

Anemia and Interleukin-10, Tumor Necrosis Factor Alpha, and Erythropoietin Levels among Children with Acute, Uncomplicated *Plasmodium falciparum* Malaria

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Anemia is an important complication of malaria, and its pathogenesis is not well understood. To gain insight into potential age-related relationships between tumor necrosis factor alpha (TNF- α), interleukin 10 (IL-10), erythropoietin, and anemia during acute malaria, 273 children of ages 12 to 120 months presenting with acute, uncomplicated malaria in Kampala, Uganda, were monitored at enrollment and 3 and 7 days later. Younger children had higher geometric mean erythropoietin, TNF- α , and α_1 -acid glycoprotein (AGP) concentrations than older children. Univariate regression analysis revealed that age, log₁₀ erythropoietin levels, IL-10/TNF- α ratio, and AGP levels were each significantly associated with hemoglobin levels at baseline. Hemoglobin concentrations were inversely correlated with the log₁₀ erythropoietin level at all three visits. For the older age groups, higher levels of TNF- α were significantly associated with higher IL-10 levels at all three visits, but this relationship was significant only at baseline for younger children. These data suggest that younger children do not maintain IL-10 production in response to the inflammatory process, and this mechanism may contribute to the more severe anemia found in younger children. Acute malaria is an illness whose incidence and severity are largely age dependent. Further studies are needed to understand the relationships between age-related immune responses to malaria and their role in the pathogenesis of malarial anemia.

Malaria is a leading cause of morbidity and mortality and accounts for an estimated one million deaths annually among children in developing countries (40). Anemia is an important complication of malaria both in asymptomatic children and in children with acute febrile episodes (4, 24, 33). The pathogenesis of malarial anemia is not well understood, but inflammatory cytokines, such as tumor necrosis factor alpha (TNF- α) and IL-10, are believed to be involved (23, 30). TNF- α , an inflammatory cytokine that plays a role in T-helper type 1-like immune responses, has been implicated in several biological actions during acute malaria, including stimulation of nitric oxide production, enhancement of the production of other cytokines, such as IL-6, and inhibition of erythropoiesis (22, 23). TNF- α is downregulated by IL-10, an anti-inflammatory cytokine that plays a role in T-helper type 2-like immune responses (30) and appears to stimulate erythropoiesis (38). It has been proposed that the balance between IL-10 and TNF- α may modulate the severity of malarial anemia in children (30).

Erythropoietin, a glycoprotein with a molecular mass of 34 kDa, is produced primarily by the kidney in response to tissue hypoxia and is the primary factor regulating red blood cell production. Elevated serum erythropoietin concentrations generally correlate inversely with decreased hemoglobin concentrations. Among children with malarial anemia, the produc-

tion of erythropoietin has been reported to be elevated in response to low hemoglobin concentrations (8, 25). In vitro studies suggest that TNF- α can inhibit the production of erythropoietin (11, 19), but it is unclear whether inhibition of erythropoietin production by TNF- α occurs during acute malaria.

In areas where malaria is endemic, the prevalence of anemia, geometric mean parasite densities, and risk of fever with *Plasmodium falciparum* decrease with increasing age among children (6, 33). The acquisition of immunity to malaria appears to be reflected in a stronger ability to limit the density of malaria parasites and the severity of anemia. To gain insight into the relationships between TNF- α , IL-10, erythropoietin, and anemia during acute malaria, we conducted a longitudinal study of children presenting with acute, uncomplicated malaria in Kampala, Uganda.

MATERIALS AND METHODS

Study population. The study population consisted of a sample of children 1 to 10 years old, seen consecutively in the acute pediatric care unit of Mulago Hospital, Kampala, Uganda, between August and December 1998. The Mulago Hospital serves urban and peri-urban Kampala, an area where *P. falciparum* malaria is endemic. Children were eligible for the study if they were between the ages of 1 and 10 years, were positive for malaria on the basis of a thick smear, had hemoglobin levels of >50 g/liter, were not admitted for transfusion, and had no evidence of cerebral malaria.

Study protocol. Children were seen by a medical officer upon presentation in the acute care unit.

Temperature was recorded using an oral thermometer. A fingerprick blood sample was taken to prepare thick and thin blood films to determine the presence or absence of malaria parasites, level of parasitemia, and hemoglobin concentrations using a HemoCue instrument (HemoCue Inc, Mission Viejo, Calif.)

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TABLE 1. Clinical and laboratory findings with children with acute malaria at baseline, day 3, and day 7^a

Characteristic	Value [n]			P value ^b
	Baseline	Day 3	Day 7	
Any parasitemia (%)	100 [273]	42.7 [218]	14.7 [190]	0.0001
Log ₁₀ parasitemia ^c	2.76 ± 0.82 [273]	1.65 ± 0.85 [93]	1.71 ± 0.80 [28]	0.0001
% with fever (oral temp > 38°C)	43.9 [273]	1.8 [218]	2.6 [190]	<0.0001
% with splenomegaly	47.2 [271]	45.4 [218]	36.8 [190]	0.026
Hemoglobin concn (g/liter)	86 ± 19 [272]	85 ± 19 [216]	89 ± 18 [186]	0.029
% with hemoglobin concn (g/liter) of:				
<110	87.1 [272]	87.9 [215]	86.0 [186]	0.73
<70	20.1 [272]	21.9 [216]	14.0 [186]	0.85
Geometric mean erythropoietin concn (IU/liter)	77 (23, 257) [257]	91 (22, 368) [177]	44 (15, 130) [179]	0.0001
Geometric mean IL-10 concn (pg/ml)	426 (131, 1380) [239]	97 (43, 218) [178]	63 (28, 138) [181]	0.0001
Geometric mean TNF-α concn (pg/ml)	25 (10, 62) [261]	8 (2, 26) [180]	5 (1, 27) [183]	0.0001

^a For continuous variables, values are given as means ± SDs. For geometric means, SDs are in parentheses (lower SD, upper SD). n, no. of samples.

^b Difference between baseline and day 7.

^c Among individuals with detectable parasitemia [n].

(18). Height was measured using a child measuring board (Shorr Productions, Olney, Md.). Weight was measured using a digital scale.

Children with malaria were enrolled in the study after written informed consent was obtained from the parent or guardian. The entire consent form was read to the parent or guardian in Luganda, the local language, or in English, as appropriate, and participants were given a copy of the consent form after signature or thumbprint was obtained. Children were examined by a medical officer at enrollment, day 3, and day 7, and spleen enlargement was assessed using the Hackett scale (12). At enrollment and on days 3 and 7, a venous blood sample was collected by venipuncture (Sarstedt Monovette, Newton, N.C.). Blood samples were shielded from bright light and immediately aliquoted and stored in cryotubes at -70°C. All children were treated with chloroquine sulfate as per guidelines of the Ugandan Ministry of Health. Children with increased parasitemia on day 3 or day 7 were treated with quinine or fansidar. The study protocol was approved by the Joint Committee for Clinical Investigation of the Johns Hopkins School of Medicine, the Mulago Hospital Ethical Committee, and the Ugandan National Council of Science and Technology.

Laboratory methods. Thick and thin Giemsa-stained blood films were analyzed for the number of parasites per 200 white blood cells. Slides were considered negative if no parasites were seen in 100 fields on the thick film. Levels of plasma α₁-acid glycoprotein (AGP) were measured using radial immunodiffusion (Bindarid; The Binding Site, Birmingham, United Kingdom) with a between-assay coefficient of variation (CV) of 10.3%. C-reactive protein (CRP) was measured by enzyme-linked immunosorbent assay (Virgo CRP 150; Hemagen Diagnostics, Waltham, Mass.) with a between-assay CV of 8.6%. Plasma erythropoietin concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (ALPCO, Windham, N.H.). Plasma TNF-α concentrations were measured using ELISA (Human TNF-α, Quantikine High Sensitivity; R & D Systems, Minneapolis, Minn.). Concentrations of IL-10 in plasma were measured using ELISA (Human IL-10; BD PharMingen, San Diego, Calif.). Pooled human standards were used to measure within-run and between-run CVs in laboratory analyses. The within-run and between-run CVs for erythropoietin, TNF-α, and IL-10 were 6.8 and 5.8%, 7.6 and 12.5%, and 6.1 and 4.5%, respectively.

Statistical analyses. Malaria parasitemia was highly skewed to higher values and was transformed by log₁₀ to normalize the distribution. Weight-for-age Z score of <-2, weight-for-height Z score of <-2, and height-for-age Z score of <-2 were considered to be consistent with underweight, wasting, and stunting, respectively. Anemia was defined as hemoglobin levels of <110 g/liter, according to standard guidelines (41). Analysis of variance was used to compare means of normally distributed variables among groups. The Mantel-Haenszel chi-square test was used to compare categorical variables among groups. Student's t test and chi-square and exact tests were used where appropriate for two group comparisons. Spearman correlation was used to measure the relationship between hemoglobin, erythropoietin, IL-10, TNF-α, acute phase proteins, and parasitemia. Univariate and multivariate linear regression models were used to examine the relationship between log₁₀ erythropoietin concentrations, hemoglobin levels, age, and other variables.

RESULTS

There were 273 children (164 boys and 109 girls), age (mean ± standard deviation [SD]) 38.9 ± 25.9 months, enrolled in the study. Mean weight-for-age, weight-for-height, and height-for-age Z scores were -0.80 ± 1.29, -0.12 ± 1.69, and -1.00 ± 1.77, respectively. The proportions of underweight, wasted, and stunted children were 15.8, 9.0, and 26.6%, respectively. At baseline, 43.9% of children presented with a fever (oral temperature, >38.0°C). The proportions of children with spleen grades of 0, 1, 2, and 3 at presentation were 52.8, 13.3, 23.6, and 10.3%, respectively. *P. falciparum* parasitemia, hemoglobin, erythropoietin, IL-10, and TNF-α levels at baseline and days 3 and 7 are shown in Table 1. *P. falciparum* parasitemia decreased from baseline to day 7. Mean hemoglobin concentrations increased from baseline to day 7, but the proportion of children who were anemic did not decrease significantly from baseline to day 7. Geometric mean erythropoietin, IL-10, and TNF-α concentrations decreased from baseline to day 7.

The clinical and laboratory findings were stratified by age (12 to <24 months, 24 to <48 months, and 48 to <120 months) and are shown at baseline, day 3, and day 7 in Table 2. There were no significant differences in malaria parasitemia by age. The data were suggestive that upon enrollment (*P* = 0.079) and at day 7 (*P* = 0.043), the proportion of children with fever was higher among younger children. At all three visits, there were significant differences in mean hemoglobin concentrations and proportions of children with hemoglobin concentrations of <70 g/liter by age. There were no significant differences in hemoglobin concentrations from baseline to day 7 within each age group. Erythropoietin concentrations decreased significantly from baseline to day 7 within each age group (all *P* values, <0.01). Geometric mean erythropoietin and geometric mean TNF-α concentrations were higher with decreasing age at all three visits. Children 12 to <24 months old had significantly higher erythropoietin and TNF-α concentrations than children 48 to <120 months old on all three visits (all *P* values, 0.002), but only erythropoietin concentrations were significantly different for children 24 to <48 months old on all three visits (all *P* values, <0.0001). Erythropoietin con-

TABLE 2. Clinical and laboratory findings by age at baseline, day 3, and day 7

Characteristic and sample category ^a	Value [n] for age group			P value ^b
	12 to <24 mo	24 to <48 mo	48 mo	
Any parasitemia (%)				
Baseline	100 [105]	100 [86]	100 [82]	1.00
Day 3	50.6 [83]	39.7 [68]	35.8 [67]	0.10
Day 7	18.8 [69]	15.0 [60]	9.8 [61]	0.26
Log ₁₀ parasitemia ^c				
Baseline	2.77 ± 0.83 [105]	2.77 ± 0.80 [86]	2.72 ± 0.83 [82]	0.94
Day 3	1.65 ± 0.74 [42]	1.81 ± 0.85 [27]	1.58 ± 1.05 [24]	0.60
Day 7	1.80 ± 0.69 [13]	1.62 ± 1.04 [9]	1.64 ± 0.74 [6]	0.85
Fever (oral temp > 38.0°C) (%)				
Baseline	50.5 [105]	41.8 [86]	37.8 [82]	0.079
Day 3	2.4 [83]	1.5 [68]	1.5 [67]	0.66
Day 7	5.6 [71]	1.7 [60]	0 [59]	0.043
Splenomegaly (%)				
Baseline	44.2 [104]	49.4 [85]	48.7 [82]	0.51
Day 3	42.1 [83]	50.0 [68]	44.7 [67]	0.71
Day 7	39.4 [71]	40.0 [60]	30.5 [59]	0.31
Hemoglobin concn (g/liter)				
Baseline	78 ± 15 [104]	86 ± 17 [86]	97 ± 20 [82]	0.0001
Day 3	75 ± 15 [83]	83 ± 18 [66]	97 ± 18 [66]	0.0001
Day 7	82 ± 18 [68]	89 ± 15 [59]	100 ± 15 [59]	0.0001
Hemoglobin concn of <70 g/liter (%)				
Baseline	33.6 [104]	13.9 [86]	12.2 [82]	0.001
Day 3	34.9 [83]	18.1 [66]	9.0 [66]	0.001
Day 7	25.0 [68]	10.1 [59]	5.0 [59]	0.001
Geometric mean erythropoietin concn (IU/liter)				
Baseline	138 (44, 389) [95]	89 (25, 309) [86]	43 (14, 134) [80]	0.0001
Day 3	177 (56, 562) [64]	104 (30, 363) [59]	43 (10, 177) [54]	0.0001
Day 7	72 (28, 181) [66]	48 (20, 114) [57]	27 (8, 89) [56]	0.0001
Geometric mean IL-10 concn (pg/ml)				
Baseline	524 (194, 1412) [87]	386 (114, 1335) [74]	371 (100, 1380) [78]	0.11
Day 3	129 (69, 241) [62]	95 (38, 239) [59]	77 (35, 169) [57]	0.003
Day 7	89 (41, 186) [65]	57 (29, 112) [58]	46 (21, 101) [58]	0.0001
Geometric mean TFN-α concn (pg/ml)				
Baseline	31 (13, 72) [97]	24 (10, 60) [83]	20 (8, 48) [81]	0.007
Day 3	11 (6, 21) [66]	10 (4, 24) [59]	4 (1, 24) [55]	0.0001
Day 7	7 (1, 35) [68]	5 (1, 26) [57]	2 (1, 17) [58]	0.0013
AGP concn (g/liter) ^d	2.15 ± 0.63 [105]	2.01 ± 0.48 [86]	1.75 ± 0.45 [82]	0.0001
CRP concn (mg/liter) ^d	44.8 ± 17.3 [105]	46.0 ± 17.2 [86]	44.1 ± 19.5 [82]	0.78

^a For continuous variables, values are given as means ± SDs. For geometric means, SDs are in parentheses (lower SD, upper SD).

^b Differences between age categories by the Mantel-Haenszel chi-square test for categorical variables and by analysis of variance for continuous variables.

^c Among individuals with detectable parasitemia [n].

^d AGP and CRP levels measured at baseline only.

centrations for children 24 to <48 months old were significantly different from concentrations for children 48 to <120 months old at baseline and day 3 (all *P* values, 0.001), whereas TNF-α concentrations for these two groups were significantly different at days 3 and 7 (all *P* values, 0.01). Geometric mean IL-10 concentrations rose with decreasing age at day 3 and day 7. Children 12 to <24 months old had significantly higher IL-10 concentrations than both older groups of children (*P* = 0.05), and IL-10 concentrations were not significantly different between the two older age groups. At baseline, mean AGP

concentrations were higher in young-aged children, but there were no significant differences between mean CRP by age.

The Spearman correlations among hemoglobin and other laboratory indicators at baseline are shown in Table 3. Erythropoietin and hemoglobin levels had a significant inverse correlation (*r* = -0.558). Hemoglobin levels were inversely correlated with those of both TNF-α (*r* = -0.174) and AGP (*r* = -0.353). Erythropoietin was significantly correlated with both TNF-α (*r* = 0.304) and AGP (*r* = 0.466). Parasitemia was significantly correlated with TNF-α (*r* = 0.375). IL-10 and

TABLE 4. Univariate and multivariate regression models for hemoglobin at baseline and age, log₁₀ erythropoietin, IL-10/TNF-α ratio, and AGP

Independent variable	Univariate analysis			Multivariate analysis		
	β	SE	P value	β	SE	P value
Age (mo)	0.3	0.04	<0.0001	0.1	0.04	0.016
Log ₁₀ erythropoietin concn	-19.6	1.90	<0.0001	-13.9	2.4	<0.0001
IL-10/TNF-α ratio	0.1	0.03	<0.0001	0.1	0.03	0.004
AGP concn (g/liter)	-12.2	1.93	<0.0001	-4.6	2.1	0.028

TNF-α were significantly correlated (*r* = 0.567). TNF-α was significantly correlated with AGP (*r* = 0.246).

There were no statistically significant differences in mean plasma erythropoietin, IL-10, and TNF-α concentrations between girls and boys at baseline (data not shown). At enrollment, among children who presented with and without a fever, geometric mean TNF-α and IL-10 concentrations (SD) were 35.4 (79.4, 15.8) and 19.0 (45.7, 7.9) (*P* < 0.0001) and 891 (2454, 323) and 251 (676, 93) pg/ml (*P* < 0.0001), respectively. Log₁₀ parasitemia was 3.06 ± 0.65 and 2.52 ± 0.87 (*P* < 0.0001) among children with and without a fever at presentation, respectively. For children presenting with and without a fever at enrollment, mean hemoglobin and plasma erythropoietin concentrations did not differ significantly (data not shown).

Univariate regression analysis revealed that age, log₁₀ erythropoietin levels, the IL-10/TNF-α ratio, and AGP levels were each significantly associated with the hemoglobin concentration at baseline (Table 4). For every unit increase in age, hemoglobin concentrations increased by 0.3 g/liter (*P* = 0.001). Higher IL-10/TNF-α ratios were associated with higher hemoglobin concentrations (*P* < 0.0001). Higher log₁₀ erythropoietin and AGP concentrations were both associated with a lower hemoglobin concentration (all *P* values, <0.0001). Each variable remained significant in the multivariate model (Table 4).

Univariate linear regression analysis showed that at baseline, an increase in hemoglobin corresponded to a decrease in log₁₀ erythropoietin (*P* < 0.0001). The model fit for the relationship at baseline was log₁₀ erythropoietin concentration = 3.21 - (0.02 × hemoglobin concentration [g/liter]) (*P* < 0.0001). This inverse relationship was present at days 3 and 7 as well. At day 3, the regression equation was log₁₀ erythropoietin concentration = (3.63 - 0.02) × hemoglobin concentration (g/liter) (*P* < 0.0001), and at day 7, it was log₁₀ erythropoietin concentration = (2.74 - 0.01) × hemoglobin concentration (g/liter) (*P* < 0.0001).

Univariate regression was used to examine the relationship between IL-10 and TNF-α concentrations for each age group at baseline, day 3, and day 7, using the model IL-10 concentration = β₀ + β₁ TNF-α concentration for each age category and visit (Table 5). TNF-α concentrations were significantly associated with IL-10 concentrations for children 12 to <24 months old at baseline only (*P* < 0.0001). For the older age groups, increasing TNF-α concentrations were significantly associated with increasing IL-10 concentrations at all three visits (all *P* values, <0.01). At baseline, the increase in IL-10 per pg of TNF-α/ml was significantly larger in children 48 to <120

TABLE 3. Spearman correlations among hemoglobin and other laboratory indicators at baseline

Parameter	Correlation (<i>r</i>) with ^a :					
	Erythropoietin concn	Parasitemia	IL-10 concn	TNF-α concn	AGP concn	CRP concn
Hemoglobin concn	-0.588 (257), <i>P</i> < 0.0001	0.070 (272), <i>P</i> = 0.24	0.094 (238), <i>P</i> = 0.14	-0.174 (261), <i>P</i> = 0.0047	-0.353 (272), <i>P</i> < 0.0001	-0.119 (272), <i>P</i> = 0.48
Erythropoietin concn		0.080 (257), <i>P</i> = 0.20	0.012 (226), <i>P</i> = 0.85	0.304 (257), <i>P</i> < 0.0001	0.466 (257), <i>P</i> < 0.0001	0.134 (257), <i>P</i> = 0.031
Parasitemia			0.442 (239), <i>P</i> < 0.0001	0.375 (261), <i>P</i> < 0.0001	0.059 (273), <i>P</i> = 0.33	0.148 (273), <i>P</i> = 0.014
IL-10 concn				0.567 (230), <i>P</i> < 0.0001	0.012 (239), <i>P</i> = 0.84	0.092 (239), <i>P</i> = 0.15
TNF-α concn					0.246 (261), <i>P</i> < 0.0001	0.144 (261), <i>P</i> = 0.019
AGP concn						0.219 (273), <i>P</i> < 0.0003

^a*n*, number of samples.

TABLE 5. Univariate regression models for relationship between IL-10 and TNF- α by age at baseline, day 3, and day 7

Age (mo)	Correlation between IL-10 and TNF- α					
	Baseline		Day 3		Day 7	
	β (SE)	<i>P</i> value	β (SE)	<i>P</i> value	β (SE)	<i>P</i> value
12 to <24	13.32 (4.16)	0.002	1.47 (2.97)	0.622	3.51 (2.35)	0.140
24 to <48	12.39 (3.68)	0.001	13.13 (3.30)	<0.0001	7.13 (2.15)	0.009
48 to <120	60.34 (6.98)	<0.0001	9.76 (2.17)	<0.0001	8.18 (1.76)	<0.0001

months old than in both younger groups (all $P < 0.0001$) but was not significantly different between the two younger age groups.

DISCUSSION

The present study demonstrates that the severity of anemia is related to age among children who present with acute, uncomplicated *P. falciparum* malaria and suggests that age-related differences in immunologic responses may play a role in the pathogenesis of malarial anemia. The younger children in our study had lower mean hemoglobin concentrations and higher plasma erythropoietin, IL-10, and TNF- α concentrations than older children. These observations from an acute care setting are consistent with community-based studies that show that the prevalence of anemia is higher among younger children (6, 33). A potential caveat in our study is that the TNF- α levels at day 3 and day 7 are lower than the suggested lower limit of the assay (13.5 pg/ml). The mean plasma concentrations of IL-10 and TNF- α in the present study, however, are consistent with those described in children with acute malaria in Gabon (27). Our study in Uganda suggests that mean plasma erythropoietin, IL-10, and TNF- α concentrations during malaria episodes differ with age in children with acute, uncomplicated malaria (Table 2). While TNF- α and IL-10 concentrations have been shown to be higher in young children with severe malaria than in older children and adults with mild malaria (29), to our knowledge, this is the first study to examine age-related differences in the relationship between TNF- α and IL-10 responses in children during acute uncomplicated malaria.

Severe malarial infection is associated with an intense T-helper type 1-like response (16). It has recently been shown that the balance between T-helper type 1-like cytokines, such as TNF- α , and T-helper type 2-like cytokines may play a role in the clinical presentation of *P. falciparum* infection (15, 23, 30). Elevated serum concentrations of TNF- α have been reported during malaria, and high TNF- α concentrations correlate strongly with increasing severity of disease (13, 21, 27, 29, 30, 35). IL-10, an anti-inflammatory cytokine mediated by the T-helper type 2-like response, is also elevated during malaria (15, 27, 30) and has been shown to down regulate TNF- α (9, 15, 21, 30, 39). Severe malarial anemia has been associated with defective IL-10 production in children (23), and animal models have shown that IL-10 gene knockout mice develop more severe disease and experience higher mortality rates than normal mice. These IL-10-deficient mice also exhibited a strong T-helper type 1-like response, while a T-helper type 2-like response predominated in the control mice (26). It has been proposed that children with *P. falciparum* infection who pro-

duce balanced levels of IL-10 to regulate excessive TNF- α are better able to control severe anemia (30), and severe anemia during malaria may be the consequence of dysregulation of immunologic inflammation (3). Recently the balance between plasma IL-10 and TNF- α concentrations, or the IL-10/TNF- α ratio, was shown to be predictive of severe malaria anemia (23, 28–30), and certain TNF promoter variants have been shown to influence the balance of IL-10 and TNF in plasma (28). The present study corroborates and extends the observations that the IL-10/TNF- α ratio is predictive of more severe malarial anemia by demonstrating that an increased IL-10/TNF- α ratio is associated with increased hemoglobin concentrations in acute, uncomplicated *P. falciparum* malaria.

In malaria-endemic areas such as Uganda, the prevalence of anemia is higher among younger children (6, 33). Our study showed an increase in IL-10, and thus an increase in the T-helper type 2-like response, with increasing TNF- α concentrations throughout the study, except in younger children (12 to <24 months). These data suggest that younger children do not maintain IL-10 production in response to the inflammatory process and are consistent with the observation that more severe anemia is more common among younger children. Luty and colleagues showed that TNF- α and IL-10 are positively correlated with parasitemia (27). In our study, TNF- α and IL-10 concentrations differed according to age, even though no significant differences in parasitemia levels were present across age groups throughout the study. In addition, at baseline, older children (48 to <120 months) had a much greater increase in IL-10 with increasing TNF- α than the two other age groups, suggesting that the ability to mount a good T-helper type 2-like response early is protective. These age-associated changes are probably related to both cumulative malaria exposure and acquisition of immunity to malaria, since children in Uganda are continually exposed to *P. falciparum* and older children are likely to have had more exposure to malaria than younger children.

The present study showed that plasma AGP had a strong negative inverse correlation with hemoglobin and a positive correlation with both erythropoietin and plasma TNF- α . Plasma AGP concentrations appeared to decrease with age. AGP is an acute-phase protein produced by hepatocytes and may play a role in nonspecific resistance to infection. AGP has been shown to stimulate TNF- α secretion in human monocytes in vitro (36), and in an experimental animal model, AGP protected mice from a lethal challenge of gram-negative bacteria, possibly through an anti-inflammatory role (17). Whether AGP plays a role in nonspecific immunity to *P. falciparum* malaria is unclear.

This study was suggestive that a higher proportion of

younger children with acute, uncomplicated *P. falciparum* malaria presented with a fever. At enrollment, children with a fever had significantly higher parasitemia, plasma TNF- α , and plasma IL-10 concentrations than children without a fever. In vitro studies suggest that after the rupture of parasitized red blood cells, the malaria pigment and other soluble antigens may stimulate production of TNF- α in human monocytes (32, 37). Serum TNF- α concentrations have been closely correlated with paroxysmal episodes of malarial fever (20).

Two previous studies have shown that plasma erythropoietin concentrations are elevated in response to malarial anemia in children (8, 25). Our results show that the log₁₀ erythropoietin concentration has a consistent inverse relationship to hemoglobin levels during and after uncomplicated malaria. Whether production of erythropoietin by renal cortical cells is normal or abnormal in various disease states is sometimes difficult to determine, since erythropoietin production is inversely proportion to hemoglobin concentrations, and the slope of this regression line should be the basis for comparison with controls (5). The slope of the regression line for log₁₀ erythropoietin versus hemoglobin concentrations among children with malaria in Uganda is similar to that reported elsewhere for normal healthy children (34).

The pathogenesis of anemia during malarial infection is usually multifactorial, and other mechanisms that are involved include abnormalities of erythropoiesis, or dyserythropoiesis, in bone marrow (1), increased red blood cell destruction (10), and hypersplenism (31). The prevalence of iron deficiency and folate deficiency may be high in many areas where malaria is endemic and may contribute to anemia in children with malaria (2, 14). In a malaria-endemic area on the east coast of Africa, anemia was found to be more severe among children with heavy hookworm infection (7). The prevalence and intensity of hookworm infection increases with age (7), and it seems unlikely that hookworm influenced the results of the present study, since younger children were more anemic than older children.

Acute malaria is an illness whose incidence and severity are largely age dependent. The recent studies outlined above have demonstrated that the T-helper type 2-like response is important in dampening potentially damaging effects of T-helper type 1-like cytokines. Our study demonstrated that younger children may have inadequate counterbalancing T-helper type 2-like responses during acute uncomplicated malaria. Further studies are needed to understand the relationships between various host immune responses and their role in the pathogenesis of malarial anemia.

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