade of the COX enzymatic site in the mature protein, and not through blockade of de novo COX-2 gene expression [24, 25]. Since we did not have access to cryopreserved PBMCs in the present study, we were unable to determine whether levels of COX-2 transcript and protein were suppressed in circulating blood mononuclear cells. Although the exact molecular mechanisms by which malaria suppresses COX-2 and PGs are presently unknown, we have

recently found that ingestion of hemozoin (malarial pigment) and β -hematin (synthetic malarial pigment) by cultured monocytes decreases COX-2 gene expression and formation of PGE_2 [26]. Thus, we postulate that acquisition of parasitic products by circulating monocytes and tissue macrophages is directly responsible for the systemic reduction of PGs in children with CM reported in the present study.

In addition to the results of the present study and those of our previous studies [13], the results of several other studies suggest that decreased production of PGs during malaria infection results in enhanced pathogenesis. In experiments by Xiao et al., treatment with aspirin (a COX-1 and COX-2 inhibitor) increased mortality in a murine model of CM [15]. In the same murine model, celecoxib, a more selective inhibitor of COX-2, was associated with an earlier onset of CM [16]. In addition, studies in Gabon have demonstrated that treatment of children with acetaminophen prolongs the time to clearance of *P. falciparum* and decreases production of oxygen radicals in mitogen-stimulated whole blood [14]. On the basis of previous studies by others that showed that the overuse of salicylates in areas where malaria is endemic contributes to meta-

bolic acidosis and hypoglycemia [27], along with our studies of Gabonese children with severe malarial anemia [13] and the present study of Tanzanian children with CM, we postulate that malaria-induced suppression of peripheral PGE₂ may be exacerbated by pharmacological interventions attempting to control the fever commonly associated with malaria. Although this effect of antipyretics may be deleterious, it may be balanced by potentially beneficial effects of antipyretics in reduction of seizure risk and reduction of the fever-induced cytoadherence of pRBCs within the cerebral microvasculature [28]. In previous studies, only toxic doses of antipyretics due to overmedication by caregivers have been linked to severe malaria [27]. We are currently in the process of examining the advantageous versus the adverse effects associated with use of antipyretics in both human and nonhuman primate malarias.

Formation of PGE₂ is determined by the relative expression of proinflammatory cytokines, which increase COX-2 gene expression (e.g., TNF- α), and anti-inflammatory cytokines, which decrease COX-2 gene expression (e.g., IL-10) [17, 29, 30]. Once formed, PGE₂ can act as a negative feedback signal to decrease production of TNF- α in monocytes [31, 32] and promote the release of IL-10 [33]. Thus, although PGE₂ is typically associated with proinflammatory events, PGE₂ has anti-inflammatory properties through its ability to down-regulate production of TNF- α and up-regulate production of IL-10. Analysis of the complex interactions between cytokines and PGE₂ in vivo, however, is largely unreported. Investigation of regulation of production of PGE₂, by cytokines, in our previous studies of children with severe malarial anemia revealed a significant inverse association between systemic levels of PGE₂ and those of IL-10 [13], which is consistent with a model suggesting that IL-10 down-regulates production of PGE₂. However, in the present study, there was a significant positive correlation between systemic levels of PGE₂ and those of IL-10, suggesting a model consistent with the work of Shinomiya et al. in which PGE₂ was found to drive increased production of IL-10 [33]. Additional studies examining the time-dependent relationship between cytokines and production of PGE₂ during the acute and convalescent stages of disease will be required to appropriately understand the complex inflammatory milieu.

Although, in the present study, there was not a significant inverse correlation between systemic levels of PGE₂ and of TNF- α , we postulate that inadequate peripheral production of PGE₂ during malaria exacerbates the overproduction of monocyte-derived TNF- α . High systemic levels of TNF- α (because of systemic suppression of production of PGE₂) would then circulate to the preoptic area of the anterior hypothalamus (POAH) in the central nervous system (CNS) and induce local tissue-specific COX-2 gene expression and production of PGE₂ in astrocytes and/or microvascular endothelial cells. This model is consistent with the fact that localized production of PGE₂ in the

POAH is responsible for generation of the febrile response [34], which is the hallmark clinical sign of malaria. This may also explain why previous immunohistochemical studies have found increased levels of COX-1 in macrophages/microglial cells within Dürcks granulomas and increased expression of COX-2 in endothelial cells and astrocytes within brain parenchyma, in expatriates with CM [35]. Consistent with the fact that the systemic immune response does not always mirror what is occurring within central brain regions, children with the lowest systemic levels of PGE₂ have the highest levels of fever—an event requiring increased COX-2–derived production of PGE₂ within the POAH. On the basis of the results presented here and those presented elsewhere, we postulate that down-regulation of PGE₂ in the periphery may allow overexpression of inflammatory mediators, such as TNF- α , which promote enhanced pathophysiological consequences in the CNS.

Although previous studies of cultured *P. falciparum* isolates have shown that the parasite is capable of biosynthesizing PGs [36], the results presented here and in our previous work in Gabon [13] illustrate that children with the highest parasite burdens have the lowest levels of systemic production of PGE₂. Thus, any PGE₂ synthesized by parasites appears to be counteracted by the down-regulation of host production of PGE₂, by the malaria disease process. The effect of the parasites' ability to synthesize PGs on the pathophysiology of malaria remains unresolved. In the present study, we found a positive, significant correlation ($r_p = 0.31$; $P = .007$) between systemic levels of PGE₂ and parasitemia, after adjustment for age and disease severity, suggesting that parasitemia influences levels of PGE₂.

PGs also have other biological activities that could mediate the pathogenesis of malaria, such as their ability to regulate erythropoiesis [18, 19]. In our previous investigations in Gabon, we found that plasma levels of PGE₂ were inversely related to hemoglobin levels [20], which is consistent with the fact that PGE₂ is an important molecule for promoting appropriate erythropoiesis [19]. The data presented here differ from our previous results in that levels of bicyclo-PGE₂ and hemoglobin levels were not significantly associated. When age, parasitemia, and disease severity were taken into account, the association between levels of bicyclo-PGE₂ and hemoglobin levels remained nonsignificant.

High levels of PGE₂ in children with asymptomatic parasitemia suggest that PGs may also contribute to the maintenance of malaria tolerance, which is the ability of children to tolerate circulating parasites without fever. We have previously found that high basal levels of COX-2 gene expression and production of PGE₂ in unstimulated circulating monocytes in healthy malaria-exposed children in Gabon are higher than those in non-malaria exposed, age-matched children in the United States (D.J.P., unpublished data). Similarly, high basal production of NO has been found in children and adults with asymptomatic

parasitemia [37, 38] and healthy malaria-exposed children who develop mild versus severe disease during acute malaria [39], suggesting that high basal production of NO may contribute to malaria tolerance in endemic areas. In fact, we have previously shown that healthy malaria-exposed children who develop mild disease and produce high basal levels of NO have significantly lower basal production of TNF- α than do children who produce low basal levels of NO and develop severe disease [39]. We therefore hypothesize that a common mechanism for both PGE₂ and NO may be their ability to inhibit monocyte production of TNF- α , one of the major fever-inducing cytokines in malaria [40]. Future studies aimed at determining the mechanisms by which *P. falciparum* suppresses systemic levels of PGE₂ and the role that PGs play in regulating malaria pathogenesis should provide important insight into our understanding of malarial immunity and may have important therapeutic implications.

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