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Sporozoite Immunization of Human Volunteers under Mefloquine Prophylaxis Is Safe, Immunogenic and Protective: A Double-Blind Randomized Controlled Clinical Trial

Else M. Bijker1,9, Remko Schats2,9, Joshua M. Obiero1, Marije C. Behet1, Geert-Jan van Gemert1, Marga van de Vegte-Bolmer1, Wouter Graumans1, Lisette van Lieshout3,4, Guido J. H. Bastiaens1, Karina Teelen1, Cornelus C. Hermse1, Anja Scholzen1, Leo G. Visser2, Robert W. Sauerwein1*

1 Radboud university medical center, Department of Medical Microbiology, PO Box 9101, 6500 HB Nijmegen, The Netherlands, 2 Leiden University Medical Center, Department of Infectious Diseases, PO Box 9600, 2300 RC Leiden, The Netherlands, 3 Leiden University Medical Center, Department of Medical Microbiology, PO Box 9600, 2300 RC Leiden, The Netherlands, 4 Leiden University Medical Center, Department of Parasitology, PO Box 9600, 2300 RC Leiden, The Netherlands

Abstract

Immunization of healthy volunteers with chloroquine ChemoProphylaxis and Sporozoites (CPS-CQ) efficiently and reproducibly induces dose-dependent and long-lasting protection against homologous Plasmodium falciparum challenge. Here, we studied whether chloroquine can be replaced by mefloquine, which is the only other licensed anti-malarial chemoprophylactic drug that does not affect pre-erythrocytic stages, exposure to which is considered essential for induction of protection by CPS immunization. In a double blind randomized controlled clinical trial, volunteers under either chloroquine prophylaxis (CPS-CQ, n = 5) or mefloquine prophylaxis (CPS-MQ, n = 10) received three sub-optimal CPS immunizations by bites from eight P. falciparum infected mosquitoes each, at monthly intervals. Four control volunteers received mefloquine prophylaxis and bites from uninfected mosquitoes. CPS-MQ immunization is safe and equally potent compared to CPS-CQ inducing protection in 7/10 (70%) versus 3/5 (60%) volunteers, respectively. Furthermore, specific antibody levels and cellular immune memory responses were comparable between both groups. We therefore conclude that mefloquine and chloroquine are equally effective in CPS-induced immune responses and protection.

Trial Registration: ClinicalTrials.gov NCT01422954

Introduction

Malaria remains one of the most important infectious diseases worldwide and still causes approximately 207 million cases and 627,000 deaths every year [1]. Anti-disease immunity against malaria is not easily induced: in endemic areas this takes many years of repeated exposure to develop [2], and sterile protection against infection does not seem to be induced at all [3]. Also candidate vaccines have shown only limited protective efficacy so far [4,5]. Novel vaccines and drugs can be tested for efficacy at an early stage of clinical development in Controlled Human Malaria Infection (CHMI) studies, exposing a small number of healthy volunteers to Plasmodium falciparum by bites from infected Anopheles mosquitoes. Immunization of healthy volunteers under chloroquine ChemoProphylaxis with Sporozoites (CPS-CQ immunization) efficiently, reproducibly and dose-dependently induces protection against homologous CHMI [6,7], shown in a subset of volunteers to last for more than 2 years [8]. CPS-CQ immunization requires exposure to bites from only a total of 30–45 P. falciparum infected mosquitoes to induce 89–95% protection [6,7,9]. In contrast, protection by immunization with radiation-attenuated sporozoites (RAS) requires a minimum of 1000 infected mosquito bites [10], or intravenous injection of five times 135,000 cryopreserved sporozoites [11]. The unprecedented efficiency of the CPS immunization regime may relate to its design: in contrast to RAS, CPS immunization allows full liver stage development and exposure to early blood-stages. Moreover, chloroquine is known for its immunomodulatory capacities [12–14] that may play a role in induction of protection, which is mediated by pre-erythrocytic immunity [9] including...
antibodies directed against sporozoites [15–17], and likely T cells targeting liver-stages [7]. Next to chloroquine, mefloquine (MQ) is the only licensed drug for chemoprophylaxis that does not affect pre-erythrocytic stage development [10]. We therefore aimed to assess whether chloroquine could be replaced by mefloquine for CPS immunization. In a double blind randomized controlled clinical trial we assessed safety, immunogenicity and protection against challenge for CPS-MQ compared to CPS-CQ.

Methods

Study subjects

Healthy subjects between 18 and 35 years old with no history of malaria were screened for eligibility based on medical and family history, physical examination and standard hematological and biochemical measurements. Urine toxicology screening was negative in all included subjects; none of the subjects were pregnant or lactating. Serological analysis for HIV, hepatitis B, hepatitis C and \textit{P. falciparum} asexual blood-stages was negative in all subjects. All subjects had an estimated 10-year risk smaller than 5% of developing a cardiac event as estimated by the Systematic Coronary Evaluation System adjusted for the Dutch population [19]. None of the subjects had travelled to a malaria-endemic area during or within 6 months prior to the start of the study. All subjects provided written informed consent before screening. The Central Committee for Research Involving Human Subjects of The Netherlands approved the study (NL 37563.058.11). Investigators complied with the Declaration of Helsinki and Good Clinical Practice including monitoring of data. This trial is registered at ClinicalTrials.gov, identifier NCT01422954. The protocol for this trial and supporting CONSORT checklist are available as supporting information (Checklist S1 and Protocol S1).

Study design and procedures

This single center, double blind randomized controlled trial was conducted at Leiden University Medical Center (Leiden, the Netherlands) from April 2012 until April 2013 (Figure 1). Twenty subjects were randomly divided into three groups by an independent investigator using a computer-generated random-number table. Subjects, investigators and primary outcome assessors were blinded to the allocation. Subjects in the CPS-CQ group (n = 5) received a standard prophylactic regimen of chloroquine consisting of a loading dose of 300 mg on the first and fourth day and subsequently 300 mg once a week for 12 weeks. Subjects in the CPS-MQ group (n = 10) and the control group (n = 5) received mefloquine prophylaxis starting with a loading split dose regimen to limit potential side-effects: 125 mg twice per week for a duration of 3 weeks and subsequently 250 mg once a week for 12 weeks. Chloroquine and mefloquine were administered as capsules, indistinguishable from each other. During this period all subjects were exposed to the bites of 8 \textit{Anopheles} mosquitoes three times at monthly intervals, starting 22 days after start of mefloquine prophylaxis and 8 days after start of chloroquine prophylaxis. Volunteers in the CPS-CQ and CPS-MQ groups received bites from mosquitoes infected with the \textit{P. falciparum} NF54 strain, control subjects received bites from uninfected mosquitoes. The immunization dose was based on our previous dose-de-escalation trial [7] and aimed to establish partial protection in the CPS-CQ group in order to enable detection of either improved or reduced protection in the CPS-MQ group. Sample sizes were calculated based on the expected difference of 4 days in prepatent period between the CPS-CQ and CPS-MQ groups, a standard deviation of 1.6 and 2.3 days respectively, an à of 5% and a power of 0.90. This calculation resulted in a CPS-CQ group of 4 and a CPS-MQ group of 8 subjects. To account for possible dropouts based on (perceived) side effects we included one and two extra volunteers in the CPS-CQ and CPS-MQ groups respectively. The control group was included as infectivity control for the challenge infection.

On days 6 to 10 after each immunization by mosquito exposure, all subjects were followed on an outpatient basis and peripheral blood was drawn for blood smears, standard hematological measurements, cardiovascular markers and retrospective qPCR.

Twenty weeks after the last immunization, sixteen weeks after discontinuation of prophylaxis, all subjects were challenged by the bites of five mosquitoes infected with the homologous NF54 \textit{P. falciparum} strain, according to previous protocols [20]. After this challenge-infection, all subjects were checked twice daily on an outpatient basis from day 5 up until day 15 and once daily from day 16 up until day 21 for symptoms and signs of malaria. Thick blood smears for parasite detection were made during each of these visits after challenge, hematological and cardiovascular markers were assessed daily. As soon as parasites were detected by thick smear, subjects were treated with a standard curative regimen of 1000 mg atovaquone and 400 mg proguanil once daily for three days according to Dutch national malaria treatment guidelines. If subjects remained thick smear negative, they were presumptively treated with the same curative regimen on day 21 after challenge infection. All subjects were followed closely for 3 days after initiation of treatment and complete cure was confirmed by two negative blood smears after the last treatment dose. Chloroquine and mefloquine levels were measured retrospectively in citrate-plasma from the day before challenge by liquid chromatography (detection limit for both chloroquine and mefloquine: 5 µg/L) [21].

\textit{Anopheles stephensi} mosquitoes for immunizations and challenge-infection were reared according to standard procedures at the insectary of the Radboud university medical center. Infected mosquitoes were obtained by feeding on NF54 gametocytes, a chloroquine- and mefloquine-sensitive \textit{P. falciparum} strain, as described previously [22]. After exposure of volunteers, all blood-engorged mosquitoes were dissected to confirm the presence of sporozoites. If necessary, feeding sessions were repeated until the predefined number of infected or uninfected mosquitoes had fed.

Endpoints

The primary endpoint was prepatent period, defined as the time between challenge and first positive thick blood smear. Secondary endpoints were parasitemia and kinetics of parasitemia as measured by qPCR, adverse events and immune responses.

Detection of parasites by thick smear

Blood was sampled twice daily from day 5 until day 15 and once daily from day 16 up until day 21 after challenge and thick smears were prepared and read as described previously [9]. In short, approximately 0.5 µL of blood were assessed by microscopy and the smear was considered positive if two unambiguous parasites were seen.

Quantification of parasitemia by qPCR

Retrospectively, parasitemia was quantified by real-time quantitative PCR (qPCR) on samples from day 6 until day 10 after each immunization and from day 5 until day 21 after challenge as described previously [23], with some modifications. Brieﬂy, 5 µL Zap-Oglobin II Lytic Reagent (Beckman Coulter) was added to 0.5 ml of EDTA blood, after which the samples were mixed and stored at −80°C. After thawing, samples were spiked with the
extraction control Phocine Herpes Virus (PhHV) and DNA was extracted with a MagnaPure LC isolation instrument. Isolated DNA was resuspended in 50 μl H₂O, and 5 μl was used as template. For the detection of *P. falciparum*, the primers as described earlier [23] and the TaqMan MGB probe AAC AAT TGG AGG GCA AG-FAM were used. For quantification of PhHV the primers GGGCGAATCACAGATTGAATC, GCG-GTTCCAAACGTACCAA and the probe Cy5- TTTTTATGTGTCCGCCACCATCTGGATC were used. The sensitivity of qPCR was 35 parasites/ml of whole blood.

**Adverse events and safety lab**

Adverse events (AEs) were recorded as following: mild events (easily tolerated), moderate events (interfering with normal activity), or severe events (preventing normal activity). Fever was recorded as grade 1 (≥37.5°C–38.0°C), grade 2 (≥38.0°C–39.0°C) or grade 3 (≥39.0°C). Platelet and lymphocyte counts were determined in EDTA-anti-coagulated blood with the Sysmex XE-2100 (Sysmex Europe GmbH, Norderstedt, Germany). D-dimer concentrations were assessed in citrate plasma by STA-R Evolution (Roche Diagnostics, Almere, The Netherlands).

**Immunological analyses**

In order to assess cellular immune memory responses, peripheral blood mononuclear cell (PBMC) re-stimulation assays were performed as described previously [7]. PBMCs were collected, frozen in fetal calf serum containing 10% dimethylsulfoxide, and stored in vapor phase nitrogen before initiation of prophylaxis (baseline; B) and one day before the challenge infection (C-1).

After thawing, PBMCs were re-exposed in vitro to *P. falciparum*-infected red blood cells (PfRBC) and incubated for 24 hours at 37°C in the presence of a fluorochrome-labeled antibody against CD107a. Uninfected red blood cells (uRBCs) were used as a negative control. During the last 4 hours of incubation, 10 μg/ml Brefeldin A and 2 μM Monensin were added, allowing cytokines to accumulate within the cells. As a positive control, 50 ng/ml PMA and 1 μg/ml ionomycin were added for the last four hours of incubation. After 24 h stimulation, cells were further stained with a viability marker and fluorochrome-labeled antibodies against CD3, CD4, CD8, CD56, CD-T cell receptor, IFNγ and granzyme B (Table S1 [7]). For each volunteer, cells from all time points were tested in a single experiment: thawed and stimulated on the same day and stained the following day. Samples were acquired on a 9-color Cyan ADP (Beckman Coulter) and data analysis was performed using FlowJo software (version 9.6.4; Tree Star). A representative example showing the full gating strategy is shown in Figure S1. Gating of cytokine-positive cells was performed in a standardized way by multiplying a fixed factor with the 75 percentile of the geometric Mean Fluorescent Intensity (MFI) of cytokine negative PBMCs for each volunteer, time point and stimulus. Responses to uRBC were subtracted from the response to PfRBC for each volunteer on every time point.

Plasma for the assessment of malaria-specific antibodies was collected and stored at baseline (B), 27 days after the first immunization (I1; one day before the second immunization), 27 days after the second immunization (I2; one day before the third immunization), and one day before the challenge infection (C-1). Antibody titers were assessed as described previously [17]. In...
Summary, serially diluted citrate plasma was used to perform standardized enzyme-linked immunosorbent assay (ELISA) in NUNC Maxisorp plates (Thermo Scientific) coated with 1 mg/ml circumsporozoite protein (CSP), liver-stage antigen-1 (LSA-1) or merozoite surface protein-1 (MSP-1) antigen, diluted in PBS. Bound IgG was detected using horseradish peroxidase (HRP) conjugated anti-human IgG (Thermo Scientific, 1/60000) and Tetramethylbenzidine (all Mabtech). Spectrophotometrical absorbance was measured at 450 nm. OD values were converted into AUs by four-parameter logistic curve fit using Auditable Data Analysis and Management System for ELISA (ADAMSEL-v1.1, http://www.malariaresearch.eu/content/software; accessed 27 October 2014). Levels of antibodies were calculated in relation to a pool of 100 sera from adults living in a highly endemic area in Tanzania (HIT serum [24]), which was defined to contain 100 arbitrary units (AU) of IgG directed against each antigen.

Statistical analyses
The proportion of protected subjects in the CPS-CQ versus CPS-MQ group was tested with the Fisher’s exact test using Graphpad Quickcalcs online and the 95% confidence interval (CI) of protection for each group was calculated by modified Wald Method [25]. Further statistical analyses were performed with GraphPad Prism 5. Differences in prepatent period and time from qPCR positivity until thick smear positivity were tested by Mann Whitney test. Antibody levels are shown as individual titers with medians and differences between time points were analyzed by Friedman test with Dunn’s multiple comparison post-hoc test. Induction of cellular immune responses was tested for CPS-CQ and CPS-MQ groups separately by Wilcoxon matched-pairs signed rank test (B versus C-1). A p-value of <0.05 was considered statistically significant. Analyses of parasitemia were performed on log transformed data, the geometric mean peak parasitemia after each immunization was calculated using the maximum parasitemia for each subject.

Results
Safety of CPS-CQ and CPS-MQ immunization
Twenty out of 36 screened subjects (median age 21 years; range 18–25) were included in the study (Figure 1). One control subject was excluded at start of prophylaxis and the first immuni-
Table 1. Protection against challenge infection after CPS-CQ and CPS-MQ immunization.

<table>
<thead>
<tr>
<th>Group</th>
<th>Protection</th>
<th>Unprotected volunteers</th>
<th>Day of positivity after challenge</th>
<th>Thick smear</th>
<th>qPCR</th>
<th>qPCR+TS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPS-CQ</td>
<td>3/5</td>
<td>60 (23–88)</td>
<td>14.0 (14.0–14.0)</td>
<td>1.3 (10.5–12.8)</td>
<td>0.01'</td>
<td>0.005'</td>
</tr>
<tr>
<td>CPS-MQ</td>
<td>7/10</td>
<td>70 (39–90)</td>
<td>12.0 (11.0–12.0)</td>
<td>8.5 (7.0–12.0)</td>
<td>0.03'</td>
<td>0.048'</td>
</tr>
<tr>
<td>Control</td>
<td>0/4</td>
<td>0% (0–55)</td>
<td>0.03 (0–3.5)</td>
<td>6.3 (5.0–9.5)</td>
<td>0.006'</td>
<td>0.005'</td>
</tr>
</tbody>
</table>

Presented as protected/total number of subjects.

Presented as % protected (95% CI by modified Wald Method).

Presented as median (range) days.

\( p \) = 0.03-0.017 of Fisher’s exact test comparing CPS-MQ versus CPS-CQ or control versus all CPS-immunized subjects.

\( q \) = 0.048 of Mann Whitney test comparing CPS-MQ versus CPS-CQ or control versus all CPS-immunized subjects (both excluding protected subjects).

None of the protected subjects showed parasitemia by qPCR at any time point during follow-up. After the first immunization, 7/10 CPS-CQ volunteers were protected against challenge infection (Fisher’s exact test \( p = 1.0 \)). All control subjects became thick smear positive (median day 8.3, range 7–12, \( p = 0.03 \) versus CPS-immunized subjects; Table 1). None of the protected subjects showed parasitemia by qPCR at any time point during follow-up (Figure 4). The median prepatent period was not significantly different between the CPS-CQ and CPS-MQ groups, neither when protected subjects were arbitrarily set at a prepatent period of 21 days (\( p = 1.00 \)), nor when comparing unprotected subjects.

Protection against challenge infection

In the CPS-CQ group 3/5 subjects and in the CPS-MQ group 7/10 volunteers were protected against challenge infection (Fisher’s exact test \( p = 1.0 \)). All control subjects became thick smear positive (median day 8.3, range 7–12, \( p = 0.03 \) versus CPS-immunized subjects; Table 1). None of the protected subjects showed parasitemia by qPCR at any time point during follow-up (Figure 4). The median prepatent period was not significantly different between the CPS-CQ and CPS-MQ groups, neither when protected subjects were arbitrarily set at a prepatent period of 21 days (\( p = 1.00 \)), nor when comparing unprotected subjects.
only \((p = 0.1)\). The median chloroquine plasma concentration on the day before challenge infection was \(9\ \text{mg/L}\) (range \(7–10\)) in the CPS-CQ group, and the median mefloquine concentration was \(24\ \text{mg/L}\) (range \(5–116\)) in the mefloquine groups.

**Immunogenicity of CPS-CQ and CPS-MQ**

Antibodies against the pre-erythrocytic antigens CSP and LSA-1 and the cross-stage antigen MSP-1 were assessed by ELISA. Antibodies against CSP were induced in both CPS-CQ and CPS-MQ immunized volunteers \((p < 0.05\) and \(p < 0.01\) respectively, on C-1; **Figure 5A and 5B**), but not significantly higher in protected compared to unprotected subjects \((p = 0.88\) and \(p = 0.48\) respectively). Antibodies against LSA-1 were only significantly induced in CPS-MQ immunized volunteers on I2 \((p < 0.001; \text{Figure } 5C \text{ and } 5D)\), although not higher in protected subjects \((p = 0.39)\). Anti-MSP-1 antibodies by CPS immunization were not statistically significant increased in either group \((\text{Figure } 5E \text{ and } 5F)\).

IFN\(\gamma\) production by both adaptive and innate cell subsets in response to \textit{in vitro} \textit{P. falciparum} re-stimulation was induced by both CPS-CQ and CPS-MQ \((\text{Figure } S2)\), without a clear quantitative or qualitative difference between the study groups. Next, CD107a expression by CD4 T cells and granzyme B production by CD8 T cells, both associated with protection in a previous CPS-CQ trial \([7]\), were assessed by flow cytometry. Four out of 5 CPS-CQ and 8/10 CPS-MQ immunized subjects showed induction of CD107a expression by CD4 T cells upon \textit{in vitro} re-stimulation after immunization \((\text{Figure } 6A \text{ and } 6B)\). Although volunteer numbers were too low to reach statistical significance, the magnitude of this response appeared to be associated with protection for CPS-CQ \((\text{Figure } 6A)\), while for CPS-MQ it was not \((\text{Figure } 6B)\). Granzyme B production by CD8 T cells was not significantly induced in either CPS-CQ or CPS-MQ group, nor was it associated with protection \((\text{Figure } 6C \text{ and } 6D)\).

After challenge, MSP-1 specific antibodies were boosted in all unprotected volunteers \((\text{median fold change } 20.4 \text{ (range } 7.1–33.6\text{), } 76.0 \text{ (5.7–106.3) and } 7.7 \text{ (2.9–15.3) for CPS-CQ, CPS-MQ and control groups respectively})\). None of the protected subjects showed an increase in MSP-1 antibody levels on C+35 compared to C-1 \((\text{median fold change } 1.0 \text{ (range } 1.0–1.3\text{) and } 1.0 \text{ (0.6–2.4) for CPS-CQ and CPS-MQ groups, respectively})\).

**Discussion**

Immunization of healthy volunteers with \textit{P. falciparum} sporozoites while taking mefloquine prophylaxis is safe, induces both humoral and cellular immune responses and protects against homologous malaria challenge.

Although most volunteers experienced AEs after the first immunization, their frequency declined after subsequent immunizations in line with a reducing number of volunteers developing parasitemia. The majority of AEs was mild, with only 10–20% of subjects experiencing a grade 3 AEs after each immunization. In general, the reported neurologic and psychiatric side effects of mefloquine are a major concern limiting its acceptability and clinical application. In this study, mild to moderate dizziness and sleep-related complaints occurred in a small number of subjects in both chloroquine and mefloquine groups. Although this study was not powered to detect differences in AEs, frequency of neuropsychiatric AEs did not appear to differ between both drugs. This is in line with most reports in literature comparing AEs of mefloquine or chloroquine \([26–29]\) although one study found more neuropsychiatric AEs in subjects taking mefloquine by retrospective questionnaire \([30]\).

Taking the small sample size into consideration, both CPS-CQ and CPS-MQ immunization was safe and induced protective immune responses despite the use of a potent antimalarial prophylaxis.
and CPS-MQ immunization regimens appear to be reasonably well tolerated and safe. In 2013, however, after completion of this study, the U.S. Food and Drug Administration (FDA) issued a boxed warning for mefloquine, stating that neurologic side effects might be permanent. This might lead to adjustment of prophylaxis guidelines and limitation of mefloquine use where

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**Figure 5. Antibody responses induced by CPS-CQ and CPS-MQ immunization.** Antibodies against CSP (A and B; in AU), LSA-1 (C and D), and MSP-1 (E and F) were analyzed at baseline (B), 28 days after the first (I1) and second (I2) immunization and one day before challenge (C-1; 20 weeks after the last immunization) for all CPS-CQ (A, C and E, n = 5) and CPS-MQ (B, D and F, n = 10) immunized volunteers. Data are shown as individual titers with medians. Open squares indicate protected subjects, filled circles indicate unprotected subjects. Differences between the time points were analyzed by Friedman test with Dunn’s multiple comparison post-hoc test. Significant differences are indicated by asterices with * (p < 0.05), ** (p < 0.01), *** (p < 0.001).

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alternatives are available, as for now it remains a recommended antimalarial prophylactic for several target groups [31].

In previous studies we showed that 19/20 subjects (95%) were protected after bites from 45 infected mosquitoes, 8/9 (89%) after bites from 30 and 5/10 (50%) after bites from 15 infected mosquitoes during chloroquine prophylaxis [6,7,9]. The 60–70% protection observed in the current CPS-CQ and CPQ-MQ groups, immunized with bites from 24 mosquitoes, demonstrates the reproducibility of CPS immunization and indicates a linear relationship between immunization dose and protection. This confirms the consistency of the CPS approach and is remarkable, given the assumed variation in the number of sporozoites injected by mosquitoes [32]. This study further establishes CPS immunization as a worthwhile immunization protocol to relatively easily induce protection and create differentially protected cohorts to study target antigens and correlates of protection, both of which would be highly valuable tools in the search for *P. falciparum* vaccines and biomarkers of protection [33].

Although the study was not powered to detect these differences, there are hints suggestive of more efficient induction of protection by CPS-CQ compared to CPS-MQ: i) the two unprotected CPS-CQ volunteers showed a longer prepatent period than the CPS-MQ subjects (14 versus 12 days, Mann-Whitney test p = 0.13); ii) induction of immunity required less immunizations in the CPS-CQ group i.e. none of these subjects showed blood-stage parasites after the second immunization while subjects in the CPS-MQ group still developed parasitemia after the second and third immunization. If there is a difference between CPS-CQ and CPS-

**Figure 6. Cellular immune responses: CD107a expression by CD4 T cells and granzyme B production by CD8 T cells.** CD107a expression by CD4 T cells after PRBC re-stimulation, corrected for uRBC background in CPS-CQ (A) and CPS-MQ (B) groups; granzyme B production by CD8 T cells after PRBC re-stimulation, corrected for uRBC background in CPS-CQ (C) and CPS-MQ (D) groups. Symbols and lines represent individual subjects before immunization (B) and one day before challenge (C-1). Open squares indicate protected subjects, filled circles indicate unprotected subjects. Differences between B and C-1 for all subjects were tested by Wilcoxon matched-pairs signed rank test.
doi:10.1371/journal.pone.0112910.g006
Mefloquine or chloroquine plasma concentrations were still detectable in all volunteers one day before the challenge infection. Possible contributing effects of these remaining drug levels to the protective efficacy outcome were considered in several ways: i) The interval between first qPCR and thick smear positivity, as protective efficacy outcome were considered in several ways; i) the two volunteers with the highest mefloquine levels (116 and 603 g/L) were control subjects who became thick smear positive during CPS-CQ and CPS-MQ immunization. This interval was similar to previous CHMI studies with the NF54 P. falciparum strain in the absence of prophylactic drug levels [7,34]; ii) the two volunteers with the highest mefloquine levels (116 and 77 g/L) were control subjects who became thick smear positive with only a minimal delay in patency within the time-frame of historical controls [35]; iii) plasma chloroquine and mefloquine levels at C-1 were in all volunteers well below the minimum therapeutic concentration (CQ: 30 µg/L [36]) or the concentration at which breakthrough infections are observed in non-immune people (MQ: ≤406-603 µg/L [37]); iv) We cannot rule out that protected subjects experienced transient parasitemia after challenge, which was cleared in the first blood-stage cycle by remaining drug levels. But because parasitemia was not detected by qPCR in any of the protected subjects at any time point after challenge potential parasitemia must have been below the qPCR detection limit of 35 parasites/ml, indicating a reduction of at least 92% in liver load, given a geometric mean height of the first peak liver load, given a geometric mean height of the first peak; v) None of the protected subjects showed a boost in anti-MSP-1 antibodies after challenge while all unprotected subjects did, suggesting that protected subjects did not experience blood-stage parasitemia after challenge. [9].

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References


Author Contributions

Conceived and designed the experiments: EMB RS LGV AS RWS. Performed the experiments: EMB RS JMO MCB GJvG MdVB WG Lvl L GJHB KT CCH AS. Analyzed the data: EMB RS JMO CCH. Contributed to the writing of the manuscript: EMB RS LGV RWS.