PHASE 1 SAFETY AND IMMUNOGENICITY TRIAL OF MALARIA VACCINE RTS,S/AS02A IN ADULTS IN A HYPERENDEMIC REGION OF WESTERN KENYA

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Abstract. We conducted a phase 1 trial of candidate malaria vaccine RTS,S/AS02A in western Kenya to determine its safety and immunogenicity in healthy adults in an area hyperendemic for malaria. Twenty adults were enrolled and received RTS,S/AS02A (50 µg of RTS,S in 0.5 mL of AS02A) by intramuscular injection on a 0-, 28-, and 178-day schedule. After 60 scheduled immunizations were given, and 18 of 20 volunteers completed the last study visit on day 210. The vaccine was safe and well-tolerated. There were no vaccine-related severe adverse events. The most common solicited adverse events associated with immunization were injection site pain and headache. The geometric mean concentration of antibodies to circumsporozoite protein was 1.9 µg/mL at baseline and it increased 2–4 weeks after each dose to 16, 17.8, and 36.6 µg/mL, respectively. These safety and immunogenicity data from adults in hyperendemic Kenya are comparable to data reported earlier from two trials in west African adults in hypo-endemic and meso-endemic areas of The Gambia. We conclude that in this small study, RTS,S/AS02A is safe and similarly immunogenic in malaria-exposed African adults of different ethnicity in different transmission settings.

INTRODUCTION

Malaria, humankind’s ancient nemesis, kills three children every minute, most of whom are in sub-Saharan Africa.1 The spread of drug-resistant Plasmodium falciparum parasites, shortages of affordable effective drugs for treatment, and the inadequate use of vector control measures such as insecticide-impregnated bed nets and household use of pesticides have resulted in a worldwide resurgence of this disease.2 Deployment of an effective vaccine could save countless lives and improve the overall quality of life in the tropics and subtropics.3

A collaboration between GlaxoSmithKline Biologicals (Rixensart, Belgium) and the Walter Reed Army Institute of Research (Washington, DC) resulted in the development of a novel malaria antigen, RTS,S, which when formulated with the AS02A adjuvant, is able to confer partial protection against experimental P. falciparum malaria challenge in malaria-naive adults.4–7 Our plan is to develop a malaria vaccine that is effective in diverse populations and epidemiologic settings.8 Accordingly, we first evaluated the safety and immunogenicity of RTS,S/AS02A in parallel phase 1 studies in malaria-exposed adults in The Gambia9 and Kenya (the presently reported trial) before proceeding to subsequent field trials in The Gambia10 and Mozambique.11,12 This study from east Africa is the first report of the safety and immunogenicity of RTS,S/AS02A in adults in an area hyperendemic for P. falciparum.13

MATERIALS AND METHODS

Study site and population. This study was conducted in the Kombewa Division, Kisumu District, Nyanza Province, Kenya. The study site has the infrastructure and staff required for a Good Clinical Practices–compliant study. Malaria in this region is hyperendemic, with two seasonal peaks after the long and short rains. Plasmodium falciparum is the most frequent species, but P. malariae and rarely P. ovale are found.13 The population is chiefly of Luo ethnicity, a Nilotic people who earlier emigrated from southern Sudan and settled the basin of Lake Victoria. Most adults engage in farming and fishing as their chief economic pursuit.

Study design. This was an open label phase 1 study in which a single group of 20 healthy adults received RTS,S/AS02A by intramuscular injection on a 0-, 1-, and 6-month schedule. The primary objective was to evaluate the safety and reactogenicity of RTS,S/AS02A, and the secondary objective was to evaluate the immune response to RTS,S/AS02A.

Ethics and monitoring. This clinical study was conducted under a protocol reviewed and approved by the Scientific Steering Committee and the National Ethical Review Committee of the Kenya Medical Research Institute (Nairobi, Kenya) and the Human Subjects Research Review Board of the Surgeon General of the U.S. Army (Falls Church, VA). The study was monitored for regulatory compliance and data quality assurance by the United States Army Medical Material and Development Activity, GlaxoSmithKline Biologicals and the World Health Organization.

Study procedures. After obtaining written informed consent, volunteers were screened by history, physical examination, and laboratory testing to determine eligibility for enrollment. Inclusion criteria allowed the enrollment of healthy men and women 18–45 years of age. Exclusion criteria included an oral temperature > 38°C, clinically significant acute or chronic disease, history of splenectomy, known or suspected immunosuppression, use of systemic steroids, history of allergic reactions to study medications, hepatomegaly, a hematocrit < 30%, a serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST) level greater than the upper limit of normal, a positive urine β-human chorionic gonadotropin test result within 48 hours prior to vaccination, use of any investigational or non-registered drug or vaccine, and simultaneous participation in any other clinical trial or receipt of immunoglobulin or any blood product transfusion within three months of study start. Subjects were not screened for the presence of antibody to human immunodeficiency virus.

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**Diagnosis and treatment of malaria.** Blood smears were obtained on day of immunization and when clinically indicated. Asexual plasmodial parasitemia was diagnosed by microscopic examination of a Giemsa-stained peripheral blood thick smear. Asymptomatic parasitemia was not treated. Volunteers with asexual parasitemia and signs or symptoms of malaria were categorized as having clinical malaria according to the judgment of the medical officer and were treated with either sulfadoxine/pyrimethamine or oral quinine and doxycycline.

**Vaccine.** RTS,S/AS02A was manufactured by GlaxoSmithKline Biologics under Good Manufacturing Practices. It consists of a mixture of two proteins, RTS, a hybrid molecule recombinantly expressed in yeast, in which the circumsporozoite protein (CSP) central tandem tetrapeptide repeat (R) and carboxyl-terminal region containing T cell epitopes (T) are fused to the surface antigen (S) of the hepatitis B virus (HBsAg), and S, an additional unfused S antigen. These two proteins are co-expressed and then self-assembled into particles, collectively referred to as RTS,S. The vaccine was provided in a two-vial presentation consisting of lyophilized RTS,S (lot no. DMA141A46) in a single dose vial, and a second vial of liquid AS02A adjuvant (lot no. DAS2005A2), a proprietary oil-in-water emulsion containing the immunostimulants 3-deacylated monophosphoryl lipid A (CorixaInc., Seattle, WA) and *Quillaja saponaria* fraction 21 (Antigenics, New York, NY). After mixing, the final delivered dose contained 50 μg of RTS,S in a 0.5-mL volume of AS02A.

**Solicited and unsolicited adverse events.** Vaccines were administered at the study center, and vaccinees observed for 30 minutes for evidence of anaphylaxis. The presence of solicited local and general signs and symptoms, including measurement of oral body temperature, were assessed after each vaccination and daily for three subsequent days. The solicited injection site adverse events were pain, swelling, and limitation of arm motion abduction at the shoulder. Solicited general adverse events were fever, nausea, headache, malaise, myalgia, and joint pain. In addition to the solicited signs and symptoms, investigators recorded any other adverse events occurring during the study period within a 28-day follow-up period (day of vaccination and 27 subsequent days) as unsolicited adverse events.

Adverse events were assessed for intensity. Injection site pain was graded as 0 = absent, 1 = painful on touch, 2 = painful when limb is moved, and 3 = spontaneously painful. Limitations of arm motion was graded according to the angle of voluntary arm abduction at 0° = 180°, 1 = 90° but < 120°, 2 = 30° but < 90°, and 3 = 30°. Solicited symptoms were graded as 0 = normal, 1 = easily tolerated, 2 = interferes with normal activity, and 3 = prevents normal daily activity. Additional grading scales were applied to visible swelling at the injection site: 0 = none, 1 = > 20 mm, 2 = 20 to 50 mm, and 3 = > 50 mm and to oral temperature: 0 = < 37.5°C, 1 = 37.5–38°C, 2 = 38 to 39°C, and 3 = > 39°C.

All adverse events were assessed for their probability of a causal relationship to vaccine administration: not causally related; unlikely, there were other, more likely causes than study vaccine administration; suspected, there is a reasonable possibility that the event was caused by the study vaccine; and probable, a direct cause and effect between the adverse event and vaccine administration is suspected.

**Serious adverse events.** Serious adverse events were reported from enrollment until study completion on day 210. They were defined as any untoward medical occurrence that resulted in death, significant disability, hospitalization, incapacity, or required intervention to prevent such outcomes.

**Clinical laboratory parameters.** Biochemical (ALT, AST, creatinine) and hematologic (hemoglobin, hematocrit, white blood cell count, platelets) were measured at screening and on days 14, 42, 178, and 194.

**Immunogenicity outcomes.** Blood samples for determining antibodies to the CSP repeat region (R32LR) concentrations and antibodies to HBsAg were obtained on study days 0, 28, 42, 178, and 194. The concentration of antibodies (μg/mL) against the CSP tetrapeptide repeats was measured by an enzyme-linked immunosorbent assay using recombinant R32LR as the capture antigen and calibrated with a standard reference antibody as a control as previously described. Concentrations of antibodies against HBsAg (mIU/mL) were measured at the GlaxoSmithKline laboratories with a commercial radioimmunoassay (Abbott Laboratories, Abbott Park, IL).

**RESULTS**

**Safety.** The study was conducted from November 3, 1998 to June 28, 1999. Twenty subjects of Luo ethnicity were enrolled, 8 men (mean age = 27.8 years, range = 19–44 years) and 12 women (mean age = 27.9 years, range = 17–43 years). All subjects received all three immunizations and 18 of 20 completed the final study visit on day 210. Two volunteers were lost to follow-up after day 194, one dropped out without giving a reason, and the other migrated from the study area. Compliance with the four-day post-immunization follow-up for solicited adverse events was > 99%, i.e., 238 of 240 scheduled visits occurred. The vaccine was well tolerated (Table 1). Pain was the most prevalent solicited local symptom. Solicited local symptoms were mainly of intensity 1 and 2. The number of reports of local swelling (any or grade 3 swelling) was greater after the third immunization than after the first or second immunizations. All 12 instances of grade 3 pain occurred during the initial 24 hours after immunization and had decreased in severity by the second day after immunization. Overall there was a 20% and 33% incidence of grade 3 injection site pain and swelling, respectively. Most local symptoms resolved within the four-day follow-up period after immunization; after the first, second, and third vaccination, only two, one, and three volunteers, respectively, reported persistence of any local symptom.

There were 20 episodes of clinical malaria in 14 volunteers during the 60 one-month post-immunization follow-up periods. Physicians determining systemic solicited adverse events during the four-day post-immunization follow-up period attributed the degree of probable causality to immunization with RTS,S/AS02A (Table 1). The most prevalent solicited general symptom was headache (78%), followed by malaise (47%) and arthralgia (45%). Most (76%) of the solicited general symptoms showed a PB or SU relationship to the study vaccine. Only two grade 3 solicited general symptoms (headache and malaise) were reported; they occurred in the same subject after dose 3, resolved within four days without sequelae and had a probable relationship to the vaccine. There were no grade 3 unsolicited symptoms deemed
to have a probable or suspected relationship to immunization. No clinically significant hematologic, biochemical, or urine abnormalities were observed.

**Serious adverse events.** Two subjects had serious adverse events and both recovered without sequelae. One was a case of paronychia and cellulitis of the right index finger, the other was a case of typhoid fever with onset 31 days after vaccine dose 2. Both were deemed not related to study vaccine by the investigator. There were no pregnancies during the study.

**Humoral immune responses.** Geometric mean and 95% confidence intervals (CIs) for repeat concentrations of antibody to CSP on days 0, 28, 42, 178, and 194 were $1.9 (95\% \text{ CI} \ 1.4–2.7)$, $16 (95\% \text{ CI} \ 9.8–26.1)$, $17.8 (95\% \text{ CI} \ 12.6–25.2)$, $12.5 (95\% \text{ CI} \ 7.8–20.1)$, and $36.6 (95\% \text{ CI} \ 24.1–55.6)$ µg/mL, respectively (Figure 1). At prevaccination, 85% of the subjects were seropositive (i.e., concentration above an arbitrarily defined cut-off of 1 µg/mL) for antibody against the CSP repeat region. A more than eight-fold increase in geometric mean antibody concentration was observed after dose 1, but no significant increase in antibody concentration was seen after dose 2. A good response was again observed after dose 3, in which the geometric mean antibody concentration increased 19-fold from pre-vaccination to day 194 (14 days after vaccine dose 3) (Figure 1). There was no relationship between the concentration of antibody in individual subjects at baseline and their concentration at the end of the study ($r = 0.07, P = 0.75$).

There was a rapid increase in geometric mean concentration of antibody to HBsAg from a baseline of 29 (95% CI = 7–125) mIU/mL to 519 (95% CI = 83–3,230), 1,417 (95% CI = 314–6,407), and 11,216 (95% CI = 6,427–19,574) after each dose (Figure 2). At pre-vaccination, 15 (75%) of 20 subjects were seropositive (> 1 mIU/mL) and 11 (55%) of 20 of the subjects were seroprotected (> 10 mIU/mL). Seroprotection increased from 55% at baseline to 80% post-dose 1, to 95% post-dose 2, and to 100% post-dose 3.

**DISCUSSION**

The RTS,S/AS02A malaria vaccine was safe in 20 semi-immune adults subject to intense malaria transmission in east

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**TABLE 1**

Solicited adverse events after each dose of RTS,S/AS02A vaccine during the four-day follow-up*

<table>
<thead>
<tr>
<th>Local symptoms</th>
<th>Dose 1 (n = 20)</th>
<th>Dose 2 (n = 20)</th>
<th>Dose 3 (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Grade 3</td>
<td>Total</td>
</tr>
<tr>
<td>Swelling</td>
<td>8</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Pain</td>
<td>20</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>Limited arm motion</td>
<td>6</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Systemic symptoms</td>
<td>Total</td>
<td>PB/SU</td>
<td>Total</td>
</tr>
<tr>
<td>Temperature ≥ 37.5°C</td>
<td>5</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Headache</td>
<td>16</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>Malaise</td>
<td>10</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Myalgia</td>
<td>8</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>12</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Nausea</td>
<td>9</td>
<td>6</td>
<td>10</td>
</tr>
</tbody>
</table>

* Grade 3 = interferes with daily activity; PB = probable relation to vaccination; SU = suspected relation to vaccination.

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**FIGURE 1.** Serum concentrations of antibody (Ab) to R32LR (µg/mL) in response to three doses of RTS,S/AS02A given on days 0, 28, and 178. Values are geometric mean concentration with 95% confidence intervals.

**FIGURE 2.** Serum concentration of antibody to hepatitis B surface antigen (mIU/mL) in response to three doses of RTS,S/AS02A given on days 0, 28, and 178. Values are geometric mean concentrations with 95% confidence intervals.
Africa. There were no serious adverse events causally related to vaccination. A high incidence of post-vaccination local and general symptoms were reported in these semi-immune adults, but all symptoms largely resolved within four days of follow-up after each vaccination and no volunteer dropped out because of an adverse event. The reactogenicity observed in this study was higher than that reported from healthy semi-immune adults in The Gambia. Further interpretation of this finding is limited by the lack of a comparator vaccine group and by the small size of this phase 1 trial. The Kenyan adults from this hyperendemic region had a strong humoral response against CSP (R32LR), which increased from a baseline geometric mean concentration of 1.9 µg/mL to a post-third dose peak geometric mean of 36.6 µg/mL (95% CI = 24.1–55.6). This result is remarkably similar to that obtained in a phase 1 trial of RTS,S/AS02A of identical design in 20 Gambian adult males subject to less intense malaria transmission, whose baseline geometric mean concentration of antibody to R32LR of 1.7 µg/mL increased to post-third dose peak geometric mean of 46.8 µg/mL (95% CI = 33.2–66.1), and to a phase 2 three-dose trial of RTS,S/AS02A in 131 Gambian adults, whose baseline anti-R32LR geometric mean concentration of antibody to R32LR of 1.6 µg/mL increased to a geometric mean of 21.79 µg/mL (95% CI = 18.44–25.75). Bojang and others completed phase 1 trials of RTS,S/AS02A in children 1–11 years of age in The Gambia, and observed that antibody responses to R32LR were inversely related to age in malaria-endemic areas. Data for adult Kenyan and antibody responses to RTS,S/AS02A measured in a phase 1 pediatric trial of RTS,S/AS02A in Mozambique in children 1–4 years of age (geometric mean concentration of antibody to of 270.4 µg/mL, 95% CI = 182.7–400.3, (Macete E, unpublished data) support the observation of Bojang and others. This inverse relationship between age and immune response has been described for serologic responses to standard vaccines against hepatitis A and hepatitis B and could be the result of children receiving relatively larger doses than adults on a per kilogram basis. Alternatively, one or more immunosuppressive factors may be more active in adults, such as cumulative exposure to malaria parasites, chronic nematode infection, or unrecognized nutritional factors. Taken together, these findings emphasize the importance of conducting specific phase 1 dose-finding studies in pediatric populations for whom the vaccine is ultimately intended.

In conclusion, this trial found RTS,S/AS02A to be safe, well-tolerated, and immunogenic in a highly malaria-exposed adult population in east Africa. Further development of this vaccine for pediatric populations is well underway in malaria-endemic regions.

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Disclosure: Joe D. Cohen, Laurence Vigneron, and Gerald Voss, and W. Ripley Ballou are employees of GlaxoSmithKline Biologicals, the manufacturer of the RTS,S/AS02A vaccine. Joe D. Cohen, Gerald Voss, and W. Ripley Ballou hold shares of stock in GlaxoSmithKline. Joe D. Cohen is listed as an inventor on patented malaria vaccines based on RTS,S/AS02A; however, he is not a holder of such patents. None of the other authors have declared conflicts of interest.


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