Glutamate-rich protein (GLURP) induces antibodies that inhibit in vitro growth of Plasmodium falciparum in a phase 1 malaria vaccine trial

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Abstract

The glutamate-rich protein (GLURP) of P. falciparum is the target of cytophilic antibodies which are significantly associated with protection against clinical malaria. A phase 1 clinical trial was conducted in healthy adult volunteers with the long synthetic peptide (LSP) GLURP85–213 combined with either Aluminum Hydroxide (Alum, 18 volunteers) or Montanide ISA 720 (ISA, 18 volunteers) as adjuvants. Immunizations with 10, 30 or 100 μg GLURP85–213 were administered subcutaneously at days 0, 30, and 120. Adverse events occurred more frequently with increasing dosage of GLURP85–213 LSP and were more prevalent in the ISA group. Serious vaccine-related adverse events were not observed.

The vaccine induced dose-dependent cellular and humoral immune responses, with high levels of (mainly cytophilic IgG1) antibodies that recognize parasites by immunofluorescence (IFA). Plasma samples collected 30 days after the last immunization induced a dose-dependent inhibition of parasite growth in vitro in the presence of monocytes. In conclusion, immunizations with GLURP85–213 LSP formulations induce adverse events but can be administered safely, generating antibodies with capacity to mediate growth-inhibitory activity against P. falciparum in vitro.

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Keywords: Malaria; Plasmodium falciparum; GLURP; Immunization; Clinical trial; Phase 1; Montanide

1. Introduction

Malaria is one of the most important infectious diseases worldwide. As part of the enlarged global efforts to control malaria, production of candidate malaria vaccines at clinical grade has significantly increased [1]. The development of a safe and effective vaccine against P. falciparum will be a major step in the fight against malaria [2].

Glutamate-rich protein (GLURP) is a P. falciparum vaccine candidate protein expressed in both the pre-erythrocytic and erythrocytic stages [3]. Immunno-epidemiological studies have shown that high levels of cytophilic (IgG1 and
IgG3) antibodies with specificity for both repeat and non-repeat regions of GLURP are associated with protection from high parasitaemia and clinical disease in Africa and Asia [4–9]. In vitro studies show that affinity-purified human IgG against the non-repeat region R0 (residues 24–489) and the repeat region R2 (residues 816–1091) can inhibit parasite growth in the presence of monocytes [10]. The target B-cell epitopes in this context show only limited degree of polymorphism between different P. falciparum strains (n = 44) [11].

The GLURP85–213 sequence (LR67) was selected for cGMP manufacturing as a long synthetic peptide (LSP), which included the immunodominant P3-epitope, antibodies to which mediating the strongest biological effect in vitro [7]. Results are presented from an open-label, randomized, dose-finding phase 1 clinical trial with GLURP85–213 conducted in 36 healthy adult volunteers comparing 2 adjuvants.

2. Materials and methods

2.1. Study subjects

The study was conducted from July 2001 to June 2003. Thirty-six healthy (25 female) volunteers (mean age 31.3 years; range 18–54) without previous history of malaria or long-term residence in endemic areas were enrolled. None of these volunteers had antibody reactivity with GLURP85–213. Blood samples showed no abnormalities for standard clinical tests and were negative for HIV, HBV or HCV. Informed consent was obtained from all volunteers enrolled into the study, which was approved by the Institutional Review Board of the University Medical Center Nijmegen (CMO 2001/063). None of the volunteers was lost to follow-up.

2.2. Peptide synthesis and formulations

GLURP85–213 peptide was produced by Dictagène SA, Epalinges, Switzerland (batch number 01FS008) and sampled by Serolab SA in Epalinges according to GMP standards. Toxicity, under GLP standards, was tested in Macaca mulata monkeys by three consecutive subcutaneous injections of 100 μg GLURP85–213 with Montanide ISA 720 (batch number 95041) and found to be safe and well tolerated (Chengdu Kuachang Science and Technology Co. Ltd., Chengdu, China) and was approved by the Institute of Medical Biology, Chinese Academy of Medical Sciences, China under supervision of SEDAC therapeutics SA, Lille, France.

GLURP85–213 LSP is presented as a lyophilized powder aliquoted by individual doses. Absorption of the peptide to Alum Hydroxide as tested in the supernatant after 15 and 45 min absorption to Alum by BCA (bicinchoninic acid) protein assay and RP-HPLC was 100%. Prior to subcutaneous injection (1 ml), the lyophilized peptide was reconstituted with sterile water and further diluted with sterile saline. One hour before the administration, the peptide was mixed with Montanide ISA 720 (SEPPIC, Paris, France) or Alum Hydroxide (Sedac Therapeutics, Lille, France). Each Montanide ISA 720 vaccine mixture contained 700 μl Montanide ISA 720.

2.3. Trial design

This trial was a single center, open-label, dose-finding, randomized, two adjuvants, three doses, safety and immunogenicity phase 1 clinical study in healthy adult volunteers. Subcutaneous 10, 30 or 100 μg GLURP85–213 doses with the adjuvants were administered on day 0 in the deltoid region and subsequently in alternate arms for the two following doses on days 30, and 120. Six volunteers were randomly assigned to each group. The study design was dose escalating with an asymptomatic interval of 6 weeks before starting immunization with the next higher concentration in the next group. All volunteers were followed for 540 days.

2.4. Assessment of safety

Volunteers were observed after each immunization for 1 h, at 24 and 48 h post-immunization. A diary was provided for documentation of adverse events (AEs). Both solicited and unsolicited adverse events were collected during the study period. Solicited adverse events were—(a) local: pain, induration, swelling, erythema and functional inabilities, or (b) systemic: diffuse erythema, exanthema, urticaria, edema, fatigue, fever, joint pain, muscle pain, headache, asthma, hoarseness, malaise, syncope, dizziness, paleness, transpiration, nausea and palpitations.

Adverse events were graded as—(i) grade 1 (mild): aware of discomfort but no disruption of daily living; (ii) grade 2 (moderate): sufficient to interfere with normal daily activity; (iii) grade 3 (severe): inability to work or perform normal daily activities.

The intensity of pain was recorded on a visual analogue scale [12] (VAS, grade 1: 1–30 mm, grade 2: 31–70 mm, grade 3: >70 mm), grading of induration and erythema was performed by measuring the size of the event (grade 1: 1–20 mm, grade 2: 21–50 mm, grade 3: >50 mm) and temperature (grade 1: <37.5 °C, grade 2: 38–39 °C, grade 3: >39 °C).

The causality of the AE with respect to the study immunization was assessed by a medical doctor and reported as not related, possibly related or probably related.

Criteria for exclusion of volunteers from further immunization, but not from follow-up, were predefined as follows: local erythema >10 cm, induration >5 cm, pain on the VAS >7 cm, or signs of necrosis.

Biological safety was assessed at days 0, 30, 60, 150 and 360 and included a complete blood cell count, sodium, potassium, creatinine, glucose, alkaline phosphatase, aspartate aminotransferase (ASAT), alanine aminotransferase (ALT), total bilirubin and gamma glutamyl transpeptidase levels.
2.5. Assay randomization

Following a dose escalating schedule, 36 volunteers were randomized into 6 groups; each group was immunized 3 times with either 10, 30 or 100 μg GLURP85–213 combined with either Alum or ISA. Volunteers were matched for sex and age following a standard procedure in the programming language SAS 8.1 for Windows by Dr. A.M. Jensen, Statens Serum Institute, Denmark.

2.6. Assessment of immunogenicity

At days 0, 30, 60, 120, 150 and 360 evaluations of the immune response were performed using whole blood samples collected on citrate in a Vacutainer CPT tube (BD, Alphen aan de Rijn, The Netherlands); fresh peripheral blood mononuclear cells (PBMC) were separated on the density gradient in the Vacutainer CPT tube, washed and used for lymphocyte proliferation assays and cytokine production. Plasma was collected after centrifugation and aliquots were stored at −20°C for evaluation.

2.7. Measurements of anti-GLURP85–213 antibodies by ELISA and IFA

Anti-GLURP85–213 and anti-P3 antibodies were measured in plasma samples by ELISA [10]. Briefly, microtiter plates (NUNC™ Maxisorp, Life Techn, The Netherlands) were coated with 0.2 μg GLURP85–213/ml. After a washing step, plasma samples were serially diluted in PBS/0.05% Tween 20/1.25% non-fat milk (PBSTM) and added to the plates. The following conjugates were diluted in PBSTM: rabbit anti-human IgG HRP (Dako, P-214) dilution 1/10,000; mouse anti-human IgG1-HRP, HP6188 (M1328, CLB, A'dam, The Netherlands), dilution 1/10,000; mouse anti-human IgG2-HRP, HP6014 (M1329, CLB, A'dam, The Netherlands), dilution 1/500; mouse anti-human IgG3-HRP, HP6095 (M1330, CLB, A'dam, The Netherlands), dilution 1/500; mouse anti-human IgG4-HRP, HP6196 (M1331, CLB, A'dam, The Netherlands), dilution 1/1000. K-Blue substrate (Neogen, Sigma, cat 010131) was used as substrate for 15 min and the absorbance was read at 450 nm in a Titertek plate reader (Labsystems, VWR, Amsterdam, The Netherlands). Day 0 data from all volunteers (n=36) were used to calculate the baseline (2 S.D. over the normal sample mean OD). Test samples were serially diluted in duplo starting at 1/125. The test sample titer was defined as the last dilution step with an OD higher than the baseline. A pool of hyperimmune plasma of African individuals (n=10) was used as a positive control.

The immunofluorescence assay (IFA) was performed with air dried and frozen cultured P. falciparum NF 54 parasites (a mixture of all asexual forms). Pre- and post-immunization samples were tested in a serial dilution starting at 1/20. A pool of hyperimmune plasma of African individuals (n=10) was used as a positive control. The scores between the two blinded examiners was never greater than one dilution and the mean of both scores was taken. Post-immunization titer was defined as the highest dilution with positive fluorescence compared to day 0 sample of each individual. All day 0 samples were negative at 1/20.

2.8. Peripheral blood mononuclear cells proliferation and cytokine production

Fresh PBMC were evaluated for proliferation in the presence of P. falciparum GLURP85–213 (1.2, 6 and 30 μg/ml) in six-fold cultures, each of 1.10⁵ cells/well. PBMC were cultured in 200 μl Dulbecco’s MEM with Glutamax-I, 2 mM pyruvate and high glucose (GIBCO BRL, cat 31966-021) supplemented with 10 mM HEPES buffer (GIBCO BRL, cat 15630-056), 100 IU/ml penicillin–streptomycin (GIBCO BRL, cat 15140-122), 100 μM non-essential amino acids

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Number of volunteers who experienced solicited signs and symptoms during the 48-h follow-up after each immunization</th>
</tr>
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<td></td>
<td>Alum (μg)</td>
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<tr>
<td>Local</td>
<td></td>
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<tr>
<td>Erythema</td>
<td>0 6 6 3 4 6</td>
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<tr>
<td>Induration</td>
<td>1 6 6 2 4 4</td>
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<tr>
<td>Pain</td>
<td>1 0 5 2 2 5</td>
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<tr>
<td>Swelling</td>
<td>0 1 2 3 1 2</td>
</tr>
<tr>
<td>Late onset</td>
<td>0 0 0 1 5 6</td>
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<tr>
<td>Systemic Contralateral</td>
<td>0 0 0 0 0 0</td>
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<tr>
<td>Vaccination #2</td>
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<tr>
<td>Group size</td>
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<tr>
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<tr>
<td>Erythema</td>
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<tr>
<td>Induration</td>
<td>4 6 6 4 3 4</td>
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<tr>
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<tr>
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<tr>
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<tr>
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<tr>
<td>Systemic Contralateral</td>
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Fig. 1. Prevalence of adverse events (AEs). Bars represent mean number of AEs subsequent to the first, second or third immunization. The black bars represent the grade 3 AEs, the grey bars represent both the grades 1 and 2 AEs. Groups of six volunteers were immunized with GLURP85–213 (10, 30 or 100 µg) with Aluminum Hydroxide (Alum) or Montanide ISA 720 (ISA). Number of subjects represent the number of volunteers immunized at, respectively, the first, second or third immunization.

Fig. 2. Production of anti-GLURP85–213 IgG. Results are expressed as box plots and whiskers that show the 5th, 25th, 50th, 75th and 95th percentile of log endpoint titer for each immunization group. Groups of six volunteers were immunized with 10 µg (A), 30 µg (B) or 100 µg (C) GLURP85–213 with Alum (grey bars) or ISA (white bars). Arrows indicate the time points of first, second and third immunization. *, # and † represent data of 5, 4 and 1 volunteer(s), respectively.
2.9. *P. falciparum* growth inhibition assay

Antibody dependent monocyte mediated inhibition of *P. falciparum* growth *in vitro* was assayed according to the method described by Bouharoun-Tayoun et al. [13] as modified by Bolad et al. [14]. Parasites (F32) were synchronized by sorbitol lyses [15] and allowed to mature to trophozoite/schizont stages (PRBC). PRBC were adjusted to 4% hematocrit with culture medium (CM: RPMI 1640 containing 25 mM HEPES, 2 mM L-glutamine, 50 μg/ml gentamycin and 0.2% sodium bicarbonate (all ingredients from Gibco, Paisley, UK) and supplemented with 1% Albumax (Gibco BRL, Grand Island, NY)) and to 1% parasitaemia with O+ uninfected erythrocytes.

For human monocyte isolation, PBMC from healthy malaria non-exposed Swedish volunteers were separated on Ficoll-Paque and resuspended in 25% autologous serum in CM. Adherent cells (97% viability) were removed from a Petri dish after 1 h incubation in autologous serum at 37°C in 5% CO2, and were then resuspended in CM.

Plasma samples from volunteers (pre- and post-immunization, day 150) were serially diluted (40–10%) in

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Fig. 3. Anti-GLURP-P3 IgG and isotypic antibody response at days 60 and 150. Responses are calculated as log endpoint titer for IgG and the isotypes IgG1, IgG3 and expressed as described in legend of Fig. 2. IgG2 and IgG4 were below baseline (data not shown). Groups of six volunteers were immunized with the combinations GLURP<sub>85–213</sub>/Alum (grey bars) 10 μg (A10), 30 μg (A30) or 100 μg (A100) or GLURP<sub>85–213</sub>/ISA (white bars) 10 μg (ISA 10), 30 μg (ISA 30) or 100 μg (ISA 100). Day 150 data of ISA 100 are not shown because it represents only 1 volunteer. * and # represent data of 5 and 4 volunteers, respectively, ○ = outlier.
CM without Albumax. Diluted plasma (100 μl) was added to an equal volume of the PRBC suspension in duplicate and incubated at 37 °C for 42 h in the presence or absence of 1.5 × 10⁸ to 2 × 10⁹ monocytes. After incubation, cells were harvested, washed and monolayers were prepared and fixed. Parasites were stained with Acridine Orange (Fluka Chemie AG, Buchs, Switzerland) and the percentage of newly infected erythrocytes was determined per 40,000 erythrocytes in a fluorescence microscope. The reading of the slides was performed blindly with regard to the donor tested. The starting parasitaemia of 1% increased after incubation to a mean of 3.2% (range 2.7–3.6). A serum pool from malaria immune Burkinabes was used as a positive control and a plasma pool from Swedish blood bank donors as negative control. The percentage growth inhibition was determined as follows:

\[
\frac{\% \text{ parasitaemia in control wells} - \% \text{ parasitaemia in test wells}}{\% \text{ parasitaemia in control wells}} \times 100
\]

2.10. Statistical evaluation

Box plots were made by using the SPSS-10 program. The Pearson correlation coefficient (R) was calculated to detect significant relationships. P-values<0.05 were considered to be significant.

3. Results

3.1. Safety assessment

Three dosages of GLURP85–213 (10, 30 and 100 μg) were tested after formulation with either Alum or ISA. The Alum group received the complete immunization protocol, but 8/18 volunteers of the ISA group had to be excluded during the study. Seven were excluded from further immunization because they met the predefined criteria for exclusion after the first (n = 3) or second (n = 4) immunization (Table 1). One volunteer (ISA 100 μg) developed a possible related infection of the chin after the first immunization.

Serious adverse events did not occur but all volunteers reported some degree of AEs related to immunization (possible and/or probable), adding up to a total number of 291 AEs. In general, ISA induced more frequent and more pro-

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**Fig. 4.** (A) Immunofluorescence on asexual parasites of *P. falciparum* NF54. Responses (day 150) in the different treatment groups are expressed as mean log endpoint titer (95% CI) for positive fluorescence. *P* < 0.05. For detailed information see legend of Fig. 3. (B) Immunofluorescence of a plasma sample on asexual parasites of *P. falciparum* NF54. As a representative sample immunofluorescence reactivity of a volunteer in the Alum 100 μg group is shown. B1, plasma of day 0; B2, plasma of day 150 at a dilution of 1:320.
nounced AEs than Alum and prevalence and severity clearly increased with increasing dosage (Fig. 1). Only Alum volunteers showed an increase in prevalence and severity of AEs with the number of immunizations (Fig. 1). One volunteer developed abnormal laboratory tests, showing both an elevated glucose (8.2 mmol/l) and ALAT (73 U/l) at 1 year after the third immunization. Further analysis by his general practitioner did not reveal any abnormalities.

Three volunteers reported late onset local AEs at a mean of 17 days (range 12–28) after immunization in the ISA group after the first immunization (2 in the 30 μg and 1 in the 100 μg group).

Remarkably, 10 volunteers developed local contralateral adverse reactions after the second (n = 1) or third (n = 9) immunization at the previous immunization site (possibly or probably related). These AEs occurred in nine volunteers in the Alum group and one ISA volunteer. The intensity of these AEs ranged from mild to severe and occurred on average 6 h post-immunization.

3.2. Anti-GLURP85–213 antibody response

Immunization elicited specific IgG antibodies in 97% of the volunteers with one non-responder in the Alum 10 μg group. The highest anti-GLURP85–213 IgG antibody response was observed after two immunizations in the ISA 30 μg group and after three immunizations in the ISA 10 μg and Alum 100 μg groups (Fig. 2). A dose related increase in antibody responses was observed after each immunization, with earlier and higher titers in the ISA groups. The ISA 30 μg group reached its peak antibody response after two immunizations without further increase after the third immunization. By day 360, ~8 months after the third immunization, the mean IgG antibody titer had decreased by 78% (range 43–98%) in both Alum and ISA groups. Anti-GLURP85–213 IgG1 was the predominant isotype induced and IgG3 to a lesser extent (data not shown) with a similar outcome for the P3 epitope (Fig. 3). IgG2 and IgG4 were virtually absent.

Comparison of anti-GLURP85–213 and anti-GLURP-P3 IgG titers at both days 60 and 150 showed a strong correlation (R = 0.83, P = <0.0001; R = 0.63, P = <0.001).

3.3. Antibody reactivity against P. falciparum parasites

Antibody reactivity against asexual parasites at day 150, was demonstrated by IFA in 21 of the 28 volunteers receiving the full course of 3 immunizations. The IFA-titer was dose-dependent showing no significant difference between Alum and ISA groups (Fig. 4). The highest responses were measured in the ISA 30 μg and Alum 100 μg groups, while only two volunteers responded in the Alum 10 μg group. Comparison of IgG and IgG1 Elisa titers with titers obtained by IFA showed a significant correlation (R = 0.50, P = 0.007, n = 28 and R = 0.73, P = <0.001, n = 27, respectively).

Fig. 5. Monocyte-dependent inhibition of parasite growth in vitro. Results are expressed as a mean inhibition percentage ± S.E.M. of parasite growth in the presence of plasma (day 150) with or without monocytes. (A) Six volunteers immunized with ISA 30 μg, post-immunization plasma without monocytes [□], pre-immunization [■] and post-immunization [▲] plasma with monocytes; (B) six volunteers immunized with Alum 100 μg, post-immunization without monocytes [□], pre-immunization [■] and post-immunization [▲] plasma with monocytes; (C) pool plasma from immune adults from Burkina Faso without [□] and with [■] monocytes.
3.4. Biological effect of GLURP-induced antibodies

For testing the capacity to inhibit parasite growth in vitro in the presence of human monocytes [13,14] we selected one dosage group from each adjuvant formulation with the highest level of cytophilic antibodies, i.e. ISA 30 μg and Alum 100 μg. Significant inhibition was observed in both groups in a dose-dependent way, except the 20% plasma of the ISA 30 μg group probably due to a high non-specific growth inhibition in the pre-immunization samples of 3/6 volunteers. This dose-dependent reactivity is comparable with the reactivity found in immune plasma from African adults (Fig. 5).

3.5. PBMC response to GLURP_{85–213} peptide

PBMC proliferation to GLURP_{85–213} peptide was found in the vast majority of volunteers with generally no further increase after two immunizations (Fig. 6). ISA 30 μg and ISA 100 μg groups showed higher proliferation after the first immunization as compared to the respective Alum groups. The responses of the Alum 10 μg group were higher than the ISA 10 μg group, but the overall proliferation was comparable between both adjuvant groups.

By day 360, ~8 months after the third immunization, the stimulation index decreased by 76% in both groups.

Levels of IFNγ and IL-10 were evaluated in the Alum 30 μg, Alum 100 μg and ISA 30 μg groups. Overall, in vitro IFNγ production (Fig. 7) increased after each immunization with very low IL-10 concentrations (data not shown).

A correlation was found between the T cell proliferative response and the IFNγ production at day 150 (R = 0.50, P = <0.05) in all groups.

Fig. 6. GLURP_{85–213} induced PBMC proliferation. Expression of results and acronyms are described in legend of Fig. 2 and show the log stimulation index (S.I.) of PBMC stimulated with GLURP_{85–213} (30 μg/ml). Control S.I. with tetanus toxoid 10 μg/ml and phytohemagglutinin 5 μg/ml ranged from 2 to 605 and 10 to 3287, respectively.
4. Discussion

This study shows that GLURP\textsubscript{85–213} LSP can be administered safely but AEs are relatively frequent. Immunogenicity data show that GLURP\textsubscript{85–213} LSP may induce: (i) specific antibodies that recognize \textit{P. falciparum} parasites, (ii) cytophilic antibodies (in particular IgG1) that recognize GLURP\textsubscript{85–213} and the P3 epitope and (iii) GLURP\textsubscript{85–213} specific PBMC proliferation with concomitant high IFN-\gamma production. In addition, post-immunization plasma samples inhibit \textit{P. falciparum} parasite growth \textit{in vitro} in the presence of human monocytes.

AEs occur relatively frequently compared to other malaria vaccine trials [16–19]. The subcutaneous route of immunization may have contributed to this difference since intramuscular administration used in other trials may show less obvious and noticeable signs and symptoms.

Alum was generally better tolerated with slower development of adverse events compared to ISA, although both adjuvants induce more frequent and severe reactions with increasing dose. ISA induces more probably related local severe adverse events in particular with the 100 \(\mu\)g dose and this explains why 7/18 vaccines in the ISA group did not receive the full regimen of 3 immunizations.

Our study generated two remarkable observations: Firstly, three volunteers in the ISA group developed \textit{late onset} local adverse events up to grade 3, starting on average 17 days after the first immunization. Similar reactions have been reported previously following administration with other vaccine formulations [17,19–22]. These events are suggestive of an Arthus-type reactivity (type III hypersensitivity) induced by GLURP antibodies, which may have bound to the GLURP depot at the injection site. Microscopic analyses of the infiltrates was not possible, as biopsies were not taken. Immune responses, more specifically the IgG levels and PBMC stimulation indices, of these volunteers to GLURP\textsubscript{85–213} were similar to the other volunteers.

Secondly, 10 volunteers, mainly from the Alum group, develop local adverse events at previous immunization sites (\textit{contralateral} events), shortly after the third immunization. Previously, such \textit{contralateral} events have been reported in other malaria vaccine studies adjuvanted with Alum [19,21,23]. Suggestive of a type I hypersensitivity, specific IgE binding may take place to incompletely adsorbed antigen that persisted at these sites [21]. However, GLURP\textsubscript{85–213} was shown to be 100% bound to Alum, and volunteers with \textit{contralateral} events did not present with urticaria, elevated eosinophil counts or detectable IgE antibodies (data not shown). A type III Arthus-type reaction may be a possibility.

Remarkably, volunteers with the most frequent and in general severe \textit{contralateral} adverse events generate higher IgG responses at day 150 (data not shown), which corroborates previous results [19]. It should be emphasized that \textit{contralateral} events warrant close monitoring and that recipients susceptible to immediate type allergic reactions may be identified by low dose skin test.

In general, GLURP\textsubscript{85–213} Elisa titers increased after each immunization with titers far beyond those found in individuals living in endemic areas [6,24]. Cytophilic anti-GLURP IgG1 and IgG3 antibodies are associated with protection in humans with P3 as potentially the most important epitope for the development of clinical immunity [25]. Our study shows that P3 is well recognized by the predominant IgG1 subclass antibodies. Affinity-purified anti-P3 antibodies from semi-immune subjects and IgG preparations with high specific levels of IgG3, can mediate monocyte-dependent inhibition of \textit{P. falciparum} growth in a biological \textit{in vitro} assay [25].
This prompted us to study whether anti-GLURP antibodies in the post-immunization plasma samples show a similar effect. A clear monocyte-dependent inhibition of parasite growth was found in all 12 volunteers tested. Thus, GLURP85–213 LSP can induce antibodies in humans with biological activity in vitro that is similar to the anti-GLURP IgG activity found in immune African adults.

Humoral responses in our volunteers were generally accompanied by a marked GLURP85–213-specific PBMC IgG activity found in immune African adults.

In conclusion, safe and immunogenic combinations of GLURP85–213 with Aluminum Hydroxide or Montanide ISA 720 have been identified that induces antibodies with biological activity in vitro.

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