Acquisition and longevity of antibodies to *Plasmodium vivax* pre-erythrocytic antigens in western Thailand

Running Head: Acquisition of antibodies to *Plasmodium vivax*


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ABSTRACT

*Plasmodium vivax* (Pv) is now the dominant *Plasmodium* species causing malaria in Thailand, yet little is known about naturally acquired immune responses to this parasite in this low-transmission region. The pre-erythrocytic stage of the Pv life cycle is considered an excellent target for a malaria vaccine, and in this study we assessed the stability of both the seropositivity and the magnitude of IgG responses to three different pre-erythrocytic Pv proteins in two groups of adults from an endemic region of western Thailand. These individuals were enrolled in a yearlong cohort study, and comprised one group whom remained Pv-free (by qPCR detection, n = 31) and another which experienced two or more blood-stage Pv infections during the year of follow up (n = 31).

Despite overall low levels of seropositivity, both IgG positivity and magnitude was long-lived over the one-year period in the absence of qPCR-detectable blood-stage Pv infections. In contrast, in the adults with two or more Pv infections during the year, IgG positivity was maintained but the magnitude of the response to Pv CSP210 decreased over time. These findings demonstrate that long-term humoral immunity can develop in low-transmission regions.
INTRODUCTION

The parasite species *Plasmodium vivax* (Pv) is one of the causative agents of the disease malaria. It is the most geographically widespread of the *Plasmodium* species that cause disease in humans, with an estimated 2.5 billion people currently at risk of infection (1). Clinical disease peaks in children, whereas adults are often parasitaemic but asymptomatic (2). In addition, morbidity measures tend to decrease following successive infections (3). These epidemiological observations demonstrate the impact of naturally acquired immunity against Pv. Unfortunately, Pv has been historically neglected (4), and so we have little understanding of the mechanisms and targets of such immunity.

Pv has a complicated life cycle, with stages both in human hosts and mosquito vectors. Within humans, injected sporozoites travel to the liver and the first rounds of asexual replication occur within hepatocytes, after which thousands of merozoites are released into the blood-stage. The infection of hepatocytes is known as the pre-erythrocytic, or liver, stage. This stage precedes clinical symptoms and also acts as a bottleneck in the life cycle (before parasite numbers dramatically increase), and hence is an attractive target for a malaria vaccine (5). Currently, the most advanced vaccine against *P. falciparum* is RTS,S, recently given a positive opinion for regulation by the European Medicines Agency. RTS,S is a particulate vaccine targeting the major sporozoite surface protein known as the circumsporozoite protein, or CSP (6), and is speculated to provide protection via antibodies targeting CSP and preventing sporozoite invasion of hepatocytes. Existing anti-CSP antibody titres prior to vaccination were predicted to be an important influence on the post-vaccination peak antibody titres (7), demonstrating the need to understand naturally induced antibodies in volunteers in
endemic regions prior to conducting vaccine trials. Hence, we require a greater understanding of IgG responses to potential Pv candidate vaccine antigens in naturally exposed populations.

IgG antibody responses have been assessed to a number of Pv antigens in individuals resident in malaria-endemic areas, however attention has been focused on blood-stage rather than pre-erythrocytic antigens (8). As for *P. falciparum*, CSP is also the predominant sporozoite coating antigen for Pv. The thrombospondin-related adhesion protein (TRAP) is another major sporozoite antigen, important in motility and invasion of both mosquito salivary glands and hepatocytes (9). TRAP has shown promise both as a *P. falciparum* vaccine candidate in humans (10) and a Pv vaccine candidate in mice (11).

Another recently identified pre-erythrocytic antigen expressed on sporozoites is the cell-traversal protein for ookinetes and sporozoites (CelTOS), important for cell-traversal of host cells (12). Impressive data in mice indicated cross-species protection with a *P. falciparum* CelTOS vaccine and challenge with the murine parasite species *P. berghei* (13), however recent evidence has questioned its promise as a vaccine candidate (14). IgG antibody responses to Pv CSP have been extensively studied, and they are relatively prevalent in populations in various endemic regions (8). To our knowledge, IgG antibody responses to Pv TRAP and CelTOS have not been assessed in human populations in endemic areas.

The relative longevity of antigen-specific antibody responses to Pv is also poorly understood, given most immuno-epidemiological studies conducted have been cross-sectional in design. However, some longitudinal studies have provided evidence that IgG responses to specific blood-stage proteins (i.e. DBP, AMA1, MSP1) can be well
maintained up to five months to potentially 30 years following infection (recently reviewed in (8)); conversely, for other proteins (or even the same proteins in a different transmission setting) IgG responses have been noted to quickly decline. For Pv CSP, relatively well-maintained antibody responses have been identified. In a region of Brazil that suffered an isolated malaria outbreak in 1988, anti-CSP antibody responses were assessed five months and seven years later (15). Whilst both the seropositivity (45% to 20%) and magnitude declined, some individuals were clearly still antibody positive seven years after exhibiting malarial symptoms. A study in Thailand also identified that 51/159 individuals were able to consistently produce anti-CSP antibodies for five months following enrolment (16).

Given the lack of knowledge on naturally acquired immune responses to pre-erythrocytic antigens, we aimed to determine the stability of IgG responses to Pv TRAP and CelTOS, in addition to both major CSP variants, CSP210 and CSP247 (17), in individuals living in a malaria-endemic region on the Thai-Myanmar border. The availability of well-characterized longitudinal samples from a cohort study conducted in western Thailand from 2013 to 2014 allowed us to assess the longevity of these IgG responses in both the presence and absence of blood-stage Pv infections. We provide further evidence that IgG responses to Pv CSP can be long-lived, even in the absence of re-infection, and demonstrate that Pv TRAP and CelTOS are not substantially immunogenic following exposure in this low-transmission region of western Thailand.
MATERIALS AND METHODS

Study population. A cohort study was undertaken in the Kanchanaburi and Ratchaburi provinces of western Thailand from May 2013 to June 2014. 999 volunteers were enrolled, with ages 1-82 years included (median = 23 years). May represents the beginning of the rainy season in Thailand, and roughly the beginning of the peak malaria season. 14 active case detection visits were performed over the yearlong cohort (approximately 4 weeks apart). 609 volunteers attended all visits. At each visit a finger prick blood sample of approximately 250μl was collected into an EDTA-containing microtainer (Becton Dickinson and Company), axillary temperature recorded and a questionnaire detailing the volunteer’s health for the past month administered (including: use of bed net, reported illness, antimalarial drugs taken and travel history). 200μl of whole blood was centrifuged at 800xg for 5 minutes to separate plasma and red blood cells. Genomic DNA was extracted from the blood pellet and eluted in 50μl elution buffer using FavorPrep™ 96-well genomic DNA extraction kits (Favorgen). 4μl of DNA (equivalent to 8μl of whole blood) was used in detection of malaria parasites by quantitative PCR assay using previously published primer and probe sequences (18, 19). Any volunteer with an axillary temperature ≥37.5°C, or with history of fever between visits, was checked for malaria using a SD BIOLINE Malaria Ag P.f/Pan rapid diagnosis test kit (Standard Diagnostics). Passive case detection for symptomatic illness was conducted at local health clinics within the region. Any confirmed malaria cases were referred to their local health clinics for anti-malarial drug treatment. Over the 14 active case detection visits, parasite prevalence ranged from 1.5-5% for Pv and from 0.2-1.4% for P. falciparum (W. Nguitragool, J. Sattabongkot and I. Mueller, unpublished data).
At the first and last visits of the cohort (approximately one year apart, visits 1 and 14), 5ml venous bleeds were taken from a selection of consenting volunteers (13-80 years of age) in the Kanchanaburi province in order to isolate peripheral blood mononuclear cells and to collect larger volumes of plasma. Blood was collected from 498 volunteers at the first visit and 323 matched volunteers at the last visit. Of the 323 volunteers where large volumes of plasma was collected at the first and last time points, 31 volunteers had two or more Pv infections (mostly asymptomatic) during the cohort period and attended at least 11 of the 14 visits. These volunteers were selected for antibody analysis, along with 31 age and gender matched controls with no detected (Pv or P. falciparum) infections during the entire cohort period who had attended all 14 visits (Table 1). These control volunteers were chosen at random from all available. All data and analysis presented in the current manuscript relate to this subset of 62 adult volunteers, apart from the control samples described below.

Antibody measurements were also conducted using plasma from two naïve controls (one Australian, one Thai, both male) with no known previous exposure to malaria and one historical Thai plasma sample known to be positive to CSP247. Positivity cut-offs were set based on IgG measurements in 21 children (2-3 years old, 48% male) from the same cohort. These children had no recorded blood-stage malarial infections (detected by qPCR) at any of the 14 visits of the cohort and plasma, collected by finger prick, was used from the second last visit (visit 13). Samples from these children were run in singlicate.

Informed consent and assent (for children aged 7-≤13 years) was obtained from all participants in the study and ethics approval was obtained from the Ethics Committee.
The study was clearly explained to all volunteers.

**Protein expression in HEK-293T cells.** The *P. vivax* sequences CSP210 (Belem, NCBI accession number P08677), CSP247 (PNG, NCBI Accession number M69059), TRAP (Salvador I, Uniprot Accession number A5K806) and CelTOS (Salvador I, Uniprot Accession number Q53UB7) were cloned in the expression vector pHLsec (20), flanked by the chicken β-actin/rabbit β-globin hybrid promoter with a signal secretion sequence and a Lys-His6 tag. Proteins were expressed upon transient transfection in HEK-293T cells with endotoxin-free plasmids in roller bottles (2125 cm²). Secreted proteins were purified from the supernatants by immobilized Ni Sepharose-affinity chromatography. Presence of proteins in the elution samples was confirmed using 6X-His Epitope tag antibody (HRP conjugate), monoclonal antibodies (MRA-1028K sporozoite ELISA kit) for CSP210/CSP247 and a Horseradish peroxidase monoclonal antibody (Invitrogen) in a western blot. Samples were concentrated using an Amicon® ultra centrifugal filter system (Life technologies) until reaching 10ml of final volume. Contaminant proteins and salts were removed from the concentrate by size exclusion purification (SEC) using Superdex medium in the column. Protein concentration after recovery was tested using a Bradford protein assay and purity assessed by silver staining and western blot (Figs. S1-4).

**Measurement of IgG responses.** Antigen-specific antibodies were measured in plasma samples (from the 31 uninfected and 31 exposed volunteers) at visits 1 and 14 using an enzyme-linked immunosorbent assay (ELISA), essentially as previously described (21). Briefly, 96-well flat-bottom MaxiSorp plates (Nunc) were coated with
1μg/ml antigen in phosphate buffered saline (PBS) and incubated overnight at 4°C. Plates were blocked and plasma samples diluted with PBS containing 0.05% Tween-20 and 5% skim milk. All washing steps were performed using PBS containing 0.05% Tween-20 using an automated microplate washer (HydroFlex™, Tecan). Plasma samples were run in duplicate (with individual replicates on separate plates) at a 1/100 dilution, and two naïve plasma samples and one CSP247 positive plasma sample were included as controls on each plate. To measure total IgG, horseradish peroxidase conjugated goat anti-human IgG (H+L) (Thermo Scientific Pierce) was used at 100ng/ml. ABTS (2,2'-Azinobis[3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt; Merck Millipore) was used as the substrate, with the optical density (OD) measured after 30 minutes at 405 nm. Background values from wells with no plasma were subtracted on a per-plate basis. On each plate, control wells containing no antigen (coated only with PBS) were included for each individual plasma sample: this value was subtracted from the corresponding well containing antigen. The mean of the duplicates after both background and no antigen control subtraction was then used as the final value. When the co-efficient of variation was greater than 20%, the result was repeated (unless both values were below the positivity cut-off). For each volunteer, samples from each time point were run on the same plate to allow direct comparability.

Positive cut-off values for each protein were created using relevant data from the 21 uninfected children analyzed from the same cohort who experienced no qPCR-detectable malaria infections during the yearlong follow-up period (Fig. S5). The assumption was made that these children would be young enough that it was unlikely they would have had previous unreported malaria infections before the cohort began, but
old enough that maternal antibodies would no longer be circulating (22). Cut-off
background levels were defined as the average plus two times the standard deviation and
were set at OD values of: Pv CSP247 0.68; CSP210 0.16; TRAP 0.14; and CelTOS 0.12.

**Statistical analysis.** As the data were not normally distributed non-parametric
tests were used. The change in OD value from visit 1 to visit 14 was assessed using the
Wilcoxon matched-pairs signed rank test. Statistical difference between groups was
assessed using the Mann-Whitney test. Statistical difference in rates of seropositivity
between exposed individuals with or without a current infection was assessed using
Fisher’s exact test. All statistical analysis was performed in Prism version 6 (GraphPad).
RESULTS

Effect of current *P. vivax* blood-stage infections on IgG positivity and magnitude.

We first assessed the effect of current *P. vivax* blood-stage infections on the IgG response in our exposed group of volunteers, to determine whether we could treat these individuals as one group or not. 14 of the 31 exposed individuals had a current blood-stage infection at visit 1, and 10 of the 31 at visit 14. There was no significant difference between individuals with or without a current blood-stage infection in the percentage of volunteers classed as seropositive for CSP210 at either visit, and the percentages were also very similar for CSP247 at visit 14 and CelTOS at visit 1 (Table 2). A larger (but not significant) percentage of the exposed but currently uninfected volunteers were seropositive for CSP247 at visit 1 (Fisher’s exact test, p=0.068), and the opposite was true for CelTOS at visit 14 (p=0.17). There was no detected seropositivity to Pv TRAP in both the uninfected and exposed groups, and so responses to this antigen were not included in further analysis. Overall, current blood-stage infections thus appeared to have limited impact on the seropositivity. In addition, current blood-stage infections also had little impact on the IgG magnitude of exposed volunteers, both at visit 1 (Fig. 1) and visit 14 (Fig. S6). The decision was therefore made to treat all exposed individuals as one group, regardless of the presence or absence of current infections at the time of IgG measurement.

Overall, in both exposed and uninfected volunteers, seropositivity was highest for Pv CSP210, followed by CSP247 and then CelTOS (Table 2). The magnitude of the IgG response was significantly higher in the exposed group of volunteers over both time points for Pv CSP210 and CSP247 (two-way ANOVA, p=0.0047 and p=0.019,
respectively) (Fig. 4), although not for CelTOS likely due to the small number of seropositive individuals (p=0.5). The breadth of the response was also greater in exposed individuals (Fig. S7).

Stability of the prevalence and magnitude of the IgG responses. We next assessed the longevity of IgG positivity and magnitude over the yearlong cohort period, and how this was affected by the presence or absence of blood-stage infections. Overall, there was very little change in seropositivity status over the year, whether the volunteers were exposed or not to blood-stage infections (Table 3). There was also no significant difference in the IgG magnitude between visits 1 and 14 against Pv CSP247 or CSP210 in the uninfected group of volunteers (Wilcoxon matched-pairs signed rank test, p=0.26 and p=0.46, respectively) (Fig. 2), suggesting ongoing qPCR-detected Pv blood-stage infections are not required to maintain antibody responses to these pre-erythrocytic proteins. Pv CelTOS was not assessed given the limited seropositivity in uninfected individuals. Similarly, there was no significant difference in the IgG magnitude between visits 1 and 14 against Pv CSP247 and CelTOS in the exposed group of volunteers (p>0.99 and p=0.77, respectively) (Fig. 3), however, for Pv CSP210 the magnitude of the IgG response significantly declined from visit 1 to visit 14 for the exposed volunteers (p=0.0056) (Fig. 3).

DISCUSSION

The current study presents new insights into the acquisition and stability of IgG responses to pre-erythrocytic Pv proteins in a low-transmission region. Overall, seropositivity was highest to Pv CSP210, followed by CSP247 then CelTOS, in both uninfected and
exposed individuals, with similar percentages one year later. The higher prevalence of IgG responses to CSP210 over CSP247 is consistent with the predominance of CSP210 in previous genotyping of Pv parasites in this region (23). The lack of IgG responses to Pv TRAP is consistent with the absence of or low responses to *P. falciparum* TRAP in low to moderate transmission regions (24-26). To our knowledge, this is the first time IgG responses have been assessed to Pv TRAP in naturally exposed volunteers. In addition, we report the first evidence of naturally induced IgG responses to Pv CelTOS in human volunteers. We found relatively higher and more prevalent IgG responses in volunteers exposed to Pv blood-stage parasites, as well as a greater breadth of response, at both the beginning and end of the one-year cohort, indicating that these IgG responses are most likely reflective of long-term exposure and risk of malaria, rather than recent exposure.

Antibody responses to Pv CSP have been extensively studied in the past (8), however, the majority of these studies have been cross-sectional, resulting in limited data concerning the longevity and stability of responses. We have shown that IgG positivity to both Pv CSP210 and CSP247 was well maintained over a one-year period, even in the absence of qPCR-detected blood-stage infections. This builds on previous evidence of antibody longevity in the absence of detectable exposure, both to Pv CSP (15, 16) and to other proteins in a similar low-transmission setting: Wipasa and colleagues identified long-lived IgG responses to three Pv blood-stage proteins in Northern Thailand (27). This suggests that either memory B cells or long-lived plasma cells were induced, to provide an on-going source of measurable IgG. Whether or not specific antigen stimulation is required for differentiation of memory B cells into antibody secreting cells is still
contentious (28, 29). There has been speculation that the presence of hypnozoites could provide antigenic stimulation in the absence of new Pv infections (30), however given the relapse period for South East Asia is of the ‘frequent-relapse phenotype’ (31), it seems unlikely these individuals (who had no qPCR-detectable blood-stage infections for one year) harbored the dormant liver-stages. Another potential source of antigenic stimulation could be failed Pv infections i.e. where sporozoites were inoculated but failed to establish (qPCR-detectable) blood-stage infections. To tease out the potential mechanism of such long-lived antibodies, antigen-specific B cell phenotyping will be ultimately required.

Conversely, we noted a statistically significant, albeit small, decrease in the magnitude of the IgG response to Pv CSP210 over the yearlong period in individuals who experienced two or more qPCR-detectable blood-stage Pv infections. Plasma samples from these individuals were run as matched-pairs on the same ELISA plate and hence are directly comparable over time. However, a limitation of our study is that only two time-points were assessed, so we do not know how the IgG magnitude fluctuated over the yearlong period. It is also important to note that the presence of blood-stage Pv does not necessarily indicate a new infection, as it could be due to a relapse. Hence, one plausible explanation is that the most recent pre-erythrocytic exposure was to sporozoites expressing CSP210, leading to a boosting of the IgG response that then underwent an initial rapid decay followed by a maintenance phase of long-lived IgG (32). In this setting it is unknown as to what proportion of detected blood-stage infections are due to hypnozoite activation rather than new infections, but both modeling (33) and a field trial in Papua New Guinea suggest it could be very large (34). We would also expect both short and long-lived plasma cells to be contributing to the IgG magnitude in our exposed
volunteers, creating more variability in the response. An alternate explanation for the reduced IgG magnitude of Pv CSP210 antibodies in individuals with multiple blood-stage Pv infections that cannot yet be ruled out is the presence of T regulatory cells (Tregs) (35) or atypical B cells, both of which can suppress B cell Ig production (36, 37). However, it must be noted that some of the exposed individuals only had two qPCR-detected blood-stage infections, and this may not be enough to induce suppression of the IgG magnitude by Tregs or atypical memory B cells. It will be important to determine whether the decrease in the IgG response seen is replicated in a larger sample size, and to elucidate the mechanism, in order to determine whether relapsing and/or new infections could have a detrimental impact on vaccine efficacy (if a CSP-based vaccine was introduced into this region).

In conclusion, despite the simple immunoassay we employed, our well-defined samples from a carefully designed epidemiological cohort study have provided interesting data and insights into the longevity of antigen-specific IgG responses to pre-erythrocytic Pv antigens in a low-transmission setting. Our findings have raised a number of questions about the effect of antigenic exposure on the development and maintenance of long-lived IgG responses, and hence we propose a number of directions for future research: phenotypic examination of i) memory B cells, ii) Tregs and iii) atypical memory B cells in a similar cohort of volunteers where peripheral blood mononuclear cells are available in addition to plasma samples. It will also be important to consider what effect such pre-existing IgG responses to Pv CSP might have on a vaccine targeting this antigen: based on data from Pf CSP (7), in adults the presence of IgG to Pv CSP might enhance IgG induced by a vaccine, and importantly we have demonstrated that
these responses have the potential to be long-lived, even in the absence of boosting infections. Furthermore, whilst requiring further study with a larger sample size, our results suggest that IgG responses to Pv CSP may be able to identify individuals at a higher risk of malaria in this region. It will be of interest to include these data in the overall analysis currently being conducted on this cohort study, alongside the behavioral risk factors.
FUNDING INFORMATION

This work was supported by the National Institute of Allergy and Infectious Diseases, US (NIH grant number 5R01 AI 104822) and the Foundation for Innovative New Diagnostics. Funding was also provided by a Wellcome Trust Career Development Fellowship award, Grant Number: 097395 to AR-S. The field-study was funded by the Bill and Melinda Gates foundation. IM is supported by a NHMRC Senior Research Fellowship (#1043345). AR-S is a Jenner Investigator and Oxford Martin School Fellow. SD is a Wellcome Trust Intermediate Clinical Fellow. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

ACKNOWLEDGEMENTS

We thank all the participants in the transEPI study, all members of MVRU involved in sample collection and PCR, Louis Schofield, Emily Eriksson and Danika Hill for assistance with sample processing, Rachaneeporn Jenwithisuk for laboratory support and the National Research Council of Thailand for their support.
REFERENCES


5. Reyes-Sandoval A, Bachmann MF. 2013. Plasmodium vivax malaria vaccines: why are we where we are? Hum Vaccin Immunother 9:2558-2565.


FIGURE LEGENDS

FIG 1 IgG magnitude and the effect of current blood-stage *P. vivax* infections. IgG responses to Pv (A) CSP247 (B) CSP210 and (C) CelTOS at visit 1. Volunteers are stratified into those uninfected throughout the cohort (Group A, n=31), those exposed but currently uninfected (Group B, n=17) and those exposed but currently infected (Group C, n=14). The dashed line represents protein-specific positivity cut-offs. Statistical difference between the three groups was assessed using the Kruskal-Wallis test with Dunn’s multiple comparisons test. * p<0.05.

FIG 2 Longevity of the IgG magnitude in uninfected volunteers. IgG responses to Pv (A) CSP247 and (B) CSP210 in the 31 uninfected volunteers were stratified by visits 1 and 14. The left panels show individual data points and the median, whilst the right panels show the individual change over time for individuals who were seropositive. The dashed line represents protein-specific positivity cut-offs. Statistical difference over visits was assessed using the Wilcoxon matched-pairs signed rank test: (A) p=0.26, (B) p=0.46.

FIG 3 Longevity of the IgG magnitude in exposed volunteers. IgG responses to Pv (A) CSP247, (B) CSP210 and (C) CelTOS in the 31 exposed volunteers were stratified by visits 1 and 14. The left panels show individual data points and the median, whilst the right panels show the individual change over time for individuals who were seropositive. The dashed line represents protein-specific positivity cut-offs. Statistical difference over visits was assessed using the Wilcoxon matched-pairs signed rank test: (A) p>0.99, (B) p=0.0014 and (C) p=0.77.
FIG 4 Magnitude of the IgG response and exposure to Pv infection. IgG responses to Pv proteins at visits 1 and 14 were stratified by the 31 adults who experienced no Pv infections (uninfected) and 31 adults who experienced 2 or more qPCR-detectable blood-stage Pv infections (exposed), during the one-year cohort. Individual data points and the median are shown. The dashed line represents protein-specific positivity cut-offs. Statistical difference between uninfected and exposed adults over both time-points was assessed using a two-way ANOVA: (A) \( p = 0.019 \) (B) \( p = 0.0047 \) (C) \( p = 0.5 \).

TABLES

TABLE 1 Demographic characteristics of the selected sub-sets of volunteers.

<table>
<thead>
<tr>
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<th>Exposed</th>
<th>Uninfected</th>
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<tr>
<td>Age</td>
<td>37 (19-63)a</td>
<td>39 (20-67)</td>
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<tr>
<td>Proportion male</td>
<td>0.71</td>
<td>0.71</td>
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<tr>
<td>% Infected (of total visits)b</td>
<td>50 (14.2-100)</td>
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<tr>
<td>Number of visits attended</td>
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aData are shown as the median, with the range in brackets. bPercentage of visits at which the volunteer was positive by qPCR for Pv blood-stage malaria.
TABLE 2 Percentage of volunteers seropositive for each antigen at each visit.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Uninfected (n=31)</th>
<th>Exposed (n=31)</th>
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<tr>
<td></td>
<td>V1 (n=17)</td>
<td>V14 (n=21)</td>
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<tr>
<td>CSP210</td>
<td>16</td>
<td>29</td>
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<tr>
<td>CSP247</td>
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<td>3</td>
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<tr>
<td>CelTOS</td>
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V1 = visit 1, V14 = visit 14.

TABLE 3 Percentage of volunteers who maintained sero-negativity or sero-converted, and who maintained seropositivity or sero-reverted.

<table>
<thead>
<tr>
<th>Maintained sero-negativity</th>
<th>Sero-converted</th>
<th>Maintained seropositivity</th>
<th>Sero-reverted</th>
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Group 1: Uninfected (n=31)

Group 2: Exposed + Currently Uninfected (n=17)

Group 3: Exposed + Currently Infected (n=14)