Decreasing Malaria Prevalence and Its Potential Consequences for Immunity in Pregnant Women

Andrew Teo,1,2 Wina Hasang,1,2,3 Louise M. Randall,1,2,3 Gaqian Feng,6 Lauren Bell,4 Holger Unger,1,3 Christine Langer,6 James G. Beeson,6 Peter M. Siba,7 Ivo Mueller,5,8 Malcolm E. Molyneux,9,10 Graham V. Brown,4 and Stephen J. Rogerson1,2,3

1Department of Medicine, 2Victorian Infectious Diseases Service, 3Doherty Institute, 4Nossal Institute for Global Health, University of Melbourne, 5Walter and Eliza Hall Institute of Medical Research, Parkville, and 6Burnet Institute, Melbourne, Australia; 7Papua New Guinea Institute of Medical Research, Goroka; 8Barcelona Center for International Health Research, Spain; 9Malawi-Liverpool-Wellcome Trust Clinical Research Programme College of Medicine, Blantyre; and 10Liverpool School of Tropical Medicine, United Kingdom

Background. As malaria control is intensified, pregnant women may be less exposed to malaria, thus affecting the acquisition of protective antibody.

Methods. Plasma samples were collected from Malawian and Papua New Guinean (PNG) pregnant women enrolled over 7-year periods, during which malaria prevalence fell by over two thirds. Immunoglobulin G (IgG) levels to schizont extract, merozoite antigens, and VAR2CSA-DBL5 were measured by enzyme-linked immunosorbent assay (ELISA). Levels of IgG to variant surface antigens of infected erythrocytes (IEs) and merozoites and levels of opsonizing IgG to IEs were measured by flow cytometry.

Results. In both settings, levels of antibodies in pregnant women to recombinant antigens and to intact IEs but not of opsonizing antibodies decreased over time. After adjustment for coverage with insecticide-treated bed nets (ITNs), these differences disappeared in the Malawian cohort, whereas in the PNG cohort, time was independently associated with a decrease in several antibody responses measured by ELISA.

Conclusions. The impact of falling parasite prevalence on anti-Plasmodium falciparum serological indicators in pregnant women varies by setting. Increased ITN coverage may affect development of antibodies to recombinant antigens, but levels of opsonizing IgG remained stable over time. Opsonizing IgG against placental-binding IEs may persist, thus offering longer-lasting protection against malaria during pregnancy.

Keywords. Plasmodium falciparum; pregnancy; acquisition; antibody; longer-lasting; Malawi; Papua New Guinea.

Plasmodium falciparum malaria remains entrenched in sub-Saharan Africa and the Pacific Islands. Women in their first pregnancy are particularly susceptible to malaria [1, 2], in part because of the ability of P. falciparum–infected erythrocytes (IEs) to sequester within the placenta [3, 4]. Placental-binding IEs express different variant surface antigens (VSA) from IEs that cause malaria in childhood, so they are not recognized by antibodies conferring existing immunity to malaria [5, 6].

Malaria in pregnancy (MiP) increases the risk of maternal anemia at delivery and low birth weight, which are important causes of maternal and infant mortality [7–9]. Antibodies to placental-binding IEs are associated with protection against MiP and are acquired in a gravidity-dependent manner [1, 6, 10]. Development of these antibodies is influenced by transmission intensity, human immunodeficiency virus (HIV) infection, and the use of malaria-prevention strategies, including insecticide-treated bed nets (ITNs) [11–14].

As malaria exposure declines, immunity may decrease. This is indirectly supported by data showing that, as infection prevalence fell in Malawian pregnant women, parasite densities increased among infected women [15]. Taken together, if exposure to MiP falls, antibody to placental-binding IEs may be less
developed, susceptibility to MiP may spread into later gravidi-
ties, and among the infants who are infected, the consequences
may be more severe [16].

Malaria prevalence in pregnancy has declined substantially in
Malawi and Papua New Guinea (PNG), and we hypothesized
that this is associated with reduced levels of pregnancy-specific
malaria immunity but not with immunity to non–pregnancy-
associated antigens.

MATERIALS AND METHODS

Ethics Approval
Ethics approval was obtained from the College of Medicine Re-
search Ethics Committee, University of Malawi (P99/00/91R,
P00/01/107), the PNG Institute of Medical Research Institu-
tional Review Board (08.15), the PNG Medical Research Advi-
sory Council (05.03, 10.50), and the Human Research Ethics
Committee of Melbourne Health (2001.016, 2008.162). All par-
ticipants provided informed written consent.

Study Sites and Participants
Plasma samples came from 2 cohorts. In Malawi, pregnant
women were recruited at delivery between 1999 and 2006
from the maternity unit of Queen Elizabeth Central Hospital
in Blantyre, as previously described [15]. Women were classi-
ﬁed on the basis of enrollment time into early (enrollment years,
1999–2000; parasite prevalence, 25.2%) and late (enrollment
years, 2004–2006; parasite prevalence, 6.2%) groups. In PNG.
pregnant women were recruited at the ﬁrst antenatal care
(ANC) visit and followed to delivery at rural clinics in Madang
Province between 2005 and 2012. Women were classiﬁed on
the basis of enrollment time into early (enrollment years,
2005–2007; parasite prevalence, 18.0%) and late (enrollment
years, 2010–2012; parasite prevalence, 3.1%) groups. In the lat-
ter period, women were randomized to receive 3 courses of
intermittent preventive treatment (IPTp) with sulfadoxine-
pyrimethamine (SP) and azithromycin (AZ) during pregnancy.
In PNG, women were recruited at delivery between 1999 and
2006 from the maternity unit of Queen Elizabeth Central Hospital
in Blantyre, as previously described [15]. Women were classi-
ﬁed on the basis of enrollment time into early (enrollment years,
1999–2000; parasite prevalence, 25.2%) and late (enrollment
years, 2004–2006; parasite prevalence, 6.2%) groups. In PNG.
pregnant women were recruited at the ﬁrst antenatal care
(ANC) visit and followed to delivery at rural clinics in Madang
Province between 2005 and 2012. Women were classiﬁed on
the basis of enrollment time into early (enrollment years,
2005–2007; parasite prevalence, 18.0%) and late (enrollment
years, 2010–2012; parasite prevalence, 3.1%) groups. In the lat-
ter period, women were randomized to receive 3 courses of
intermittent preventive treatment (IPTp) with sulfadoxine-
pyrimethamine (SP) and azithromycin (AZ) during pregnancy.

Samples were collected at delivery (and at the ﬁrst ANC visit
in PNG), and malaria parasite infection was deﬁned as the de-
tection of parasites by concurrent peripheral or placental blood
microscopy. Because current infection increases antibody re-
sponses [17], only samples from uninfected women were select-
ed. We did not study primigravidae because, when uninfected,
they have very low levels of antibodies against placental-binding
IEs [17]. All available samples from women experiencing preg-
nancies 2–4 were used.

In the PNG cohort, paired enrollment and delivery samples
were tested together, to determine whether declining exposure
impairs the acquisition of antibody over the course of
pregnancy. Samples were tested in duplicate and were random-
ized to minimize testing samples from the same year together.

Parasite and Cell Cultures
The laboratory-adapted P. falciparum lines CS2 (placental
binding) and E88-ICAM (endothelial binding) were cultured
as described elsewhere [18]. THP-1 monocyte-like cells [19]
were cultured as previously described [20].

Assays of Immunoglobulin G (IgG) to Schizont Extract,
Merozoite Antigens, and VAR2CSA-DLB5
Antibody responses to recombinant P. falciparum antigens were
measured by enzyme-linked immunosorbent assay (ELISA),
using established methods [21]. Microtiter plates were coated
with 50 µL of individual targets diluted in phosphate-buffered
saline (PBS) at the following dilutions and concentrations:
schizont extract [22] from CS2 IEs, 1:2000 dilution; MSP2
from FC27, 0.5 µg/mL [21]; MSP3 from 3D7 full ectodomain,
2 µg/mL [23]; PfRh2 (construct PfRh2–2030 from 3D7), 0.5
µg/mL [24]; and VAR2CSA-DLB5, from 3D7, 0.5 µg/mL [25,
26]. Plasma was added in duplicate at a 1:1000 dilution. A stan-
dard curve generated from serial dilution of our positive control
(pooled from 44 pregnant women with high antibody respons-
es) was used to convert ODs into antibody levels represented by
arbitrary units, where the positive control is equivalent to 100 U.

Merozoite Phagocytosis Assay
Antibody opsonic phagocytosis of merozoites was performed
using a recently developed assay (Osier, Feng, and Beeson, un-
published material). Whole merozoites were obtained as de-
cscribed elsewhere [27]. In brief, merozoites were stained with
10 µg/mL ethidium bromide (Sigma-Aldrich) for 30 minutes
and washed thrice with 2200 × g for 5 minutes [27]. The cell den-
sity was determined using relative counting against Count-
Bright Absolute Counting Beads (Invitrogen) as per the
manufacturer’s protocol. The merozoites were resuspended at
5 × 107 merozoites/mL in Roswell Park Memorial Institute
1640 medium (RPMI)–HEPES, and 30 µL of suspension was
opsonized with 3.5 µL of plasma (1:250 dilution) in newborn
calf serum (NCS; Gibco)–coated 96-well U-bottomed plates
for 1 hour in the dark. The cells were washed thrice, resuspend-
ed in 150 µL of THP-1 medium (RPMI supplemented with 10%
fetal bovine serum, 1% penicillin-streptomycin-glutamine, and
25 mM HEPES [Gibco]), and 50 µL of suspension was trans-
ferred in duplicate into fresh NCS-coated 96-well U-bottom
plates, followed by addition of 100 µL of THP-1 cells at
5 × 105 cells/mL (ratio of target to effector, 10:1). The cells
were incubated in 5% humidified CO2 at 37°C for 10 minutes.
Phagocytosis was stopped by centrifugation at 4°C at 350 ×
g for 5 minutes and washed thrice with FACS buffer. THP-1 cells
were ﬁxed in 2% paraformaldehyde (PFA) in PBS before acquisi-
tion with a FACScantorII flow cytometer (BD Biosciences).

The background level of nonspeciﬁc phagocytosis observed
with the no-plasma (negative) control was set to be <5%, and the data are specified as the percentage of THP-1 cells that ingested free merozoites.

Measuring Levels of IgG to VSAs
Total IgG levels against VSAs expressed on CS2 and E8B-ICAM IEs was measured as described elsewhere [28], with slight modifications. In brief, trophozoite-stage IEs at 4%–8% parasitemia were washed thrice with 1% NCS in PBS, resuspended at 0.2% hematocrit in PBS/NCS, and incubated for 30 minutes with test plasma (1:20 dilution, in duplicate) in NCS-coated 96-well U-bottomed plates at room temperature. IEs were washed, incubated with rabbit anti-human IgG (1:100 dilution, Dako), and washed 3 times. IEs were incubated in the dark with AlexaFluor 647 donkey anti-rabbit IgG (1:500 dilution, Invitrogen) containing 10 µg/mL ethidium bromide. IEs were washed and resuspended in 2% PFA. The data are specified as the relative geometric mean fluorescence intensity (MFI), calculated as the percentage of the MFI of a positive (hyperimmune pooled sera) control remaining after subtraction of the MFI of a negative (CSA-DBL5) control. The mean value ± 3SDs for negative controls was set to be <5%, and the data are specified as the percentage of THP-1 cells that ingested free merozoites.

Opsonic Phagocytosis Assay
We improved our previously established phagocytosis assay [20] to measure opsonic antibody responses to CS2 and E8B-ICAM IEs. In summary, trophozoite-stage IEs were purified by density gradient centrifugation [20], stained with 10 µg/mL ethidium bromide, washed thrice, and resuspended at 1.67 × 10⁷ IEs/mL. Thirty microliters of the IE suspension were then opsonized with 3.3 µL (1:10 dilution) of plasma in NCS-coated 96-well U-bottomed plates for 1 hour in the dark. IEs were washed thrice, resuspended in 50 µL of THP-1 medium, and aliquoted in duplicate into NCS-coated 96-well U-bottomed plates, and THP-1 cells (25 µL at 5 × 10⁵ cells/mL; ratio of target to effector, 10:1) were added. The cells were incubated at 37°C in 5% humidified CO₂ for 40 minutes. Phagocytosis was stopped, and unphagocytosed IEs were lysed [20]. THP-1 cells were washed and fixed in 2% PFA [20]. Results were represented as the percentage of THP-1 cells that ingested IEs. A sample caused phagocytosis when ingestion was greater than the mean value + 3SDs for negative controls.

Assays for total IgG and opsonic IgG to IEs were performed using a HyperCyt CyAn flow cytometer (Beckman Coulter), and in each assay discordant samples were reanalyzed using our published rules [28].

Antibody Responses in the PNG Cohort
To assess whether antibody levels changed between enrollment and delivery, we used the antibody levels for each sample and used our published rules to resolve discordant results [28]. Samples with antibody levels exhibiting an adjusted mean variance of <20% and a mean difference of <10% between enrollment and delivery were categorized as having unchanged antibody responses, whereas samples with an adjusted mean variance of >20% and a mean difference of >10% were classified as having either decreased or increased antibody responses.

Statistical Analyses
Data obtained from assays were combined with clinical information about the study participants and were analyzed using Stata v11.2 (Stata). In some instances, analyses were done in GraphPad Prism v5 (GraphPad Software).

The Mann–Whitney U test was performed on continuous nonparametric variables, and categorical variables were assessed using χ² tests. Multiple linear regression models were performed to determine the association between continuous and categorical variables. Potential confounders, including gravidity, age, IPTp and ITN use, and maternal characteristics, were included.

RESULTS
Study Populations Characteristics
In Malawi, samples from 184 pregnant women enrolled early and 148 women enrolled late were assayed for antibody responses against P. falciparum antigens (Table 1). At recruitment, women enrolled later were significantly heavier than the women enrolled earlier. The percentage of secundigravid women varied between 31.5% (early) and 46.6% (late; *P* = .005). In the PNG cohort, samples from 131 pregnant women who were recruited early and 281 women recruited late were used (Table 2). At the first ANC visit, women enrolled later were significantly older and had a greater mid-upper-arm circumference. Use of ITNs and malaria preventive drugs were significantly higher in women enrolled late (Table 2).

Antibody Responses Against Schizont Extract, Merozoite Antigens, and VAR2CSA-DBL5
To determine malaria exposure, we assayed the antibody response to schizont extract. In both cohorts, women enrolled later exhibited significantly lower levels of antibody to schizont extract, indicative of reduced exposure. Prior studies have identified the merozoite antigens MSP2, MSP3, and PfRh2 [21, 23, 24] as targets of antibodies associated with immunity in nonpregnant individuals and VAR2CSA-DBL5 [29] as a highly immunogenic target associated with protection from low birth weight. Antibody responses to merozoite antigens and VAR2-CSA-DBL5 were significantly lower in later-enrolled women in both study population (Figures 1A and 2A). A similar significant decrease over time was observed in plasma samples collected at the first ANC visit in the PNG cohort (data not shown).
Opsonic Antibody Responses Against Whole Merozoites

The difference in antibody responses to each merozoite antigen observed in the PNG cohort led to the evaluation of the opsonic IgG response to whole merozoites. We restricted this analysis to delivery plasma from women in second and third pregnancies because the assays are resource intensive. Although women recruited later had lower IgG antibody responses to merozoite antigens, levels of opsonic IgG to whole merozoites did not vary over time ($Z = 0.6$; $P = 0.5$; Figure 2B).

Total Antibodies Levels Against Infected Erythrocytes

In Malawian women, median levels of anti-VSA IgG antibody to E8B-ICAM declined over time ($P < 0.01$), but responses against CS2 did not vary (Figure 1B). In PNG, analysis of the same subset of women for opsonic IgG responses against whole merozoites revealed that median levels of anti-VSA IgG antibodies to both E8B-ICAM and CS2 declined over time ($P < 0.01$; Figure 2C).

Opsonic Antibodies Against Infected Erythrocytes

Levels of opsonic IgG responses against E8B-ICAM and CS2 did not vary significantly over time in either study population (Figures 1C and 2D). In Malawi, the proportion of women with opsonic IgG antibodies to CS2 (early group, 85.2%; late group, 88.7%; $P = 0.3$) or E8B-ICAM (early group, 96.7%; late group, 96.0%; $P = 0.7$) did not differ between the 2 enrollment periods. In PNG, similar results were observed for CS2 (early group, 72.4%; late group, 63.0%; $P = 0.08$) and E8B-ICAM (early group, 90.8%; late group, 94.1%; $P = 0.2$). To determine whether differences in antibody titers were obscured by performing assays at concentrations above the saturation level, pooled plasma samples from each cohort and enrollment period were titrated. No significant differences in the dilution curves generated were observed (data not shown).

Analysis of Antibodies Against P. falciparum Antigens, Adjusted for Confounders

The changes in antibody responses over time, adjusted for confounding and interaction variables, are presented in Tables 3 and 4. In the Malawian cohort, ITN use was associated with lower levels of IgG to schizont extract. The levels of IgG antibodies to MSP2, MSP3, VAR2CSA-DBL5e, and VSA-CS2 were significantly lower as maternal weight increased. Additionally, levels of opsonic IgG against CS2 increased with gravidity, and women enrolled later who received a single dose

---

### Table 1. Study Population Characteristics of Malawian Women, by Enrollment Period

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>26.0 (21.0–27.0)</td>
<td>24.0 (21.0–26.0)</td>
<td>0.2</td>
</tr>
<tr>
<td>Maternal weight, kg</td>
<td>55.0 (51.0–60.0)</td>
<td>57.0 (53.0–65.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>Maternal BMI</td>
<td>23.0 (21.2–25.8)</td>
<td>23.7 (22.2–25.6)</td>
<td>0.5</td>
</tr>
<tr>
<td>Gravida 2</td>
<td>58 (31.5)</td>
<td>69 (46.6)</td>
<td></td>
</tr>
<tr>
<td>Gravida 3</td>
<td>89 (48.4)</td>
<td>54 (36.5)</td>
<td></td>
</tr>
<tr>
<td>Gravida 4</td>
<td>37 (20.1)</td>
<td>25 (16.9)</td>
<td></td>
</tr>
<tr>
<td>Bed net user</td>
<td>35 (19.0)</td>
<td>81 (54.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Received SPa</td>
<td>0 doses 20 (10.9)</td>
<td>11 (7.4)</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>1 doses 84 (45.7)</td>
<td>55 (37.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 doses 63 (34.2)</td>
<td>56 (37.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥3 doses 15 (18.6)</td>
<td>35 (17.6)</td>
<td>0.5</td>
</tr>
<tr>
<td>Hb level at delivery, g/dL, mean ± SD</td>
<td>11.9 (1.7)</td>
<td>11.8 (1.8)</td>
<td>0.5</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>3.1 (2.8–3.3)</td>
<td>3.1 (2.9–3.4)</td>
<td>0.8</td>
</tr>
<tr>
<td>Parasite prevalence, women, %</td>
<td>25.2</td>
<td>6.8</td>
<td></td>
</tr>
</tbody>
</table>

Data are median (interquartile range) or no. (%) of women, unless otherwise indicated. $P$ values of <0.05 were considered statistically significant.

Abbreviations: Hb, hemoglobin; SP, sulfadoxine-pyrimethamine.

a During pregnancy.

b Prevalence of placental malaria at each time point, as identified by light microscopy of placental blood smear (overall incidence of placental malaria at each time point).

### Table 2. Study Population Characteristics of Papua New Guinean Women, by Enrollment Period

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>26 (24–29)</td>
<td>27 (24–30)</td>
<td>0.02</td>
</tr>
<tr>
<td>Maternal weight, kg</td>
<td>53.0 (50.0–57.0)</td>
<td>53.0 (48.0–59.0)</td>
<td>0.2</td>
</tr>
<tr>
<td>Mid-upper-arm circumference, cm</td>
<td>22.0 (21.0–23.0)</td>
<td>23.0 (22.0–25.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gravida 2</td>
<td>44 (33.6)</td>
<td>95 (33.8)</td>
<td></td>
</tr>
<tr>
<td>Gravida 3</td>
<td>52 (39.7)</td>
<td>96 (34.2)</td>
<td></td>
</tr>
<tr>
<td>Gravida 4</td>
<td>35 (26.7)</td>
<td>80 (32.0)</td>
<td></td>
</tr>
<tr>
<td>Bed net user</td>
<td>80 (61.0)</td>
<td>264 (94.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Received SPa</td>
<td>No 7 (5.3)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes 123 (94.7)</td>
<td>277 (100)</td>
<td></td>
</tr>
<tr>
<td>Hb level at delivery, g/dL, mean ± SD</td>
<td>9.2 ± 1.6</td>
<td>10.0 ± 1.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>2.6 (2.9–3.7)</td>
<td>3.1 (2.8–3.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>Parasite prevalence, women, %</td>
<td>18.0</td>
<td>3.1</td>
<td></td>
</tr>
</tbody>
</table>

Data are median (interquartile range) or no. (%) of women, unless otherwise indicated. $P$ values of <0.05 were considered statistically significant.

Abbreviations: Hb, hemoglobin; SP, sulfadoxine-pyrimethamine.

a During pregnancy.

b Prevalence of placental malaria at each time point, as identified by light microscopy of placental blood smear (overall incidence of placental malaria at each time point).
of SP had significantly lower levels of IgG antibody to MSP2 (Table 3).

In the PNG cohort, later enrollment was associated with significantly lower levels of IgG antibodies to schizont extract, MSP2, and MSP3 in the adjusted analyses. ITN use was associated with lower levels of IgG to schizont extract and MSP2. There was an important interaction between enrollment period and ITN use, such that women who enrolled later and who used an ITN had particularly low levels of IgG to MSP2 (Table 4).

Changes in Antibody Responses Between the First ANC Visit and Delivery

We compared differences in antibody responses between the first ANC visit and delivery in the PNG cohort of women enrolled early or late. There was significantly more change between enrollment and delivery values in the early group, compared with the late group, for antibody responses to schizont extract, MSP2, MSP3, PfRh2, and VAR2CSA-DBL5. Interestingly, women enrolled in the later period had an increased variability in levels of opsonizing antibody to CS2, which was largely confined to women in the SP and AZ treatment arm. In contrast, significantly more women who received SP and CQ had increases in their levels of opsonizing antibodies to E8B-ICAM, compared with women who received SP and AZ, Table 5.

DISCUSSION

As malaria control intensifies, it is important to understand its impact on the development and maintenance of naturally acquired immunity to malaria during pregnancy. Examination of a range of antibody responses in 2 different settings revealed evidence of lower levels of humoral immunity in the more recent cohorts of pregnant women since intensification of malaria-control interventions, particularly in PNG. However, our results suggest that any decline in immunity with reduction in malaria transmission was modest and not evident across all measures of humoral immunity and that established immunity may be maintained for intervals at least as long as the time frame in our study. Although levels of some malaria antibodies measured by ELISA decreased, levels of opsonizing antibodies

Figure 1. Levels of immunoglobulin G (IgG) antibody to Plasmodium falciparum antigens over time in pregnant Malawian women. White bars are data for pregnant women enrolled between 1999 and 2000 (early group), and gray bars are data for pregnant women enrolled between 2004 and 2006 (late group). A, Levels of IgG antibody to schizont extract, PfRh2, MSP2, and VAR2CSA-DBL5 (175 from the early group and 145 from the late group). Antibody levels are standardized using positive and negative controls and are presented as arbitrary units. B, Levels of IgG antibody to endothelial-binding and placental-binding variant surface antigens (VSAs; 141 from the early group and 124 from the late group). Data are presented as mean fluorescence intensity relative to the positive and negative controls. C, Levels of opsonic IgG antibody to VSAs of both endothelial-binding and placental-binding infected erythrocytes (IEs; 172 in the early group and 139 in the late group). Data are presented as the percentage of THP-1 cells that have ingested IEs. *P<.05 and **P<.01 by the Mann-Whitney U test. Error bars show 95% confidence intervals. Abbreviation: Etbr, ethidium bromide.
to intact parasites or IEs uniformly did not. Both settings experienced similar decreases in parasite prevalence, associated with increases in use of ITN and IPTp. Constant exposure may be more crucial in maintaining the levels of antibodies to specific antigenic determinants, such as the recombinant antigens used in our ELISAs, than to intact merozoites or IEs [17, 25]. In PNG women, a decline over time in total IgG levels to IEs may be due to reduced malaria exposure, suggesting that repeated exposure may be required to maintain antibodies against malaria [30, 31]. On the other hand, the development of opsonizing antibodies may be a better surrogate for protective immunity in areas with lower transmission.

Antibody responses to merozoite antigens are short-lived in young children [32, 33] but were better sustained in pregnant women from Papua New Guinea (PNG) women. Levels of immunoglobulin G (IgG) to Plasmodium falciparum antigens over time in pregnant women from PNG women. White bars are data for pregnant women enrolled between 2005 and 2007 (early group), and gray bars are data for pregnant women enrolled between 2010 and 2012 (late group). A, Levels of IgG antibody to schizont extract, PfRh2, MSP2, MSP3, and VAR2CSA-DBL5 (118 in the early group and 265 in the late group). Antibody levels are standardized using positive and negative controls and are presented as arbitrary units. B, Levels of opsonic IgG antibody to whole merozoites (85 in the early group and 95 in the late group). Data are presented as the percentage of THP-1 cells that have ingested whole merozoites. C, Levels of IgG antibody to endothelial-binding and placental-binding variant surface antigens (VSAs; the median level of IgG to placental-binding infected erythrocytes [IEs] is 0 for the gray bar; 85 in the early group and 95 in the late group). D, Levels of opsonic IgG antibody to VSAs that were or were not pregnancy specific (119 in the early group and 263 in the late group). Data are presented as the percentage of THP-1 cells that have ingested IEs. **P < .01, ***P < .001, ****P < .0001 by the Mann–Whitney U test. Error bars show 95% confidence intervals. Abbreviation: Etbr, ethidium bromide.
Table 3. Associations Between Time of Enrollment, Maternal Characteristics, Malaria-Prevention Strategies, and Antibody Responses to *Plasmodium falciparum* Antigens in Malawian Women

<table>
<thead>
<tr>
<th>Variables</th>
<th>IgG Schizont Extract</th>
<th>IgG RH2A9</th>
<th>IgG MSP2</th>
<th>IgG MSP3</th>
<th>IgG VAR2CSA-DBL5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coeff (95% CI)</td>
<td>P Value</td>
<td>Coeff (95% CI)</td>
<td>P Value</td>
<td>Coeff (95% CI)</td>
</tr>
<tr>
<td>Early and late enrollment</td>
<td>−7.6 (−27.3 to 12.1)</td>
<td>.4</td>
<td>−7.1 (−23.5 to 9.3)</td>
<td>.4</td>
<td>12.8 (−4.3 to 29.8)</td>
</tr>
<tr>
<td>Maternal weight</td>
<td>−0.3 (−6 to 1)</td>
<td>.1</td>
<td>−0.02 (−3 to .3)</td>
<td>.9</td>
<td>−0.4 (−7 to −2)</td>
</tr>
<tr>
<td>Gravida</td>
<td>−1.7 (−5.7 to 2.2)</td>
<td>.4</td>
<td>−1.3 (−4.7 to 2.0)</td>
<td>.4</td>
<td>−1.6 (−5.7 to 11.9)</td>
</tr>
<tr>
<td>Bed net use</td>
<td>−12.3 (−22.3 to −2.2)</td>
<td>.02</td>
<td>−6.2 (−14.8 to 2.3)</td>
<td>.2</td>
<td>−4.7 (−13.6 to 4.1)</td>
</tr>
<tr>
<td>SP dose</td>
<td>1.0 (−8.9 to 11.0)</td>
<td>.8</td>
<td>0.6 (−7.9 to 9.0)</td>
<td>.9</td>
<td>3.1 (−5.7 to 11.9)</td>
</tr>
<tr>
<td>Late enrollment and bed net use</td>
<td>10.1 (−2.6 to 22.9)</td>
<td>.1</td>
<td>6.6 (−4.2 to 17.4)</td>
<td>.2</td>
<td>1.3 (−10.0 to 12.5)</td>
</tr>
<tr>
<td>Late enrollment and SP dose</td>
<td>2.7 (−18.1 to 23.4)</td>
<td>.8</td>
<td>2.6 (−14.6 to 19.8)</td>
<td>.8</td>
<td>−20.4 (−38.3 to −2.5)</td>
</tr>
<tr>
<td>1</td>
<td>4.1 (−17.1 to 25.4)</td>
<td>.7</td>
<td>3.8 (−13.8 to 21.4)</td>
<td>.7</td>
<td>−15.5 (−33.8 to 2.8)</td>
</tr>
<tr>
<td>2</td>
<td>8.3 (−18.7 to 35.2)</td>
<td>.5</td>
<td>5.3 (−17.3 to 27.9)</td>
<td>.6</td>
<td>1.5 (−22.0 to 25.0)</td>
</tr>
<tr>
<td>SP dose</td>
<td>7.1 (−3 to 14.6)</td>
<td>.06</td>
<td>−1.0 (−6.7 to 4.7)</td>
<td>.7</td>
<td>0.5 (−12.9 to 13.9)</td>
</tr>
<tr>
<td>Late enrollment and bed net use</td>
<td>5.3 (−9.1 to 19.6)</td>
<td>.5</td>
<td>10.7 (−8.2 to 22.2)</td>
<td>.07</td>
<td>9.2 (−8.9 to 27.3)</td>
</tr>
</tbody>
</table>

Data were calculated using multiple linear regression models. A positive coefficient implies an increase of antibody levels. A negative coefficient implies a decrease of antibody levels. *P* values of <.05 were considered statistically significant.

Abbreviations: CI, confidence interval; Coeff, coefficient; IgG, immunoglobulin G; SP, sulfadoxine-pyrimethamine.
Table 4. Associations Between Time of Enrollment, Maternal Characteristics, Malaria-Prevention Strategies, and Antibody Responses to *Plasmodium falciparum* Antigens in Papua New Guinean Women

<table>
<thead>
<tr>
<th>Variables</th>
<th>IgG-Schizont Extract Coeff (95% CI)</th>
<th>P Value</th>
<th>IgG-RH2A9 Coeff (95% CI)</th>
<th>P Value</th>
<th>IgG-MSP2 Coeff (95% CI)</th>
<th>P Value</th>
<th>IgG-MSP3 Coeff (95% CI)</th>
<th>P Value</th>
<th>IgG-VAR2CSA DBL5 Coeff (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early and late enrollment</td>
<td>−49.8 (−70.6 to −29.1)</td>
<td>&lt;.0001</td>
<td>2.0 (−19.6 to 23.6)</td>
<td>.9</td>
<td>−49.6 (−74.4 to −24.9)</td>
<td>&lt;.0001</td>
<td>−26.4 (−5.4 to .5)</td>
<td>.05</td>
<td>−26.4 (−56.0 to 3.3)</td>
<td>.08</td>
</tr>
<tr>
<td>Age</td>
<td>0.2 (−.4 to .7)</td>
<td>.5</td>
<td>0.2 (−.4 to .8)</td>
<td>.5</td>
<td>0.2 (−.4 to .9)</td>
<td>.5</td>
<td>0.1 (−.8 to .5)</td>
<td>.7</td>
<td>0.1 (−.6 to .9)</td>
<td>.7</td>
</tr>
<tr>
<td>Mid-upper-arm circumference</td>
<td>0.3 (−.8 to 1.3)</td>
<td>.5</td>
<td>−0.1 (−1.2 to 1.2)</td>
<td>1.0</td>
<td>−0.01 (−1.2 to 1.2)</td>
<td>1.0</td>
<td>0.2 (−1.0 to 1.4)</td>
<td>.7</td>
<td>−0.5 (−1.8 to .8)</td>
<td>.5</td>
</tr>
<tr>
<td>Bed net use</td>
<td>−10.0 (−18.7 to −1.1)</td>
<td>.03</td>
<td>−4.8 (−13.9 to 4.4)</td>
<td>.3</td>
<td>−19.1 (−29.6 to −8.6)</td>
<td>&lt;.0001</td>
<td>0.4 (−10.0 to 10.8)</td>
<td>.9</td>
<td>−6.0 (−17.5 to 5.6)</td>
<td>.3</td>
</tr>
<tr>
<td>SP use</td>
<td>15.0 (−3.3 to 33.3)</td>
<td>.1</td>
<td>0.1 (−19.0 to 19.1)</td>
<td>1.0</td>
<td>16.4 (−5.5 to 38.2)</td>
<td>.1</td>
<td>1.1 (−20.5 to 22.8)</td>
<td>.9</td>
<td>11.8 (−17.5 to 5.5)</td>
<td>.3</td>
</tr>
<tr>
<td>Late enrollment and bed net use</td>
<td>18.3 (−3.3 to 39.8)</td>
<td>.09</td>
<td>−10.9 (−33.3 to 11.5)</td>
<td>.3</td>
<td>28.1 (2.4 to 53.7)</td>
<td>.03</td>
<td>3.5 (−24.3 to 31.3)</td>
<td>.8</td>
<td>11.3 (−11.9 to 35.6)</td>
<td>.5</td>
</tr>
<tr>
<td>Opsonic IgG Whole Merozoite</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early and late enrollment</td>
<td>−5.5 (−14.0 to 3.1)</td>
<td>.2</td>
<td>−3.1 (−7.4 to 1.3)</td>
<td>.2</td>
<td>−11.6 (−54.0 to 30.8)</td>
<td>.6</td>
<td>9.9 (−22.7 to 42.5)</td>
<td>.6</td>
<td>−8.8 (−37.6 to 20.0)</td>
<td>.5</td>
</tr>
<tr>
<td>Age</td>
<td>−0.2 (−1.1 to .7)</td>
<td>.7</td>
<td>−0.02 (−4.4 to .8)</td>
<td>.9</td>
<td>−0.2 (−.9 to .5)</td>
<td>.6</td>
<td>−0.1 (−.9 to .7)</td>
<td>.8</td>
<td>−0.1 (−.8 to .6)</td>
<td>.8</td>
</tr>
<tr>
<td>Mid-upper-arm circumference</td>
<td>0.9 (−9.2 to 2.6)</td>
<td>.3</td>
<td>0.2 (−4.4 to 8.4)</td>
<td>.4</td>
<td>0.1 (−1.3 to 1.5)</td>
<td>.9</td>
<td>−1.0 (−2.4 to .5)</td>
<td>.2</td>
<td>0.2 (−1.1 to 1.4)</td>
<td>.8</td>
</tr>
<tr>
<td>Bed net use</td>
<td>0.4 (−10.5 to 11.3)</td>
<td>.9</td>
<td>3.4 (−4.4 to 8.8)</td>
<td>.05</td>
<td>4.3 (−3.6 to 12.2)</td>
<td>.3</td>
<td>−3.7 (−16.3 to 8.8)</td>
<td>.6</td>
<td>2.7 (−8.4 to 13.8)</td>
<td>.6</td>
</tr>
<tr>
<td>SP use</td>
<td>6.8 (−10.5 to 11.3)</td>
<td>.5</td>
<td>0.6 (−35.4 to 4.8)</td>
<td>.8</td>
<td>−0.2 (−12.3 to 8.5)</td>
<td>.7</td>
<td>−5.4 (−16.3 to 8.8)</td>
<td>.7</td>
<td>−0.02 (−23.1 to 23.0)</td>
<td>1.0</td>
</tr>
<tr>
<td>Late enrollment and bed net use</td>
<td>. . .</td>
<td></td>
<td>. . .</td>
<td></td>
<td>−2.8 (−44.2 to 38.6)</td>
<td>.9</td>
<td>−7.2 (−40.8 to 26.3)</td>
<td>.7</td>
<td>4.3 (−25.3 to 34.0)</td>
<td>.8</td>
</tr>
</tbody>
</table>

Data were calculated using multiple linear regression models. A positive coefficient implies an increase of antibody levels. A negative coefficient implies a decrease of antibody levels. P values of <.05 were considered statistically significant.

Abbreviations: CI, confidence interval; Coeff, coefficient; IgG, immunoglobulin G; SP, sulfadoxine-pyrimethamine.
women [25], and their prevalence is influenced by transmission intensity [34–36]. In the PNG cohort, a decrease in merozoite antibodies, as detected by ELISA, contrasted with no change in the levels of opsonic IgG against whole merozoites. This is the first assessment of changes in these functional opsonizing antibodies over time, and it suggests that opsonic IgG to merozoites may be more stable than antibodies to individual merozoite antigens. Given that opsonic IgG was recently correlated with protection from clinical malaria in children [37], persistence of these responses may be critical for maintenance of functional protective immunity in the face of declining transmission.

Of clinical importance for the development of a vaccine targeting pregnancy-specific VSAs [38], levels of IgG against placental-binding IEs declined in the PNG cohort only, while levels of opsonic IgG to placental-binding IEs did not change in either cohort. The stability of the latter may be important given that opsonic IgG has been associated with protection against maternal anemia [39] and low birth weight [40].

Adhesion-inhibiting antibodies are also associated with protection from complications of MiP [41]. We did not measure the ability of plasma to inhibit adhesion of IEs, but antibodies to VAR2CSA-DBL5ɛ may inhibit adhesion of IEs to placental cells (although results are conflicting [26, 42]). Pregnant women have a wide repertoire of antibodies against the VAR2CSA-DBL5ɛ domain, and these appear to be cross-reactive against placental isolates [16, 43]. Given that levels of IgG against VAR2CSA-DBL5ɛ were lower in the later time points in both cohorts, this may indicate reduced ability to block placental sequestration of IEs at the population level. IgG against VAR2CSA-DBL5ɛ has been suggested to have a long half-life [25], and consistent with this, individuals’ responses to this antigen rarely decreased between enrollment and delivery. The lower levels seen in the late populations, compared with those in the early

<table>
<thead>
<tr>
<th>Variable, Response</th>
<th>Early Enrollment (2005–2007) (n = 81)</th>
<th>Late Enrollment (2010–2012) (n = 242)</th>
<th>P Value</th>
<th>Late Enrollment, SP + AZ</th>
<th>Late Enrollment, SP + CQ</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG to schizont extract</td>
<td></td>
<td></td>
<td>&lt;.0001</td>
<td>6</td>
<td></td>
<td>.6</td>
</tr>
<tr>
<td>Decrease</td>
<td>27 (33.3)</td>
<td>30 (12.2)</td>
<td></td>
<td>15 (11.7)</td>
<td>15 (12.8)</td>
<td></td>
</tr>
<tr>
<td>No change</td>
<td>39 (48.1)</td>
<td>202 (82.4)</td>
<td></td>
<td>108 (84.4)</td>
<td>94 (80.3)</td>
<td></td>
</tr>
<tr>
<td>Increase</td>
<td>15 (18.5)</td>
<td>13 (5.3)</td>
<td></td>
<td>5 (3.9)</td>
<td>8 (6.8)</td>
<td></td>
</tr>
<tr>
<td>IgG to MSP2</td>
<td></td>
<td></td>
<td>.005</td>
<td>.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decrease</td>
<td>19 (23.5)</td>
<td>40 (16.1)</td>
<td></td>
<td>19 (14.5)</td>
<td>21 (17.9)</td>
<td></td>
</tr>
<tr>
<td>No change</td>
<td>51 (63.0)</td>
<td>196 (79.0)</td>
<td></td>
<td>106 (80.9)</td>
<td>90 (76.9)</td>
<td></td>
</tr>
<tr>
<td>Increase</td>
<td>11 (13.5)</td>
<td>12 (4.8)</td>
<td></td>
<td>6 (4.6)</td>
<td>6 (5.1)</td>
<td></td>
</tr>
<tr>
<td>IgG to MSP3</td>
<td></td>
<td></td>
<td>&lt;.0001</td>
<td>.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decrease</td>
<td>21 (26.3)</td>
<td>51 (21.1)</td>
<td></td>
<td>23 (18.4)</td>
<td>28 (23.9)</td>
<td></td>
</tr>
<tr>
<td>No change</td>
<td>37 (46.3)</td>
<td>168 (69.4)</td>
<td></td>
<td>91 (72.8)</td>
<td>77 (65.8)</td>
<td></td>
</tr>
<tr>
<td>Increase</td>
<td>22 (27.5)</td>
<td>23 (9.5)</td>
<td></td>
<td>11 (8.8)</td>
<td>12 (10.3)</td>
<td></td>
</tr>
<tr>
<td>IgG to RH2A9</td>
<td></td>
<td></td>
<td>.003</td>
<td>.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decrease</td>
<td>23 (28.4)</td>
<td>39 (15.8)</td>
<td></td>
<td>16 (12.2)</td>
<td>23 (19.8)</td>
<td></td>
</tr>
<tr>
<td>No change</td>
<td>45 (55.6)</td>
<td>187 (75.7)</td>
<td></td>
<td>102 (77.9)</td>
<td>85 (73.3)</td>
<td></td>
</tr>
<tr>
<td>Increase</td>
<td>13 (16.0)</td>
<td>21 (8.5)</td>
<td></td>
<td>13 (9.9)</td>
<td>8 (6.9)</td>
<td></td>
</tr>
<tr>
<td>IgG to DBL5</td>
<td></td>
<td></td>
<td>&lt;.0001</td>
<td>.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decrease</td>
<td>12 (18.8)</td>
<td>25 (10.4)</td>
<td></td>
<td>14 (11.0)</td>
<td>11 (10.0)</td>
<td></td>
</tr>
<tr>
<td>No change</td>
<td>37 (53.4)</td>
<td>201 (84.5)</td>
<td></td>
<td>108 (85.0)</td>
<td>93 (83.8)</td>
<td></td>
</tr>
<tr>
<td>Increase</td>
<td>15 (21.7)</td>
<td>12 (5.0)</td>
<td></td>
<td>5 (4.0)</td>
<td>7 (6.3)</td>
<td></td>
</tr>
<tr>
<td>Opsonic IgG to E8B-ICAM</td>
<td></td>
<td></td>
<td>.07</td>
<td>&lt;.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decrease</td>
<td>11 (14.7)</td>
<td>50 (20.7)</td>
<td></td>
<td>29 (22.8)</td>
<td>21 (18.4)</td>
<td></td>
</tr>
<tr>
<td>No change</td>
<td>46 (61.3)</td>
<td>111 (45.9)</td>
<td></td>
<td>71 (56.9)</td>
<td>40 (35.1)</td>
<td></td>
</tr>
<tr>
<td>Increase</td>
<td>18 (24.0)</td>
<td>80 (33.4)</td>
<td></td>
<td>27 (21.3)</td>
<td>53 (46.5)</td>
<td></td>
</tr>
<tr>
<td>Opsonic IgG to CS2</td>
<td></td>
<td></td>
<td>.03</td>
<td>.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decrease</td>
<td>10 (12.7)</td>
<td>50 (22.9)</td>
<td></td>
<td>33 (28.2)</td>
<td>17 (16.8)</td>
<td></td>
</tr>
<tr>
<td>No change</td>
<td>59 (74.7)</td>
<td>126 (57.8)</td>
<td></td>
<td>59 (50.4)</td>
<td>67 (66.3)</td>
<td></td>
</tr>
<tr>
<td>Increase</td>
<td>10 (12.7)</td>
<td>42 (19.3)</td>
<td></td>
<td>25 (21.4)</td>
<td>17 (16.8)</td>
<td></td>
</tr>
</tbody>
</table>

Data are no. (%) of women. P values of <.05 were considered statistically significant.

Abbreviations: AZ, azithromycin; CQ, chloroquine; IgG, immunoglobulin G; SP, sulfadoxine-pyrimethamine.
populations, may instead reflect reduced malaria exposure resulting in slower acquisition of anti-VAR2CSA-DBL5. Prospective studies, performed over the course of a women’s reproductive life, of the development of antibodies that opsonize IEs or merozoites or that block IEs adhesion, as well as their relationship to protection from infection, would be of great value [1].

In the Malawi cohort, enrollment period was not significantly associated with antibody levels after adjustment for confounders. Instead, ITN and maternal weight were independently associated with decline in levels of IgG against 1 or more antigens. Decreased exposure from increased ITN coverage may be driving the decline in antibody responses. The direct relationship between maternal weight and antibody was surprising. Women enrolled later were on average heavier, perhaps reflecting robust economic growth [44] and the declining prevalence of HIV infection [45] in Malawi. Maternal weight may be a proxy for other indicators of decreased parasite exposure and improved maternal health, including access to healthcare services. A declining prevalence of HIV infection could mitigate the drop in levels of IgG to placental-binding IEs, which are decreased by HIV infection [14]. As expected, levels of opsonic IgG to CS2 were gravidity dependent [1, 28]. Despite the lower force of infection, the gravidity-dependent acquisition of opsonic IgG to CS2 suggests that opsonic antibody to placental-binding IEs may be carried forward to subsequent pregnancies.

In the PNG cohort, a significant association between antibody levels to some antigens and enrollment period remained after adjustment for confounders. Similarly, ITN use was associated with lower levels of antibodies, and for MSP2 antibodies there was evidence of an important interaction between enrollment period and ITN use. Both IPTp and ITNs have previously been shown to decrease development of pregnancy-specific immunity (but not of other measures of malaria immunity) [11–13]. Women who begin using ITNs early during pregnancy may be protected against malaria during early fetal development, but increased ITN use by women in the later group may have reduced malaria exposure and led to a decline in antibody responses.

Antibody levels vary over a malaria season [34] or a single pregnancy [25, 28]. In PNG, among women who were negative for parasitemia at antenatal booking and at delivery, a high proportion of in the early group experienced changes in levels of antibody to merozoite antigens and VAR2CSA-DBL5, whereas more responses were unchanged among women in the later group, consistent with a lower force of infection. By contrast, levels of opsonic IgG to CS2 became more variable with time. When we compared changes in antibody responses by treatment arm, responses measured by ELISA rarely changed, and this did not differ by intervention arm, but levels of opsonic IgG to E8B-ICAM increased more frequently among women receiving a single course of SP with CQ. By contrast, women receiving more-intensive IPTp with SP and AZ more often had declining levels of opsonic IgG against placental-binding IEs [11], probably reflecting decreased exposure to malaria.

Pregnant women infected with HIV have reduced levels of antibodies to P. falciparum antigens [14, 44]. HIV infection is uncommon in pregnant women in PNG, and we lacked information regarding HIV status in Malawian women, so we could not address this factor. Evidence of past malaria detected by placental histologic analysis is associated with higher levels of antibodies [46]. We did not examine this, but in the PNG cohort, in which samples were obtained at ANC booking and delivery, the decline in antibody responses between periods was similar, making it unlikely that past infection confounded our findings. Future studies should address these questions.

In conclusion, the impact of falling parasite prevalence on anti-P. falciparum serological indicators in pregnant women differed between settings. There were limited changes over time in Malawian women after adjustment for other important cofactors, but in PNG, decreases in several antibody responses remained significant after adjustment. For antibodies to IEs, findings varied depending on the assay used. The parasite prevalences in PNG and Malawi were quite similar in both the early and late groups. ITN use was associated with lower levels of antimalarial antibodies and might increase women’s susceptibility to malaria in subsequent pregnancies. Whether malaria transmission has declined overall in these 2 populations is currently unknown, but differences in local transmission might explain differences between the sites. Continued monitoring of the immunity of susceptible populations, including assays that measure level of antibodies to intact cells, will be important as malaria control intensifies.

Notes

Acknowledgments. We thank the participants in Malawi and PNG; E. Chaluluka, L. Njiragoma, A. Munthali, M. Kanjala, and V. Uzalii, for assisting with patient recruitment and sample processing Malawi; staff in the maternity unit of Queen Elizabeth Central Hospital, for their assistance; F. Baitog, D. Stanisic, Sr Valsi, and staff of Alexishafen Health Centre, for assisting with enrollment of the first cohort of patients in PNG; Dr Maria Ome, Dr Regina Wangnapi, and the IPTp clinical and laboratory staff, for assisting with collection and processing of specimens from the second cohort of patients in PNG; Dr Robin Anders, for providing recombinant protein MSP2; and Dr Joseph Smith, for providing recombinant protein VAR2CSA-DBL5.

Financial support. This work was supported by the National Health and Medical Research Council of Australia (grant 1024441 to S. J. R. and G. V. B., grant 1047715 to J. G. B. and S. J. R., and fellowship to J. G. B.); the Victorian state government (Operational Infrastructure Support to J. G. B.); the Australian Research Council (Future Fellowship to J. G. B.); and the Malaria in Pregnancy Consortium, which receives funding from the Bill and Melinda Gates Foundation (for sample collection in PNG).

Disclaimer. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


