

EFFECTS OF PERMETHRIN-TREATED BED NETS ON IMMUNITY TO MALARIA IN WESTERN KENYA I. ANTIBODY RESPONSES IN PREGNANT WOMEN AND CORD BLOOD IN AN AREA OF INTENSE MALARIA TRANSMISSION

SIMON K. KARIUKI, FEIKO O. TER KUILE, KATHLEEN WANNEMUEHLER, DIANNE J. TERLOUW, MARGARETTE S. KOLCZAK, WILLIAM A. HAWLEY, PENELOPE A. PHILLIPS-HOWARD, ALLOYS S. S. ORAGO, BERNARD L. NAHLEN, ALTAFA A. LAL, AND YA PING SHI

Centre for Vector Biology and Control Research, Kenya Medical Research Institute, Kisumu, Kenya; Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; Department of Infectious Diseases, Tropical Medicine & AIDS, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; Zoology Department, Kenyatta University, Nairobi, Kenya

Abstract. As part of a community-based group-randomized trial on the impact of permethrin-treated bed nets (ITNs) on malaria in pregnancy in a holoendemic area of western Kenya, we assessed their effects on antibody responses to *Plasmodium falciparum* pre-erythrocytic antigens (recombinant circumsporozoite protein [CSP] and peptides complementary to the repeat region of the liver stage antigen-1 [LSA-1]) and blood stage antigen (recombinant C-terminal domain of the merozoite surface protein-1 [MSP-1₁₉ kD]) in paired maternal/cord plasma samples obtained from 296 deliveries (157 from ITN villages and 139 control villages). Levels of total IgG and IgG subclasses 1–3 to LSA-1 and total IgG and IgG3 to MSP-1 were lower, whereas those of total IgG to CSP were significantly higher in women from ITN villages than those from control villages. In cord plasma, levels of total IgG and IgG2 to LSA-1 and IgG3 to MSP-1 were lower in ITN villages than in control villages, but antibody responses to CSP were similar. Our results suggest that the use of ITNs decreases antibody responses to LSA-1 and MSP-1 antigens in pregnant women with associated reductions in levels of the same antibodies in cord blood. In contrast, ITN use was found to be associated with increased antibody responses to CSP in pregnant women, but had no effect on antibody levels to CSP in cord blood.

INTRODUCTION

Pregnant women in sub-Saharan Africa are at increased risk of malaria caused by *Plasmodium falciparum* compared with their non-pregnant counterparts.¹ In areas of high transmission, the increased risk of malaria infection has been found to be particularly high during the first and second pregnancies, but diminishes with increasing gravidity.^{2,3} The underlying biologic mechanisms responsible for the increased risk have not been elucidated. Non-specific immunosuppression and hormonal changes such as increased cortisol levels in primigravidae women have been implicated.⁴ Although there is some evidence of a general immunosuppression in cellular immunity in pregnant women,⁵ two studies have shown no difference in antibody responses to malaria antigens between pregnant and non-pregnant women.^{6,7} Recent evidence suggests that gravidity-dependent changes in cytokine levels,⁸ and the acquisition of antibodies that block parasite binding to the placenta could play an important role in protection against placental malaria.⁹

It is well recognized that infants in areas of stable malaria transmission have a reduced risk of clinical consequences of *P. falciparum* infection during the first few months of life. The presence of fetal hemoglobin, passively acquired malaria-specific antibodies, dietary absence of *p*-aminobenzoic acid and breast milk constituents such as lactoferrin may account for this phenomenon.^{10–12} Among these, transplacentally acquired antibodies are thought to play an important role.¹³

Although malaria in pregnancy is amenable to intervention through existing strategies such as intermittent preventive treatment,¹ this may be undermined by resistance of *P. falciparum* to antimalarials, poor compliance, and other logistical problems.¹⁴ In our study area in western Kenya, we demonstrated that in an area with intense malaria transmission, insecticide (permethrin)-treated bed nets (ITNs) reduce the densities of malaria-transmitting mosquitoes by 90%.¹⁵ The ITNs were also found to be a promising tool for malaria con-

trol in pregnancy, since they markedly reduced malaria and its adverse effects during the first four pregnancies.¹⁶

One concern with the use of ITNs, especially in high-transmission areas, is whether their use might interfere with the development and maintenance of antibody responses to malarial antigens in pregnant women, thereby potentially affecting passive immune protection in early infancy. Previous studies in children and non-pregnant adults have found that use of ITNs was associated with a reduction in antibody responses to malarial antigens.^{17–19} In a companion study conducted in young children, we found that ITNs were associated with a reduction in IgG responses to pre-erythrocytic circumsporozoite surface protein (CSP) and liver stage antigen-1 (LSA-1), but not to the blood stage antigen merozoite surface protein-1 (MSP-1).²⁰ The current investigation focused on the antibody responses in mothers at delivery and cord blood samples to determine the effect of ITNs on antibody responses to *P. falciparum* pre-erythrocytic (CSP and LSA-1) and erythrocytic (MSP-1) antigens. A subsidiary goal of the investigation was to evaluate the effect of ITNs on the transplacental transfer of malaria-specific IgG.

MATERIALS AND METHODS

Study site and participants. This study was conducted as part of a community-based cohort study of the impact of ITNs on malaria in pregnancy in Asembo Bay in western Kenya, which experiences intense perennial malaria transmission. A detailed description of the study site, study population, and methods of the cohort study have been reported elsewhere.¹⁶ Briefly, half of the cohort villages were randomized by public lottery to receive ITNs in the fourth quarter of 1996 and the remaining half acted as controls and received ITNs in April 1999 at the completion of the trial. All women who delivered during a one-year period between September 1997 and October 1998 were eligible for the current study. Exclusion of

women who were pregnant during the first nine months of the ITN trial ensured that all women in the ITN villages had been protected by ITNs throughout the entire pregnancy. In this region, the prevalence of peripheral and placental malaria during the first four pregnancies is approximately 30% and 25%, respectively.¹⁶ In addition, up to one-third of all infants are born preterm, intra-uterine growth retarded (small-for-gestational age), or with low birth weight.¹⁶ Ethical clearance for the ITN trial was obtained from the Ethical Review Committee of the Kenya Medical Research Institute (Nairobi, Kenya) and the Institutional Review Board of the Centers for Disease Control and Prevention (Atlanta, GA).

Sample collection. At delivery, approximately 500 μ l of maternal capillary blood and 5 mL of cord blood were collected in EDTA-containing tubes and heparinized tubes, respectively. Blood samples were transported to the laboratory at 4°C for determination of hemoglobin levels and immunologic studies. Two thick blood smears on the same slide and a thin blood smear on a separate slide were prepared from maternal, cord, and placental blood for detection of malaria parasites.

Antigens. The same antigens used in our study in young children²⁰ were used for the assessment of antibody reactivity in maternal and cord plasma samples. These are *Escherichia coli*-expressed full-length recombinant CSP, synthetic peptides corresponding to the repeat region of LSA-1 and *Saccharomyces cerevisiae*-expressed MSP-1₁₉kD protein. *Clostridium tetani* tetanus toxoid (TT) (Accurate Chemical and Scientific Corp., Westbury, NY) was used as a non-malarial control antigen to test for any underlying difference in the general antibody responses in maternal and cord plasma from ITN and control villages.

Laboratory procedures. *Sample processing, hematologic, and parasitologic examinations.* A complete blood cell count was done on maternal and cord blood using an AcT 10 Coulter-Counter[®] (Coulter Corporation, Miami, FL). Maternal and cord plasma were obtained by centrifuging blood at 700 \times g for five minutes in a Micro 7[®] microcentrifuge (Fisher Scientific, Pittsburgh, PA), aliquoted into sterile vials, and stored at -70°C until testing for antibodies. Maternal, cord, and placental blood smears were stained with Giemsa and examined for the presence of malaria parasites and/or pigment with a 100 \times oil-immersion objective.

Enzyme-linked immunosorbent assay (ELISA). Total IgG reactivity to CSP, LSA-1, and MSP-1 antigens was determined by a standard ELISA as described elsewhere.²⁰ The IgG subclass 1-4 reactivity to LSA-1 and MSP-1 was as described for total IgG with minor modifications. Briefly, mouse anti-human monoclonal antibodies diluted in phosphate-buffered saline containing 0.05% Tween-20 and 2.5% nonfat milk powder (PBS-T-M): IgG1 at 1:2,000, IgG2 at 1:6,000, IgG3 at 1:50,000, and IgG4 at 1:20,000 were used as the primary antibodies. After four washes with PBS containing 0.05% Tween-20 (PBS-T), peroxidase-conjugated goat anti-mouse antibodies (Biosource International, Camarillo, CA) diluted at 1:10,000 in PBS-T-M was added and allowed to react for one hour at room temperature. The rest of the procedure was as described for the total IgG ELISA.²⁰ The procedure for evaluating IgG reactivity to TT antigen was similar to that described for total IgG to the malarial antigens CSP and LSA-1,²⁰ except that the coating antigen was used at a concentration of 1 μ g/ml and in 0.01 M PBS, pH 7.2. A posi-

tive control, consisting of pooled hyperimmune sera was included in each experiment. The cut-off values for each antigen were determined by assaying samples from 40 malaria-naive individuals. Samples that showed an optical density (OD) greater than the mean plus two standard deviations of the negative control plasma were scored as positive.

Statistical analyses. To eliminate zeros and negative ELISA OD values, we added the sum of the lowest negative value for each antigen plus 0.001 to each of the corresponding antibody data. The resulting ELISA OD values were log transformed to normalize the data. Analyses were performed using SUDAAN version 8.0, SAS callable version (Research Triangle Institute, Research Triangle Park, NC) and SAS version 8.0 (SAS Institute, Cary, NC). Since in multivariate analysis there was no effect modification by gravida, the data were pooled for all gravidae. To assess differences in mean levels of antibodies (maternal and cord blood) between those from ITN and control villages, analysis of variance methods were implemented. The association between maternal and corresponding cord blood antibody levels and possible differences in transplacental transfer of antibodies between women from the ITN and control villages were assessed through fitting multiple regression models. Log-binomial regression models were used to estimate ratios of antibody prevalence for ITN versus control. Models were fit using GEE methods in the GENMOD procedure in SAS. Since the data were collected as part of a group randomized trial, reported 95% confidence intervals are based on empirical SE estimates obtained by adjusting for clustering at the village level. A two-sided *P* value < 0.05 was considered statistically significant.

RESULTS

Maternal and birth characteristics. Paired maternal/cord blood samples were available from 296 deliveries (ITN = 157, control = 139). The baseline characteristics of the women are shown in Table 1. Overall, the demographic characteristics in women from both intervention and control villages were comparable, with the exception of the number of years of education, which was significantly higher in women living in ITN villages. The prevalence of maternal anemia and parasitemia was lower in women from the ITN villages, although the differences were not statistically significant. The birth characteristics of infants from ITN and control villages were also comparable with the exception of cord blood hemoglobin levels, which were lower in infants from ITN villages (*P* = 0.06) (Table 2).

Antibody response to non-malaria control antigen (TT) and *P. falciparum* antigens. There was no significant difference in the prevalence and levels of total IgG to non-malarial antigen TT in maternal and cord plasma from ITN and control villages (Figure 1A and Table 3). For CSP, only total IgG reactivity was assessed. The frequency and levels of total IgG to CSP were significantly higher in women from ITN villages than in women from control villages. In contrast, there was no significant difference in the prevalence and levels of IgG antibodies to CSP in cord blood samples (Figure 1A and Table 3).

Samples were tested for total IgG and subclasses 1-4 reactivity to the LSA-1 and MSP-1. Since only a few samples showed IgG4 reactivity to LSA-1 and IgG2 and IgG4 reactivity to MSP-1, these data were not included in the final

TABLE 1
Maternal characteristics by intervention group*

Characteristic	ITN (n = 157)	Control (n = 139)	P or OR (95% CI)†
Age in years, mean (SE)	25.1 (0.44)	25.4 (0.54)	0.57
Marital status			
Married, No. (%)	129 (83.8)	110 (82.7)	
Never married, No. (%)	21 (13.6)	19 (14.3)	
Widowed, No. (%)	3 (2.0)	3 (2.3)	
Divorced/separated, No. (%)	1 (0.6)	1 (0.7)	0.99
SES rank score, median (quartiles)‡	52.3 (26.6–71.5)	40.7 (19.8–74.3)	0.65
Years of schooling, median (quartiles)	7.1 (5.3–8.0)	6.4 (5.0–7.5)	0.049
Gravida, median (quartiles)	3.1 (1.5–5.3)	2.8 (1.3–5.4)	0.61
Previous child death, No. (%)§	18 (11.5)	25 (18.0)	0.59 (0.32–1.10)
Height in cm, mean (SE)	50.1 (0.24)	49.9 (0.46)	0.62
Maternal parasite rate at delivery, No. (%)	26 (16.8)	36 (26.1)	0.57 (0.31–1.07)
Hemoglobin level at delivery <11 g/dL, No. (%)	64 (50.0)	66 (60.0)	0.67 (0.44–1.00)

* ITN = insecticide-treated bed net, OR = odds ratio; CI = confidence interval.

† P were determined by t-test for continuous variables reporting mean (SE), non-parametric Wilcoxon rank sum test for continuous variables reporting median (first and third quartiles), OR (95% CI) for dichotomous categorical variables, and Cochran Mantel-Haenszel chi-square test for variables with more than 2 categories.

‡ SES = Socioeconomic status graded according to the rank position of a computed wealth index.

§ Includes deaths among children who were born alive but died before the start of the ITN trial.

analysis. Unlike the antibody responses to CSP, the prevalence and levels of total IgG and IgG subclasses 1–3 to LSA-1 were either significantly or marginally significantly lower in women from ITN villages than in women from control villages (Figure 1B and Table 3). The pattern of cord blood antibody reactivity to LSA-1 was similar to that seen in maternal samples, although the difference was only of marginal statistical significance for total IgG and IgG2 levels and prevalence of total IgG (Figure 1B and Table 3).

Similar to the LSA-1 antigen, the prevalence and levels of IgG3 to the blood stage antigen MSP-1 were significantly lower in maternal and cord plasma from ITN villages than control villages. There was no significant difference in the prevalence and levels of total IgG and IgG1 to MSP-1 antigen in both maternal and cord blood samples from ITN villages and those from control villages (Figure 1C and Table 3).

Association between maternal and cord blood antibody levels. To further examine the effect of ITNs on the association between maternal and cord blood antibody levels, we assessed the slope estimates of ITN and control groups from

a regression model fitting maternal antibodies to cord blood antibodies while controlling for gravida and rainfall 90 days prior to birth. There was a significant positive association between maternal and cord blood total IgG levels to TT, LSA-1, and MSP-1 in both ITN and control villages (Table 4). Furthermore, the slopes for total IgG to these three antigens between ITN and control villages were not significantly different (Table 4). In contrast, there was a significant negative association between maternal and cord blood total IgG levels to CSP in ITN villages and no significant association in control villages (Table 4). The slopes for total IgG to CSP were significantly different between ITN and control villages (Table 4), suggesting that ITNs modified the relationship between maternal and cord blood antibody levels.

DISCUSSION

This study was performed in the context of a cohort study to determine the impact of ITNs on malaria in pregnancy. The purpose of the study was to assess the effect of reduced ma-

TABLE 2
Birth characteristics by intervention group*

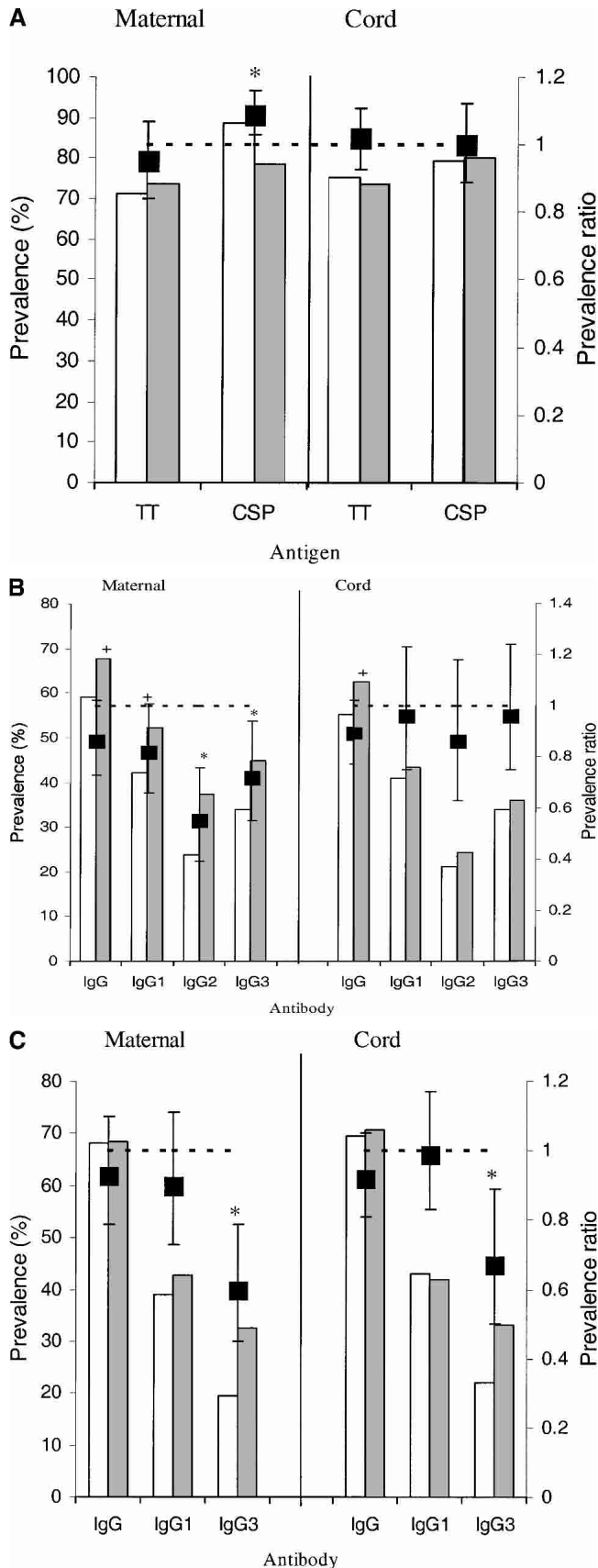
Characteristic	ITN (n = 157)	Control (n = 139)	P or OR (95% CI)
Gestational age at delivery, mean (SE)†	39.9 (0.09)	39.7 (0.18)	0.30
Birth weight in kg, mean (SE)	3.2 (0.02)	3.1 (0.05)	0.11
Birth height in cm, mean (SE)	48.8 (0.51)	48.2 (0.51)	0.41
Birth MUAC in cm, mean (SE)‡	11.3 (0.18)	10.8 (0.2)	0.14
Males, No. (%)	78 (49.7)	61 (43.9)	1.26 (0.92–1.72)
Cord blood parasitemia, No. (%)	4 (2.6)	3 (2.20)	1.19 (0.33–4.37)
Cord blood hemoglobin level in g/dL, mean (SE)	14.9 (0.24)	15.5 (0.25)	0.06
Placenta malaria, No. (%)	20 (13.6)	27 (20.6)	0.61 (0.28–1.32)
Season at delivery			
Driest, No. (%)§	53 (46.0)	46 (47.9)	
Intermediate, No. (%)	44 (38.3)	29 (30.2)	
Wettest, No. (%)	18 (15.7)	21 (21.9)	0.17
Hemoglobin genotype			
AA, No. (%)	97 (76.4)	83 (76.9)	
AS, No. (%)	28 (22.1)	25 (23.2)	
SS, No. (%)	2 (1.6)	0 (0.0)	0.24

* ITN = insecticide-treated bed net; OR = odds ratio; CI = confidence interval.

† Gestational age was assessed using a modified Dubowitz score as described elsewhere.¹⁶

‡ MUAC = mid upper arm circumference.

§ Based on mean daily rainfall 90 days before birth.



alaria parasite exposure on antibody responses to well-characterized *P. falciparum* malaria vaccine candidate antigens and antigenic determinants in paired maternal and cord blood samples. To our knowledge, this is the first study on the impact of ITNs on antibody responses to malaria antigens in pregnant women in an area of intense perennial malaria transmission.

We found lower antibody levels against the MSP-1 and against peptides complementary to the pre-erythrocytic antigen LSA-1 and higher antibody response to the pre-erythrocytic antigen CSP in maternal samples from ITN villages compared with control villages. This effect was not dependent on pregnancy order and was observed in all gravida groups. The lower antibody response to the LSA-1 antigenic determinant is consistent with the antibody reactivity to LSA-1 observed in young children in our cross-sectional surveys,²⁰ and presumably indicates reduced exposure to malaria parasites in women from ITN villages compared with women from control villages. Our observation is also in agreement with a previous study showing a relatively lower prevalence of antibodies to the repeat region of LSA-1 in individuals residing in two areas of low malaria transmission compared with those from an area of moderately higher transmission.²¹

The increased antibody responses to CSP and decreased responses to MSP-1 in this study differ from the immune response we observed in children.²⁰ A possible explanation for these inconsistencies are differences in parasite densities and/or the predominant parasite stage-specific immune effector mechanism in children vs. adults. Several studies have shown that parasite densities decrease as a function of age.^{22,23} In endemic areas, young children are more likely to have high parasite densities compared with adults, who develop protective immunity and are capable of limiting malaria blood stage parasite densities to very low levels.²⁴ This observation has led to the hypothesis that children first develop anti-toxic (disease) immunity, followed by the development of anti-parasite immunity with an increase in age.²⁵ The predominant immune effector mechanisms in adults could mainly be directed at limiting parasite invasion/multiplication at the pre-erythrocytic stage, while in children the mechanisms could be directed at the blood stages, which are respon-

FIGURE 1. **A**, Prevalence of total IgG to tetanus toxoid (TT) and circumsporozoite protein (CSP) in maternal and cord plasma in insecticide-treated bed net (ITN) (solid bars) and control (shaded bars) villages. Solid squares represent the prevalence ratios for ITN and control villages (right y-axis), error bars represent 95% confidence intervals, and the horizontal dotted line represents a prevalence ratio of 1. The asterisk indicates a statistically significant difference between ITN and control villages ($P < 0.05$). **B**, Prevalence of antibodies to liver stage-1 antigen in maternal and cord plasma in (ITN) (solid bars) and control (shaded bars) villages. Solid squares represent the prevalence ratios for ITN and control villages (right y-axis), error bars represent 95% confidence intervals, and the horizontal line represents a prevalence ratio of 1. Asterisks indicate a statistically significant difference between ITN and control villages ($P < 0.05$). Maternal plasma: total IgG; $P = 0.086$, IgG1; $P = 0.059$. Cord plasma: total IgG; $P = 0.104$. **C**, Prevalence of total IgG to merozoite surface protein-1 in maternal and cord plasma in (ITN) (solid bars) and control (shaded bars) villages. Solid squares represent the prevalence ratios for ITN and control villages (right y-axis), error bars represent 95% confidence intervals, and the horizontal line represents a prevalence ratio of 1. Asterisks indicate a statistically significant difference between ITN and control villages ($P < 0.05$).

TABLE 3
Antibody levels to tetanus toxoid (TT) and *Plasmodium falciparum* antigens in maternal and cord plasma*

Antigen	Antibody	Geometric mean†		Ratio of geometric means (95% CI)	P	
		ITN	Control			
TT	IgG	Maternal	0.48	0.54	0.89 (0.64–1.22)	0.448
		Cord	0.49	0.51		
CSP	IgG	Maternal	0.43	0.37	1.13 (1.01–1.26)	0.035
		Cord	0.34	0.31		
LSA-1	IgG	Maternal	0.20	0.28	0.72 (0.56–0.91)	0.009
		Cord	0.18	0.21		
	IgG1	Maternal	0.07	0.09	0.76 (0.59–0.98)	0.035
		Cord	0.06	0.07		
	IgG2	Maternal	0.02	0.03	0.61 (0.45–0.83)	0.001
		Cord	0.02	0.03		
IgG3	Maternal	0.04	0.05	0.83 (0.60–1.14)	0.245	
	Cord	0.05	0.04			
MSP-1	IgG	Maternal	0.27	0.35	0.76 (0.57–1.03)	0.078
		Cord	0.29	0.35		
	IgG1	Maternal	0.07	0.08	0.85 (0.58–1.26)	0.420
		Cord	0.08	0.08		
	IgG3	Maternal	0.09	0.12	0.73 (0.63–0.84)	0.000
		Cord	0.08	0.11		

* ITN = insecticide-treated bed net; CI = confidence interval; CSP = circumsporozoite surface protein; LSA-1 = liver stage antigen-1; MSP-1 = merozoite surface protein-1.
† Geometric means adjusted for gravida and rainfall 90 days prior to birth.

sible for clinical disease. This hypothesis is supported by recent findings in Dielmo, Senegal, suggesting that adults are able to resist a higher sporozoite challenge than are children.²⁶

The observation of increased levels of antibody to CSP in women in the ITN group was surprising, but may be explained by cross-regulation of immune responses by different parasite stages, which may be further complicated by immune suppression during pregnancy. Studies in both humans and animal models have shown that blood stage malaria parasites cause an immunosuppressive effect on the anti-sporozoite antibody response.^{27,28} In addition, a negative correlation has been shown between malaria-induced polyclonal B cell activation and anti-sporozoite antibody responses *in vitro*.²⁹ However, whether and how the higher parasitemia rates resulting from the more intense malaria transmission in control villages lead to immunosuppressive effects on antibody responses to CSP in pregnant women remains unclear and requires further investigation.

Our previous studies conducted in children from the same area have demonstrated that MSP-1₁₉ kD antibody responses, particularly IgG1, are associated with clinical protection.^{30,31} There was no evidence from our epidemiologic investigations that the diminished antibody responses to MSP-1₁₉ kD had any clinical implications. Pregnant women in their first four pregnancies from ITN villages were significantly less likely to have maternal or placental malaria parasitemia resulting in a reduced risk of maternal anemia and increased birth weight in the newborn.¹⁶ It is of note that IgG1 responses were not affected by bed net use, and that only IgG3 responses to MSP-1₁₉ kD were diminished. It is possible that switching antibody subclasses may occur during pregnancy. Consequently, the antibody subclasses to MSP-1 could play different roles in malaria infection in pregnant women, in whom IgG3 may be associated with exposure.

Consistent with other studies, we found that antibody reactivity to the malarial antigens LSA-1 and MSP-1 in cord blood were positively and linearly correlated with the maternal responses^{32,33} and thus lower in cord blood from new-

borns in ITN villages than in control villages. The ITNs did not affect this relationship between maternal/cord blood antibodies to LSA-1 and MSP-1. Interestingly, there was no significant difference in the levels of IgG to CSP in cord blood responses despite the difference observed in maternal plasma. We further observed no association between maternal and cord blood total IgG antibodies to CSP in control villages, which is consistent with other studies.³³ However, we found a negative linear correlation in ITN villages. This difference between ITN and control villages was statistically significant, suggesting that ITNs modified the maternal/cord blood anti-CSP antibody relationship.

It is well accepted that malaria-specific transplacental antibodies play an important role in the passive immune protection in newborns, and concentrations of antibody to MSP-1₁₉ kD at birth have been associated with protection against parasitemia, clinical malaria, and anemia in young infants.^{30,34} Similar to the results in pregnant women,¹⁶ there was no indication from our larger birth cohort analysis that, with continued ITN use, newborns from ITN villages were at an increased risk of malaria morbidity in the first few months of life.³⁵ Follow-up of these newborns showed they had 70% less clinical malaria episodes and anemia than young infants in control villages.³⁵ However, the direct relationship between malaria clinical end points during early and late infancy and

TABLE 4
Comparison of relationship between maternal and cord antibody levels in ITN and control villages*

Antigen	Slope estimates (95% confidence intervals)†		P
	ITN	Control	
TT	0.88 (0.74–1.02)	0.97 (0.83–1.10)	0.3791
CSP	-0.24 (-0.47–-0.008)	0.05 (-0.09–0.20)	0.0377
LSA-1	1.03 (0.76–1.29)	0.94 (0.80–1.09)	0.5898
MSP-1	0.78 (0.61–0.94)	0.84 (0.72–0.96)	0.4853

* ITN = insecticide-treated bed net; TT = tetanus toxoid; CSP = circumsporozoite protein; LSA-1 = liver stage antigen-1; MSP-1 = merozoite surface protein-1.
† Slope estimate represents the association between maternal and cord antibodies on the natural log scale. The model was controlled for gravida and rainfall 90 days prior to birth.

the consequences of the effect of ITNs on transplacental transfer of the different subclasses of antibodies requires further investigation.

In summary, we have observed that the use of ITNs leads to decrease in antibody levels to LSA-1 and MSP-1 antigenic determinants in pregnant women with associated reductions in levels of the same antibodies in cord blood. In contrast, ITN use was found to be associated with increased antibody responses to CSP in pregnant women, but had no effect on antibody levels to CSP in cord blood. The marked improvements in clinical outcomes observed in mother and infant^{15,35} do not suggest that the altered levels of antibodies resulting from ITN use adversely affect the risk of malaria in pregnant women and their newborns.

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Authors' addresses: Simon K. Kariuki, Centre for Vector Biology and Control Research, Kenya Medical Research Institute, PO Box 1578, Kisumu, Kenya. Feiko O. ter Kuile, Kathleen Wannemuehler, Dianne J. Terlouw, Margarette S. Kolczak, William A. Hawley, Penelope A. Phillips-Howard, Altaf A. Lal, and Ya Ping Shi, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Mailstop F-22, 4770 Buford Highway, Atlanta, GA 30341. Alloys SS. Orago, Zoology Department, Kenyatta University, PO Box 43844, Nairobi, Kenya. Bernard L. Nahlen, Roll Back Malaria, World Health Organization, Avenue Appia 20, 1211 Geneva 27, Switzerland.

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