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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) $GLURP_{85–213}$ combined with either Aluminum Hydroxide (Alum, 18 volunteers) or Montanide ISA 720 (ISA, 18 volunteers) as adjuvants. Immunizations with 10, 30 or 100 μg $GLURP_{85–213}$ were administered subcutaneously at days 0, 30, and 120.

Adverse events occurred more frequently with increasing dosage of $GLURP_{85–213}$ 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 $GLURP_{85–213}$ LSP formulations induce adverse events but can be administered safely, generating antibodies with capacity to mediate growth-inhibitory activity against *P. falciparum in vitro*.

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]. Immuno-epidemiological studies have shown that high levels of cytophilic (IgG1 and

Abbreviations: GLURP, glutamate-rich protein; Alum, Aluminum Hydroxide; ISA, Montanide ISA 720; IFA, immunofluorescence; AEs, adverse events

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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 $GLURP_{85–213}$ 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 $GLURP_{85–213}$ 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 $GLURP_{85–213}$. 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

$GLURP_{85–213}$ 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 $GLURP_{85–213}$ 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.

$GLURP_{85–213}$ 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 $GLURP_{85–213}$ 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 (ALAT), total bilirubin and gamma glutamyl transpeptidase levels.