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The Effects of Varying Exposure to Malaria Transmission on Development of Antimalarial Antibody Responses in Preschool Children. XVI. Asembo Bay Cohort Project

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In areas of intense malaria transmission, malaria morbidity and mortality is highest in children 3–18 months old. Interventions that reduce malaria exposure early in life reduce morbidity but may also delay development of clinical immunity. We assessed the relationship between intensity of malaria exposure and development of antibody responses. Thirty-nine children were monitored monthly, from birth to ≥ 2.5 years old (1238 observations), and were divided into 3 exposure categories, on the basis of parasitemic episodes or entomological data. Children with low exposure during the first 2 years of life had higher subsequent levels of antibody to merozoite surface protein-1_{19-kDa} (a marker of blood-stage responses) by months 24–35 ($P < .05$). This inverse relationship decreased as children aged. There was no consistent relationship between exposure early in life and subsequent levels of antibody to circumsporozoite protein (a marker of sporozoite-stage responses). These data suggest that, in areas of intense malaria transmission, during the first 3 years of life, interventions that either reduce the number of asexual parasitemic episodes or lower entomological exposure do not delay the development of antibody responses to blood-stage malarial antigens.

Of the estimated 1 million deaths annually attributed to malaria in sub-Saharan Africa, almost all are children < 5 years old [1]. In areas of endemicity, the age-specific distribution of malaria-associated morbidity and mortality is a function of malaria transmission pressure and the rate of development of naturally acquired immunity. In areas of intense malaria transmission, children

3–18 months old have the most malaria-associated morbidity and mortality, and older children who sur-

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Informed consent was obtained from all patients associated with this study, and human subjects guidelines of the US Centers for Disease Control and Prevention Institutional Review Board and the Kenya Medical Research Institute Ethical Review Committee were strictly followed. Use of trade names is for identification purposes only and does not imply endorsement by the US Department of Health and Human Services. This work was published with the permission of the director of the Kenyan Medical Research Institute.

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vive have typically acquired sufficient immunity to be protected from severe disease [2, 3].

A number of studies have shown that, in these areas of intense malaria transmission, short-term (≤ 2 years) use of insecticide-treated bednets (ITNs) reduces malarial infection and associated morbidity [4–6]. Of concern is whether the long-term use of ITNs would delay the development and maintenance of naturally acquired immunity and cause a change in disease pattern, shifting the burden of disease to older age groups [7–9]. Knowledge of the explicit role that exposure plays in the development of naturally acquired immunity is required to predict the long-term impact of exposure-reducing interventions on the development and maintenance of naturally acquired immunity. However, these issues are difficult to address directly, because of the ethical limitations of maintaining a control group long term in randomized, controlled trials, particularly if the intervention is perceived to be beneficial in the short term [10].

To assess the role of exposure in the development of naturally acquired immunity, several epidemiological studies have modeled relationships between transmission intensity and the following: infection [10, 11], parasite density [12], malaria-associated morbidity [13, 14], malaria-associated mortality [7, 8], and, most recently, all-cause mortality [15]. Although Charlwood et al. [10] clearly show that incidence of malarial infection increases with entomological inoculation rate (EIR), until saturation at ~ 1 infectious bite/person/night, the dynamics of the relationship between exposure and malaria-associated morbidity and mortality has been a subject of much debate. Data from southern Tanzania demonstrate a linear relationship between EIR and malaria-associated morbidity, defined as fever with parasitemia [14]. Other studies comparing sites with different transmission levels suggest a decrease in severe malaria-associated morbidity above certain transmission levels [13]. Community-based studies from Senegal suggest that transmission intensity affects the age distribution of malaria attack rates but not the total number of lifetime attacks [8]. Similar to this, a comparative study of malaria-associated mortality rates in locations with different transmission intensities proposes that malaria-associated mortality plateaus or declines above certain transmission levels [7].

Subsequent to that, several groups have questioned the validity of the previous comparisons of malaria-specific morbidity and mortality across study sites [16, 17]. In partial response to these criticisms, Smith et al. [15] compared EIR and all-cause mortality, rather than malaria-specific mortality, in areas of different transmission intensity. This comparison shows that infant mortality increases linearly with EIR and that there is no clear relationship between all-cause mortality and EIR in 1–4-year-olds.

All of these studies underscore the need to understand the

potential impact of transmission-reducing interventions on the development of naturally acquired immunity. Only 2 studies have addressed the relationship between exposure and specific markers of naturally acquired immunity, in areas of intense malaria transmission. Kitua et al. [18] examined the relationship between concomitant exposure and antibody responses, and Branch et al. [19] compared antibody responses with cumulative number of parasitemic episodes detected since birth. However, the specific dynamics of the relationship between exposure and subsequent development or maintenance of effective humoral response remains unclear. The relationship may be linear (that is, increased exposure always leads to more rapid development of immunity); there may be saturation (that is, above a certain transmission intensity there is no further increase in the rate of development of immunity); or, most interesting, there may be an exposure threshold (that is, above a certain transmission intensity the immune system is overwhelmed, resulting in an ineffective humoral response).

To examine the role that exposure plays in subsequent development of immune responses, we compared antibody responses to 2 malaria vaccine candidate antigens, merozoite surface protein-1_{19kDa} (MSP-1_{19kDa}) and circumsporozoite surface protein (CSP), as a function of the intensity of exposure to *Plasmodium falciparum*, in a birth cohort of infants monitored from birth to ≥ 2.5 years old, in an area with intense perennial malaria transmission. These data are useful in the prediction of the potential long-term impact of exposure-reducing and/or parasitemia-decreasing control strategies.

SUBJECTS, MATERIALS, AND METHODS

Study Site and Sample Selection

This study was conducted in the context of the Asembo Bay Cohort Project (ABCP), a longitudinal, prospective birth cohort study of the epidemiology and immunology of malaria in this area of intense transmission. Thirty-nine children who had ≥ 2.5 years of follow-up were selected for the current study. Detailed descriptions of methodologies of the ABCP and the epidemiology of malaria in the study area have been reported elsewhere [2, 20]. Asembo Bay is located in western Kenya on the shores of Lake Victoria and has holoendemic malaria transmission. Pregnant women and their delivered infants were enrolled in the study. Participants were visited in their homes every 2 weeks. At each visit, a clinical history and body temperature were recorded. Blood samples were taken every other visit and at any other time that participants thought their children were ill. Parasite densities were quantified by counting asexual parasites per 300 leukocytes, on a Giemsa-stained blood smear, and are expressed as parasites per microliter, assuming a leukocyte count of 8000/mm³. Any participant with docu-

mented fever (axillary temperature $\geq 37.5^{\circ}\text{C}$) and malaria parasitemia was treated with sulfadoxine-pyrimethamine.

MSP-1_{19kDa} Antigen and Antibody Assays

Standard ELISA techniques described elsewhere were used to determine IgM and total IgG responses to MSP-1_{19kDa} antigen [21]. Microtiter Immunlon-2 plates were coated with MSP-1_{19kDa} (200 ng/mL) in borate buffer solution overnight at 4°C . Plates were washed with PBS, were blocked with 200 μL /well 5% nonfat lyophilized milk in PBS for 1–2 h at room temperature, and were washed with PBS containing 0.05% Tween 20 (PBST). Serum samples were diluted 1:50 in casein, for storage in deep-well titer plates. Fifty microliters of diluted serum and 100 μL of high-salt PBS (PBS containing 150 mM sodium phosphate, 500 mM NaCl, 0.05% Tween 20, 1.5% nonfat lyophilized milk, 0.05% bovine serum albumin, and 0.1% Nonidet P-40 [pH 7.4]; HSPBST) were added in duplicate to microtiter plates and were incubated with shaking, for 1.5 h at room temperature. Each plate also contained ≥ 3 negative controls of serum samples from healthy, malaria-naive persons from the Centers for Disease Control and Prevention (CDC) blood bank and a positive control of an amalgam of serum samples from 4 hyperimmune healthy adults from ABCP. To remove unbound antibody, plates were washed 4 times with HSPBST. Antibodies were detected by the addition of peroxidase-conjugated goat anti-human antibodies diluted in HSPBST. IgG was detected with a 1:8000 dilution (Gibco); IgM was detected with a 1:500 dilution (Southern Biotech). Secondary antibody was incubated with shaking, at room temperature for 1 h. Plates were washed 4 times with PBST and were developed, by the addition of 150 μL of 3,3',5,5'-tetramethylbenzidine, for 5–10 min. The reaction was terminated by the addition of 50 μL of 1 M phosphoric acid. Plates were read at 450 nm on an ELISA reader (Molecular Devices SpectraMax 340 and SoftMax Pro Software). Day-to-day and plate-to-plate variation were normalized by addition of a standardization factor calculated by the following equation, and, because of variation in background, 0.415 was added to each optical density (OD) value after standardization, to make all values positive: standardized OD = [(total mean positive control) – (total mean negative control)]/[(plate mean positive control) – (plate mean negative control)].

In the present study, we used a yeast-expressed E-KNG variant of MSP-1_{19kDa} [22]. E-KNG is the most prevalent parasite genotype in the study area [23], and serum samples from adults in the study area react more frequently with the E-KNG variant than they do with the other 3 variants [24].

CSP Antigen and Antibody Assays

ELISA was used to determine total IgG response to CSP [4]. Microtiter Immunlon-2 plates were coated with 70 μL /well CSP (150 ng/mL) in borate buffer solution overnight at 4°C .

Plates were washed with PBS, were blocked with 200 μL /well PBST containing 2.5% nonfat lyophilized milk (PBST-M), for 1–2 h at room temperature, and were washed with PBST. Serum samples were diluted 1:50 in casein, for storage in deep-well titer plates. Fifty microliters of diluted serum and 100 μL of PBST-M were added in duplicate to microtiter plates, and then plates were incubated with shaking, for 2 h at room temperature. Each plate also contained at least 3 negative controls of serum samples from healthy, malaria-naive persons from the CDC blood bank and a positive control of an amalgam of serum samples from 4 hyperimmune healthy adults from ABCP. To remove unbound antibody, plates were washed 4 times with PBST. Antibodies were detected by the addition of peroxidase-conjugated goat anti-human antibodies diluted 1:8000 in PBST-M. After incubation for 1 h at room temperature, plates were washed 8 times with PBST and were developed by the addition of 150 μL of 3,3',5,5'-tetramethylbenzidine. The reaction was terminated after 5–10 min by the addition of 50 μL of 1 M phosphoric acid. Plates were read at 450 nm on an ELISA reader. Day-to-day and plate-to-plate variation were normalized by addition of a standardization factor calculated by the following equation, and, because of variation in background, 0.558 was added to each standardized OD value to make all values positive: standardized OD = [(total mean positive control) – (total mean negative control)]/[(plate mean positive control) – (plate mean negative control)].

We used a full-length *Escherichia coli*-expressed recombinant CSP. It has been reported elsewhere that the majority of anti-CSP antibodies are directed against the (NANP)₄₀ repeat region [25].

Exposure Group Categorization

Children were ranked into 3 exposure groups (low, moderate, and high), on the basis of cumulative exposure to malaria, by years 1, 2, or 3. Three separate exposure measures were used to categorize children: EIR, incidence of parasitemia, and incidence of parasitemia at ≥ 5000 parasites/ μL . Children were included if they had ≥ 11 exposure observations/year; that is, children included in the 0–3-year-old age window were required to have had 33 exposure observations before age 3 years.

EIR. The EIR is the total number of *Anopheles gambiae* sensu lato and *Anopheles funestus* collected by bednet traps, at weekly visits, for each individual child, and the proportion of infected mosquitoes. Mosquito numbers varied considerably, both geographically within the study site and temporally, during the study, whereas the proportion of mosquitoes infected by sporozoites varied much less. Furthermore, because the number of mosquitoes collected per child was, in most cases, <100 specimens, estimates of sporozoite rates per child are subject to severe sampling error. For the entire ABCP, during 1996, the sporozoite rate in *A. gambiae* sensu lato was 5.8%

Table 1. Characteristics of study participants, by exposure measure and infant exposure group.

Exposure measure, parameter	Low	Moderate	High	Total
EIR				
No. of children	10	11	11	32
EIR ^a	28.6 (18.0–34.7)	61.3 (45.0–65.4)	118.6 (85.8–263.7)	61.3 (34.7–85.8)
Incidence				
No. of children	12	12	12	36
Incidence/1000 person-months ^a	376 (249–494)	758 (639–948)	1344 (1252–1620)	758 (494–1252)
Incidence \geq 5000 parasites/ μ L				
No. of children	12	12	12	36
Incidence/1000 person-months ^a	127 (95–210)	352 (299–402)	649 (583–795)	352 (210–583)

NOTE. EIR, entomological inoculation rate.

^a Data are median (25th and 75th quartiles).

and in *A. funestus* was 5.1% [26]. In the current study population, 66.5% of mosquitoes captured were *A. gambiae* sensu lato. We therefore multiplied total mosquito numbers by a sporozoite rate of 5.6%. Entomological data were collected during a 2.5-year period only, and the relationship between exposure and subsequent antibody responses are presented for cumulative exposure by years 1 and 2 only.

Incidence. Incidence was calculated by dividing the cumulative number of parasitemic episodes in each age window by time at risk, in person-months. Time at risk was calculated as time until the first episode, within an interval of 35 days (4 weekly routine visits could be no more than 6 days late). All events and observation times occurring within 28 days from the previous parasitemia episode were excluded from the incidence calculation.

Incidence \geq 5000. Incidence \geq 5000 was calculated by dividing the cumulative number of episodes of parasitemia at \geq 5000 parasites/ μ L in each age window by time at risk in person-months. Time at risk was calculated by use of the same methods as were used for any parasitemia.

Statistical Analysis

All analyses included antibody measurements taken after children reached 1 year old. To analyze the effects of exposure on a child's antibody level, we used a mixed model (PROC Mixed; version 8.01) [27] to account for the within-subject and between-subject variation, over time, of the repeated antibody measurements. The mixed model includes both fixed and random effects of predictors on the outcome variable. We assessed, over time, in 3 age windows, the effect of exposure on antibody measurements. The exposure variable is a time-independent covariate. For this set of models, we modeled a random intercept to account for the between-subject variation [28]. Least-squared means were calculated for each age group and exposure group combination. Differences between high and low exposure groups were calculated within each age group and were tested

for statistical significance. Significance was set at $P < .05$. In all of the models, children were assumed to be independent from one another. Because the antibody data were not normally distributed, we transformed the antibody measurements by use of a square-root transformation. Missing observations were assumed to be missing at random. Parameter estimates were estimated by the maximum likelihood method, and we used an empirical (robust) variance estimator.

RESULTS

Antibody development over time was compared in 39 children (1238 observations). Table 1 shows the exposure-group characteristics, on the basis of infant exposure. In our study, the median EIR in the low-exposure category was 29/year, compared with a median EIR of 119/year in the high-exposure category. The median incidence of parasitemia per 1000 person-months was 376 in the low-exposure category, compared with 1344 in the high-exposure category. In the low-exposure category, the median incidence of parasitemia of \geq 5000 parasites/ μ L/1000 person-months was 127, compared with 649 in the high-exposure category. These divisions of the children included in the study are roughly similar to distribution of these exposure groups in the total ABCP; each infant exposure group is representative of \geq 20% of the total cohort. No associations were found between exposure groups and sex, transmission season of birth month, or genetic factors, including sickle cell trait, nitric oxide synthase 2, tumor necrosis factor- α , and Fc γ receptor II (data not shown).

Anti-MSP-1_{19kDa} response. Anti-MSP-1_{19kDa} antibody levels varied considerably over time, generally parallel with parasite density. Figure 1A describes the parasitemia and antibody responses over time of a child with low infant exposure; figure 1B shows the parasitemia and antibody responses over time of a child with high infant exposure. Mean MSP-1_{19kDa} antibody levels during the subsequent year, as a function of cumulative

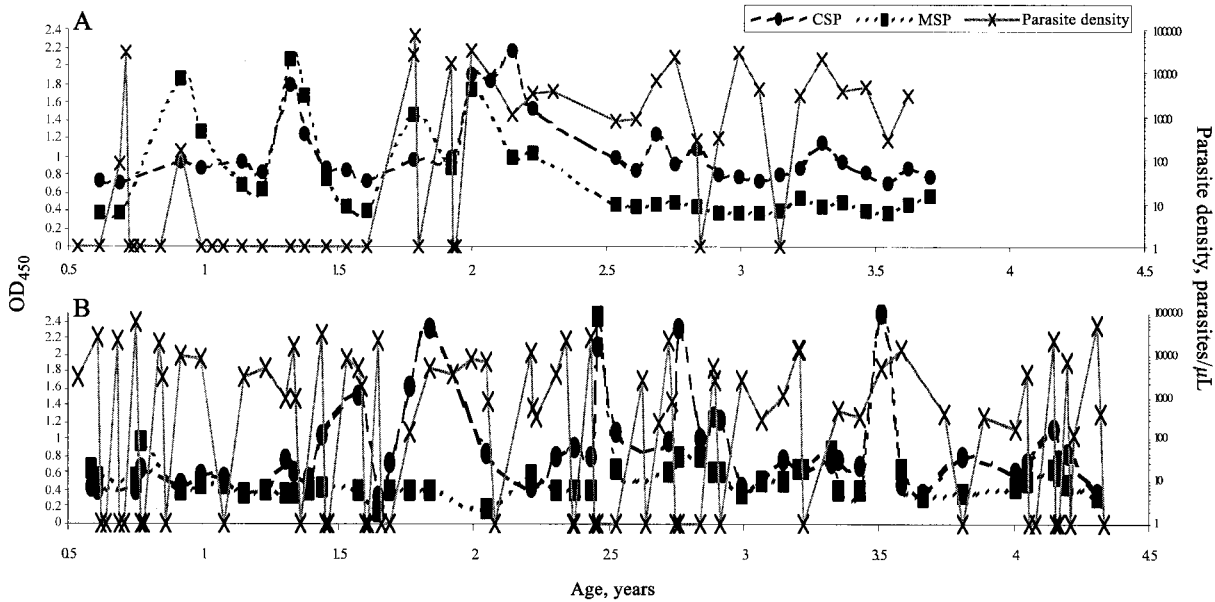


Figure 1. Examples of antibody and parasitemia life histories of children with either low exposure (*A*) or high exposure (*B*) to malaria during infancy. CSP, circumsporozoite surface protein; MSP, merozoite surface protein; OD, optical density.

exposure by years 1, 2, and 3, are shown in figure 2. On the basis of EIR and incidence ≥ 5000 , children with low exposure during infancy had statistically significant higher levels of anti-MSP-1_{19kDa} antibodies between year 1 and year 2 than did children with high exposure during infancy ($P < .01$). On the basis of EIR, children with low exposure between birth and year 2 had statistically significant higher levels of anti-MSP-1_{19kDa} antibodies between year 2 and year 3 than did children who had high exposure between birth and year 2 ($P < .05$). By year 3, there was no further evidence of a relationship between exposure.

Anti-CSP response. Anti-CSP antibody levels varied considerably over time, also generally parallel with parasite density (figure 1). There were no significant relationships between either infant exposure or exposure between birth and year 2 and anti-CSP antibody responses for any of the exposure measures. Children in the low-exposure group between birth and year 3, based on incidence of parasitemia, had lower levels of antibodies between year 3 and year 4 than did children who had high exposure to blood-stage infections ($P < .05$). On the basis of incidence of parasitemia ≥ 5000 between birth and year 3, there was no statistically significant difference between the anti-CSP antibody levels, at year 3 and year 4, of children with low exposure and children with high exposure (figure 3).

DISCUSSION

Our study has shown that reduction of exposure during infancy does not impair children's subsequent ability to mount an antibody response to MSP-1_{19kDa}. In fact, children with high

exposure produced lower levels of anti-MSP-1_{19kDa} antibodies. During the first 2 years of life, there was no consistent relationship between previous exposure and anti-CSP antibody levels, but, by year 3, children with the highest cumulative number of blood-stage infections had significantly higher anti-CSP antibodies than did those with the lowest number of infections.

The exposure measures used in this study model the possible effects of a number of different control measures. Incidence and incidence ≥ 5000 are relevant to control measures that would reduce asexual blood-stage parasitemia. EIR is representative of control measures that would reduce mosquito transmission intensity. It is important to note that, in this study, the relative differences between high-exposure and low-exposure groups are roughly equivalent to the degree of reduction in exposure found in a randomized controlled trial of ITNs conducted after the current study in the same area. In our study, the median EIR in the low-exposure category was 29/year, compared with a median EIR of 119/year in the high-exposure category, which is a 76% difference in exposure. The median incidence of parasitemia $\geq 5000/1000$ person-months in the low-exposure category was 127, compared with 649 in the high-exposure category, which is an 80% difference in exposure. In the ITN trial, the incidence of high-density parasitemia per 1000 person-months was 56 in ITN villages, compared with 202 in control villages, which is a 72% reduction in incidence of high-density parasitemia. The ITN trial also showed a 74% reduction in force of infection during infancy [29]. Thus, in the present study, the differences between low-exposure and high-exposure groups are similar to reductions in exposure demonstrated by the use of ITNs. It must be rec-

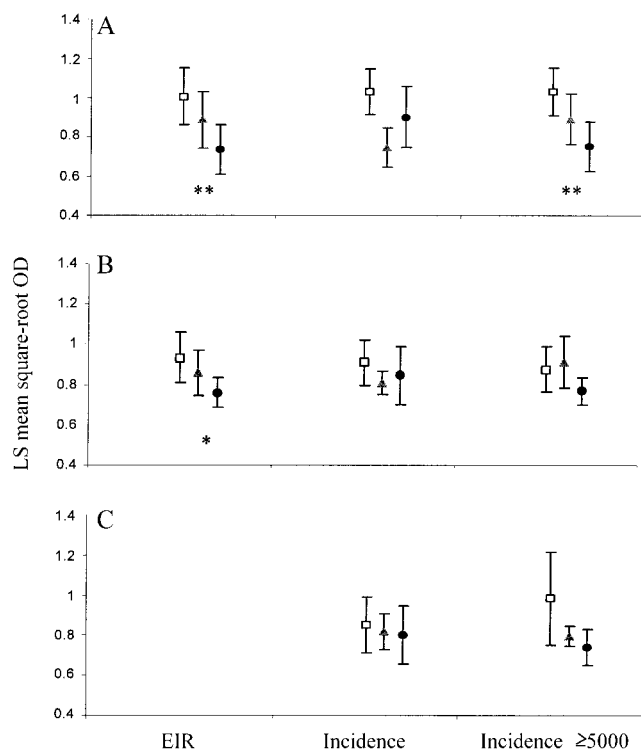


Figure 2. Comparison of merozoite surface protein-1_{19kDa} antibody levels at year 2 (A), by exposure from birth to year 1 (infant exposure); at year 3 (B), by exposure from birth to year 2; or at year 4 (C), by exposure from birth to year 3. Data are least-squared (LS) mean square-root optical densities (ODs) with 95% confidence intervals. Low-exposure (squares), moderate-exposure (triangles), and high-exposure (circles) groups are presented. Low-exposure and high-exposure groups were compared for statistical significance (* $P < .05$; ** $P < .01$). EIR, entomological inoculation rate.

ognized, however, that even the low-exposure group in this study, as measured by EIR, received relatively high exposure, compared with transmission intensity in most of the world in which malaria is endemic.

The fact that reductions in exposure, as indicated by any of the exposure measures investigated in this study, did not result in impaired anti-MSP-1_{19kDa} responses does not support the hypothesis that reductions in exposure will delay the development of acquired immunity and will possibly shift the burden of malaria morbidity and mortality to older children. Our results are consistent with those in other similar studies. When comparing concurrent sporozoite exposure and antibody levels, Kitua et al. [18] suggest that there is no strong relationship between transmission and antibody titer. Branch et al. [19] showed that anti-MSP-1_{19kDa} antibody levels are higher in children with fewer parasitemias detected since birth. Several studies have also compared IgG antibody responses to a variety of antigens, between children who used ITNs and control children. The most recent one, a cross-sectional study in the same study

site as the present study, was conducted as part of a large-scale randomized controlled ITN trial. That study measured antibody concentrations in children <3 years old from ITN and control villages, 14 and 22 months after ITNs had been introduced in the intervention villages, and found an increase in anti-MSP-1_{19kDa} antibody responses in ITN users, but only after the rainy transmission season. In contrast, decreases in anti-CSP and anti-liver stage antigen-1 antibody responses were found in ITN users, compared with those in control children [30], a finding that possibly suggests that the similar relationship we observed between cumulative incidence and anti-CSP antibody responses by age 3 years is not a result of chance. However, there is some question of the clinical relevance of this difference in anti-CSP antibody levels. As seen in a recombinant CSP vaccine study by Stoute et al. [31], extremely high titers of anti-CSP antibodies are required for protection.

Studies in The Gambia, which has much lower and more seasonal transmission of malaria than does western Kenya, have reported no differences in prevalence or titers of antibody to (NANP)₄₀ (the repeat region of the CSP antigen) when

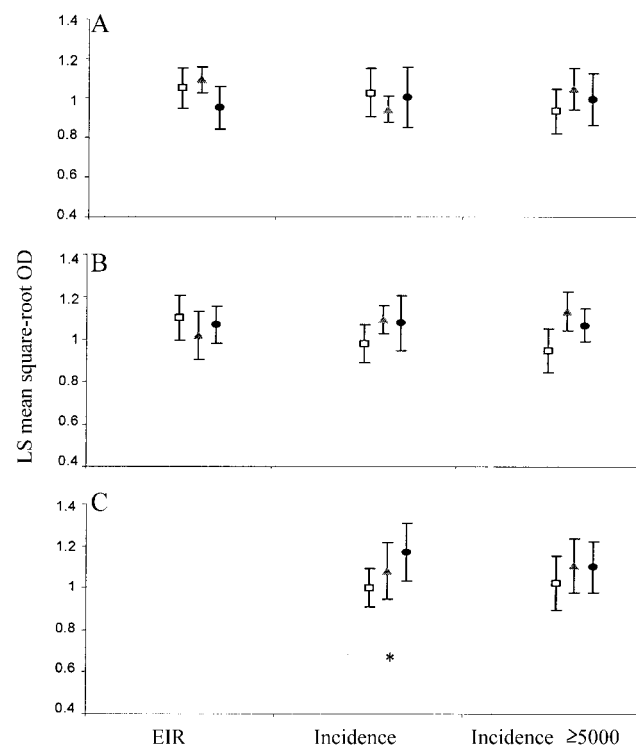


Figure 3. Comparison of merozoite surface protein-1_{19kDa} antibody levels at year 2 (A), by exposure from birth to year 1 (infant exposure); at year 3 (B), by exposure from birth to year 2; or at year 4 (C), by exposure from birth to year 3. Data are least-squared (LS) mean square-root optical densities (ODs) with 95% confidence intervals. Low-exposure (squares), moderate-exposure (triangles), and high-exposure (circles) groups are presented. Low-exposure and high-exposure groups were compared for statistical significance (* $P < .05$). EIR, entomological inoculation rate.

comparing children 1–9 years old who sleep without nets, with untreated nets, or with ITNs [32]. Two nonrandomized or quasi-randomized studies have found lower antibody responses to blood-stage antigens and infected erythrocytes in bednet users, compared with those of control children, in areas with intense malaria transmission in northern Tanzania and in Papua New Guinea [33, 34]. Evaluation of our results in terms of these studies, with the exception of the study by Kariuki et al. [30], is complicated by differences in antigens, age of study population, method of exposure reduction, and transmission intensity.

It is important to acknowledge that the present study and each of the aforementioned studies has used antibody responses to various antigens as markers of immunity. Passive-transfer studies have demonstrated the general importance of humoral responses in immunity to malaria [35]. However, the specific molecular correlates of immunity have not been convincingly described. In the absence of this reference standard, we chose to study CSP and MSP-1_{19kDa}, 2 vaccine candidate antigens, as markers of anti-sporozoite and anti-blood stage immune responses [31, 36]. It is, of course, possible that antibody responses to these 2 antigens are not the strongest markers of immunity. They are certainly not the only markers, and many other antigens have also been found to be important in generation of immunity. We assessed total IgG responses, because, in this population, total anti-MSP-1_{19kDa} IgG has been demonstrated to be representative of anti-MSP-1_{19kDa} IgG1, which has been shown to be protective [19, 37].

An important difference between our study and other studies is the longitudinal study design of our study. Each child in our study was followed approximately monthly, from birth to ≥ 2.5 years old. This allowed us to examine antibody levels, over time (that is, subsequent antibody response in addition to concurrent responses), within individuals. Our demonstration of the temporal variation in children's antibody levels (figure 1) supports the conclusion of Stirnadel et al. [38] that a child's antibody level measured at a single time is not representative of the child's acquired immune response. Therefore, a longitudinal study is a more informative method than are previous cross-sectional studies, to examine the relationship between exposure and concurrent, as well as subsequent, antibody development.

Similar to other studies [30, 33], our study also demonstrates differences in development of humoral responses to different malarial antigens—in this case, CSP and MSP-1_{19kDa}. Of interest, during the first 2 years of life, lower exposure during infancy correlated with an increase in the level of anti-MSP-1_{19kDa} responses but did not have an impact on the level of anti-CSP responses. Two explanations for these differences are antigen characteristics and antigen dynamics. MSP-1_{19kDa} is a T cell-dependent antigen [24], whereas CSP, although containing several well-defined T cell determinants, may also induce a T

cell-independent response, because of the presence of the (NANP)₄₀ repeat region. Other studies have suggested that the repeat region of CSP can directly activate B cells without T cell help [39]. Such a T cell-independent activation of B cells can lead to poor memory response. Thus, it is expected that MSP-1_{19kDa} will elicit a more long-term memory-based response, and the repeat region of CSP will drive a short-term response. The dynamics of CSP and MSP-1_{19kDa} also contrast, depending on the stage at which they are expressed. MSP-1_{19kDa}, an asexual erythrocytic-stage antigen, is produced continuously and in large number, because the blood-stage parasite goes through several cycles of multiplication. During the course of a single infection, CSP, a pre-erythrocytic antigen, is available to the immune system when the sporozoites shed this antigen into circulation. In consequence, the availability of CSP to the immune system is of much shorter duration than is that of MSP-1_{19kDa} (minutes rather than days or weeks). Therefore, reductions in parasite exposure will much more drastically reduce the amount of CSP antigen than it will the amount of MSP-1_{19kDa} antigen seen by the immune system.

This study has provided further support for the hypothesis that, in areas of intense transmission, children's immune systems can be overwhelmed by the large number of blood-stage parasites encountered during the course of their numerous malarial infections [30]. We propose that this may occur through induction of a state of high zone tolerance, resulting in an inadequate mounting of an immune response to MSP-1_{19kDa}. High zone tolerance is T cell anergy or apoptosis caused by overstimulation, usually as a result of exposure to high concentrations of antigen [40]. Although there are no published data on high zone tolerance in the in vivo human malaria system, there is evidence, in the mouse model, for clonal deletion induced by immunodominant repeat regions [41] and apoptotic deletion of MSP-1_{19kDa}-specific Th cells [42]. This hypothesis is supported by our findings that children with low incidence of parasitemia ≥ 5000 during infancy had higher anti-MSP-1_{19kDa} antibody levels than did children with high incidence of parasitemia ≥ 5000 during infancy, whereas there was no difference between children with low or high incidence of all parasitemia. Thus, this relationship appears, to some extent, to be density dependent.

In summary, this study shows that, in an area of intense malaria transmission, decreased exposure to *P. falciparum* during the first 2 years of life does not negatively affect children's ability to mount anti-CSP or anti-MSP-1_{19kDa} antibody responses. In fact, decreased exposure during the first 2 years of life was found to be associated with higher levels of subsequent anti-MSP-1_{19kDa} antibody responses. These results suggest that, in areas of intense transmission, malaria control measures that reduce, but do not eliminate, exposure early in life are unlikely to delay the acquisition of naturally acquired immunity to blood-stage parasitemia.

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