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Differential Antibody Responses to *Plasmodium falciparum* Merozoite Proteins in Malawian Children with Severe Malaria

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Cerebral malaria (CM) and severe malarial anemia (SMA) are 2 major causes of death in African children infected with *Plasmodium falciparum*. We investigated levels of naturally acquired antibody to conserved and variable regions of merozoite surface protein (MSP)–1 and MSP-2, apical membrane antigen (AMA)–1, and rhoptry-associated protein 1 in plasma samples from 126 children admitted to the hospital with CM, 59 with SMA, and 84 with uncomplicated malaria (UM) in Malawi. Children with SMA were distinguished by very low levels of immunoglobulin (Ig) G to the conserved C-terminus of MSP-1 and MSP-2 and to full-length AMA-1. Conversely, children with CM had significantly higher levels of IgG to the conserved regions of all antigens examined than did children with UM (for MSP-1 and AMA-1, $P < .005$; for MSP-2, $P < .05$) or SMA (for MSP-1 and MSP-2, $P < .001$; for AMA-1, $P < .005$). These distinct IgG patterns might reflect differences in age, exposure to *P. falciparum*, and/or genetic factors affecting immune responses.

Plasmodium falciparum malaria kills 0.5–2 million people each year, mainly young children in sub-Saharan Africa. Why only a small proportion of infected children develop severe malaria is unknown [1]. In African children, severe malarial anemia (SMA) and cerebral ma-

laria (CM) are 2 of the most life-threatening presentations of severe malaria [2].

Children who develop different clinical manifestations of malaria could have different preceding levels of naturally acquired immunity. Antibodies probably contribute to immune protection, as suggested by immunoglobulin passive-transfer experiments [3]. Protein targets of such immunity, many of which are putative vaccine candidates, include the asexual blood-stage proteins merozoite surface protein (MSP)–1 [4, 5] and MSP-2 [6], apical membrane antigen (AMA)–1 [7], and internal rhoptry-associated protein (RAP)–1 [8]. In malaria-endemic areas, numerous seroepidemiological studies of responses to MSP-1 and, to a lesser extent, MSP-2, AMA-1, and RAP-1 have shown that these antigens are naturally immunogenic in humans [9–13]. Some studies have suggested a role for IgG antibodies in protection from infection and/or clinical disease [6–8, 14, 15], although results have been inconsistent [16, 17]. Several studies have investigated the relationship between IgG antibody responses to *P. falciparum* antigens and disease severity [18–28], but none has distinguished

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Table 1. Clinical and parasitological data for the patients with malaria.

Type of malaria	Acute ^a	Mean age, years ^b	Area of residence ^c				Geometric mean parasite density (95% CI) ^d	Duration of symptoms, h ^b	Prior treatment ^e	Sequelae	Deaths	Convalescent ^f
			A	B	C	D						
CM	126	3.42 ± 2.03	49	52	13	10	25,428 (14,820–43,636)	54 ± 41	50	25	17	100
SMA	59	2.37 ± 1.37	17	17	16	7	15,075 (6728–33,778)	82 ± 50	20	1	7	48
UM	84	2.76 ± 1.52	71	13	0	0	24,233 (14,504–40,487)	53 ± 38	7	0	0	29

NOTE. CI, confidence interval; CM, cerebral malaria; SMA, severe malarial anemia; UM, uncomplicated malaria.

^a No. of patients from whom a blood sample was obtained on admission to the hospital. Other data in this table refer to these patients.

^b Arithmetic means ± SDs. The duration of symptoms (fever, vomiting, cessation of eating, cessation of drinking/sucking, diarrhea, convulsions, and unconsciousness) was recorded by interviewing parents.

^c No. of patients by area of residence (A, town of Blantyre; B, periurban areas; C, villages; D, towns other than Blantyre); data were unavailable for 4 patients (2 with CM and 2 with SMA).

^d Parasite density was calculated as parasites (ring forms) per microliter of blood. There were no significant differences between groups.

^e No. of patients with a history of antimalarial treatment (sulfadoxine-pyrimethamine and/or quinine) in the past week.

^f No. of patients who came back to the hospital for a follow-up visit 1 month after admission.

between CM and SMA syndromes within the same population.

We investigated whether children who develop CM, SMA, or uncomplicated malaria (UM) are characterized, at the time of their admission to the hospital, by different levels of antibodies to *P. falciparum* merozoite antigens implicated in immune protection by earlier studies [6–8, 14, 15]. To make this determination, we measured levels of specific IgG antibodies by means of recombinant proteins based on domains of MSP-1, MSP-2, AMA-1, and RAP-1 in 269 Malawian children infected with *P. falciparum* with well-defined and distinctly different malaria disease phenotypes.

METHODS

Patient samples. Blood samples were collected from children consecutively admitted to the pediatric research ward between 1996 and 1997. Patients had CM (Blantyre coma score <3 [29], with asexual parasitemia and no other obvious cause of coma) or SMA (hemoglobin level <5 g/dL or hematocrit <15%). All children admitted to the research ward were treated with intravenous quinine, according to standardized protocols [30]. Control patients with UM were also recruited sequentially, from 3 sources: (1) ambulant children screened for enrolment in malaria drug studies at Ndirande Health Centre, (2) patients discharged from the hospital with a final diagnosis of UM, and (3) ambulant children attending the hospital outpatient clinic for a febrile illness who were found to have malaria with no other identifiable illness and were treated as outpatients. None of the control patients had a recent history of coma or convulsions, and all were fully conscious (Blantyre coma score of 5). Informed consent was obtained from parents or guardians, and the study obtained ethical approval from the investigators' institutions.

Five milliliters of venous blood was drawn in lithium heparin or EDTA on admission, and plasma was separated and stored at –70°C for serological analyses. Acute-phase plasma samples were obtained from a total of 356 children ($n = 34$ in 1996; $n = 322$ in 1997). Clinical and parasitological data were recorded for

all children, including state of consciousness, history of convulsions, prior drug treatment, parasite density, hematocrit, and basic demographic information. Patients were asked to return for a follow-up sample after a month, or earlier if they were sick. A total of 237 convalescent-phase blood samples were obtained ($n = 22$ in 1996; $n = 215$ in 1997). For this study, children with overlapping CM and SMA ($n = 53$) and children with severe nonmalarial diseases ($n = 34$) were excluded.

A summary of patient characteristics is given in table 1. Negative control serum samples were obtained from 50 Scottish adults who had not been exposed to malaria. A pool of serum samples from immune African individuals was used as a positive control in ELISAs.

Recombinant proteins and ELISA. Expression, purification, and characterization of the recombinant proteins used here have been reported elsewhere, as specified below. MSP-1 constructs were derived from block 2, and from the C-terminal region of the molecule. Three glutathione S-transferase (GST) fusion proteins represented block 2 of RO33, K1, and MAD20 types of MSP-1 [31]. Two constructs represented the MSP-1 C-terminal regions: a GST fusion protein containing most of block 17 of the Wellcome isolate, corresponding to the 19-kDa fragment [32], and a baculovirus-expressed recombinant protein representing the 42-kDa fragment of the CAMP isolate (donated by J. Lyon). Three GST fusion proteins were derived from MSP-2 [11]. The protein denoted K1 17/14 represented a highly conserved sequence from the C-terminus (aa 207–263 in MSP-2 of the K1 isolate). Two proteins represented the 2 major dimorphic types of MSP-2, IC1/3D7 and FC27. The IC1/3D7-type protein T9/96 13/14 comprised almost the full-length MSP-2 of the T9/96 isolate (aa 22–286). The FC27-type protein contained aa 151–237 from isolate T9/105 (T9/105 12/6). The C2 fragment of RAP-1, a GST fusion protein corresponding to aa 169–366 of the antigen, included an inhibitory monoclonal antibody epitope [33]. Recombinant *P. falciparum* AMA-1 antigen consisted of the full ectodomain of the 3D7 form of AMA-1, expressed in

Escherichia coli as an N-terminal His₆ fusion protein [34] (donated by R. F. Anders). The GST protein alone was purified from cultures transformed with pGEX-2T vector (without *MSP1*, *MSP2*, or *RAP1* inserts) and was used as a negative control antigen.

The ELISA procedure was as described elsewhere [10, 35]. In brief, wells of microtiter plates (Immunolon-4; Dynatech) were coated with 0.05 µg/100 µL of antigen per well in 0.1 mol/L carbonate buffer (pH 9.6), with GST protein, or with buffer alone. The plates were washed 3 times with PBS–0.05% Tween 20 and blocked with skimmed milk powder (1% wt/vol) in PBS–Tween. Malawian plasma and control samples were diluted 1:500 in the same blocking buffer. Plates were washed again, and 100 µL of diluted serum/plasma per well was added to duplicate wells and incubated overnight at 4°C. IgG bound to the wells was detected with horseradish peroxidase–conjugated rabbit anti-human IgG (Dako) at 1:5000 dilution in PBS–Tween. The reactions were developed with 0.012% H₂O₂ as the substrate and *o*-phenylenediamine (Sigma) as the chromagen (100 µL/well) and stopped after 10 min with 20 µL of 2 mol/L H₂SO₄ per well. Optical density was measured at 492 nm.

Specific reactivity of plasma/serum IgG with a recombinant protein was calculated by subtracting optical density values for the GST or buffer controls from the value obtained for the recombinant protein, thus obtaining specific optical density values. These values were then used as a continuous variable for statistical analysis or were converted to a binary variable (positive vs. negative), using a cutoff defined for each antigen as the mean for the 50 Scottish control serum samples plus 2 SDs.

Statistical analysis. IgG antibody responses to distinct regions of *P. falciparum* MSP-1, MSP-2, AMA-1, and RAP-1 were compared among groups of children presenting with different malaria syndromes. Using multiple regression analysis, we looked for statistically significant associations between disease severity and levels of antibodies, assessed here by concentrations (expressed as optical density values read at 492 nm) and prevalence rates of antibody-positive individuals (those with optical density values above the cutoff). Antibody levels were compared between different clinical forms of malarial disease in the acute stage and were measured again at 1 month of follow-up, to assess the ability of children with different malaria symptoms to mount antibody responses to acute infection and to estimate the duration of these antibodies. Paired *t* tests were used to compare antibody levels between acute and convalescent phases, using pairs of plasma samples from 73 patients with CM, 39 with SMA, and 15 with UM.

Data were analyzed using standard multiple regression techniques, with the SAS statistical analysis package (SAS Institute, 1990). Associations between antibody levels, age, and parasite density were analyzed by fitting a generalized linear model (PROC GLM) appropriate for continuous dependent variables. The relationship between host disease status as a binary trait and

antibody levels (optical density values or prevalence rates) as an explanatory variable was analyzed by PROC GENMOD (categorical linear model), using a binomial distribution for the dependent variable. The models were adjusted for the possible confounding effects of parasite density, age, sex, area of residence, admission date, duration of symptoms, history of prior antimalarial treatment, and disease outcome (death, neurological sequelae, or full recovery). Antibody levels in each disease group are reported as least-squares means of corrected optical density values with 95% confidence intervals, as calculated by PROC GLM. Significance was defined at the 5% level.

RESULTS

Levels of antibody to MSP-1. A differential pattern of levels and prevalence rates of antibodies was found in clinically different forms of malaria. Children who presented with SMA were distinguished from children with CM or UM by lower mean levels of antibodies to the C-terminal regions of MSP-1 (19- and 42-kDa fragments) (table 2). Children admitted with CM had significantly higher mean levels of IgG to these proteins than did children with UM ($P < .05$ for 19 and 42 kDa; PROC GENMOD) or SMA ($P < .01$ for 19 and 42 kDa) (figure 1). When analyzed as a binary trait, the proportion of children with positive IgG to the C-terminus was also significantly higher in children with CM than in those with UM ($P < .001$ for 19 kDa; $P < .05$ for 42 kDa) or SMA ($P < .01$ for 19 kDa; $P < .05$ for 42 kDa) (table 2). These differences remained significant after allowances had been made for differences in parasite density, age, sex, area of residence, duration of symptoms, and prior antimalarial treatment between the disease groups.

The same trend was found for levels and prevalence of antibodies specific for the least variable of the MSP-1 block 2 types, the RO33 type (table 2). Children with CM had significantly higher mean levels of antibodies to the RO33 block 2 than children with UM ($P < .05$) or SMA ($P < .01$). In addition, a higher proportion of patients with CM than of patients with UM had antibodies to this antigen ($P < .05$). IgG levels and prevalence rates to the other 2 allelic forms of block 2, K1 and MAD20 types, were also higher in patients with CM than in those with SMA, but the difference was not significant (data not shown). For the patients with CM, we analyzed antibody levels in relation to outcome (death, neurological sequelae, or full recovery). High levels of antibodies to RO33 type MSP1 block 2 were significantly associated with full recovery ($P = .01$); none of the other IgG responses were associated with the outcome of CM.

From acute to convalescent phases, there was a significant decline in mean levels of antibody to the conserved C-terminal regions of MSP-1 in patients with either form of severe malaria ($P < .001$; paired *t* test) (figure 1) and in antibody responses to MSP-1 block 2 RO33 ($P = .05$) among patients with CM.

Table 2. Levels and prevalence rates of antibodies to *Plasmodium falciparum* merozoite antigens in acute malaria.

Type of malaria	MSP-1				MSP-2				AMA-1, full length				RAP-1, C2			
	19 kDa		42 kDa		Block 2 RO33		K1 17/14		T9/105 12/6		T9/96 13/14		AMA-1, full length		RAP-1, C2	
	Mean (95% CI)	% pos.	Mean (95% CI)	% pos.	Mean (95% CI)	% pos.	Mean (95% CI)	% pos.	Mean (95% CI)	% pos.	Mean (95% CI)	% pos.	Mean (95% CI)	% pos.	Mean (95% CI)	% pos.
CM (n = 126)	1.49 (1.34 to 1.64)	86.5	1.12 (0.99 to 1.25)	84.1	0.16 (0.11 to 0.21)	10.3	0.23 (0.15 to 0.31)	36.5	0.27 (0.19 to 0.36)	37.3	0.79 (0.67 to 0.91)	46.8	0.74 (0.60 to 0.88)	71.8	0.57 (0.49 to 0.66)	54.0
SMA (n = 59)	1.04 (0.83 to 1.25)	69.5	0.69 (0.50 to 0.88)	64.4	0.06 (-0.01 to 0.13)	1.7	0.03 (-0.08 to 0.1)	23.7	0.06 (-0.07 to 0.2)	22.0	0.60 (0.42 to 0.77)	22.0	0.45 (0.25 to 0.64)	42.9	0.52 (0.40 to 0.64)	49.1
UM (n = 84)	1.09 (0.92 to 1.26)	58.0	0.90 (0.75 to 1.06)	66.7	0.05 (-0.01 to 0.10)	3.7	0.10 (0.004 to 0.2)	28.4	0.17 (0.06 to 0.27)	24.7	0.71 (0.56 to 0.85)	29.6	0.55 (0.39 to 0.70)	52.5	0.41 (0.31 to 0.51)	35.8

NOTE. Recombinant antigens are described in the Methods section. Antibody levels are reported as least-squares means of corrected optical density values (95% confidence intervals), calculated by use of PROC GLM (SAS Institute). Prevalences of antibodies are reported as percentages of patients with detectable antibodies to each antigen. Significant differences between disease groups and *P* values are reported in the text. AMA, apical membrane antigen; CM, cerebral malaria; MSP, merozoite surface protein; pos., positive; RAP, rhoptry-associated protein; SMA, severe malarial anemia; UM, uncomplicated malaria.

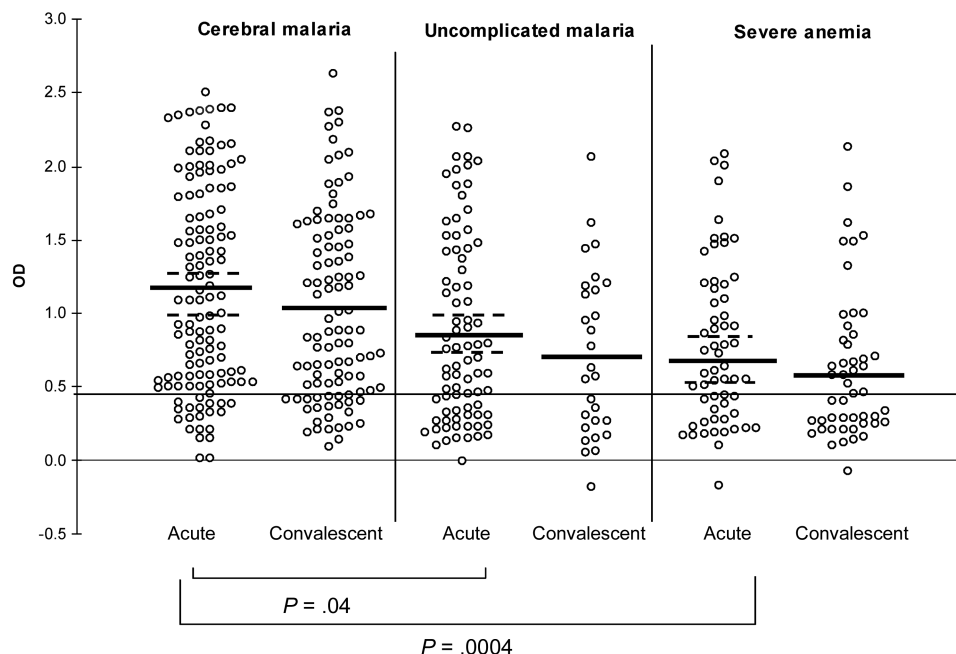


Figure 1. Antibody levels against MSP-1 (42 kDa). Dot plots show that mean antibody levels (optical density at 492 nm) in patients with cerebral malaria (126 acute and 100 convalescent) were higher than those in patients with uncomplicated malaria (84 acute and 29 convalescent) or severe malarial anemia (59 acute and 48 convalescent). The least-squares mean optical density value and 95% confidence interval for each group are indicated by a thick horizontal line and 2 dashed lines, respectively; P values were calculated by use of PROC GLM (SAS Institute). The cutoff level (OD of 0.45) for positive serum samples is indicated by a thin horizontal line.

Among patients with UM, there were no significant changes in antibody levels between presentation and convalescence, but numbers were small. Convalescent-phase samples were not obtained from all patients, and only results for pairs of acute- and convalescent-phase samples were included in these analyses.

Levels of antibody to MSP-2. Children who had SMA were distinguished by almost universally undetectable antibodies to the conserved C-terminal region of MSP-2 (K1 17/14 construct). Patients with SMA had significantly lower mean levels of antibodies to this region than did children with CM ($P < .05$) or UM ($P = .01$) (figure 2), after adjustment for confounders. The percentage of children with antibodies to K1 17/14 was significantly higher in patients with CM than in those with SMA ($P < .05$) (table 2). Consistently, antibody detectable with a protein containing a short part of the dimorphic region of the FC27 type, but mostly consisting of sequences from the conserved C-terminus, followed the same trend (protein T9/105 12/6) (table 2). Thus, adjusted antibody levels detected with the T9/105 12/6 protein were lower in patients with SMA than in those with UM ($P = .01$) or CM ($P < .01$). The prevalence of antibodies to this protein was also higher in patients with CM than in those with UM ($P < .05$) or SMA ($P = .02$) (table 2). A significantly higher proportion of patients with CM than of those with SMA had antibodies reactive with the IC1/3D7-type protein T9/96 13/14 ($P = .01$) (table 2); this difference was probably largely attributable to antibodies directed to the con-

served C-terminus of this protein. No significant differences were found in antibodies to a full-length FC27-type protein or to polymorphic regions of MSP-2 (data not shown).

Comparing acute- and convalescent-phase samples, there was a significant decrease in levels of antibody to all MSP-2 antigens examined in patients with CM (P values between .05 and .001; paired t test). No significant decline in anti-MSP-2 antibody levels was found in patients with SMA, who had the lowest levels at admission. In patients with UM, there was no significant decrease in IgG levels, except for IgG directed to the conserved C-terminus ($P < .01$) (figure 2).

Levels of antibody to AMA-1 and RAP-1. Consistent with the above findings, mean levels of antibodies to AMA-1 ectodomain were significantly higher in patients with CM than in those with SMA ($P < .05$), after adjustment for confounders (table 2). The prevalence rate of anti-AMA-1 antibody was also higher in CM than in SMA ($P < .05$), but that difference was not significant after adjustment for age. With regard to RAP-1, children with CM had higher anti-C2 levels and prevalence rates than did children with UM, but there was no significant difference with levels in children with SMA (table 2).

In convalescence, there was a significant decrease in anti-AMA-1 and anti-RAP-1 antibody levels in patients with CM ($P = .05$; paired t test) and a significant decrease in anti-RAP-1 antibodies in patients with SMA ($P < .001$). Antibody levels remained stable in patients with UM.

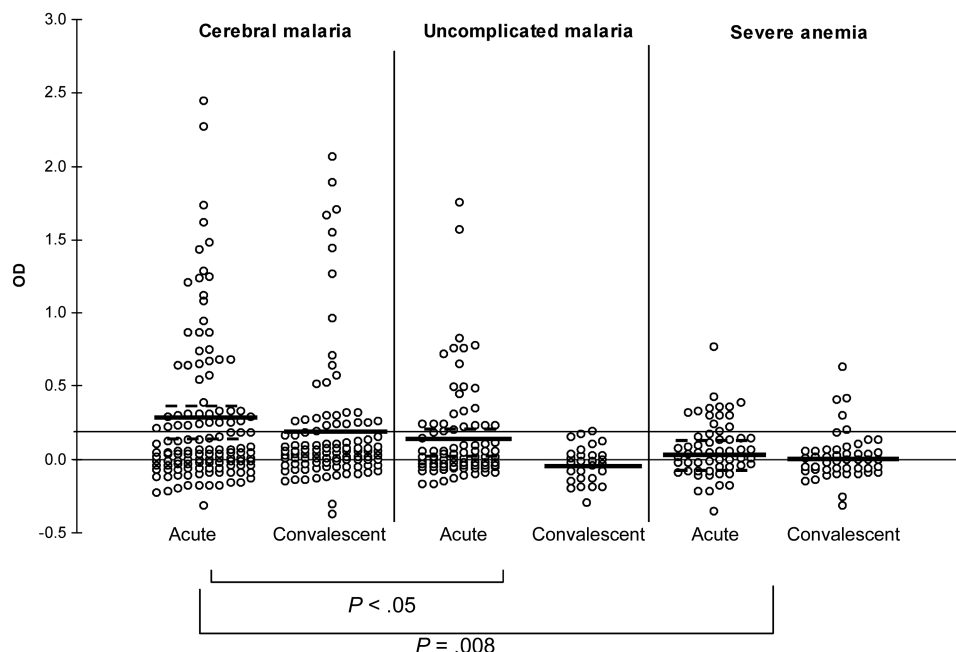


Figure 2. Antibody levels against MSP-2 K1 17/14 (conserved C-terminus). Dot plots show that mean antibody levels (optical density at 492 nm) in patients with cerebral malaria (126 acute and 100 convalescent) were higher than in patients with uncomplicated malaria (84 acute and 29 convalescent) or severe malarial anemia (59 acute and 48 convalescent). The least-squares mean optical density value and 95% confidence interval for each group are indicated by a thick horizontal line and 2 dashed lines, respectively; P values were calculated by PROC GLM (SAS Institute). The cutoff level (OD of 0.18) for positive serum samples is indicated by a thin horizontal line.

DISCUSSION

We investigated the relationship between malarial disease in pediatric patients in Malawi and levels of IgG antibodies specific for defined regions of the asexual merozoite antigens MSP-1, MSP-2, AMA-1, and RAP-1. Antibodies were measured at admission, at a point when *P. falciparum* infection has already made a child sick enough to come to the hospital. Limitations to such a study include a lack of knowledge about the participants' history of exposure to *P. falciparum* infection and uncertainty regarding the duration and progression of the current infection. We do not know whether antibody levels at admission reflect preexisting immune responses or whether they are a response to the current infection. Because these unknown factors could differ between the different disease groups, we have interpreted our results with caution.

We find that children suffering from different disease phenotypes are distinguished by different presenting patterns and concentrations of antibodies directed against various merozoite antigens tested. Children with CM are distinguished by higher antibody levels to all antigens tested. In particular, antibody levels against conserved rather than variable regions of these proteins are significantly higher in the CM group than in the UM group. In contrast, these antibody levels are lower in the SMA group than in the UM group. Children with SMA are characterized by lower IgG levels against relatively conserved regions of the merozoite proteins, whereas IgG levels against variable re-

gions appear not to differ between clinical syndromes. These results are compatible with studies in Thailand showing that patients with CM had higher mean ELISA titers than patients with UM, and a subgroup of patients with CM who had complications (e.g., anemia) had reduced antibody responses compared with patients with uncomplicated CM or UM [18].

Two general observations arise from these data. First, CM and SMA are characterized by different IgG antibody patterns, and thus it is not appropriate to pool them together, as most previous seroepidemiological studies have done [18, 19, 21–25, 27]. Second, there is a consistent general trend for antibody levels to be highest in children with CM, intermediate in children with UM, and low in children with SMA.

The differences in antibody patterns between clinical groups could reflect differences antedating the current infection or differences in response to it. We cannot exclude the possibility that levels are higher in CM because of a larger or more protracted antigenic stimulus with the current infection, although mean parasite densities in peripheral blood at admission were similar between CM and UM groups (table 1). For example, children in the CM group could harbor more sequestered parasites, and this difference could have contributed to their greater immune response.

Epidemiological studies indicate that in areas of high malaria transmission, antibody levels tend to increase with age and exposure as immunity is acquired [36–39], and, within a malaria-

endemic area, antibody levels vary in relation to malaria seasonality [38]. It has been proposed that immunity to severe malaria (particularly noncerebral) is acquired rapidly [40], after only 1 or 2 infections, and may depend on conserved antigens [41]. A possible confounder in our analyses could be differences in intensity of exposure to *P. falciparum* between patients with severe malaria and those with UM, related to area of residence. Children from urban areas (which included most patients with UM) could have had less exposure to malaria than rural children. When we controlled for area of residence as a surrogate for exposure in our analysis, this factor did not appear to alter significance. However, we cannot rule out differences in antibodies due to differences in prior exposure, because our population is quite mobile, frequently traveling between Blantyre and their traditional homes.

Epidemiological studies also suggest that the incidence rates of CM and SMA vary with age and depend on endemicity [42]. In African children, the peak incidence of CM occurs later in life than the peak of SMA [42], and this difference was seen in our study (table 1). However, mean IgG levels in children with CM remained significantly higher than levels in children with SMA, after adjustment for age.

Differential antibody patterns might also be related to differences in age at first exposure. Early *P. falciparum* infections, occurring when the immune system is immature, may result in less efficient IgG responses on subsequent boosts, as suggested by serological studies in the context of trials of intermittent preventive antimalarial treatment in infants (D. Quelhas [Barcelona Centre for International Health Research] and C.D., unpublished data). Conversely, malarial infections at a later age may induce a better priming of the immune system, which could translate into more efficient memory responses to subsequent infections. A difference of ~1 year in the age at first encounter with *P. falciparum* antigens might account for the different IgG levels observed at the time when SMA and CM diseases manifest. Again, the persisting differences between CM and SMA after adjustment for age makes this unlikely to be a sufficient explanation for the observed differences.

Clinical features of SMA are consistent with successive or chronic infections due to poorly developed antimalarial immunity. In light of published data and the information in table 1, it is plausible to presume that children with SMA have a more prolonged course of infection than do children with CM or UM. Studies in Gabon also found significantly lower levels of IgG antibodies to sporozoite and schizont antigens [22], to variant surface antigens [23], and to MSP-1₁₉ [24] in children with SMA than in those with UM. Taken together, these data suggest that the low antibody levels seen in children with SMA might be explained by some sort of immunological “tolerance” or antigen-specific B and T cell anergy [43]. Different regions of MSP-1 have previously been shown to elicit different types of antibody responses [44], and symptomatic malaria has been associated with impaired antibody responses to epitopes within MSP-1₁₉

but not to epitopes located elsewhere in the antigen [45]. This suggests the presence of different immunoregulatory mechanisms in clinical malaria controlling antibody responses in an epitope-specific manner.

Findings of other studies suggest that a degree of immunological sensitization by prior malarial infections could predispose an individual to developing CM. In Thai patients, previous UM infections were more frequent in subjects with CM than in those presenting with noncerebral forms of malaria [46]. Although in previous studies IgG responses to *P. falciparum* antigens did not differ significantly between patients with and without CM [18–20, 26], in many other studies blood levels of several humoral factors were found to be raised in patients with CM, compared with those in patients with other forms of falciparum malaria. Differences in antibody level may reflect differences in Th1/Th2 cytokine balance [47], which may be due to disease [48] or host genetics [49]. Thus, increased levels of specific antibodies in children presenting with CM may reflect a history of numerous infections that could be related to genetic factors, HIV status, or nutrition, or simply to environmental factors.

Antibody levels declined significantly from the acute to the convalescent phase, and this change was more pronounced in children with severe malaria than in those with UM; admission levels in patients with SMA were already very low. Previous studies suggest that IgG responses to blood-stage antigens tend to be short-lived [10, 13, 50], but the reasons for this finding remain speculative (e.g., severity may contribute to the shut off of plasma and/or memory B cells, or IgG catabolism may be increased, resulting in shortened antibody persistence). Given the short life of this antibody, the most plausible interpretation of the data is that the high IgG levels measured at admission in patients with CM are indeed predominantly reflective of the current infection rather than of preexisting antibodies.

To elucidate more fully the relationship between naturally acquired antibody responses to blood stages of *P. falciparum* infection and malarial disease, large prospective cohort studies should be performed, including detailed immunological analyses of IgG isotypes, fine specificity, inhibitory activity and affinity/avidity of antibodies, and assessment of cytokine responses, HIV and nutritional status, and host genetic factors. Immunological studies conducted in the context of randomized placebo-controlled trials of malaria intervention strategies that control exposure to *P. falciparum* infection at certain ages, or longitudinal newborn cohort studies in areas with demographic and morbidity surveillance systems, could help clarify the role that antibodies to these malaria-vaccine candidate antigens may have in the development of protective immunity to severe malaria.

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