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A Randomized, Controlled, Phase 1 Study of the Safety and Immunogenicity of the AMA1-C1/Alhydrogel® + CPG 7909 Vaccine for *Plasmodium falciparum* Malaria, in Semi-immune Malian Adults

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Abstract

A double blind, randomized, controlled Phase 1 clinical trial was conducted to assess the safety and immunogenicity in malaria exposed adults of the *Plasmodium falciparum* blood stage vaccine candidate Apical Membrane Antigen 1- Combination 1 (AMA1-C1)/Alhydrogel® with and without the novel adjuvant CPG 7909. Participants were healthy adults 18–45 years old living in the village of Donéguébougou, Mali. A total of 24 participants received 2 doses one month apart of either 80 μg AMA1-C1/Alhydrogel or 80 μg AMA1-C1/Alhydrogel + 564 μg CPG 7909. The study started in October 2007 and completed follow up in May 2008. Both vaccines were well tolerated, with only mild local adverse events and no systemic adverse events judged related to vaccination. The difference in antibody responses were over 2 fold higher in the group receiving CPG 7909 for all time points after second vaccination and the differences are statistically significant (all $p<0.05$). This is the first use of the novel adjuvant CPG 7909 in a malaria exposed population.

Keywords

Malaria; Vaccine; AMA1-C1; CPG

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1. Introduction

According to the most recent World Health Organization (WHO) report [1], half of the world's population is at risk of malaria, and an estimated 250 million cases led to nearly 1 million deaths in 2006, mostly in children under 5 years. A total of 109 countries were endemic for malaria in 2008, 45 within the WHO African region. While malaria elimination is being more and more considered as a possibility, the development of parasite and vector mosquito resistance to medicines and insecticides are potential stumbling blocks for these critical antimalarial interventions [2]. An effective malaria vaccine is needed to combat this disease. During the last 10 years, several malaria vaccine candidates have reached the stage of clinical testing in malaria exposed populations, and one vaccine has shown 35% efficacy against uncomplicated malaria [3] and is currently entering a phase 3 clinical trial (www.clinicaltrials.gov NCT00866619). Apical membrane antigen-1 (AMA1) is a surface protein expressed during the asexual blood stage of *Plasmodium falciparum*, and is a leading vaccine candidate, with several formulations being tested in malaria endemic areas in Africa [4–6]. Preclinical studies have shown that vaccination with AMA1 induces antibodies and protection against homologous parasite challenge in both rodent and monkey models of malaria infection [7–10]. The target population for this and other blood stage vaccines is young children and infants, primarily in Africa.

The AMA1-Combination 1 (C1) vaccine was developed by the Malaria Vaccine Development Branch of the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health, USA. The vaccine is a combination of an equal mixture of the correctly folded ectodomain portion of recombinant AMA1 from two divergent clones of *P. falciparum*, FVO and 3D7, adjuvanted with Alhydrogel®. The combination vaccine was chosen because of the sequence polymorphism of the AMA1 gene and the strain specific antibody response to recombinant AMA1 [11,12]. Several phase 1 studies have shown that the vaccine was well tolerated and relatively immunogenic [13–15]. Moreover previous phase 1 studies in malaria naïve adults have shown markedly enhanced antibody responses when the toll-like receptor (TLR) agonist CPG 7909 was added, with no significant safety issues identified [16,17]. Aluminum hydroxide gel (Brenntag Biosector, Denmark) has been extensively used as an adjuvant in many licensed human vaccines, as have other aluminum-containing adjuvants. CPG 7909 (Coley Pharmaceutical Group, Wellesley, MA) is being studied extensively in humans as a cancer therapeutic agent and as a vaccine adjuvant [18,19].

The primary aim of this study was to assess the safety of the AMA1-C1/Alhydrogel® + CPG 7909 vaccine to malaria exposed adults. A secondary objective was to compare the immunogenicity in this population of the vaccine with and without CPG 7909.

2. Methods

This was a double blind, randomized, controlled clinical trial (www.clinicaltrials.gov NCT00414336).

2.1. Participants

Participants were healthy adults 18–45 years old living in the village of Donéguébougou, Mali, an area with intense seasonal malaria transmission from July – November [20]. Enrolled volunteers were available for the duration of the trial (30 weeks) and were in good general health, with normal physical examination and screening labs (complete blood count, alanine aminotransferase (ALT), creatinine, urinalysis, anti-double stranded DNA (dsDNA) and hepatitis B and C rapid diagnostic tests). Specific exclusion criteria included pregnancy or breast feeding (if female), severe asthma, known immunodeficiency syndrome, use of systemic

corticosteroids or immunosuppressive drugs within 30 days of starting this study, receipt of a live vaccine within past 4 weeks or a non-live vaccine within past 2 weeks prior to entry into the study, history of a surgical splenectomy, receipt of blood products within the past 6 months, previous receipt of an investigational malaria vaccine, history of a known allergy to vaccine components, and history of use of chloroquine or related compounds (amodiaquine or primaquine) within 8 weeks of study entry. Chloroquine and related compounds have the potential to interfere with CPG-induced activation of B cells and plasmacytoid dendritic cells [21]; urine was checked for chloroquine at the time of first and second vaccination to confirm no recent use by vaccine recipients. Female participants were required to use reliable contraceptive methods prior to enrollment until 3 months after the second vaccination.

2.2. Ethics

Community and individual consent were obtained prior to screening and enrollment. The study protocol was reviewed and approved by the National Institute of Allergy and Infectious Diseases (NIAID) Institutional Review Board, and by the Ethics Committee of the Faculty of Medicine, Pharmacy and Dentistry, University of Bamako. The study was conducted under Investigational New Drug application BB-IND 13228, and was monitored for regulatory compliance by the Regulatory Compliance and Human Subject Protection Branch of NIAID. Safety data were reviewed by the Data Safety Monitoring Board (DSMB) of NIAID and by the local medical monitor.

2.3. Interventions

AMA1-C1 is a combination of two recombinant allelic proteins (AMA1-FVO and AMA1-3D7), consisting of amino acids 25 through 545 of the published sequences of each line's AMA1 gene (GenBank accession number AJ277646 for FVO and accession number U65407 for 3D7). Protein production and vaccine formulation are described in detail elsewhere [13,16]. CPG 7909 (VaxImmune®) was manufactured according to cGMP standards and was supplied in sterile single dose vials of 0.08 mL at 10 mg/mL in saline for IM administration.

Each dose of AMA1-C1/Alhydrogel contains about 424 μg of aluminum (Alhydrogel®, Brenntag Biosector, Denmark) onto which 80 μg of recombinant AMA1-C1 was bound. For volunteers receiving the vaccine with CPG, CPG 7909 in saline was added in a point of injection formulation as described previously [16]. Briefly, 0.7 mL of AMA1-C1/Alhydrogel was withdrawn and added to a single dose CPG 7909 vial. After mixing, 0.55 mL was withdrawn into a syringe and the vaccine was injected. Both vaccines were administered IM in the deltoid muscles on Days 0 and 28, in alternating arms. For safety a group of 6 volunteers was vaccinated first, followed by the remaining 18 volunteers approximately one week later. Volunteers were enrolled in October 2007 with vaccinations completed in November, and were followed for 30 weeks.

2.4. Outcomes

2.4.1. Safety and Tolerability—Volunteers were observed for 30 minutes after each vaccination to evaluate immediate adverse events, and were seen for follow up 1, 2, 3, 7 and 14 days after each vaccination, and at study weeks 12, 22 and 30. At all the times during the study a clinician was available on site so that participants could report adverse events at any time. Solicited adverse events included injection site pain, erythema, swelling, fever, headache, nausea, malaise, myalgia, arthralgia and urticaria. Solicited adverse events other than fever and urticaria were graded as follows: 0=absent/none, 1=easily tolerated, 2=interferes with daily activity or treatment given, 3=prevents daily activity. Fever was graded as follows: 0=oral temperature ≤37.5°C, 1=oral temperature between 37.6°C – 38.0°C, 2= oral temperature between 38.1°C – 39.0°C and 3= oral temperature >39.0°C. Urticaria was graded as follows: 0= none, 1= requiring no medications, 2= requiring oral or topical treatment or intra venous

medication or steroids for <24 hours and 3= requiring intra venous medication or steroids for >24 hours. Injection site erythema, swelling and induration were graded based on the maximum diameter as follows: mild = > 0 to ≤ 20mm, moderate = 21 – ≤ 50mm, and severe = > 50mm. Unless otherwise specified, non-solicited adverse events were graded as 0=none, 1=no effect on activities of daily living and no treatment given, 2=partial limitation in activities of daily living or treatment given, 3=activities of daily living limited to <50% of baseline or medical evaluation/therapy required. Hematological (hemoglobin, white blood cell counts, and platelets) and biochemical (ALT and creatinine) laboratory parameters were measured at screening and on days of immunization, and 3 and 14 days after each vaccination; hematological parameters were also checked 7 days after each vaccination. Anti-dsDNA was checked as a marker for autoimmunity at screening, at first vaccination, 14 days after the second vaccination, and at the end of the study. All adverse events were graded for severity and relationship to study product. Serious adverse events (SAEs) were defined as any adverse event resulting in death, life threatening, requiring hospitalization, resulting in disability or incapacity or congenital anomaly or birth defect, or any other event which required intervention to prevent such outcomes.

2.4.2. Immunogenicity—Antibody responses to the AMA1 antigens were measured in plasma by ELISA at Days 0, 14, 28, 42, 60, 90, and 210. The ELISA technique was described previously [22]. A human anti-AMA1 standard was made using a pool of plasma from individuals enrolled in a previous US vaccine trial [16]. The standard using this pool was assigned 460.9 units on AMA1-FVO and 578.0 units on AMA1-3D7. The minimal detection level of this assay was 26 ELISA units and all data below that limit of detection were assigned a value of one half the limit of detection (i.e., 13 units) for analysis. The following conversion factors were used: for FVO 1 ELISA unit=0.0294 μg/mL, for 3D7 1 ELISA unit=0.0329 μg/mL.

The method for assessing *in vitro* growth inhibition has been described previously [13]. GIA were performed using purified IgG from Days 0 and 42 (two weeks post second vaccination) to assess biologic activity of the induced antibody against *P. falciparum* FVO and 3D7 parasites. In this assay, purified antibody was added to the parasite cultures at approximately the same concentration as present in the corresponding serum sample (10.5 mg/mL in GIA well).

2.5. Randomization and Blinding

Twenty four participants were randomized 1:1 in blocks of 6 to receive 80 μg AMA1-C1/Alhydrogel® (Alum group) or 80 μg AMA1-C1/Alhydrogel® + CPG 7909 (Alum+CPG group). Randomization codes were created by a NIAID statistician, and randomization occurred at the time of first vaccination. A copy of the randomization code was provided to the pharmacist who used coded labels for the vaccines, and to the medical monitor and DSMB. Participants and investigators conducting clinical and immunologic assessments were blinded as to the participant's allocation to either Alum or Alum+CPG group to minimize the possibility of bias in assessment of adverse events.

2.6. Statistical Methods

Adverse events (AEs) were summarized by grade and assessed relationship to vaccination; all subjects receiving any vaccination were included in the analysis. One subject did not receive both vaccinations and was therefore excluded from the immunogenicity analysis; all other subjects were included.

For each subject included, the arithmetic average of the FVO and 3D7 ELISA responses at each day was used as that subject's AMA1-C1 antibody response for that day, because the

ELISA responses for the two allelic AMA1 were highly correlated (data not shown), as in previous studies [13–17]. To test for differences in ELISA response between the vaccine groups, we used the Day 42 adjusted response, which was defined for each subject as the AMA1-C1 antibody responses on Day 42 minus the AMA1-C1 antibody responses on Day 0. By using these adjusted ELISA values, we increased power to detect differences since there is variability in the baseline ELISA values in the Malian study population [14]. We compared groups using the adjusted ELISA values by exact Wilcoxon-Mann-Whitney (WMW) test and present the associated Hodges-Lehmann estimates and confidence intervals. When the adjusted ELISA values are all greater than 0, then fold-increases can be estimated by using those same methods after applying the log transformation. Geometric means and the associated t-test confidence intervals are used in Figure 1.

For GIA results, to compare the growth-inhibitory activity on Day 0 and 42 for each group for each parasite strain, an exact Wilcoxon signed rank-test was performed. Comparisons between Alum and Alum+CPG groups were performed using adjusted GIA response (Day 42 minus Day 0) by exact WMW test.

Clinical data were double entered and reconciled using Microsoft Access. Immunologic data were analyzed using PRISM (GraphPad Software, Inc., CA 92037 USA) and the coin R package [23].

2.6.4. Sample size—This study was powered to provide initial safety data for the use of AMA1-C1/Alhydrogel with CPG 7909 in an adult population living in a malaria endemic area. A group size of 10 volunteers per dose give a probability of 0.80 for detecting one or more AEs that occurred with a frequency of 0.15 per volunteer; we included 12 per group in case of withdrawals or loss to follow-up. Thus, a total of 24 volunteers were enrolled (12 in the Alum and 12 in the Alum+CPG group).

3. Results

3.1. Participant Flow and Baseline Data

Eighty subjects were screened for inclusion in the study, of whom 24 (19 males and 5 females) were enrolled. Reasons for exclusion were concurrent illness (n=13), positive serology for hepatitis B or C (n=13), or other abnormal screening laboratory tests (n=21; among them 4 with positive anti-dsDNA), use of chloroquine (n=1), and subjects who came late or did not come on the enrollment day (n=8). Group 1 (6 volunteers) received their first vaccination in October 18th, 2007, and Group 2 (18 volunteers) received their first vaccination in October 27th, 2007; second vaccinations were 28 days later for all volunteers. One subject who received the vaccine with CPG 7909 was withdrawn after the first vaccination due to an adverse reaction, as described below. Baseline characteristics at enrollment were similar in the two groups as shown in Table 1. All volunteers completed follow up to Day 210.

3.2. Safety

Adverse events related to vaccination are summarized in Table 2. Both vaccines were well tolerated. All local adverse events were mild, and there were no solicited systemic adverse events reported. Injection site pain was the most common local adverse event: 5/12 subjects after the first vaccination and 3/11 subjects after the second vaccination for Alum+CPG group *vs* 3/12 subjects after the first vaccination and 4/12 subjects after the second vaccination for Alum group. Three volunteers had mild leukopenia 3 days after first vaccination with the vaccine with CPG 7909 and one of these volunteers had a moderate (Grade 2) decrease in granulocytes. All white blood cell counts returned to normal by day 7. Two additional volunteers who received CPG 7909 had mild leukopenia 7 days after second vaccination; these

also returned to normal, one after 7 days and the other after 25 days. There was no increase in AE frequency or severity with the second dose of vaccine. No serious adverse events and no elevations in anti-dsDNA or clinical autoimmunity occurred. No anemia (hemoglobin <8.6 g/dL) was seen; the lowest hemoglobin at any time point for any volunteer was 11.0 g/dL.

Twelve clinical malaria cases (fever or other malaria symptoms with any asexual malaria parasitemia detected on thick smear) occurred during the follow-up period showing a non-significant trend of fewer in the Alum+CPG group: 3 cases in the Alum+CPG group (one was a *Plasmodium ovale* infection) and 9 in the Alum group having *P. falciparum* malaria, with 2 subjects each having 2 malaria episodes in the Alum group. The 3 cases of malaria in the Alum +CPG group all occurred within 14 days (day 1, 12 and 14) after the first vaccination while 8 episodes in the Alum group occurred between 20 and 100 days after the first vaccination and 1 occurred 8 days after the first vaccination.

One volunteer had moderate generalized pruritus the same day after receiving the first dose with AMA1-C1/Alhydrogel +CPG 7909. No rash or other symptoms were noted, and symptoms resolved within a few hours after treatment with an oral antihistamine. This volunteer had an undisclosed history of allergies requiring treatment with steroids, which would have precluded enrolment if known. He was followed to study completion with no additional study related adverse events.

3.3. Immunogenicity

AMA1-C1 antibody responses over time for both vaccine groups are shown in Figure 1. Peak antibody levels for both groups were seen at Day 42, two weeks after second vaccination. Because there is variability in the baseline ELISA values (range from 0.40 to 96.46 μg/mL) in this study population, to increase power to detect differences between the two groups, we used the adjusted response (subtracting the Day 0 ELISA value for each subject) for the following analysis. On Day 42 (Figure 2), there is a significant difference (Wilcoxon-Mann-Whitney test, p=0.013) with the Alum+CPG group having a higher response by on average 52.09 μg/mL (95% confidence interval; 7.44 – 76.52). Adjusted antibody responses of the Alum+CPG group were significantly higher than those of Alum group for all time points after second vaccination: fold increase was 2.78, 2.91, 3.18 and 2.52 on Days 42, 60, 90 and 210, respectively.

Growth-inhibitory activity of each IgG is shown in Figure 3. In the Alum+CPG group, there is a significant increase between Days 0 and 42 against FVO parasites (Fig. 3B; Wilcoxon signed rank-test, p=0.010), but not against 3D7 parasites (p=0.532). There is no significant difference in the Alum group. When adjusted (i.e., Day 42-Day 0) growth-inhibitory activity was used to compare Alum and Alum+CPG groups, there was no significant difference against FVO Wilcoxon-Mann-Whitney test, p=0.586) or 3D7 (p=0.868) parasites.

Analysis of memory B cell responses was performed and published separately [24].

4. Discussion

In a previous Phase 1 study in US malaria naïve adults, local and solicited adverse events were more severe when CPG 7909 was added to the AMA1-C1/Alhydrogel vaccine [16]. Another Phase 1 study in US adults showed the AMA1-C1/Alhydrogel vaccine with CPG to be well tolerated, with only one severe local adverse event of short duration reported and no volunteers withdrawn from vaccinations due to adverse events [17]. In this study both vaccine groups experienced only mild local adverse events and no systemic adverse events judged related to vaccination. The only significant related adverse event seen in this study was the generalized pruritus seen in one subject after receiving the first dose of AMA1-C1/Alhydrogel +CPG 7909. However, this occurred in a volunteer who had an undisclosed history of allergies requiring

treatment with steroids, which would have precluded enrolment if known. Autoimmunity is also a concern with the use of CPG adjuvants [19]. No positive anti-dsDNA was detected after vaccination and no clinical autoimmune events occurred, although given the small sample size the occurrence of such rare adverse events would be unlikely.

The AMA1-C1/Alhydrogel vaccine has previously been shown to be well tolerated by malaria naïve adults, malaria exposed adults, and malaria exposed children, but with only moderate immunogenicity [13–15], and with no impact on parasite density or clinical malaria demonstrated in a Phase 2 trial [25]. Higher antibody levels against AMA1 may be needed for protection against disease. In this study geometric means of the change in anti-AMA1 antibody unit levels were more than 2.5 fold higher in the group receiving the vaccine with CPG 7909 at all time points after second vaccination. This compares with 11–14 fold higher antibody levels in US adults when CPG 7909 was added to AMA1-C1/Alhydrogel when tested on malaria naïve subjects [16]. These differences between the two studies (2.5–3.2 fold higher vs. 11–14 fold higher) appear to be largely due to differences in the responses of the Alum groups (compare Figure 1 shown here with Figure 2 of Mullen et al [16]). Specifically, the Alum+CPG groups in both populations are similar with respect to geometric mean anti-AMA1 antibody levels after vaccination, while in the Alum alone groups, the Malian adults have much higher anti-AMA1 antibody levels than the comparable group of malaria naïve subjects. High baseline antibody levels in malaria exposed adults may also limit the extent to which antibody responses can be boosted with vaccination. An additional possible reason for the reduced enhancement of antibody levels with CPG in Malian adults could be extensive previous malaria exposure and down regulation of TLR9 due to the malaria pigment hemozoin, which has been reported to be a TLR9 agonist [26], although there is also evidence that parasite DNA may provide this stimulus [27]. The relative lack of reactogenicity in this study, and reduced effect on white blood cells compared to the previous studies in US volunteers (where all volunteers experienced decreases in neutrophils on Day 3) [17], may also be related to down regulation of TLR9, through chronic exposure to bacterial and viral pathogens. It is also possible that Malians are hyporesponsive to CPG adjuvants regardless of malaria exposure. Chloroquine and related antimalarials have been shown to reduce the effect of CPG in vitro [21,28], but volunteers with a history of recent use of these medications were excluded from enrollment and no chloroquine or similar products were detected in the urine of participants at enrollment or second vaccination. The extent to which young African children (who are the target population for this vaccine) will respond to CPG adjuvants remains to be demonstrated. Previous studies of AMA1-C1/Alhydrogel have shown immunogenicity in Malian children to be intermediate to that of US and Malian adults [13–15]. If the reduced enhancement of antibody response with CPG in this study is related to previous exposure to malaria or other pathogens then it is likely that children who have had less cumulative exposure will have enhancement with CPG more similar to US adults than Malian adults.

In the Phase 2 trial of AMA1-C1/Alhydrogel an imbalance was seen in the frequency of anemia, with more anemia occurring in the group receiving the study vaccine versus the comparator vaccine (Hiberix) [25]. No anemia cases were found in the adults in this study, with the lowest hemoglobin after vaccination 11.0 g/dL, well within the normal range for this population. Vaccinations took place during the malaria season, and there was an imbalance between the groups in the number of cases of clinical malaria, with more episodes in the Alum group than in the Alum+CPG group. This observation needs to be validated in a larger study, with a comparator vaccine that does not contain malaria antigens.

Despite the enhanced antibody levels in both the Alum and Alum+CPG groups after immunization, consistent increases were seen only in the FVO *in vitro* growth inhibition of homologous parasites in the Alum+CPG group (see Figure 3) by Wilcoxon signed rank-test. However, when we compared adjusted growth-inhibitory activity, there is no significant

difference between the Alum and Alum+CPG groups. Similarly, in our previous human trial with AMA1-C1/Alhydrogel in Malian adults [14], vaccination did not impact growth-inhibitory activity. We have shown that malaria-specific IgGs from Malian sera interfere with the growth-inhibitory activity of anti-AMA1 IgG in GIA [29]. Therefore, it is possible that such interfering IgGs masked the effect of AMA1-C1 immunization in this assay, even though a stronger adjuvant was utilized in this trial. A relationship between *in vitro* growth inhibition and reduction of parasite multiplication *in vivo* needs to be demonstrated.

Although the AMA1-C1 vaccine contains two divergent proteins, it is possible that vaccination with additional allelic proteins is required for protection in the field, or that an AMA1 protein designed to target conserved epitopes is needed. To further counter both antigen polymorphism and individual variability in responses a blood stage vaccine may require a combination of both alleles and antigens [30–38]. A vaccine combining FVO and 3D7 alleles of AMA1 and $MSP1_{42}$ and adjuvanted with Alhydrogel with CPG 7909 is entering clinical trials. Thus far the safety profile of CPG 7909 with these antigens is favorable, although experience in malaria exposed populations is limited and clinical benefit has not yet been demonstrated. Additional studies in malaria exposed adults and children are needed to further extend the safety profile of these vaccines, confirm the enhancement of antibody responses with CPG 7909, and demonstrate benefit against clinical outcomes in the target population.

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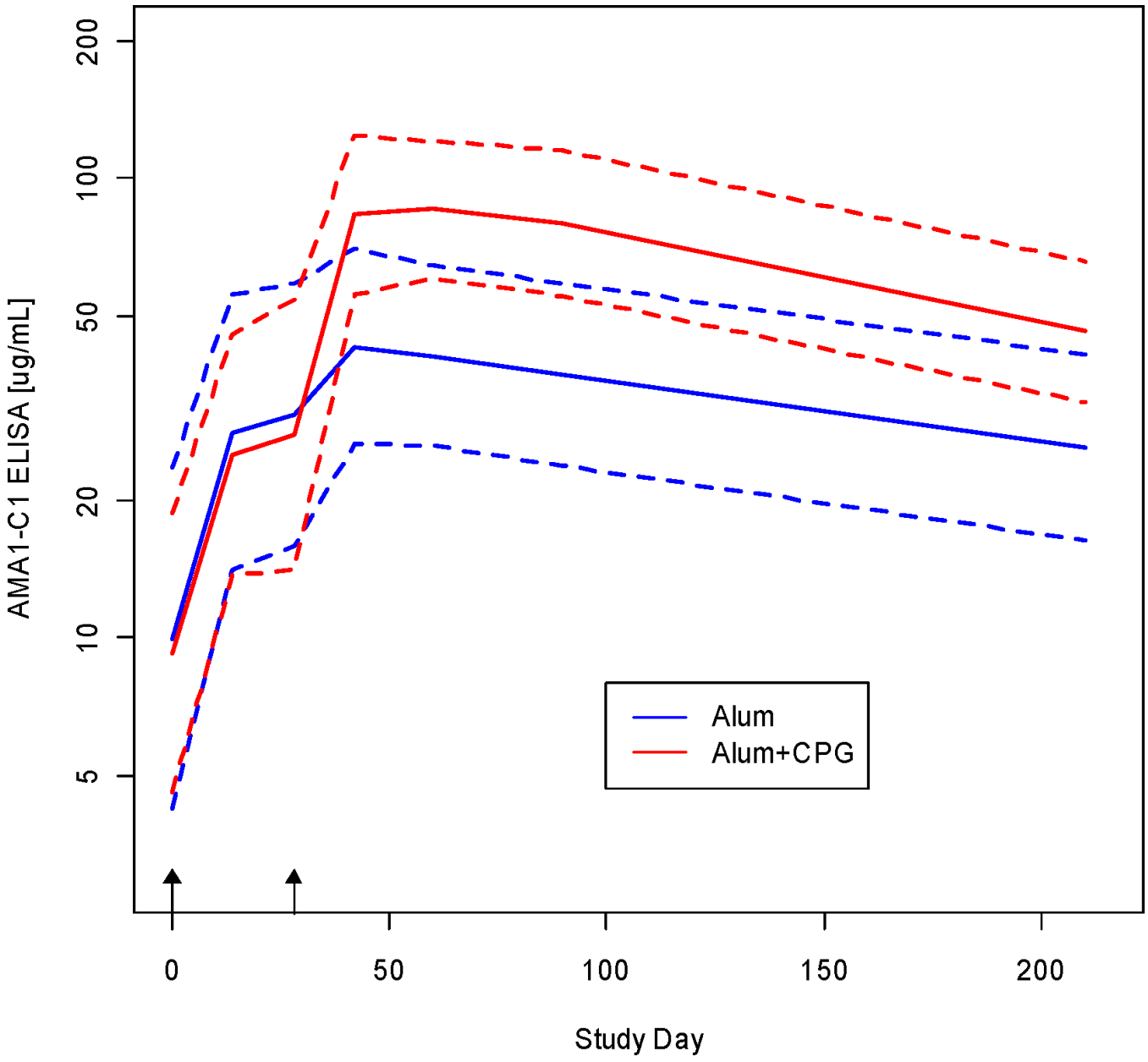

Figure 1.
AMA1-C1 antibody response over time. Geometric mean and 95% pointwise confidence intervals for each group are shown. Participants were immunized on Days 0 and 28, and antibody responses were measured on Days 0, 14, 28, 42, 60, 90, and 210. Arrows indicate immunization days.

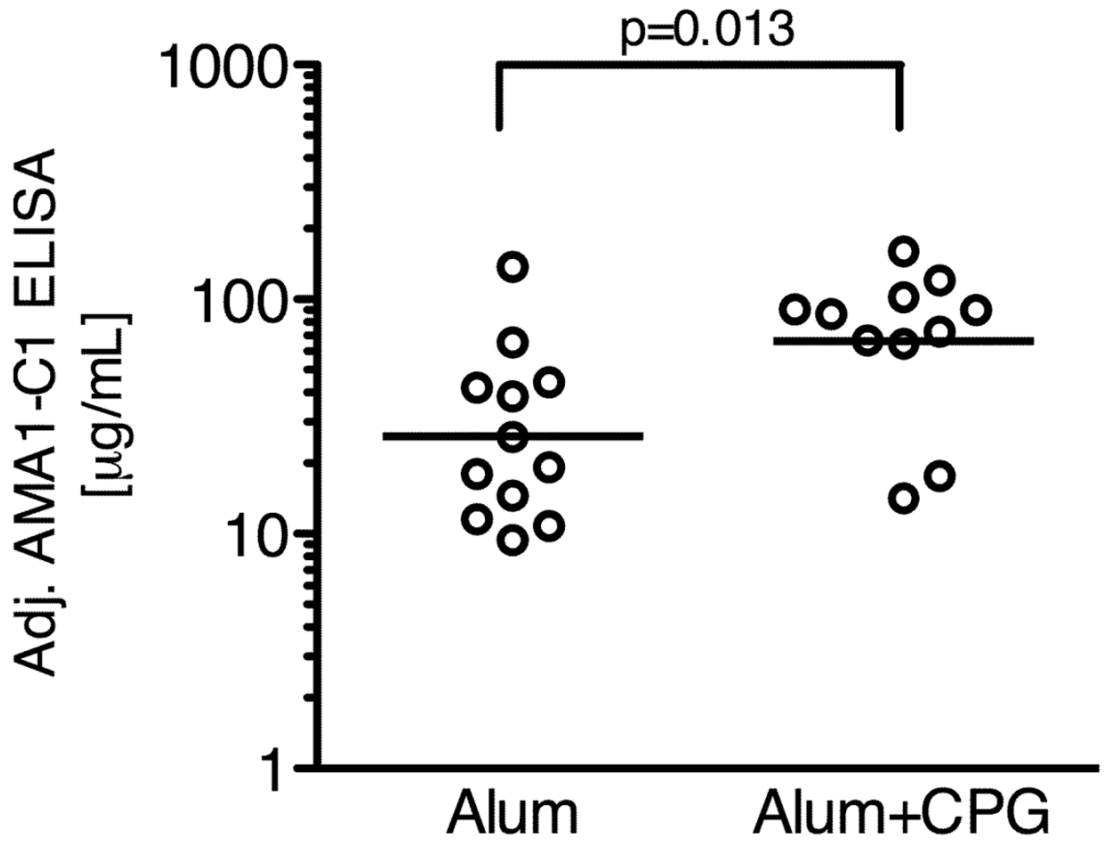

Figure 2.
Adjusted AMA1-C1 antibody response on Day 42. Adjusted antibody response was calculated by subtracting Day 0 response from Day 42 response for each person. Adjusted response for all individuals and the geometric mean are shown.

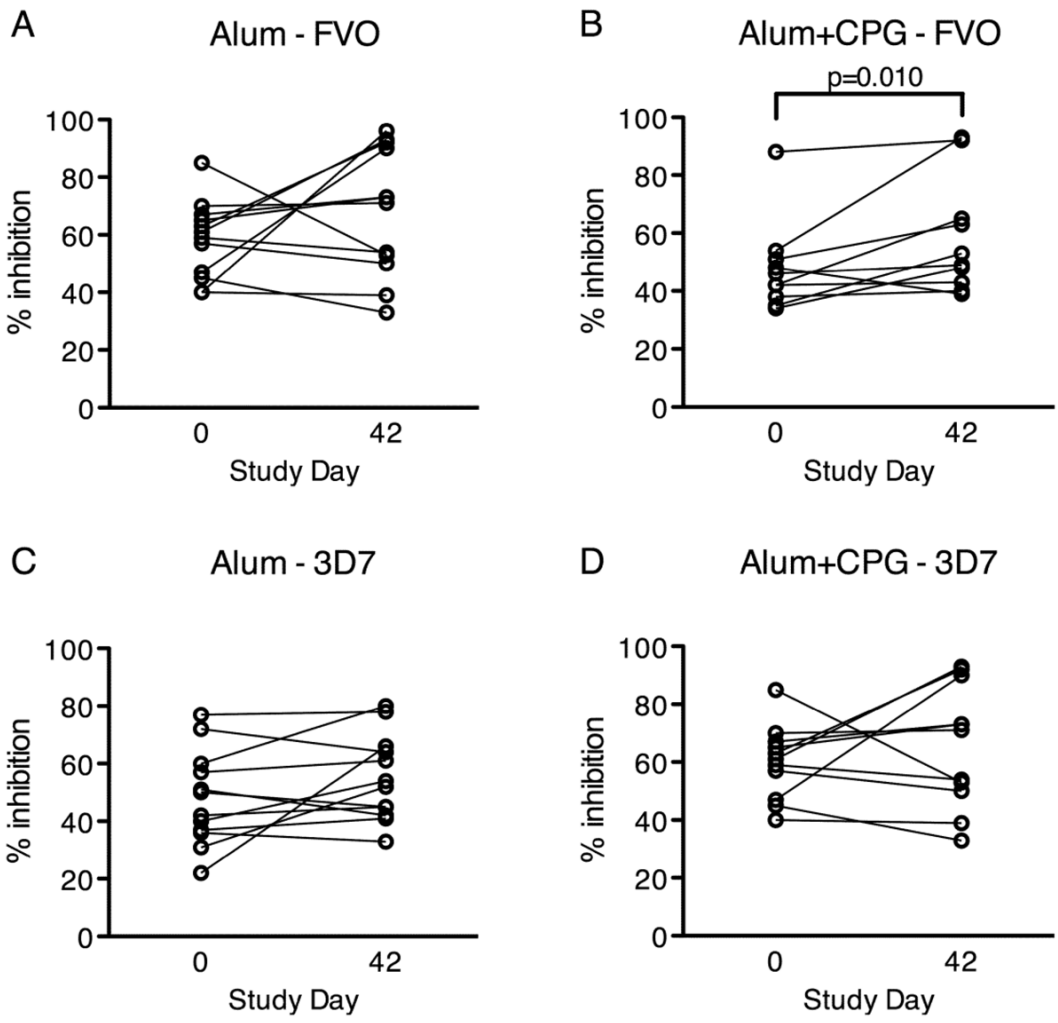

Figure 3.

Growth-inhibitory activity of Days 0 and 42 IgGs. Total IgG was purified individually by Protein G purification. IgGs from Alum group (A and C) and Alum+CPG group (B and D) were tested against either *P. falciparum* FVO (A and B) or 3D7 (C and D) parasites at 10.5 mg/mL in GIA well.

Table 1

Baseline characteristics at enrolment

	AMA1-C1/Alhydrogel+CPG 7909 n=12	AMA1-C1/Alhydrogel n=12
Age in years[1]	28.17 (19–41)	26.33 (19–44)
Gender-Male (%)[2]	9 (75.0%)	10 (83.33%)
Ethnic groups		
Bambara (%)[2]	2 (16.67%)	5 (41.67%)
Sarakolle (%)[2]	10 (83.33%)	7 (58.33%)
Parasitemic[2,3]	7 (58.33%)	5 (41.67%)
Hemoglobin[1]	13.25 (11.8–15.5)	13.17 (11.3–14.8)

[1] mean(Range)

[2] number (%)with characteristic.

[3] *P. falciparum* parasites detected by microscopy

Table 2

Frequency of local injection site, systemic and laboratory adverse events judged definitely, probably, or possibly related to vaccination

	After Vaccination #1 but before Vaccination #2		After Vaccination #2	
	AMA1-C1/Alhydrogel+CPG 7909	AMA1-C1/Alhydrogel	AMA1-C1/Alhydrogel+CPG 7909	AMA1-C1/Alhydrogel
Local				
Pain	5/12	3/12	3/11	4/12
Erythema	0	0	0	0
Swelling	1/12	1/12	0	0
Systemic				
hypersensitivity reaction	1/12	0	0	0
Laboratory				
Low granulocytes	1/12	0	0	2/12
Low leukocytes	3/12	0	2/11	1/12

All adverse events were mild except for one low granulocytes count in AMA1-C1/Alhydrogel+CPG after the first vaccination which was moderate.