Randomized, Double-Blind, Phase 2a Trial of Falciparum Malaria Vaccines RTS,S/AS01B and RTS,S/AS02A in Malaria-Naive Adults: Safety, Efficacy, and Immunologic Associates of Protection


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(See the editorial commentary by Bremen and Plowe, on pages 317–20, and the article by Sacarlal et al, on pages 329–36.)

Background. To further increase the efficacy of malaria vaccine RTS,S/AS02A, we tested the RTS,S antigen formulated using the AS01B Adjuvant System (GlaxoSmithKline Biologicals).

Methods. In a double-blind, randomized trial, 102 healthy volunteers were evenly allocated to receive RTS,S/AS01B or RTS,S/AS02A vaccine at months 0, 1, and 2 of the study, followed by malaria challenge. Protected vaccine recipients were rechallenged 5 months later.

Results. RTS,S/AS01B and RTS,S/AS02A were well tolerated and were safe. The efficacy of RTS,S/AS01B and RTS,S/AS02A was 50% (95% confidence interval [CI], 32.9%–67.1%) and 32% (95% CI, 17.6%–47.6%), respectively. At the time of initial challenge, the RTS,S/AS01B group had greater circumsporozoite protein (CSP)–specific immune responses, including higher immunoglobulin (Ig) G titers, higher numbers of CSP-specific CD4+ T cells expressing ≥2 activation markers (interleukin-2, interferon [IFN]–γ, tumor necrosis factor–α, or CD40L), and more ex vivo IFN-γ enzyme-linked immunospots (ELISPOTs) than the RTS,S/AS02A group. Protected vaccine recipients had a higher CSP-specific IgG titer (geometric mean titer, 188 vs 73 μg/mL; P < .001), higher numbers of CSP-specific CD4+ T cells per 10⁶ CD4+ T cells (median, 963 vs 308 CSP-specific CD4+ T cells/10⁶ cells; P < .001), and higher numbers of ex vivo IFN-γ ELISPOTs (mean, 212 vs 96 spots/million cells; P < .001). At rechallenge, 4 of 9 vaccine recipients in each group were still completely protected.

Conclusions. The RTS,S/AS01B malaria vaccine warrants comparative field trials with RTS,S/AS02A to determine the best formulation for the protection of children and infants. The association between complete protection and immune responses is a potential tool for further optimization of protection.

Trial registration. ClinicalTrials.gov identifier NCT00075049.
developed by GlaxoSmithKline Biologicals (GSK) and the Walter Reed Army Institute of Research (WRAIR) after less-immunogenic formulations of RTS,S failed to confer meaningful protection [9]. RTS,S/AS02A consists of the *P. falciparum* circumsporozoite protein (CSP)–based antigen RTS,S, which is formulated with AS02A, an adjuvant system by GSK that contains an oil-in-water emulsion, and the immunostimulants monophosphoryl lipid A (MPL) and QS21 [10]. AS01B is a related liposome-based adjuvant system that also contains MPL and QS21. In the rhesus model, RTS,S/AS01B elicited equal CSP-specific antibody, greater CSP-specific delayed-type hypersensitivity responses, and greater and more sustained CSP-specific ex vivo CD4 T cell responses and interferon (IFN)–γ production by ex vivo enzyme-linked immunospots (ELISPOTs) than did RTS,S/AS02A [11–13]. In the present study, we compared RTS,S/AS01B with RTS,S/AS02A in terms of safety, immunogenicity, and short- and long-term efficacy against challenge with homologous *P. falciparum* in healthy, malaria-naive adults.

**SUBJECTS, MATERIAL, AND METHODS**

**Study Design**

This phase 2a, double-blind, randomized challenge trial consisted of 2 cohorts of 52 adults each who received RTS,S/AS01B or RTS,S/AS02A in a 1:1 ratio. Immunizations were given according to a 0-, 1-, and 2-month schedule, with *P. falciparum* challenge occurring 2–3 weeks after the last immunization and rechallenge occurring 5 months later for vaccine recipients who were protected at the time of the initial challenge (figure 1). The study protocol was approved by the WRAIR Human Use Review Committee and the US Army Medical Research and Materiel Command’s Human Subjects Research and Review Board (Fort Detrick, Maryland).

**Study Subjects and Eligibility**

All volunteers provided written, informed consent before screening and enrollment. The study subjects were men and women 18–45 years of age. Study inclusion criteria were good health, no history of malaria, and the ability to comply with the study schedule. Preexisting antibody to hepatitis B surface antigen (HBsAg) was not a criterion for exclusion from the study.

**Vaccines**

The RTS,S vaccine antigen has been described elsewhere [10]. The final lyophilized pellet contained 62.5 μg of RTS,S per single-dose vial. Just before administration, the pellet was reconstituted with liquid AS01B or AS02A such that the final 0.5-mL dose contained 50 μg of RTS,S. AS01B is a liposome-based formulation that contains 50 μg of MPL and 50 μg of QS21 per 0.5-mL dose [14, 15]. AS02A contains 50 μg of MPL, 50 μg of QS21, and 250 μL of an oil-in-water emulsion per 0.5-mL dose. Vaccines were administered by personnel not involved in the subsequent assessment of adverse events (AEs).

**Safety Assessments**

Vaccine safety was measured by assessment of reactogenicity, general symptoms, and hematologic and biochemical parameters. Subjects were observed for 30 min and again at 1, 2, 3, and 7 days after each vaccination. During these 7 days, a standardized questionnaire was used to capture solicited local symptoms and solicited general symptoms. All unsolicited AEs that were reported during the 30 days after each immunization were recorded.

Serious AEs (SAEs) were captured throughout the study. Complete blood counts and blood urea nitrogen, creatinine, alanine aminotransferase, and aspartate aminotransferase levels were assessed on days 0, 14, 28, 42, 56, and 70 after vaccination, on the day of challenge (DOC), and at month 5.
Figure 2. Summary of solicited local and systemic adverse events (AEs). Paired columns denote the frequency of specific solicited AEs occurring within 7 days of immunization, for recipients of RTS,S/AS02A (shaded columns) and RTS,S/AS01B (open columns). Pain at the injection site was graded as follows: 0 (absence of pain), 1 (painful on touch), 2 (painful when limb is moved), or 3 (spontaneously painful). Redness and swelling were independently measured at the greatest surface diameter and were assigned the following grades: 0 (denoting 0 mm), 1 (10–20 mm), 2 (20–50 mm), or 3 (>50 mm). Fever was defined according to oral temperature, which was assigned one of the following grades: 0 (<37.5°C), 1 (37.5°C–38°C), 2 (38°C–39°C), or 3 (>39°C). Other general symptoms were graded as follows: 0 (normal), 1 (easily tolerated), 2 (interferes with normal activity), or 3 (prevents normal activity). Total nos. of grade 1, 2, and 3 AEs are depicted without determination of their association with immunization. Grade 3 AEs associated with immunization are denoted by the hatched area, and grade 3 AEs thought to be unassociated with immunization are denoted by the solid black area. A, associated; GI, gastrointestinal AE; NA, not associated.

Immunologic Outcomes

Serologic findings. Antibodies to CSP were determined by evaluating immunoglobulin (Ig) G responses to the *P. falciparum* CSP-repeat region, as measured using standard enzyme-linked immunospot assay (ELISA) with R32LR as the capture antigen [16–18]. Seropositivity was defined as ≥1.0 μg/mL anti-CSP immunoglobulin (Ig) G. Antibodies to HBsAg were measured using a commercial assay (AUSAB; Abbott). The cutoff level for seropositivity was ≥3.3 mIU/mL, and that for seroprotection was ≥10 mIU/mL. Serum samples obtained on the day of sporozoite challenge and preimmune serum samples were analyzed by an indirect fluorescence antibody assay that used 3D7 strain air-dried sporozoites, as described elsewhere [17].

Peripheral blood mononuclear cell collection and antigens. Peripheral blood mononuclear cell (PBMCs) were isolated and maintained in liquid nitrogen until use. The following peptides were used: a pool of 15–amino acid (aa) CSP peptides that overlapped by 11 aa and corresponded to the CSP domain of RTS,S in the intracellular cytokine staining (ICS) assay; a pool of 15-aa peptides that corresponded to the C-terminal of the CSP domain of RTS,S in the ELISPOT assay; a pool of 15-aa peptides that overlapped by 11 aa, representing the HBsAg domain of RTS,S; P2, the 44-aa peptide EEPSDHIKEYLNKIQNLSTEWSPCSVTGNGIQVRKPSAN, which represents the Th2R and a part of the Th3R regions of CSP; and P4, the 35-aa peptide KPKDELDYANDIEKKICKMEKCSSVFNVNNSIGL, which represents the remainder of the Th3R region of CSP [19, 20].

Ex vivo ELISPOTs. The ELISPOT assay was performed as described elsewhere [4]. Culture medium consisted of RPMI supplemented with 100 IU/mL penicillin, 100 μg/mL streptomycin, 8 mmol/L glutamax, 1 mmol/L nonessential amino acid, 1 mmol/L sodium pyruvate, and 50 mmol/L 2-mercaptoethanol. After thawing, 100 μL of PBMCs at 2 × 10^6 per milliliter of culture medium containing 20% human AB serum (Gemini Bioproducts) were added in triplicate to 96 U-bottom wells that contained either culture medium alone or a pool of the 15-aa peptides at 2.5 μg per peptide per milliliter or the P2 or P4 peptides at 40 μg/mL. The plates were incubated at 37°C for 24 h. Results were measured as the number of spot-forming units per 200,000 PBMCs and are expressed as “spots per million” (spm) PBMCs.

ICS. PBMCs were washed twice, resuspended to 2 × 10^6 cells in culture medium, and stimulated for 2 h with the CSP peptide pool (final concentration, 1.25 μg/mL each peptide per well) in the presence of anti-CD28 and anti-CD49d (dilution, 1/250). Brefeldin A (BD Pharmingen), in a final concentration of 1 μg/mL per well, was added for the last 18 h of culture. A negative control (anti–human CD28 and CD49d) and a positive control (1 μg/mL staphylococcal enterotoxin B; Sigma-Aldrich) were included in each assay. After incubation, the cells were washed (in phosphate-buffered saline plus 1% fetal calf serum) and stained with anti–CD4–peridinin chlorophyll protein
Anti–circumsporozoite protein (CSP) antibody responses, by vaccine received and by protection. Points depict geometric mean antibody concentrations (GMCs). Bars denote 95% confidence intervals.

**Figure 3.**

**A,** Time (expressed in months) since the first immunization, as shown on the x-axis. The dotted line denotes the RTS,S/AS02A group, and the solid line denotes the RTS,S/AS01B group. *P* values for specific time points are as follows: month 0, *P* < .008; month 1, *P* < .918; month 2, *P* < .001; day of challenge (DOC), *P* < .008; and month 5, *P* = .017. **B,** Data from unprotected subjects (dotted line) and protected subjects (solid line). *P* values for specific time points are as follows: month 0, *P* < .654; month 1, *P* < .008; month 2, *P* < .001; DOC, *P* < .001; and month 5, *P* < .017.

Vaccine Efficacy Outcomes

**Malaria challenge.** Immunized volunteers underwent standardized malaria challenge ~2–3 weeks after the third immunization. Nonimmunized, malaria-naive infectivity controls were recruited specifically for each malaria challenge. Subjects found to be parasitemic were treated with oral chloroquine under direct physician observation.

**Statistical analyses.** Analyses adhered to a prospective reporting and analysis plan. All statistical tests were 2-tailed. Vaccine efficacy was assessed by comparing the incidence of parasitemia. Efficacy was defined as $1 - RR$ (relative risk [RR] of parasitemia in the treatment group vs the control group), and exact 95% confidence intervals (CIs) were calculated. Volunteers who did not have parasitemia by 28 days after challenge were considered to be protected.

RESULTS

**Study flow.** The present study, which was conducted at the WRAIR Clinical Trials Center from 2003 through 2006, involved 102 vaccine recipients. A total of 36 malaria-naive volunteers underwent challenge as infectivity controls, such that 6 volunteers were challenged on each day that vaccine recipients were challenged (figure 1).

**Safety.** Both vaccines were safe and well tolerated (figure 2). Pain, the most frequently reported solicited local AE, occurred after administration of 81% and 82% of RTS/AS01B and RTS,S/AS02A doses, respectively. The incidence of grade 3 pain was lower in the RTS,S/AS01B group than in the RTS,S/AS02A group (2.8% vs 9.0% of doses; *P* = .043). Instances of grade 3 redness and swelling were not associated with functional impairment. All grade 3 solicited local events resolved within the 7 day follow-up period. Fatigue, headache, and malaise were the most frequent solicited general AEs in both groups, occurring with a similar frequency in both groups (for fatigue, after 35.9% and 44.1% of doses; for headache, after 35.2% and 33.1% of doses; and for malaise, after 24.6% and 28.3% of doses, respectively). Only 1 solicited general AE did not resolve within the 7-day follow-up period: 1 occurrence of grade 3 malaise, which developed after dose 2 of RTS,S/AS02A, lasting for 1 additional day. Solicited general AEs of grade 3 that were considered to be associated with the study vaccine occurred infrequently after ~4.2% of doses. There was no trend toward an increase in the incidence of solicited AEs associated with subsequent vaccination in either group. Two AEs (both occurred in the RTS,S/AS01B group) led to withdrawal: (1) a generalized urticarial reaction that involved only the skin, was considered to be associated with the study vaccine occurred infrequently after ≤4.2% of doses. There was no trend toward an increase in the incidence of solicited AEs associated with subsequent vaccination in either group. Two AEs (both occurred in the RTS,S/AS01B group) led to withdrawal: (1) a generalized urticarial reaction that involved only the skin, was considered to be associated with the study vaccine, and from which the subject rapidly recovered without sequelae, and (2) a cerebral infarction that was considered to be unassociated with the study vaccine.

**SAEs.** Three nonfatal SAEs (tendon rupture and cerebral
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infarction in the RTS,S/AS01B group and acute myeloid leukemia in the challenge control group) unassociated with the study vaccines were reported in separate subjects during the study.

**Antibody response to CSP.** All subjects were seropositive for anti-CSP antibodies at 1 month after dose 2. The highest geometric mean concentrations (GMcs) of anti-CSP antibody were observed on the DOC (2 weeks after dose 3), when GMcs were 143.5 µg/mL (95% CI, 100.9–203.9 µg/mL) in the RTS,S/AS01B group and 82.8 µg/mL (95% CI, 66.8–102.6 µg/mL) in the RTS,S/AS02A group. GMCs of anti-CSP antibody were significantly higher in the RTS,S/AS01B group than in the RTS,S/AS02A group at 3 time points (at day 56, P < .001; at the DOC, P < .001; and at month 5, P = .017) (figure 3).

**Antisporozoite indirect fluorescence antibody assay.** The antisporozoite IgG titer on the DOC was higher in the RTS,S/AS01B recipients (median, 25,600; first quartile, 12,800; third quartile, 51,200) than in the RTS,S/AS02A recipients (median, 25,600; first quartile, 6400; and third quartile, 51,200) but not significantly so (P < .053, Wilcoxon test).

**Anti-HBsAg.** At baseline, 51.0% of the subjects in the RTS,S/AS01B group and 40.0% of the subjects in the RTS,S/AS02A group were seroprotected (hepatitis B surface antibody [HBsAb] titer, >10 IU). In both groups, 100% of the subjects had seroprotective HBsAb titers at 1 month after dose 2 (ie, at day 56). Anti-HBsAb GMcs were greatest at the DOC (ie, 2 weeks after dose 3) and trended higher in the RTS,S/AS01B group (GMC, 45,290 mIU/mL [95% CI, 24,492–83,750 mIU/mL]) than in the RTS,S/AS02A group (GMC, 27,746 mIU/mL [95% CI, 14,744–52,213 mIU/mL]).

**ELISPOT response to CSP, by group.** Both RTS,S/AS01B and RTS,S/AS02A elicited an IFN-γ recall response to CSP peptides in vitro that was statistically greater for the CSP peptide pool on the DOC (P = .004) and for P4 (P = .004). No vaccine group differences were seen for CSP peptide pool IL-2 responses. With regard to efficacy, as determined by ELISPOT, a greater IFN-γ response to the CSP 15–amino acid peptides and to the P2 peptide was observed in protected subjects and was statistically significant for CSP at day 42 (P < .021), on the DOC (P < .001), and at month 5 (P < .003) and for the P2 peptide at day 42 (P < .021) and on the DOC (P < .002). A higher IL-2 response was observed in protected subjects and was statistically significant on the DOC (P < .002) and at month 5 (P < .002).

**ICS response to CSP, by group.** The responses of CSP-specific dsCD4+ T cells, defined as cells that express ≥2 biomarkers (CD40L, IL-2, TNF-α, or IFN-γ), were detected in both vaccine groups at all measured time points. Specific CD4+ T cells predominantly expressed CD40L and IL-2 and, to a

Figure 4. Ex vivo interferon (IFN)-γ and interleukin (IL)-2 enzyme-linked immunospot (ELISPOT) responses to circumsporozoite protein (CSP) peptides, by vaccine and by protection. Vaccines were given at 0, 1, and 2 months. Cellular data are presented for the following time points: baseline, 1.5 months, the day of challenge (DOC), and 5 months. The y-axis bars denote the no. of spot-forming units per peripheral blood mononuclear cells (PBMCs) expressed as the no. of spots per million cells (spm). Data are expressed as the median value ± the first and third quartiles. A and B, IFN-γ responses to CSP peptide pools, by vaccine group and by protection status. C and D, IFN-γ responses to P2 peptide, by vaccine group and by protection status. E and F, IFN-γ responses to P4 peptide, by vaccine group and by protection status. G and H, IL-2 responses to the P2 peptide, by vaccine group and by protection status. ELISPOT response to CSP, by group. The IFN-γ recall response to CSP peptides in vitro was greater in the RTS,S/AS01B group on the DOC, for the CSP peptide pool (P = .041) and for P4 (P = .004). No vaccine group differences were seen for CSP peptide pool IL-2 responses. With regard to efficacy, as determined by ELISPOT, a greater IFN-γ response to the CSP 15–amino acid peptides and to the P2 peptide was observed in protected subjects and was statistically significant for CSP at day 42 (P < .021), on the DOC (P < .001), and at month 5 (P < .003) and for the P2 peptide at day 42 (P < .021) and on the DOC (P < .002). A higher IL-2 response was observed in protected subjects and was statistically significant on the DOC (P < .002) and at month 5 (P < .002).
lesser extent, TNF-α and IFN-γ. The total responses of dsCD4+ T cells peaked on the DOC and were consistently higher in the RTS,S/AS01B recipients but did not achieve statistical significance (figure 5A). By contrast, on the DOC, a statistically higher response in the RTS,S/AS01B group was noted with respect to dsCD4+ T cells producing IFN-γ plus one other cytokine or marker (P < .040) or TNF-α plus one other cytokine or marker (P < .050) (not shown). No CD8+ T cell responses were detected using ICS in either vaccine group.

**ICS responses to HBsAg, by group.** There was a trend toward greater HBsAg-specific CD4+ T cell responses in the RTS,S/AS01B group than in the RTS,S/AS02A group (data not shown).

**Vaccine efficacy, by group.** After initial malaria challenge, 18 (50.0%) of 36 subjects in the RTS,S/AS01B group and 30 (68.2%) of 44 subjects in the RTS,S/AS02A group became infected (figure 6). All infectivity control subjects became infected. Vaccine efficacy tended to be higher in the RTS,S/AS01B group than in the RTS,S/AS02A group (50.0% [95% CI, 32.9%–67.1%] vs 31.8% [95% CI, 17.6%–47.6%; P = .11]). In nonprotected subjects, the mean prepatent period in the RTS,S/AS01B, RTS,S/AS02A, and infectivity control groups was 14.4, 13.6, and 10.8 days, respectively. Five months later, a subset of 9 protected subjects from each group underwent a second malaria challenge. After rechallenge, 5 (55.6%) of 9 subjects in the RTS,S/AS01B and RTS,S/AS02A groups were infected; all infectivity control subjects became infected. Point estimates for vaccine efficacy were 44.4% (95% CI, 10.9%–79.2%; P = .022) in both vaccine groups. The mean prepatent period in nonprotected subjects was similar in the RTS,S/AS01B group (14.0 days) and the RTS,S/AS02A group (13.6 days); the mean time to infection of control subjects was 11.8 days.

**Vaccine efficacy, by antibody.** GMCs of anti-CSP antibodies were higher in subjects who were protected than in those who were not protected after initial challenge at 1 month after receipt of dose 1 (at day 28), at 1 month after receipt of dose 2 (at day 56), on the DOC, and at 3 months after dose 3 (at month 5). This finding is statistically significant at all 4 time points (at day 28, P < .008; at day 56, P < .001; on the DOC, P < .001; and at month 5, P < .002) (figure 3). On the DOC, the median antispore indirect fluorescence antibody assay for protected subjects (51,200; first quartile, 25,600; and third quartile, 51,200) was significantly higher (P < .001, Wilcoxon test) than that for subjects who were not protected (median, 12,800; first quartile, 12,800; and third quartile, 25,600). There was no difference in anti-HBsAg antibody levels between protected and nonprotected subjects.

**Vaccine efficacy, by ELISPOT assay.** A greater IFN-γ response to the CSP 15-aa peptides and to the longer P2 peptide was observed in subjects who were protected (after initial challenge) than in subjects who were not protected. This finding was statistically significant for CSP at 2 weeks after dose 2 (at day 42, 70.0 vs 26.5 spm; P < .021), on the DOC (at 2 weeks after dose 3, 90.0 vs 21.5 spm; P < .001), and at 3 months after dose 3 (at month 5, 46.5 vs 18.5 spm; P < .001); it was also statistically significant for the P2 peptide at 2 weeks after receipt of dose 2 (at day 42, 61.0 vs 21.5 spm; P < .001), and at 3 months after dose 3 (at month 5, 53.5 vs 18.5 spm; P < .001). A higher IL-2 response was observed in protected subjects than in nonprotected subjects; this response was statistically significant on the DOC (at 2 weeks after receipt of dose 3, 61.0 vs 20.0 spm; P < .002). A higher IL-2 response in protected subjects than in nonprotected subjects; this response was statistically significant on the DOC (at 2 weeks after receipt of dose 3, 53.5 vs 20.0 spm; P < .002) and at 3 months after dose 3 (at month 5, 40.0 vs 14.0 spm; P < .002) (figure 4).

**Vaccine efficacy, by ICS assay.** The total number of CSP-specific dsCD4+ T cell responses was greater in protected sub-
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Figure 6. Kaplan-Meier curve for the time to onset of parasitemia after initial challenge. Data are for pooled cohorts 1 and 2 for RTS,S/AS02A and RTS,S/AS01B vaccine recipients and for infectivity control subjects. The y-axis denotes the cumulative incidence of parasitemia. The x-axis denotes the time (expressed in days) from challenge. Lower rows denote the nos. of people at risk, by category.

jects than in nonprotected subjects after initial immunization and was statistically significant at 2 weeks after dose 2 (at day 42; P < .005), on the DOC (P < .001), and at 3 months after receipt of dose 3 (at month 5; P < .050) (figure 5B). The frequencies of dsCD4+ T cells that expressed any combination of 2 of the immune markers analyzed were also significantly higher at the DOC in the protected versus the nonprotected groups.

Vaccine efficacy, by antibody and CD4+ T cell response. In an exploratory analysis, we plotted the magnitude of the CSP-specific antibody and CSP-specific CD4+ T cell responses on logarithmic axes, for each subject who underwent initial challenge (figure 7). There was no correlation between the humoral and cellular responses (R = .017).

Rechallenge and immune responses. Of the 18 protected subjects who underwent rechallenge, 8 were protected and 10 were not. Among the protected subjects, the GMC of the anti-CSP antibody was 58.2 μg/mL (95% CI, 15.8–214.9 μg/mL), and, among the nonprotected subjects, it was 18.4 μg/mL (95% CI, 7.6–44.5 μg/mL). In 7 of the 8 samples from the protected group that were analyzed, only CSP-specific CD4+ T cells that elaborated IFN-γ plus either CD40L, TNF-α, or IL-2 had higher levels (median, 54 CSP-specific CD4+ T cells/10^6 CD4+ T cells; first quartile, 33 CSP-specific CD4+ T cells/10^6 CD4+ T cells; and third quartile, 111 CSP-specific CD4+ T cells/10^6 CD4+ T cells) than did 5 of the 10 samples from unprotected subjects (median, 1 CSP-specific CD4+ T cell/10^6 CD4+ T cells; first quartile, 1 CSP-specific CD4+ T cell/10^6 CD4+ T cells; and third quartile, 39 CSP-specific CD4+ T cells/10^6 CD4+ T cells). No pattern distinguished the ex vivo ELISPOT responses to CSP peptides, P2, or P4 for IFN-γ production for the protected versus unprotected groups.

DISCUSSION

We report what is the first clinical trial of the candidate malaria vaccine antigen RTS,S with the AS01B Adjuvant System. This formulation, designated RTS,S/AS01B, was well tolerated, appeared to be safe, and elicited stronger immune responses than did the current formulation, RTS,S/AS02A. The observed 50% efficacy of RTS,S/AS01B was not statistically different than the 32% efficacy of RTS,S/AS02A (P = .11), but it may represent a true improvement in efficacy. The highly significant, independent correlation of both CSP-specific antibody responses and cell-mediated immune responses with complete protection suggests that additional enhancement of these responses as the result of new vaccine strategies might further improve vaccine efficacy.

The AE profile of the RTS,S/AS01B formulation was indistinguishable from that of RTS,S/AS02A (figure 2). We noted equivalent rates of local AEs. Likewise, the most common solicited general AEs—fatigue, headache, and malaise—occurred at a similar rate. The one study withdrawal that resulted from a vaccine-related AE—urticarial rash—occurred in the RTS,S/AS01B group after a second dose and resolved without sequelae; thus, the true incidence of this AE cannot be predicted. The 2 serious AEs in the RTS,S/AS01B group were deemed to be unrelated to immunization.

The superior adjuvant activity of RTS,S/AS01B observed in
Figure 7. The circumsporozoite protein (CSP)–specific dsCD4⁺ T cell and antibody (Ab) responses and their relation to protection against malaria-infected mosquito challenge. The log-log plot shows the CSP Ab titer on the y-axis and the no. of CSP-specific dsCD4⁺ T cells on the x-axis. Vaccine recipients were further categorized according to challenge outcome. Diamonds, triangles, and circles denote subjects who are not protected and who have no delay in patency (NP), subjects who are not protected but who have at least a 2-day delay in the prepatent period (DL), or subjects who are fully protected (PR), respectively. A large cross (+) denotes the median CSP-specific Ab concentration (112 μg/mL) and CSP-specific CD4⁺ T cell count (575 CSP-specific CD4⁺ T cells/10⁶ CD4⁺ T cells) for all subjects. Data points above and below the median denote high and low CSP Ab responses and CSP-specific CD4⁺ T cell responses, respectively. The no. of efficacy responses by volunteers was distributed among the 4 quadrants: left upper quadrant (high Ab and low CD4⁺ responses: among NP subjects, 1; DL subjects, 6; and PR subjects, 10); right upper quadrant (high Ab and high CD4⁺ responses: among NP subjects, 2; DL subjects, 5; and PR subjects, 13); left lower quadrant (NP subjects, 15; DL subjects, 4; and PR subjects, 0); right lower quadrant (NP subjects, 5; DL subjects, 7; and PR subjects, 5).

the rhesus model has been confirmed and extended in this trial—namely, greater anti-CSP antibody responses (figure 3A) and even greater CSP-specific multifunctional CD4⁺ T cell immune responses (figure 5A). We found a strong association of anti-R32LR antibody with protection, with no overlap of the 95% CIs for protected subjects versus nonprotected subjects on the DOC (GMC, 187.8 μg/mL [95% CI, 140–251 μg/mL] vs 72.7 μg/mL [95% CI, 58.1–91.0 μg/mL]; P = .008) (figure 3B), a result that mirrors the highly significant association between antisporozoite IgG on the DOC and protection. The present study is consistent with earlier trials of RTS,S/AS02A in malaria-naive adults [1–4] that demonstrated an association between anti-R32LR antibody concentrations and protection against malaria challenge. The role of anti-R32LR antibody in field efficacy studies, conducted with different efficacy end points and over different periods of time, is less clear. Potential explanations for the lack of correlation in some field studies [6, 7], but not in others [5, 8], include the use of a different efficacy end point (ie, clinical malaria rather than infection), a much longer period over which vaccine efficacy was determined, and a hypothesized indirect effect of RTS,S/AS02A vaccine–enhanced acquisition of blood-stage immunity affecting the efficacy end point of clinical malaria [21]. The latter idea stems from observations in insecticide-treated bed net trials in which a reduction in transmission intensity was associated with a reduction in the incidence of both severe and clinical malaria [22].

The present study confirms our preliminary report that adjuvanted RTS,S (RTS,S/AS02A) elicited T cells capable of CSP-specific IFN-γ production in an ex vivo ELISPOT assay that was associated with protection of malaria-naive subjects against malaria challenge (figure 4B, 4D, and 4F) [23]. We now report, for the first time, a strong association between the frequency of multifunctional CSP-specific CD4⁺ T cells and complete protection against malaria challenge (figure 5B).

CSP-specific CD4⁺ T cells may themselves confer some protection against malaria challenge. First, subjects with both high CSP-specific antibody titers and high CD4⁺ T cell responses were more likely to be protected than were subjects who developed high antibody titers without high CD4⁺ T cell responses (figure 7). Second, subjects with low CSP-specific antibody titers and high CD4⁺ T cell responses were more likely to be
protected than were subjects with low antibody titers and low CD4+ T cell counts. Third, CSP-specific CD4+ T cells do not appear to be a surrogate marker for other protective immune mechanisms, because the CSP-specific CD4+ T cell responses of individual subjects did not correlate with their respective anti-CSP antibody levels (R = .017), and CSP-specific CD4+ T cells were not a surrogate for CSP-specific CD8+ T cells, because the latter were not detected upon short-term in vitro stimulation. Fourth, multifunctional pathogen-specific T cells correlated with the control of intracellular pathogens. Animal models of leishmaniasis have correlated the presence of vaccine-induced antigen-specific multifunctional CD4+ T cells with protection against the intracellular pathogen *Leishmania major* [24]. Likewise, in humans, the presence of multifunctional antigen-specific CD4+ and CD8+ T cells correlates with the ability to control intracellular viral infections [25]. Interestingly, as shown by the results of the rechallenge, the average anti-CSP antibody levels just before rechallenge were higher for subjects with long-term protection than for those who lost protection, but they were lower than the levels seen in the nonprotected subjects before the initial challenge.

Interpretation of the CSP-specific CD4+ T cell data associated with the day of rechallenge is limited by the number of subjects analyzed. In contrast to the differences seen in protected versus nonprotected subjects at the time of the first challenge, volunteers who remained protected at rechallenge had a lower percentage of CSP peptide-specific CD4+ T cells and a lower percentage of CSP-specific CD4+ T cells elaborating any 2 of the following biomarkers (CD40L, TNF-α, or IL-2) than did the subjects who had lost protection, but they did have greater CSP-specific CD4+ T cells identified as elaborating IFN-γ plus at least another immune marker (CD40L, TNF-α, or IL-2) than did those who had lost protection. It is plausible that the use of such markers as CCR7, CD27, and CD45RO to segregate the CD4+ T cells into effector and memory phenotypes may reveal some correlation with longer-term protection [26].

Larger parallel efforts are under way to evaluate adjuvanted RTS,S for licensure in pediatric populations that are at greatest risk [27]. Meanwhile, we are evaluating additional antigens and combined vaccine platforms with the goal of developing a next-generation RTS,S-based malaria vaccine [28]. Studies are under way to evaluate the protective effect of adjuvanted recombinant merozoite surface protein–1 or apical merozoite antigen–1 for RTS,S based malaria vaccine against *Plasmodium falciparum* malaria. RTS,S Malaria Vaccine Evaluation Group. N Engl J Med 1997; 336:86–91.


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