The anti-merozoite surface protein-1 19-kDa IgG (anti-MSP1 19KD) IgG responses of 33 parasitemic infants, aged 6–14 months, were compared with those of their mothers at the time of the infant's delivery and at the time the infants were sampled; the antimalaria protection associated with these responses was also compared. IgG1 and IgG3 were the predominant subclasses. Infants <300 days old and pregnant mothers had the lowest cytophilic-to-non-cytophilic IgG ratio. By 300 days of age, the infants had IgG subclass compositions and levels similar to those of their mothers at the same date. Among infants, older infants with only 1 or 2 detected asexual parasitemias had the highest cytophilic-to-noncytophilic IgG ratio and IgG1 levels. IgG1 level was negatively correlated with protection. The findings suggest that the MSP119KD antibody response develops with age, not with multiple experiences with parasitemia, and, thus, that an antimalaria vaccine strategy for pregnant mothers could delay infants’ first parasitemias until they are more capable of mounting a favorable anti-MSP119KD response.

More than 90% of the 3 million annual malaria-related deaths occur in children <5 years old [1]. Therefore, it is especially important to understand how protection against malaria develops in young children, how to boost these protective factors, and how to protect this age group with vaccination. Several epidemiologic investigations, including ours, have shown that individuals living in malaria-endemic regions develop immunity to infection and disease with age and increased experience with blood-stage parasitemia. This naturally acquired immunity enables an individual to control high-density infections and severe manifestations of illness. Humoral immune responses have been strongly implicated in blood-stage-based protective immunity. However, the antibodies in question may increase with age independently of protection, and different levels of both specific and nonspecific immunity may develop with age. Here, we present the results of a study of naturally acquired antibody responses and their relationship to the participants’ clinical and parasitologic manifestations over time.

Among the several asexual Plasmodium falciparum antigens identified by studying the immune responses of protected individuals, both naturally and experimentally exposed, and by in vitro and in vivo testing, the merozoite surface antigen-1 (MSP1) has emerged as a promising blood-stage vaccine candidate antigen. Antibodies to the C-terminal 19-kDa fragment of MSP1 (MSP119KD) have been shown to be protective in passive-transfer studies, preclinical vaccine studies, in vitro parasite inhibition studies, and primate vaccination studies [2–16]. Immunoepidemiologic studies have shown a correlation between anti-MSP119KD antibodies, age, and acquisition of naturally acquired protective immunity [9–16]. Finding a low mean anti-MSP119KD IgG level in young age groups [10–16], as compared with clinically immune adults, led to a theory that the conserved determinants of MSP119KD are not strongly immunogenic and that high antibody levels develop only after many exposures to this antigen [10, 14].

The longitudinal, comprehensive, and prospective nature of our Asembo Bay Cohort Project (ABCP) in western Kenya has allowed us to investigate immune responses more closely and correlate them with protection. We previously reported that infants could produce significant anti-MSP119KD antibodies upon their first detected episode of blood-stage parasitemia and
upon parasitemia episodes detected throughout their first year of life [9]. A high anti-MSP1<sub>19KD</sub> total IgG response just before a documented infection was associated with decreased clinical illness and low parasitemia level. The infants’ anti-MSP1<sub>19KD</sub> responses peaked after parasitemia and had an average half-life of <45 days. The infants experienced a lower level of illness and parasite densities during the short time this anti-MSP1<sub>19KD</sub> response remained detectable. The short-lived responses can explain why infrequent testing of children’s anti-MSP1<sub>19KD</sub> antibody levels cannot predict protection over long periods of time [17].

In addition to a low antibody level and a short-lived antibody response, a different isotype composition to MSP1<sub>19KD</sub> may be a factor in children’s susceptibility to malaria. Several studies, including ours, have shown that adults who are protected against malaria have an abundance of anti–blood-stage cyto philic isotypes, IgG1 and IgG3 [9, 11, 12, 14, 18–21]. Bouharoun-Tayoun and Drulhe [21] found that persons protected against malaria had an abundance of antischizont (asexual blood stage) IgG1 and IgG3, whereas unprotected persons (children and naïve adults) had an abundance of IgG2 and IgM. The IgG from the protected group could inhibit erythrocyte invasion (by antibody-dependent cellular inhibition [ADCI]), whereas the IgG from the unprotected group could abrogate this monocyte-IgG–mediated protection [21, 22].

We determined the anti-MSP1<sub>19KD</sub> IgG level and isotype composition of parasitic infants aged 6–14 months. The antibody levels were compared with those of their mothers at the time the respective infant’s sample was tested and with that of the mother at the time of the infant’s delivery. Comparing the infants’ antibody responses with those of their mothers at the same time (6–14 months after delivery) enabled us to study the confounding effects of host genetics, malaria transmission, and maternally acquired antibodies. By using extensive parasitologic histories before, during, and after each serum sample was tested, we examined the quantitative and qualitative differences between infants’ and mothers’ anti-MSP1<sub>19KD</sub> antibody responses and how they correlate with protection against parasitemia and malaria. Monthly parasitologic data collected on each child since birth enabled us to study whether the development of the anti-MSP1<sub>19KD</sub> antibody response was dependent upon either increasing age or increasing exposure to asexual parasitemia. This work has helped us to interpret our earlier study of anti-MSP1<sub>19KD</sub> responses throughout infants’ first year of life [9] and has enabled us to suggest how vaccines could control infection in pregnant mothers and young children.

Methods

Study site. The ABCP is an ongoing study in a holoendemic, rural region of western Kenya near Lake Victoria. The sampled points for this study spanned June 1992–August 1993. Entomologic inoculation rates (EIRs; infected bites per person per month) were determined by capturing mosquitoes with one-half bed net traps in the households of the participants and by using human-bait methods. *P. falciparum* parasites were transmitted throughout the year, with the highest transmission occurring during April–August and December–January (average EIR, 10). The EIR did not fall below an average of 2 for any month in this study.

Mothers from their last trimester of pregnancy and their infants, from their date of birth, were enrolled and monitored. Participants were visited at least every 2 weeks, were examined, and had their clinical symptoms and other epidemiologic information recorded. Blood samples were taken at least once a month and when the participants presented with symptoms of malaria. For the participants in this study, treatment with sulfadoxine/pyrimethamine was administered if a participant was both parasitemic by microscopy and febrile (axillary temperature >37.5°C). The serum was stored at −70°C until used.

Participants. Fifty 6–14-month-old infants, who were parasitic during the 1993 high transmission season (May–August), were randomly selected from the ABCP serum bank. Our earlier study showed that antibody transferred to the infant in utero became undetectable by the time the infant was 5 months old [9]. Therefore, the antibody detected in the selected infants was most likely autogeneous. These serum samples are referred to as the infant’s samples (ISs). For each infant, the mother’s serum sample obtained closest to the delivery date, but within 30 days prior to delivery, was selected. These samples will be referred to as the mother’s-at-delivery samples (mDSs). Only 33 of the mothers had this sample available. We also selected the mothers’ serum samples collected on the day on which their infants’ samples were selected. Thirty-two of the 33 mothers had this sample available. These samples will be referred to as the mother’s-at-infant’s samples (mISs). The 33 ISs and the matched mDSs and mISs were tested for serum antibody responses as detailed herein.

**MSP1<sub>19KD</sub> antigen and antibody assays.** Yeast-expressed MSP1<sub>19KD</sub> corresponding to the E-KNG type was used in an ELISA to determine IgG antibody responses. The ELISA method and more details on the antigen are described elsewhere [9]. The IgG response was measured without determining the isotype composition (referred to as total IgG), and the IgG responses were measured for each subclass of IgG (IgG1, IgG2, IgG3, and IgG4). The secondary goat antihuman antibodies were diluted 1:2000 for total IgG and 1:400 for the 4 IgG subclasses (Fisher Scientific, Pittsburgh). We found in an earlier study that of the 4 predicted MSP1<sub>19KD</sub> alleles, the E-KNG MSP1<sub>19KD</sub> variant, which is the most frequent genetic type detected in the ABCP area, is recognized by serum from 90% of the adult residents [23]. Also, an antibody response to E-KNG predicted a response to the 2 other allelic forms found in this area, Q-KNG and E-TSR, 84% and 78% of the time, respectively [23]. Therefore, we used E-KNG MSP1<sub>19KD</sub> in the present study.

Thirty serum samples from donors with no previous exposure to malaria were obtained from the Centers for Disease Control and Prevention blood bank. The normal serum samples were assayed at 1:100 dilution. The mean optical density (OD) plus 2 SDs of the normal group serum samples was used as the cutoff to determine the end point of the antibody titers. An OD less than the cutoff value at a 1:100 dilution was scored as a negative response. A positive control, an amalgam of 5 hyperimmune healthy adults
from the ABCP, was tested in every plate. The study was done on 4 different days. The positive and negative control values were similar for each day. The arithmetic OD mean cutoffs for total IgG, IgG1, IgG2, IgG3, and IgG4 were 0.227, 0.155, 0.047, 0.019, and 0.121, respectively.

Data analysis. Statistical analysis was performed with Statistical Analysis Software (SAS Institute, Cary, NC). Type I errors (alpha) were set to 0.05. Two-tailed tests were used for all comparisons. Statistical tests were performed on hypotheses generated before the experiment, and all tests investigating associations with protection are reported. Antibody titers and ELISA ODs at the first dilution point (1:100) were normalized with a logarithmic transformation (ln) by adding 0.001. Parasite densities were also logarithmically transformed (log_{10}) by adding 1. The $\chi^2$ test of independence was used to compare all proportions or rates for categorical variables. The nonparametric Wilcoxon rank sum test and analysis of variance with Duncan grouping were used to determine if 1 group’s antibody level was different from another’s. The Wilcoxon matched-pair signed rank test was used to compare mothers’ antibody levels with infants’ antibody levels. General linear models (GLMs) were performed to study the correlation between antibody level and parasitologic history, while also considering the confounding factors: age, sickle cell status, sex, parasite density at the time of sampling, and date of sample.

Results

Anti-MSP1_{19KD} IgG OD at a 1:100 dilution versus titer. End-point titer and antibody OD were strongly correlated (total IgG $R = .9301$, $P < .0001$; IgG1 $R = .9287$; IgG2 $R = .9062$; IgG3 $R = .9182$; and IgG4 $R = .8675$). We performed the analyses on both the end-point titer and the OD, and all results were concordant. Because the ln OD values had a more normal distribution than did the logarithmic titer values, and the ln OD values enabled us to consider the antibody levels of participants with a titer <1:100, we report the ln OD values in our analyses.

Anti-MSP1_{19KD} IgG levels. Because earlier results have shown that infants’ antibody responses are short-lived (half-life <45 days) and temporal with parasitemia [9], the 33 infants were selected at a time when they were parasitemic. Of the 33 samples from mothers at the time of mDS, 10 were parasitemic at the time of delivery. Of the 32 mothers at the time of mIS, 8 were parasitemic at the time of sample. Mothers’ ages ranged from 16 to 39 years at the time of delivery. Of the 33 ISs, 25 (75%) had a positive (antibody level greater than the negative cutoff) total IgG anti-MSP1_{19KD} response. The IgG subclass responder frequencies for the 33 infants were 63%, 18%, 66%, and 16% for IgG1, IgG2, IgG3, and IgG4, respectively. In the mDS group, the response frequencies were 94%, 73%, 30%, 70%, and 33% for total IgG, IgG1, IgG2, IgG3, and IgG4, respectively. The response frequencies for mISs were 97%, 69%, 3%, 53%, and 47%, respectively. The total IgG, IgG2, and IgG4 response frequencies of ISs, mDSs, and mISs were significantly different ($P < .027$).

The geometric means of the total IgG, IgG1, IgG2, IgG3, and IgG4 levels in the IS, mDS, and mIS groups, along with the statistical comparisons, are shown in figure 1. Overall, the infants had lower total IgG and IgG1 levels than did their mothers both at the time of delivery (mDS) and at the time of the infants’ sample (IS) ($P = .016$). However, there were increases in the older infants’ antibody levels (analyzed later).

There was no association between the mothers’ antibody levels (for any isotype) at time of delivery and the infants’ antibody levels (for any isotype) at 6–14 months of age ($P > .8$). However, of the 6 mothers who did not have significant anti-MSP1_{19KD} IgG in mDSs or mISs, 5 had infants who did not have any significant anti-MSP1_{19KD} IgG response.

Age, detected exposure to asexual parasitemia, and antibody levels. The ISs and mISs were tested during the high-transmission season (May 1993–August 1993). The mothers’ antibody levels and isotype compositions were not different for the 11 mDSs in the high-transmission season versus the 21 mDSs in the lower-transmission season ($P > .2$). Therefore, in the context of this study, transmission did not confound our results.

The number of detected parasitemia episodes since birth was
Figure 2. Correlation between (A) age with total IgG and (B) parasitemic episodes detected prior to the sample date with antibody to merozoite surface protein–1 C-terminal 19 kDa fragment (anti-MSP119KD) total IgG in infants. An estimate of infants’ exposure to asexual blood stage parasites was determined by counting the number of asexual parasitemias detected during intensive follow-up (every 2 weeks) since birth. The individual general linear model (GLM) regressions are shown. (A), \( R = 0.3846 \), \( P = .027 \). (B), \( R = 0.4694 \), \( P = .006 \). When age and exposure are considered in a GLM, the correlation was with and , \( R = 0.6349 \), \( P = .005 \) respectively.

Figure 3. Cytophilic-to-noncytophilic isotype composition of infants’ serum (IS) when 6–9 months of age (right crosshatch) and when 10–14 months of age (left crosshatch), along with their mothers’ serum (mother’s-at-delivery sample [mDS; solid black] and mother’s-at-infant’s sample [mIS; shaded]). The ratios were calculated as shown. The 6–9-month-old infants’ (\( n = 14 \)), 10–14-month-old infants’ (\( n = 19 \)), mDS (\( n = 33 \)), and mIS (\( n = 32 \)) geometric means are shown. The groups had different cytophilic (IgG1 and IgG3)–to–noncytophilic (IgG2 and IgG4) antibody ratios: (IgG1 + 3)/(IgG2 + 4), \( P = .014 \); (IgG1)/(IgG2 + IgG4), \( P = .022 \); (IgG3)/(IgG2 + 4), \( P = .852 \).

used to determine the correlation between the increase in antibody level and the increase in detected experience with asexual parasitemia (and, presumably, the MSP119KD antigen) or the age of the child. The results are shown in figure 2A and B. We found a positive correlation between infants’ age and anti-MSP119KD IgG antibodies (figure 2A). We found a negative correlation between the number of parasitemias detected since birth and antibody level (figure 2B). We expected to see an increased response with increased experience with the antigen. However, we found that a single infection was apparently capable of eliciting a high antibody response, and multiple parasitemia episodes were not required for the increase in antibody level with age. A similar result was seen for IgG1 (but not IgG2, IgG3, or IgG4), where a GLM resulted in \( R = .5869 \), with age \( (P = .035) \) and detected episodes \( (P = .002) \) both independently correlating with IgG1 level. Summarizing the data, we found that the infants who had the highest antibody levels (total IgG and IgG1) were the older infants who had 5 detected parasitemia episodes since birth.

We divided the infants into 2 groups to further investigate the change in antibody level and subclass composition with age: 6–9 months of age and 10–14 months of age, with sample sizes of 19 and 14, respectively. The geometric mean of the total IgG level was 0.251 (95% confidence interval [CI], 0.221–0.285) in the younger infants and 0.717 (95% CI, 0.588–0.874) in the older infants. The geometric mean IgG1 level was 0.049 (95% CI, 0.041–0.059) in the younger infants and 0.137 (95% CI, 0.105–0.178) in the older infants (see figure 2 for comparison with mDS and mIS groups). The 10–14-month-old infants’ total IgG and IgG1 levels were higher than those of the younger infants (total IgG, \( P = .0001 \); IgG1, \( P = .023 \)).

Anti-MSP119KD IgG subclass compositions. The antibody responses were mostly IgG1 and IgG3. In the 98 mothers and infants, IgG1 and IgG3 were both present 47% of the time. There was neither IgG1 nor IgG3 17% of the time. IgG1 and IgG3 response frequencies were associated \( (P = .003) \).

We compared the relative IgG subclass composition by calculating each sample’s cytophilic and noncytophilic antibody ratios. As shown in figure 3, both of the infant groups had a lower geometric mean (IgG1 + IgG3)/(IgG2 + IgG4) ratio than the mothers at time of the infants’ sample (mIS), and the mother’s ratio was lower at delivery (mDS) than at 6–14 months after delivery (mIS); \( (P = .001) \). Only the (IgG1)/(IgG2 + IgG4) ratio could explain the different cytophilic antibody ratios (see figure 3). The number of detected parasitemias since birth was not associated with the cytophilic antibody ratio \( (P = .931) \).

Anti-MSP119KD and parasitemias. We investigated the association between antibody response and protection by considering parasitemia densities ± 30 days from the sample date for mDS, mIS, and IS. Considering the parasitemia before and
after the day the antibody was tested enabled us to better consider the dynamics of parasitemia and antibody levels. We limited the parasitologic histories to ± 30 days from the sample date because infants have been shown to mount short-lived anti-MSP1<sub>19KD</sub> IgG responses [9]. The correlation between antibody level and geometric mean parasite density was analyzed for mothers (mDS and mIS) and infants (IS) separately to avoid the known association between age and protection against malaria [24].

As shown in figure 4, an increased anti-MSP1<sub>19KD</sub> IgG1 level in ISs (5–14-month-old infants), mDSs, and mISs was correlated with a lower mean parasite density during the 30 days before and 30 days after the day the antibody level was tested. Antimalaria drug treatment (administered to 14 infants), mothers’ or infants’ ages, sickle cell status, and/or sex did not correlate with the parasitemias; therefore, these factors were removed from the GLM analysis. The high malaria transmission seen in this study (~10 infected bites/month) resulted in no significant difference in mean parasitemia in the treated versus not treated groups during the 30 days before and 30 days after the day the antibody level was tested. IgG1 level was negatively correlated with mean parasite density during the 30 days before and 30 days after the sample point in mDSs, ISs, and mISs. The change in parasite density with IgG1 level (the slope) was significantly greater than the change seen in the IS group (P = .048). Total IgG, but not IgG2, IgG3, or IgG4 subclasses, showed similar results (data not shown).

IgG1 (but not IgG3) was consistently associated with protection in all the analyses of the parasitemia described above. We found that participants having both IgG1 and IgG3 had associations with protection similar to those of the participants having only IgG1 (data not shown). Conversely, the participants who had only the IgG3 anti-MSP1<sub>19KD</sub> and not IgG1, had parasitemia histories similar to those of the participants without IgG1 or IgG3. Therefore, only the IgG1 anti-MSP1<sub>19KD</sub> subclass was associated with parasitemia ± 30 days from the sample date.

Discussion

In this study, we investigated whether a lower anti-MSP1<sub>19KD</sub> antibody level or different IgG subclass response could explain the short-lived responses detected in infants [9] and investigated how the level and isotype composition correlate with protection.

We found that infants were capable of mounting significant anti-MSP1<sub>19KD</sub> IgG responses. Infants had lower total IgG and IgG1 levels than their mothers had at the time of delivery (mDS) or at the time the infants’ serum was sampled (mIS). However, by ~300 days of age, the infants’ total IgG and IgG1 anti-MSP1<sub>19KD</sub> levels were similar to those of the mDS group. The infants had more noncytophilic isotypes (IgG2 and IgG4) than did their mothers at the time of the infants’ sample (mIS). Interestingly, we also found that the mothers had a lower cytophilic-to-necytophilic isotype composition when pregnant (mDS) than when not pregnant (mIS). Although the lower total IgG level of mothers at time of delivery may be explained by the mothers’ having increased blood volume when pregnant [25], the difference in the ratio of cytophilic-to-noncytophilic antibodies cannot be accounted for by this explanation. The apparent anti-MSP1<sub>19KD</sub> isotype imbalance during pregnancy needs to be further investigated, especially in the light of pregnant women’s increased susceptibility to malaria infection. The greatest isotype imbalance was detected in infants <300 days old, which is consistent with this age group’s increased susceptibility to malaria (P. McElroy, unpublished data).

In addition to characterizing infants’ and mothers’ anti-MSP1<sub>19KD</sub> isotype responses, we also investigated the correlation between anti-MSP1<sub>19KD</sub> response and protection against malaria. Importantly, the correlations between antibody level and various measures of protection were consistent among both mothers and their infants. The finding that only cytophilic isotypes were correlated with protection is consistent with the ADCI of the parasites [22]. It is interesting to point out that IgG1 and not IgG3 correlated with protection in both infants and mothers.

What can account for IgG1’s dominance in the association with protection? Whereas IgG3 has a half-life of only 8 days, IgG1 has a half-life of 28 days. IgG1 has a greater ability to bind Fc-gamma II and Fc-gamma I receptors on monocytes.
and macrophages than does IgG3 [26, 27], suggesting that IgG1 may more effectively induce ADCI. Another possibility for detecting a protective association only with IgG1 is that the anti-MSP1_{19KD} IgG1 and IgG3 antibodies have different binding specificities. Shi et al. [11] showed that IgG3 could differentiate between the 3 different known alleles of MSP1_{19KD}, whereas IgG1 bound to all 3 indiscriminately. If anti-MSP1_{19KD} IgG1 was longer lasting, more capable of binding to different parasites, and more capable of binding to monocytes, then IgG1 would be expected to be more protective than IgG3 against malaria infections.

We found that infants experiencing their second infections could mount a significant anti-MSP1_{19KD} IgG1 response; however, infants’ ages positively correlated with anti-MSP1_{19KD} levels. At the same time, the number of previous episodes of parasitemia detected since birth negatively correlated with anti-MSP1_{19KD} total IgG and IgG1 levels. It is possible that a high antibody level in the serum sample reflects a high antibody response just prior to the date sampled. This might result in a lower total number of parasitemia episodes detected since birth. However, our previous studies suggest that this effect would be minimal, since the anti-MSP1_{19KD} IgG levels at 1 point cannot predict the antibody level in the past (i.e., the antibody is short-lived). Therefore, age might affect the level of antibody production.

Multiple exposures to blood-stage parasitemia in infants were not necessary for the detected increase in antibody levels with age. This is in agreement with the finding that naïve adults develop immunity against malaria more quickly than do young children [24]. Studies have shown that infants are ineffective at antigen presentation and instigation of interleukin (IL)–2–dependent cytotoxic T cell proliferation and are deficient in IL-4 and interferon-γ production [28, 29]. These age-based immunologic phenomena may explain why the infant’s age, rather than detected exposures to blood-stage parasitemia, is an important factor in the development of natural immune responses. Alternatively, the presentation of malarial antigens to the immune system, and not an overall immature immune response in children, might impede the development of the immune response upon multiple asexual parasitemias [13, 30].

We undertook this study to better understand the nature (i.e., quantitative and qualitative) of anti-MSP1_{19KD} immune responses mounted by infants. Certainly, other antigens are involved in protection against malaria, and additional studies of naturally acquired immunity must be performed on other antigens to understand the development of immunity and to guide the selection of antigenic determinants for vaccine development. The observation that infants can produce significant anti-MSP1_{19KD} IgG1 is promising for vaccine strategies. However, the short-lived nature of the infants’ responses must be taken into consideration. We propose that vaccinating pregnant women could provide infants and pregnant women with increased protection against malaria-related morbidity and mortality. Immunizing mothers not only would increase the level of maternally transferred antibody but also could increase the development of newborns’ memory T cells via maternally transferred cytokines [28]. We have previously shown that anti-MSP1_{19KD} IgG levels in mothers are correlated with decreased placental malaria and a delay in infants’ first-detected infections [9]. In this study, older infants had an enhanced anti-MSP1_{19KD} IgG1 response that could not be attributed to multiple infections. Delaying infants’ first exposures to parasitemia, via maternal antibodies or other interventions, may provide protection for this age group while their ability to respond to a vaccine and/or infection increases.

Acknowledgments

We thank all the volunteers for their participation in this study. We also wish to thank Dr. Davy Koech, the director of the Kenya Medical Research Institute, for his approval with regard to publication of the manuscript. We thank Allen Hightower for his statistical advice and Dr. Ya Ping Shi for her comments.

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