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ANTIBODY RESPONSES TO REPETITIVE EPITOPES OF THE CIRCUMSPOROZOITE PROTEIN, LIVER STAGE ANTIGEN-1, AND MEROZOITE SURFACE PROTEIN-2 IN INFANTS RESIDING IN A *PLASMODIUM FALCIPARUM*–HYPERENDEMIC AREA OF WESTERN KENYA. XIII. ASEMBO BAY COHORT PROJECT

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Abstract. The present study was initiated to characterize antibody responses to repetitive epitopes of the circumsporozoite protein (CSP), liver stage antigen-1 (LSA-1), and merozoite surface protein-2 (MSP-2) of *Plasmodium falciparum* in infants residing in a *P. falciparum*–hyperendemic area of western Kenya. In this study, development and maintenance of these antibody responses in 28 infants were studied longitudinally by use of monthly serum samples collected from birth to age 1 year. Mother plasma and infant umbilical cord plasma were also tested to assess the transplacental transfer of maternal antibodies. Results showed that antibodies passively transferred from mothers were detectable for CSP, LSA-1, and MSP-2 repeat epitopes. Infants were able to mount and maintain a strong antibody response against LSA-1 in their first year of life. Infants often responded to CSP repeats, but with a much lower antibody titer. Antibody responses in infants against Fc27 and 3D7 repeats of MSP-2 were low throughout their first year. In addition, 51 infants whose first detected infection occurred at $>$ 4 months of age were selected to determine antibody responses to the antigens tested upon their first and second detected infections. Antibody responses to LSA-1 and, to a lesser degree, CSP increased in positivity rates and titer upon second infection. Antibody responses to Fc27-type and 3D7-type repeats of MSP-2 were low upon both infections. There was no association between maternally transferred anti–LSA-1, anti-CSP, or anti–MSP-2 antibodies and an infant's first detected infection. No significant correlation was found between an infant's antibody responses to the 4 antigen repetitive epitopes and protection against malarial parasitemia during the first year of life.

INTRODUCTION

Plasmodium falciparum is a major cause of morbidity and mortality in children in tropical countries. Protection of the children born to immune mothers has been attributed to various mechanisms, but mainly to passively transferred specific antibodies.[1–4] Infants as young as 4 months, however, are at high risk of clinical attack. The high rate of clinical diseases in children in comparison with adults might be because of their history of lower exposure to malaria antigens, poor ability to mount immune responses, or other age-related factors.[5–7] The mechanisms involved in the development of naturally acquired immunity against malaria parasites are still not clear. The identification of parasite antigens and epitopes that induce protective antibody responses, and the elucidation of immune responses to malaria parasites in infants would be important steps toward the understanding of naturally acquired immunity to malaria.

The presence of extensive repetitive regions is a feature of many *P. falciparum* proteins. Antibody responses induced by parasites infection are in large part directed against these repetitive epitopes.[8,9] Antibody responses to T-cell–independent antigens are usually mounted against epitopes with a highly repetitious structure, which contributes to the immunodominance of repetitive epitopes.[10–12] Many studies have identified the presence of B-cell epitopes in the repeats of circumsporozoite protein (CSP), liver stage antigen-1 (LSA-1), and merozoite surface protein-2 (MSP-2) of *P. falciparum.*[13–16] These antigens are also vaccine candidate antigens against sporozoite, liver, and blood stage parasites, respectively. *In vitro* studies have revealed that antibodies directed against the CSP repeats can inhibit sporozoite invasion into cultured liver cells and administration of such antibodies can completely protect mice and monkeys against sporozoite-induced malaria.[17–19] There is also *in vivo* evidence suggesting the presence of protective antibodies induced by the antigen repeats. Antibody responses to CSP repeats of *P. vivax* are protective in Saimiri monkeys.[20,21] Mice immunized with a peptide (EQQSDLEQERLAKEKLQ) from LSA-1 repeat of *P. falciparum* are protected against challenge with *P. berghei* sporozoites.[22] Monoclonal antibody against the epitope of Ser-Thr-Asn-Ser (STNS) from the MSP-2 Fc27 repeat region (ADTIASGSQRSTNSASTSTTNNGESQTTTPTA) inhibit the growth of the asexual blood stages of *P. falciparum in vitro.*[23,24]

The repetitive epitopes, on the other hand, may play a role in immunosuppression. It has been suggested that they serve as a smokescreen by interfering with the maturation of an effective immune response to the nonrepetitive region of the antigens.[25] This suggestion has led to the hypothesis that the repetitive antigens are not protective, and that the immune response to the repetitive antigens interferes with the development of immune responses to other antigens.[26,27] Immunoepidemiologic studies of repetitive antigens have yielded contradictory results. For instance, in the case of the CSP, some studies have shown that the parasite rate was significantly higher in anti-Asn-Ala-Asn-Pro $(NANP)_6$ seropositive children, which reflected a recent exposure to malaria.[5,28] Conversely, other studies suggest that the CSP is poorly immunogenic in children because anti-$(NANP)_{50}$ titer remains very low throughout the first year of life.[29]

The purpose of the present study was to characterize antibody responses to CSP, LSA-1, and MSP-2 repetitive epitopes and to investigate the development and maintenance of these responses in naturally exposed young children. These data could be useful in the understanding of naturally acquired immunity to malaria and in the development of a vaccine against malaria.

MATERIALS AND METHODS

Study design and participants. This study was part of a prospective malaria project, the Asembo Bay cohort project, in a rural region of western Kenya hyperendemic for malaria. Malaria transmission was high throughout the year (2–10 infected bites per person per month), with the highest transmission in May–July. In the cohort study, pregnant women were enrolled in their last trimester of pregnancy. The pregnant women, and then the women and their delivered infants, were prospectively followed every other week. Blood was collected every month, or more often if illness was detected or reported. The blood was examined by microscopy for *Plasmodium* parasites and sera were stored at −70°C. If a participant had asexual parasites > 5,000/μL or a temperature ≥ 37.5°C with any parasitemia, they were treated with sulfadoxine-pyrimethamine. The term ''detected parasitemia'' refers to any slide positivity for falciparum parasites in microscopy.

From the cohort of mothers and children, we selected participants for this study in 2 ways. First, to study the antibody response dynamics of infants during their first year, we selected 28 infants who were followed monthly from birth to age 1 year. Second, to determine if children could respond to the antigenic determinants tested upon their first and second detected infection, we selected 51 infants whose first detected infection occurred at ≥ 4 months of age. Children < 4 months of age were excluded to minimize the detection of maternal antibodies. To control for malaria transmission, we only studied children born during January–March 1993. Because the children were followed at least every other week, and because treatments were documented, we could use the detection of asexual parasites by microscopy as an indication of an infection that resulted in asexual parasitemia. The seroprevalence of the repetitive epitopes of CSP, LSA-1, and MSP-2 in the general population in the study area was examined by use of serum samples from 30 volunteers aged 17–60 years.

Peptides. A 20-mer peptide containing 5 copies of tandem repeating domain (NANP) of the CSP, a 34-mer peptide containing 2 copies of tandem repeating domain (AKEKLQEQQSDLEQERL) of LSA-1, a 32-mer peptide (ADTIASGSQRSTNSASTSTTNNGESQTTTPTA) representing one copy of 32 amino acid repeat of MSP-2 FC27, and a 20-mer peptide (GAGGTAGGSAGGSAGGSAGG) containing 3 copies of MSP-2 3D7-type repeat were synthesized at the Biotechnology Core Facility, the National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia.

Enzyme-linked immunosorbent assay (ELISA). Antibodies to CSP, LSA-1, and MSP-2 repeats were tested via ELISA. The tests were performed in flat-bottom microtiter plates (Dynex Technologies, Chantilly, VA). Synthetic peptides were coated to the well surface by incubating 100 μL of individual peptide (10 μg/mL in 0.01 M phosphate-buffered saline [PBS] pH 7.2) overnight at 4°C. The plates were then washed 4 times with PBS plus 0.05% Tween-20 (PBS-Tween) and blocked at 37°C for 1 hr with 5% nonfat milk in PBS. Plasma samples were initially tested for the presence or absence of anti-repeat antibodies at dilution of 1:100. The plasma samples that were positive for CSP, LSA-1, or MSP-2 repeats were titrated subsequently with 2-fold serial dilutions up to 1:20,4800. After incubation at 37°C for 1 hr, unbound material was washed away with PBS-Tween. Horseradish peroxidase–conjugated goat anti-human immunoglobulin (Ig) G (Fisher Scientific, Pittsburgh, PA) diluted 1:6000 was added to each well. After incubation for 1 hr at 37°C, the wells were washed, and 150 μL of the peroxidase substrate, 3,3,′5,5,′- tetramethylbenzidine (Kirkegaad and Perry Laboratories, Gaithersburg, MD) was added to each well. Fifteen minutes later, the reaction was stopped by adding 50 μL of 1 M phosphoric acid. The plates were read at an absorbency of 450 nm with an ELISA reader (Molecular Devices, Sunnyvale, CA). Cutoff points were set at mean optical density value plus 3 times the standard deviation generated with plasma from 15 people from North America with no history of exposure to malaria.

Data analysis. Statistical analysis was conducted with SAS software (SAS Institute, Cary, NC). All data were normalized by logarithmic transformation before the analysis. Spearman's correlation test was used to assess the relationship between antibody level and parasitemia. Wilcoxon matched pairs signed-rank (mWPR) test was used to compare antibody levels between first and second infections. McNemar's test or Fisher's exact test were used to compare all proportions or rates of antibody responders; $P \leq 0.05$ or mWPR $Z \leq 0.05$ was considered statistically significant.

RESULTS

Antibody responses against the repetitive epitopes of CSP, LSA-1, and MSP-2 in the general population of the study area. To test the validity of the use of repetitive epitopes in examining immune response to CSP, LSA-1, and MSP-2, antibody responses to overlapping peptides from these antigens were assessed in 30 people living in this study area. The prevalence of IgG antibody was 90% for CSP repeat, 90% for LSA-1 repeat, 80% for MSP-2 Fc27, and 93% for MSP-2 3D7 repeats.

Maternal antibodies against the repetitive epitopes of CSP, LSA-1, and MSP-2. In this study, mother's plasma was available for only 14 and cord plasma available for only 20 of 28 infants used to characterize longitudinal antibody responses. Mothers had strong antibody responses to CSP, LSA-1, and MSP-2 3D7 repetitive epitopes, with highest responses to CSP repeat and lowest responses to MSP-2 Fc27 repeat (Table 1). There was no positive or negative association in maternal antibody responses to one antigen with another ($P > 0.05$). A high level of transplacental transfer of maternal anti-CSP, LSA-1, and MSP-2 antibodies occurred in these mothers as judged by the prevalence and titer of antibodies in cord blood. The titer of antibodies to LSA-1– and 3D7-type MSP-2 in cord blood were strongly correlated to the titer of antibodies in the peripheral blood of mothers ($P = 0.002$ and $P = 0.0001$, respectively), whereas antibody titer to CSP and Fc27-type MSP-2 were not significantly correlated ($P = 0.058$ and $P = 0.10$, respectively). Passively transferred maternal antibodies waned quickly in infants at 1 month, as evidenced by a stepwise decline in antibody titer to 0 in successive weeks when the infant's first infection occurred after age 3 months. This was especially notable for MSP-2 repeats (geometric mean titer, 271.1–

TABLE 1
Passivie Transfer and de novo production of IgG antibodies against repetitive epitopes of 3. *P. falciparum* antigens

Phases	CSP	LSA-1	MSP-2 Fc27	MSP-2 3D7
Mother plasma				
Positive (%)	14/14 (100)	14/14 (100)	12/14 (85.7)	13/14 (93)
Titer (geomean)	4306.9	2498.3	915.0	1889.2
(95% CI)	(2184.7–8490.6)	(754.9–8267.2)	(173.0–4838.0)	(460.5–7750.6)
Cord blood				
Positive (%)	19/20 (100)	18/20 (90)	15/20 (75)	18/20 (90)
Titer (geomean)	3675.8	848.9	271.1	1242.9
(95% CI)	(1836.8–7358.9)	(242.7–2969.7)	(55.7–1319.1)	(350.5–4407.6)
At 1 month of Age				
Positive (%)	22/22 (100)	17/22 (77)	15/22 (68)	15/22 (68)
Titer (geomean)	854.1	248.1	126.8	76.6
(95% CI)	(476.2–1531.6)	(59.6–1033.7)	(27.6–582.8)	(20.5–286.0)
At 1st infection				
Positive (%)	29/45 (64)	33/49 (67)	16/45 (35.5)	11/25 (40)
Titer (geomean)	38.4	123.3	8.6	12.5
(95% CI)	(16.7–88.4)	(43.4–350.6)	(3.5–20.8)	(3.9–40.7)
At 2nd infection				
Positive (%)	29/40 (73)	41/44 (93)	14/40 (35)	8/20 (40)
Titer (geomean)	69.6	684.0	8.9	11.8
(95% CI)	(29.5–164.3)	(330.6–1415.4)	(3.4–23.3)	(2.8–50.2)

126.8 for Fc27 type and 1242.9–76.6 for 3D7 type; see Table 1).

Antibody responses of infants at first and second infections against the repetitive epitopes of CSP, LSA-1, and MSP-2. We selected 51 infants whose first detected infection occurred after 4 months of age to test antibody responses at the first and second infection. Table 1 shows the geometric mean antibody titer and positivity rates for the 4 antigen epitopes. At the first infection, the positivity rate of anti-CSP repeat antibodies was 64%, which was higher than the positivity rate for either anti–MSP-2 Fc27 type (35.5%, $P = 0.011$) or 3D7 type (40%, $P = 0.16$) The positivity rate of anti–LSA-1 repeat antibodies (67%, $P = 0.004$) was also higher than the positivity rate for either anti–MSP-2 Fc27 or 3D7 ($P = 0.09$), but not significantly different from the anti-CSP repeat antibody response ($P = 0.93$). The geometric mean titer of antibodies to CSP and MSP-2 repeats was low, whereas the titer to LSA-1 was moderate (Table 1). Compared with the antibody response at the first detected infection, there was a significant increase in anti–LSA-1 repeat antibody response at the second infection for both positivity (67% versus 93%, $P = 0.003$) and titer (123.3 versus 684.0, mWPR $Z = 0.006$). Antibody response to CSP increased slightly in both positivity rate and titer at second infection. Antibody responses to Fc27-type and 3D7-type repeats of MSP-2 were low at the first and second infections. There was also no obvious difference between antibody responses against the peptides representing 2 allele families of MSP-2 at the first and second infections.

Antibody responses throughout the first year of life. Antibody responses against CSP, LSA-1, and MSP-2 Fc27 repetitive epitopes were tested monthly in 28 infants throughout their first year of life. Positivity rate of antibodies against CSP and LSA-1 repetitive epitopes were high (> 60%) during the first year of life. In contrast, positivity rates of antibodies against MSP-2 Fc27 repeat decreased steadily during the first 6 months. Afterward, anti–MSP-2 Fc27 antibody prevalence remained low (Figure 1). Figure 2 summarizes antibody titer by antigen epitope group and the parasite density by month of

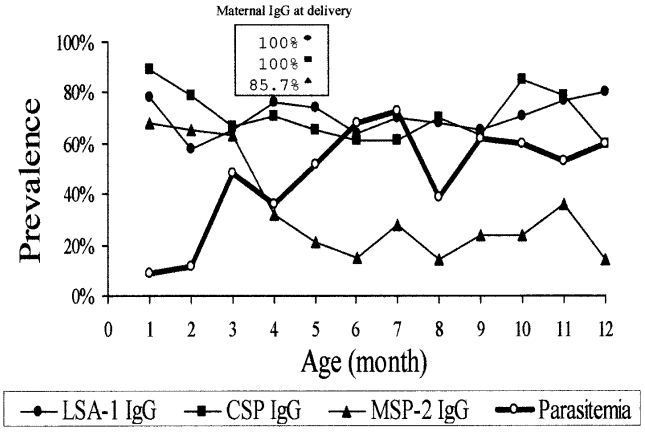

FIGURE 1. Positivity rates of IgG against repetitive epitopes of CSP, LSA-1, and MSP-2 Fc27 in infants during the first year of life.

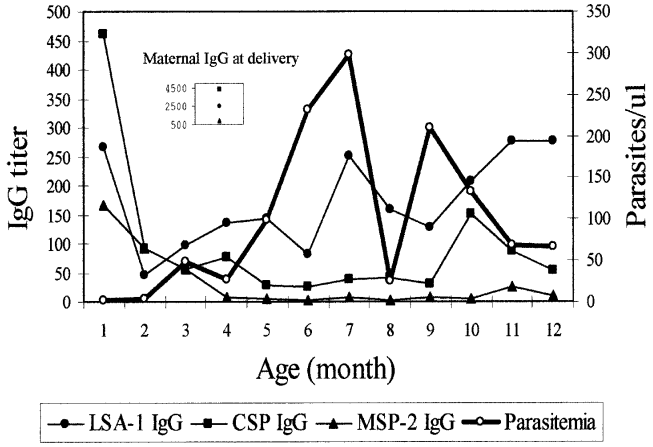

FIGURE 2. Geometric mean titer of IgG against repetitive epitopes of CSP, LSA-1, and MSP-2 Fc27 in infants during the first year of life.

TABLE 2

Percent of antibody responses of the infants followed throughout their first year of life

Antibody response	CSP	LSA-1	MSP-2 Fc27	MSP-2 3D7
Total of infants	28	28	28	19
POS, constant (%)	7 (25)	19 (68)	7 (25)	4 (21)
No. with <2 Parasitemias	4	8	3	1
LOW, constant (%)	14 (50)	4 (14)	1 (4)	7 (37)
No. with <2 Parasitemias	4	0	1	1
SL, short-lived (%)	3 (11)	1 (4)	5 (18)	ND
No. with <2 Parasitemias	0	1	0	0
NEG (%)	4 (14)	4 (14)	15 (54)	8 (42)
No. with <2 Parasitemias	4	3	8	5

Children's antibody response histories were summarized as being: POS (consistently positive following 1–2 detected parasitemias), LOW (consistently positive with an antibody titer ≤400), SL (short-lived antibody responses temporal with parasitemia), NEG (consistently negative). The number of infants in each category having less than 1–2 parasitemias detected in their first is noted.

age. Titer of IgG to LSA-1 repeats increased with the age of infants, whereas the titer of IgG to CSP and MSP-2 repeats remained low during the first year of life.

To further characterize the antibody responses in an infant's first year of life in the context of parasitemia, we categorized each child's antibody responses for each of the antigens as one of the following: (1) positive (consistently positive, with antibody titer reaching > 400 after 1–2 detected infections); (2) low (consistently positive with antibody titer never reaching > 400 after 1–2 detected infections); (3) short-lived (periods of a positive antibody response followed by a negative antibody response throughout the first year of life); or (4) negative (consistently negative). In addition, the number of distinct parasitemia episodes were estimated: if multiple parasitemias were interspersed with periods of aparasitemia, the term ''intermittent parasitemia'' was used; if parasitemia was detected throughout the 2–4 weekly follow-up sessions, the term ''constant parasitemia'' was used.

Table 2 shows the percentage of infants in each antibody response category for each of the antigens. Infants could mount consistently high antibody responses to LSA-1 repeats. Infants mounted consistent antibody responses to CSP repeats; however, their antibody titer was low. Approximately half (42–54%) of the infants did not have antibody responses to either allelic form of the MSP-2 repeat peptides, and several of the infants that did respond to the MSP-2 repeats had short-lived or low antibody responses. Exposure was important in the development of antibody responses, as evidenced by the fact that 4 of the infants who were found to be negative to all antigens tested had < 2 detected parasitemias. However, only 1–2 detected parasitemias could be sufficient to cause a significant, long-lasting antibody response against LSA-1 because 8 of 19 constant responders had ≤ 2 detected parasitemias (Table 2).

Correlation between the antibody responses. We investigated whether there was a correlation between the antibody responses against all 4 repeats detected in each sample of infants. The antibody titer to the different antigens was not correlated ($P > 0.05$). However, there were associations between the antibody positivity rates of each of the antigens. When considering all of the samples, including samples that had maternal antibody present, there was a positive association between an antibody response to the MSP-2 Fc27 antigen and the MSP-2 3D7 antigen. However, after excluding sample points that contained maternally transferred antibody, there was no association between antibody positivity to the 2 different MSP-2 alleles: 45% were negative to both alleles, 25% were positive to Fc27, 12% were positive to 3D7, and 17% were positive to both alleles. Similarly, the anti–LSA-1 and anti-CSP responses were not correlated ($P > 0.05$). Excluding time periods that included the maternally transferred antibody, a sample was positive to both LSA-1 and CSP 57% of the time, 13% of the sampled points were negative to both, 15% were positive to only LSA-1, and 15% were positive to only CSP.

Association with parasitemia. We determined the presence or absence of an association between either the maternal antibodies at the time of delivery and the first detected infection, or the infants' antibodies at the time of first detected infection and the level of asexual parasitemia. There was no correlation between the maternal antibody level and time of the first infection ($P > 0.05$ for each antigen tested). In 17 of the 28 children, the first detected parasitemia occurred at ≤ 3 months, a period when maternal antibody might be present. In 11 children, the level of antibodies transferred went below detectability before the child's first detected parasitemia. The day in which infant's maternal antibody appeared to wane was compared with time of first detected infection. Only 47, 35, 53, and 59% of the infant's maternal anti–LSA-1, anti-CSP, anti–MSP-2 Fc27, and anti–MSP-2 3D7 antibody waned, respectively, before the infant's first detected infection. These frequencies are not significantly different than what would be expected by chance (50%) ($P > 0.05$). This further confirmed that there was no association between maternally transferred LSA-1, CSP, or MSP-2 antibody and the infant's first detected asexual parasitemia. In addition, no significant correlation was found between the infant's antibody responses to any of the 3 antigen repeats and density of first detected asexual parasitemia ($P > 0.05$).

DISCUSSION

Results of this longitudinal study indicated that antibody responses to the different antigen repetitive epitopes in infants evolved differently during the first year of life. After a drop in levels of anti–LSA-1 antibody at the first month, the IgG titer remained low until the age of 4 months, suggesting a waning of maternally acquired antibodies. From the fourth month on, IgG titer increased until the 11th month. Exposure was important in the development of antibody responses, but only 1–2 detected parasitemias were sufficient to cause a significant, long-lasting antibody response to the LSA-1 repeat. During the children's first year of life, positivity rate to CSP repeat was as high as to LSA-1, but the antibody titer was low. Antibody positivity rates and titer to MSP-2 Fc27 repeats were at very low level throughout the first year of life. Thus, infants can mount a better antibody response to LSA-1 repeats and maintain this response at high levels than to CSP and MSP-2 repeats.

The difference in immune responses to these antigens may be due to the different structure of repetitive epitopes. Another possible explanation is that anti-(NANP)n antibodies are unstable and short-lived.[29,30] We also found that infants

who responded to MSP-2 had short-lived or low antibody responses. Apparently, a long period of exposure (> 1 year) to malaria is required before a stable, significant anti-CSP and anti–MSP-2 response occurs. Taken together, immune responses to distinct antigens of *P. falciparum* may evolve differently and may be differentially regulated.

IgG against (NANP)n has been used as a marker for exposure to sporozoites.[31,32] In the present study, antibody responses to $(NANP)_5$ remained low during the first year of life in infants despite repeated exposure to malaria infection. In contrast, antibody response to LSA-1 repeat increased dramatically after the first infection. Although the antibody response to CSP and MSP-2 repeats decreased sharply during an intermittent period of aparasitemia, the antibody response to the LSA-1 repeat remained high after the first infection. The immune response to LSA-1 showed high IgG prevalence rate and gradual increase in antibody titer during the first year of life, indicating antibody responses to LSA-1 required limited exposure and persisted over time. It seems that this antibody response is a better marker of first exposure than protection.

Results of the present study also suggest that the maternal antibodies to CSP, LSA-1, and MSP-2 repeats were not associated with protection. IgG antibodies passively transferred from mothers were detectable for CSP, LSA-1, and MSP-2 repetitive epitopes. IgG titer, however, declined during the first few months from the relatively high values, indicating a fast waning of maternal antibodies against those repetitive epitopes. We did not find a correlation between levels of maternal antibodies and time of first infection for any of the antigen repeats. Maternal antibodies transferred across the placenta are considered the main contributor for protection against malaria in very young infants in highly endemic areas.[1,2,4,7,33,34]

Our previous study showed that maternal and infant antimerozoite surface protein-1 (MSP-1) 19kDa antibodies are associated with protection against malaria infection in the same population.[35] In the present study, antibody responses to the tested antigenic determinants were not associated with protection. This further indicated that the immune responses to conserved epitopes and repetitive epitopes might be differentially regulated. It is also possible that the high level of maternal antibodies against CSP, LSA-1, and MSP-2 repeats might be protective for the newborn, but because maternal antibodies waned quickly, a positive correlation between antibody titer and protection against the first infection could not be detected. The protective mechanism of passive immunity against malaria in infants is not well known. Some previous studies have shown conflicting results on the protective role of maternally transferred antibodies and many other factors may also involve in resistance to malaria in infants.[36]

Several previous studies failed to show a protection against malaria by natural or vaccine-induced antibodies against CSP repeats either because of lack of positive antibody or because of lack of protective function of antibodies.[37–40] In this study, antibodies to CSP and MSP-2 repeats remained at very low levels through the first year of life, preventing the assessment of protective effect of these antibodies. After the waning of maternal antibodies, the titer of IgG antibody to LSA-1 repeat increased with age of infants, but no significant correlation was found between the level of antibodies and levels of malaria parasitemia. A negative association between antibody response to MSP-2 nonrepeat regions and risk of malarial fever and anemia was observed in a seroepidemiologic study in older children and adults of Papua New Guinea, suggesting a possible protective role for anti-MSP-2 antibodies in natural infection.[41] However, the protective role of antibodies against the repeat regions of MSP-2 has not been documented and was not found in this study. For the nonrepeat regions of other malaria antigens, an infant's antibody responses to MSP-1 19-kDa antigen have been associated with protection in this study population.[35]

The present study was an initial step in the characterization of the pattern of antibody responses to malaria repetitive epitopes in infants who live in hyperendemic areas in western Kenya. It provides useful profiles on antibody response to CSP, LSA-1, and MSP-2 in the population that may be useful in monitoring the effects of a malaria vaccine in the future.

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REFERENCES

1. Cohen S, McGregor IA, Carrington SC, 1961. Gamma-globulin and acquired immunity to malaria. *Nature 192:* 733–734.
2. Sehgal VM, Siddiqui WA, Alpers MP, 1989. A seroepidemiological study to evaluate the role of passive maternal immunity to malaria in infants. *Trans R Soc Trop Med Hyg 83:* 105–106.
3. Brabin B, 1990. An analysis of malaria parasite rates in infants: 40 years after MacDonald. *Trop Dis Bull 87:* 1–21.
4. Chizzolini C, Trottein F, Bernard FX, 1991. Isotopic analysis, antigen specificity, and inhibitory function of maternally transmitted *Plasmodium falciparum*–specific antibodies in Gabonese newborns. *Am J Trop Med Hyg 45:* 57–64.
5. Kitua AY, Smith T, Alonso PL, 1996. *Plasmodium falciparum* malaria in the first year pf life in an area of intense and perennial transmission. *Trop Med Int Health 1:* 475–485.
6. Baird JK, Purnomo HB, Bangs MJ, 1993. Age-specific prevalence of *Plasmodium falciparum* among six populations with limited histories of exposure to endemic malaria. *Am J Trop Med Hyg 49:* 707–719.
7. Snow RW, Nahlen B, Palmer A, Donnelly CA, Gupta S, Marsh K, 1998. Risk of severe malaria among African infants: direct evidence of clinical protection during early infancy. *J Infect Dis 177:* 819–822.
8. Zavala F, Masuda A, Graves PM, Nussenzweig V, Nussenzweig RS, 1985. Ubiquity of the repetitive epitope of the CS protein in different isolates of human malaria parasites. *J Immunol 135:* 2790–2793.
9. Hollingdale MR, Nardin EH, Tharavany S, 1984. Inhibition of entry of *Plasmodium falciparum* and *P. vivax* sporozoites into cultured cells, an in vitro assay of protective antibodies. *J Immunol 132:* 909–913.
10. Dintzis HM, Dintzis RZ, Vogelstein B, 1976. Molecular determinants of immunogenicity: the immune on model of immune response. *Proc Natl Acad Sci U S A 73:* 3671–3675.

11. Mond JJ, Lees A, Snapper C, 1995. T cell–independent antigens type 2. *Ann Rev Immunol 13:* 655–692.
12. DeFranco AL, 1999. B-lymphocyte activation. Paul WE, ed. *Fundamental Immunology.* Lippincott-Raven, 225–261.
13. Kaur P, Sharma P, Kumar A, Chauhan VS, 1990. Synthetic, immunological and structural studies on repeat unit peptides of *Plasmodium falciparum* antigens. *Int J Pept Protein Res 36:* 515–521.
14. Fidock DA, Gras-Masse H, Lepers J-P, Brahimi K, Benmohamed L, Mellouk S, Uerin-Marchand C, Londono A, Raharimalala L, Meis JFGM, Langsley G, Roussilhon C, Tartar A, Druilhe P, 1994. *Plasmodium falciparum* liver stage antigen-1 is well conserved and contains potent B and T cell determinants. *J Immunol 153:* 190–205.
15. Taylor RR, Smith DB, Robinson VJ, McBride JS, Riley EM, 1995. Human antibody response to *Plasmodium falciparum* merozoite surface protein 2 is serogroup specific and predominantly of the IgG3 subclass. *Infect Immun 63:* 4382–4388.
16. Ranford-Cartwright LC, Taylor RR, Asgari-Jirhandeh N, Smith DB, Roberts PE, Robinson VJ, Babiker HA, Riley EM, Walliker D, McBride JS, 1996. Differential antibody recognition of FC27-like *Plasmodium falciparum* merozoite surface protein MSP2 antigens which lack 12 amino acid repeats. *Parasite Immunol 18:* 411–420.
17. Mazier DS, Mellouk RJ, Beaudoin RL, Texier B, Druilhe P, Hockmeyer J, Trosper J, Paul C, Charoenvit Y, Young J, Miltgen F, Chedid L, Chigot JP, Galley B, Brandicourt O, Genttilini M, 1986. Effect of antibodies to recombinant and synthetic peptides on *P. falciparum* sporozoites in vitro. *Science 231:* 156–159.
18. Egan JE, Weber JL, Ballou WR, Hollingdale MR, Majarian WR, Gordon DM, Maloy WL, Hoffman SL, Wirtz RA, Schneider I, Woollett GR, Young JF, Hockmeyer WT, 1987. Efficacy of murine malaria sporozoite vaccines: implications for human vaccine development. *Science 236:* 453–456.
19. Charoenvit Y, Mellouk S, Cole C, Bechara R, Leef MF, Sedegah M, Yuan LF, Robey FA, Beaudoin RL, Hoffman SL, 1991. Monoclonal, but not polyclonal, antibodies, protect against *Plasmodium yoelii* sporozoites. *J Immunol 146:* 1020–1025.
20. Yang C, Collins WE, Xiao L, Saekhou AM, Reed RB, Nelson CO, Hunter RL, Jue DL, Fang S, Wohlhueter RM, Udhayakumar V, Lal AA, 1997. Induction of protective antibodies in Saimiri monkeys by immunization with a multiple antigen construct (MAC) containing the *Plasmodium vivax* circumsporozoite protein repeat region and a universal T helper epitope of tetanus toxin. *Vaccine 15:* 377–386.
21. Collins WE, Sullivan JS, Morris CL, Galland GG, Jue DL, Fang S, Wohlhueter R, Reed RC, Yang C, Hunter RL, Lal AA, 1997. Protective immunity induced in squirrel monkeys with a multiple antigen construct against the circumsporozoite protein of *Plasmodium vivax. Am J Trop Med Hyg 56:* 200–210.
22. Hollingdale MR, Aikawa M, Atkinson CT, Ballou WR, Chen G, Li J, Meis JFGM, Sina B, Wright C, Zhu J, 1990. Non-CS preerythrocytic protective antigens. *Immunol Lett 25:* 71–76.
23. Ramasamy R, Jones G, Lord R, 1990. Characterization of an inhibitory monoclonal antibody-defined epitope on a malaria vaccine candidate antigen. *Immun Lett 23:* 305–310.
24. Epping RJ, Goldstone SD, Ingram LT, Upcroft JA, Ramasamy R, Cooper JA, Bushell GR, Geysen HM, 1999. An epitope recognised by inhibitory monoclonal antibodies that react with a 51 kilodalton merozoite surface antigen in *Plasmodium falciparum. Mol Biochem Parasitol 28:* 1–10.
25. Anders RF, 1986. Multiple cross-reactivities amongst antigens of *Plasmodium falciparum* impair the development of protective immunity against malaria. *Parasite Immunol 8:* 529–539.
26. Schofield L, 1991. On the function of repetitive domains in protein antigens of *Plasmodium* and other eukaryotic parasites. *Parasitol Today 7:* 99–105.
27. Ramasamy R, 1998. Molecular basis for evasion of host immunity and pathogenesis in malaria. *Biochim Biophys Acta 1406:* 10–27.
28. Hough B, 1996. Clinical and parasitological studies on immunity to *Plasmodium falciparum* malaria in children. *Scand J Infect Dis 102(Suppl):* 1–53.
29. Kitua AY, Urassa H, Wechsler M, Smith T, Vounatsou P, Weiss NA, Alonso PL, Tanner M, 1999. Antibodies against *Plasmodium falciparum* vaccine candidates in infants in an area of intense and perennial transmission: relationships with clinical malaria and with entomologic inoculation rates. *Parasite Immunol 21:* 307–317.
30. Marsh K, Hayes RH, Carson DC, Otoo L, Shenton F, Byass P, Zavala F, Greenwood BM, 1988. Anti-sporozoite antibodies and immunity to malaria in a rural Gambian. *Trans R Soc Trop Med Hyg 82:* 532–537.
31. Del Guidice G, Engers HD, Tougne C, 1987. Antibodies to the repetitive epitope of *Plasmodium falciparum* circumsporozoite protein in a rural Tanzanian community: a longitudinal study of 132 children. *Am J Trop Med Hyg 36:* 203–212.
32. Kilombero Malaria Project, 1992. The level of anti-sporozoite antibodies in a highly endemic malaria area and its relationship with exposure to mosquitoes. *Trans R Soc Trop Med Hyg 86:* 499–504.
33. Edozian JC, Gilles HM, Udeozo IOK, 1962. Adult and cord blood gammaglobulin and immunity to malaria in Nigerians. *Lancet ii:* 951–955.
34. Desowitz RS, Elm J, Alpers MP, 1993. *Plasmodium falciparum* specific immunoglobulin G (IgG) IgM, and IgE antibodies in paired maternal-cord sera from East Sepik Province, Papua New Guinea. *Infect Immun 61:* 988–993.
35. Branch OH, Udhayakumar V, Hightower AW, Oloo AJ, Hawley WA, Nahlen BL, Bloland PB, Kaslow DC, Lal AA, 1998. A longitudinal investigation of IgG and IgM antibody responses to the merozoite surface protein-1 19-kilodalton domain of *Plasmodium falciparum* in pregnant woman and infants: associations with febrile illness, parasitemia, and anemia. *Am J Trop Med Hyg 58:* 211–219.
36. Riley EM, Wagner GE, Akanmori BD, Koram KA, 2001. Do maternally acquired antibodies protect infants from malaria infection? *Parasite Immunol 23:* 51–59.
37. Kaur P, Sharma P, Kumar A, Chauhan VS, 1990. Synthetic, immunological and structural studies on repeat unit peptides of *Plasmodium falciparum* antigens. *Int J Pept Protein Res 36:* 515–521.
38. Hoffman SL, Oster CN, Plowe CV, Woollett GR, Beier JC, Chulay JD, Wirtz RA, Hollingdale MR, Mugami M, 1987. Naturally acquired antibodies to sporozoites do not prevent malaria: vaccine development implications. *Science 237:* 639–642.
39. Facer CA, Tanner M, 1997. Clinical trials of malaria vaccines: progress and prospects. *Adv Parasitol 39:* 1–68.
40. Metzger WG, Haywood M, D'Alessandro U, Drakeley CJ, Weiss H, Bojang K, Targett GA, Greenwood BM, 1999. Serological responses of Gambian children to immunization with the malaria vaccine SPF66. *Parasite Immunol 21:* 335–340.
41. Al-Yaman F, Genton B, Anders RF, Falk M, Triglia T, Lewis D, Hii J, Beck H-P, Alpers MP, 1994. Relationship between humoral response to *Plasmodium falciparum* merozoite surface antigen-2 and malaria morbidity in a highly endemic area of Papua New Guinea. *Am J Trop Med Hyg 51:* 593–602.