

ORIGINAL ARTICLE

Safety and Immunogenicity of RTS,S/AS02D Malaria Vaccine in Infants

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ABSTRACT

BACKGROUND

The RTS,S/AS malaria vaccine is being developed for delivery through the World Health Organization's Expanded Program on Immunization (EPI). We assessed the feasibility of integrating RTS,S/AS02D into a standard EPI schedule for infants.

METHODS

In this phase 2B, single-center, double-blind, controlled trial involving 340 infants in Bagamoyo, Tanzania, we randomly assigned 340 infants to receive three doses of either the RTS,S/AS02D vaccine or the hepatitis B vaccine at 8, 12, and 16 weeks of age. All infants also received a vaccine containing diphtheria and tetanus toxoids, whole-cell pertussis vaccine, and conjugated *Haemophilus influenzae* type b vaccine (DTPw/Hib). The primary objectives were the occurrence of serious adverse events during a 9-month surveillance period and a demonstration of noninferiority of the responses to the EPI vaccines (DTPw/Hib and hepatitis B surface antigen) with coadministration of the RTS,S/AS02D vaccine, as compared with the hepatitis B vaccine. The detection of antibodies against *Plasmodium falciparum* circumsporozoite and efficacy against malaria infection were secondary objectives.

RESULTS

At least one serious adverse event was reported in 31 of 170 infants who received the RTS,S/AS02D vaccine (18.2%; 95% confidence interval [CI], 12.7 to 24.9) and in 42 of 170 infants who received the hepatitis B vaccine (24.7%; 95% CI, 18.4 to 31.9). The results showed the noninferiority of the RTS,S/AS02D vaccine in terms of antibody responses to EPI antigens. One month after vaccination, 98.6% of infants receiving the RTS,S/AS02D vaccine had seropositive titers for anticircumsporozoite antibodies on enzyme-linked immunosorbent assay (ELISA). During the 6-month period after the third dose of vaccine, the efficacy of the RTS,S/AS02D vaccine against first infection with *P. falciparum* malaria was 65.2% (95% CI, 20.7 to 84.7; $P=0.01$).

CONCLUSIONS

The use of the RTS,S/AS02D vaccine in infants had a promising safety profile, did not interfere with the immunologic responses to coadministered EPI antigens, and reduced the incidence of malaria infection. (ClinicalTrials.gov number, NCT00289185.)

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MALARIA PERSISTS AS A MAJOR PUBLIC health problem, and new tools for control of the disease are needed to facilitate the current renewed commitment for its control or elimination.^{1,2} The malaria vaccine contains the RTS,S antigen formulated with one of two adjuvant systems (AS), AS01 or AS02, and targets the pre-erythrocytic stage of *Plasmodium falciparum* parasite. This vaccine also has the potential to provide protection against infection with hepatitis B virus, since it contains the hepatitis B surface antigen. Studies conducted thus far show that the vaccine has a promising safety profile, is immunogenic, and confers partial protection against infection in adults who have not had malaria infection in the challenge model^{3,4} and in adults with partial immunity in the Gambia.⁵ A proof-of-concept study in children between the ages of 1 and 4 years in Mozambique also demonstrated protection against clinical malaria and severe disease lasting more than 18 months.^{6,7}

The RTS,S/AS candidate malaria vaccine, which is being developed for infants and children in regions in sub-Saharan Africa in which malaria is endemic, will ideally be delivered through the Expanded Program on Immunization (EPI) of the World Health Organization. Therefore, the two main aims of the development plan are to demonstrate prevention of malaria, which occurs in infants who have reached about 4 months of age (when maternally acquired immunity wanes⁸) and to support the inclusion of the RTS,S/AS vaccine in the EPI, which has successfully expanded the coverage of basic vaccines across the developing world.⁹

The clinical development plan for the RTS,S/AS02D vaccine (with the letter D indicating the pediatric formulation) has followed a two-step approach after the proof-of-concept trial.⁶ As a first step, the vaccine was tested in infants in Mozambique with a staggered administration of malaria vaccine and EPI vaccines.¹⁰ This trial showed that the malaria vaccine candidate had a promising safety profile, was immunogenic, and conferred 65% protection against malaria infection in infants.¹⁰ We describe the results of the coadministration of this vaccine with EPI vaccines in infants living in an area of perennial malaria transmission in Tanzania.

METHODS

STUDY DESIGN

This phase 2B, single-center, double-blind, controlled trial was conducted between July 2006 and February 2008 by the Bagamoyo Research and Training Centre, a branch of the Ifakara Health Institute in Bagamoyo, Tanzania. The protocol was approved by the Ifakara Health Institute, the Western Institutional Review Board in the United States, the National Institute of Medical Research in Tanzania, the Institutional Review Board of the London School of Hygiene and Tropical Medicine, and the Swiss Tropical Institute through the local government ethics committee in Basel, Switzerland. The trial was undertaken in accordance with the provisions of the International Conference on Harmonisation and Good Clinical Practice guidelines and was monitored by the sponsor, GlaxoSmithKline Biologicals, which provided both the RTS,S/AS02D vaccine and the hepatitis B vaccine. Vaccine containing diphtheria and tetanus toxoids, whole-cell pertussis vaccine, and conjugated *Haemophilus influenzae* type b vaccine (DTPw/Hib) (TETRActHib) was purchased from Aventis Pasteur.

The design, conduct, and results of the trial were overseen by a formally constituted data and safety monitoring board, operating under a charter. Written informed consent in Swahili was obtained from parents of infants before study entry; parents who were not able to write indicated consent using a thumbprint, with a signature from a literate witness to the consent procedure. All authors vouch for the completeness and accuracy of the data presented. For further methodologic details, see the Supplementary Appendix, available with the full text of this article at www.nejm.org.

STUDY VACCINES

We randomly assigned infants to receive three doses of either the RTS,S/AS02D vaccine or the hepatitis B vaccine (Engerix-B) through intramuscular injection in the left anterolateral thigh and the DTPw/Hib vaccine through intramuscular injection in the right anterolateral thigh at 8, 12, and 16 weeks of age. Each dose of the RTS,S/AS02D vaccine (0.5 ml) contained 25 μ g of RTS,S and

the adjuvant system AS02D, as described previously.¹¹ Oral polio vaccine was provided and administered at birth with sequential doses of DTPw/Hib.

SAFETY ASSESSMENTS

After each vaccination, infants were observed for 1 hour for general adverse events. Trained field workers visited the children at home every day for the following 6 days to record solicited reports of adverse events. Unsolicited reports of adverse events were recorded for 30 days after each dose, and serious adverse events were recorded throughout the study with the use of the morbidity surveillance system in place at Bagamoyo District Hospital. In addition, all enrolled infants were visited monthly at home by field workers to maximize identification of serious adverse events. As part of safety monitoring at the initial screening, at 1 week after the first dose of a study vaccine, and at 1 month after the third dose, we measured hemoglobin, hematocrit, platelets, and white cells, along with creatinine for assessment of renal function and alanine aminotransferase and bilirubin for assessment of hepatic function. The intensity of symptoms was graded on a scale of 0 to 3, with higher scores indicating greater intensity. Grade 3 symptoms were defined as crying when the limb was moved or a spontaneously painful limb (local pain), injection-site redness or swelling measuring more than 20 mm in diameter, an axillary temperature of more than 39.0°C, or other symptoms preventing normal daily activities (for details, see the Supplementary Appendix).

MONITORING FOR CLINICAL MALARIA EPISODES

Surveillance for malaria infections by *P. falciparum* was undertaken by both active detection of infection and passive case detection. Any infant presenting with a documented fever (axillary temperature, $\geq 37.5^\circ\text{C}$) within the preceding 24 hours underwent a blood draw for the determination of malaria parasites. For active detection of infection, home visits were conducted every 2 weeks after the administration of the third dose of a study vaccine. Four weeks before the start of surveillance for malaria infection (i.e., 2 weeks before the third dose), asymptomatic parasitemia was cleared with artemether-lumefantrine, with

the dose determined according to body weight. Each infant received a total of six doses of this drug during a 3-day period. The absence of parasitemia was confirmed by the analysis of a blood sample obtained 2 weeks later. Infants who continued to have parasitemia were retreated and excluded from the analysis. (For further details regarding the active detection of infection and determination of parasitemia, see the Supplementary Appendix.)

LABORATORY ANALYSIS

Antibody titers for anticircumsporozoite and anti-hepatitis B surface antigen were determined at screening and 1 month after the second and third doses of vaccine. Antibodies against diphtheria and tetanus toxins, polyribosylribitol phosphate for Hib, and *Bordetella pertussis* were measured at baseline and 1 month after the third dose of vaccine. The noninferiority of responses to hepatitis B, diphtheria, tetanus, Hib, and whole-cell pertussis were determined 1 month after the third dose (see the Supplementary Appendix for details).

STATISTICAL ANALYSIS

The analysis was based on a prospectively defined report and analysis plan. The primary end point for safety was the occurrence of serious adverse events during the first 9 months of the study in the intention-to-treat population, which included all infants for whom data were available. The primary end point of immunogenicity was the demonstration of noninferiority with respect to antibody responses to all antigens in the EPI vaccines at 1 month after the third dose of vaccine. This analysis was conducted in the per-protocol population for immunogenicity, which included all infants who could be evaluated — in other words, those who met all eligibility criteria, who had full compliance with the procedures (as defined in the protocol), who had no elimination criteria during the study, and for whom data concerning immunogenicity end-point measures were available. Noninferiority criteria were predefined and set to exclude more than a 10% decrease in protective antibody levels to diphtheria, tetanus, Hib, and hepatitis B surface antigen or to rule out a decrease by more than a factor of 1.5 in average antibody titers to whole-cell pertussis after vac-

ination. Secondary end points included further analyses of safety, reactogenicity, and immunogenicity and an estimation of vaccine efficacy against incident infections.

Vaccine efficacy was estimated for the per-protocol population (for details, see the Supplementary Appendix). Cases of infection were first or only infections with asexual *P. falciparum*, as detected by either active or passive means during the follow-up period, starting 14 days after the third dose of a study vaccine and continuing for approximately 7 months. The study evaluated efficacy against clinical malaria, which had a primary case definition of fever (axillary temperature, $\geq 37.5^{\circ}\text{C}$) with an asexual parasitemia of 500 parasites per microliter or more as an exploratory end point. This case definition had a reported sensitivity and specificity of more than 90%.¹² A secondary case definition for clinical malaria included fever or a history of fever in the previous 24 hours plus any asexual *P. falciparum* parasitemia. The measure of person-years at risk was adjusted for absences from the study area and for the use of antimalarial drugs. Estimates of vaccine efficacy for the intention-to-treat population included all infants who had received at least one dose of a study vaccine. Measurement of the time at risk started at the administration of the first dose. Time at risk was not corrected for absences or for the use of antimalarial drugs.

Vaccine efficacy was defined as 1 minus the hazard ratio and was adjusted according to the village of residence and the distance to Bagamoyo District Hospital. The adjusted vaccine efficacy was assessed with the use of Cox regression models.

The sample size of 170 infants per study group was calculated to have a power of 90% to reach the noninferiority of the RTS,S/AS02D vaccine, as compared with the hepatitis B vaccine, with respect to EPI antigen responses after vaccination. The trial also had a power of 80% to detect a difference of 7 to 13% between the two study groups in reports of serious adverse events (for varying rates in the control subjects) and a power of more than 90% to detect a significant vaccine efficacy under the assumption of an attack rate of 50% in control subjects and of 45% true vaccine efficacy. A two-sided P value of less than 0.05 was considered to indicate statistical significance.

Figure 1 (facing page). Enrollment and Outcomes.

Because of unavailability of vaccine at the study site, some infants who received the first dose of a study vaccine did not receive the second dose, the third dose, or both doses. Six recipients of the RTS,S/AS02D vaccine and five recipients of the hepatitis B vaccine received incomplete vaccination but were included in the 9-month follow-up. EPI denotes Expanded Program on Immunization.

RESULTS

SUBJECTS

The first mother was screened on July 21, 2006. The first infant was enrolled on September 27, 2006, and the last infant was enrolled on May 4, 2007. A total of 378 infants were screened, and 340 were vaccinated with the first dose of a study vaccine (Fig. 1). The same number of infants in each study group (153) completed the final study visit at 9 months.

The demographic profiles of the group receiving the RTS,S/AS02D vaccine and the group receiving the hepatitis B vaccine in the intention-to-treat population were balanced in terms of sex, mean age, and distance from the health center (Table 1). The mean age of infants when they received the first dose of a study vaccine was 7.8 weeks.

SAFETY AND REACTOGENICITY

From the time of the first vaccination until 9 months after the first dose, at least one serious adverse event was reported in 31 of 170 infants receiving the RTS,S/AS02D vaccine (18.2%; 95% confidence interval [CI], 12.7 to 24.9) and in 42 of 170 infants receiving the hepatitis B vaccine (24.7%; 95% CI, 18.4 to 31.9) (Table 2). When serious adverse events caused by malaria were excluded, similar numbers of subjects in the two groups reported serious adverse events: 29 receiving the RTS,S/AS02D vaccine (17.1%) and 40 receiving the hepatitis B vaccine (23.5%).

Pneumonia was the most frequently reported serious adverse event in both groups, occurring in 10 infants receiving the RTS,S/AS02D vaccine (5.9%; 95% CI, 2.9 to 10.6) and in 28 infants receiving the hepatitis B vaccine (16.5%; 95% CI, 11.2 to 22.9) (P=0.003). The next two most frequently reported serious adverse events were ane-

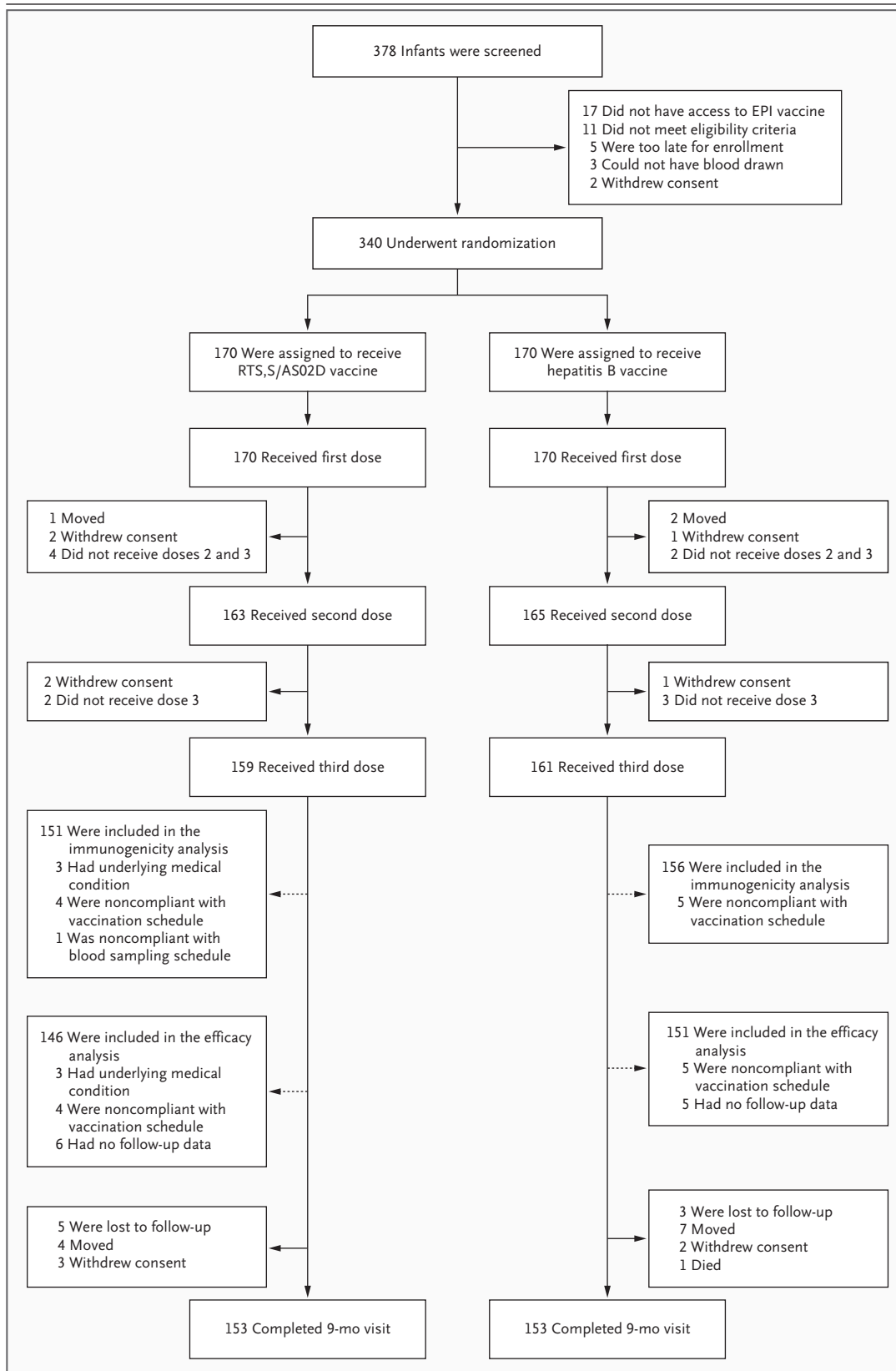


Table 1. Demographic Characteristics of the Subjects (Intention-to-Treat Population).*

Characteristic	Hepatitis B Vaccine (N=170)	RTS,S/AS02D Vaccine (N=170)	All Subjects (N=340)
Age at the time of first dose of vaccine — wk	7.9±0.8	7.8±0.8	7.8±0.8
Sex — no. (%)			
Female	85 (50.0)	91 (53.5)	176 (51.8)
Male	85 (50.0)	79 (46.5)	164 (48.2)
Distance from hospital to home — km			
<5.0	59 (34.7)	45 (26.5)	104 (30.6)
5.0–9.9	16 (9.4)	20 (11.8)	36 (10.6)
10.0–14.9	42 (24.7)	51 (30.0)	93 (27.4)
≥15.0	53 (31.2)	54 (31.8)	107 (31.5)

* Plus-minus values are means ±SD. Percentages may not total 100 because of rounding.

mia (in two infants receiving the RTS,S/AS02D vaccine and in eight receiving the hepatitis B vaccine) and gastroenteritis (in eight infants receiving the RTS,S/AS02D vaccine and in five infants receiving the hepatitis B vaccine). All other serious adverse events occurred infrequently. One serious adverse event was fatal: a case of severe pneumonia and symptomatic seizures in an infant receiving the hepatitis B vaccine. Observers who were unaware of study-group assignments determined that none of the serious adverse events were related to vaccination.

Solicited reports of pain and swelling at the injection site occurred with a similar incidence in the two study groups (Table 2). No solicited reports of grade 3 injection-site symptoms were reported after the administration of either study vaccine. In comparison, the local reactogenicity associated with the DTPw/Hib vaccine was 85.0% of doses that were followed by pain and 3.8% of doses that were followed by swelling.

Table 2. Incidence of Serious Adverse Events, Unsolicited Reports of Adverse Events, and Solicited Reports of Injection-Site and General Adverse Events (Intention-to-Treat Population).*

Event	Hepatitis B Vaccine		RTS,S/AS02D Vaccine	
	No.	Percent (95% CI)	No.	Percent (95% CI)
Serious adverse event†				
Total no. of subjects	170		170	
No. of subjects with event				
Any	42	24.7 (18.4–31.9)	31	18.2 (12.7–24.9)
<i>Plasmodium falciparum</i> infection	7	4.1 (1.7–8.3)	2	1.2 (0.1–4.2)
In absence of <i>P. falciparum</i> infection	40	23.5 (17.4–30.6)	29	17.1 (11.7–23.6)
Pneumonia	28	16.5 (11.2–22.9)	10	5.9 (2.9–10.6)
Gastroenteritis	5	2.9 (1.0–6.7)	8	4.7 (2.1–9.1)
Anemia	8	4.7 (2.1–9.1)	2	1.2 (0.1–4.2)
Death‡	1	0.6 (0.0–3.2)	0	0 (0.0–2.1)
Unsolicited report of adverse event§				
No. of subjects with event				
Any	141	82.9 (76.4–88.3)	137	80.6 (73.8–86.2)
Cough	80	47.1 (39.4–54.9)	80	47.1 (39.4–54.9)
Pneumonia	54	31.8 (24.8–39.3)	49	28.8 (22.1–36.3)
Rhinorrhea	73	42.9 (35.4–50.7)	56	32.9 (25.9–40.6)
Severity grade 3	16	9.4 (5.5–14.8)	7	4.1 (1.7–8.3)
Related to vaccine	2	1.2 (0.1–4.2)	0	0 (0.0–2.1)

Table 2. (Continued.)

Event	Hepatitis B Vaccine		RTS,S/AS02D Vaccine	
	No.	Percent (95% CI)	No.	Percent (95% CI)
Solicited report of adverse event				
Total no. of doses	492		490	
Injection-site reaction				
Pain				
Any	416	84.6 (81.1–87.6)	416	84.9 (81.4–88.0)
Severity grade 3	0	0 (0.0–0.7)	0	0 (0.0–0.8)
Swelling				
Any	18	3.7 (2.2–5.7)	19	3.9 (2.4–6.0)
Severity grade 3	0	0 (0.0–0.7)	0	0 (0.0–0.8)
General				
Drowsiness				
Any¶	4	0.8 (0.2–2.1)	3	0.6 (0.1–1.8)
Related to vaccine	2	0.4 (0.0–1.5)	0	0 (0.0–0.8)
Irritability				
Any¶	96	19.5 (16.1–23.3)	112	22.9 (19.2–26.8)
Related to vaccine	94	19.1 (15.7–22.9)	109	22.2 (18.6–26.2)
Loss of appetite				
Any¶	5	1.0 (0.3–2.4)	5	1.0 (0.3–2.4)
Related to vaccine	3	0.6 (0.1–1.8)	2	0.4 (0.0–1.5)
Fever				
Any	67	13.6 (10.7–17.0)	145	29.6 (25.6–33.9)
Severity grade 3	1	0.2 (0.0–1.1)	0	0 (0.0–0.8)
Related to vaccine	62	12.6 (9.8–15.9)	144	29.4 (25.4–33.6)
Severity grade 3 and related to vaccine	1	0.2 (0.0–1.1)	0	0 (0.0–0.8)

* Subjects could have more than one adverse event.

† For serious adverse events, the four most frequently reported events and all deaths are listed.

‡ One infant died of pneumonia and symptomatic seizures.

§ For all adverse events, those occurring in more than 10% of subjects in any group and those related to a study vaccine are listed.

¶ No grade 3 adverse events were reported.

|| Fever was defined as an axillary temperature of 37.5°C or greater.

Among solicited reports, fever (axillary temperature, $\geq 37.5^\circ\text{C}$) and irritability were the most frequently reported general symptoms in the two vaccine groups (Table 2). Fever was reported more frequently after the administration of the RTS,S/AS02D vaccine with DTPw/Hib than after the administration of the hepatitis B vaccine with DTPw/Hib (29.6% and 13.6%, respectively). Most

fevers were grade 1 or grade 2 in intensity ($< 39^\circ\text{C}$); only one grade 3 fever ($\geq 39^\circ\text{C}$) was reported, after the third dose of hepatitis B vaccine.

Irritability, drowsiness, and loss of appetite were recorded after similar proportions of doses of the two vaccines; none of these symptoms were grade 3 in intensity.

During the first 30 days after vaccination, un-

solicited reports of symptoms occurred in 80.6% of infants receiving the RTS,S/AS02D vaccine and in 82.9% of those receiving the hepatitis B vaccine (Table 2); the most common symptoms were cough (47.1% in both groups), rhinorrhea (32.9% and 42.9%, respectively), and pneumonia (28.8% and 31.8%, respectively). There was a trend toward a higher incidence of rash in recipients of the RTS,S/AS02D vaccine (7.1%; 95% CI, 3.7 to 12.0) than in recipients of the hepatitis B vaccine (0.6%; 95% CI, 0 to 3.2); none of these events were of severity grade 3. There was no other imbalance in other adverse events between the two study groups, and all events were those expected for the population. Two infants receiving the hepatitis B vaccine had an unsolicited report of an adverse event that was considered to be related to a study vaccine: injection-site erythema of moderate intensity and rash of mild intensity. Unsolicited reports of grade 3 events were infrequent and occurred in similar proportions in recipients of the RTS,S/AS02D vaccine and hepatitis B vaccine (4.1% and 9.4%, respectively). The most frequently reported grade 3 adverse event was pneumonia (2.4% and 6.5%, respectively).

Only 12 hematologic or biochemical values were outside the acceptable range in the two study groups. The abnormalities, which were all of grade 1 toxicity, included six tests of hemoglobin (in four recipients of the RTS,S/AS02D vaccine and in two recipients of the hepatitis B vaccine) and one test of alanine aminotransferase in a recipient of hepatitis B vaccine 1 week after the first dose and four tests of hemoglobin (one in a recipient of the RTS,S/AS02D vaccine and three in recipients of the hepatitis B vaccine) and one test of the white-cell count in a recipient of the RTS,S/AS02D vaccine 1 month after the third dose.

IMMUNOGENICITY RESULTS

Noninferiority of the humoral responses to all EPI antigens was demonstrated in a comparison of the RTS,S/AS02D vaccine with the hepatitis B vaccine coadministered with DTPw/Hib, as indicated by the value of -10 or more for the lower limit of the 95% confidence interval of difference in seroprotection rates or a value of more than 0.66 for the ratio of geometric mean titers between the two groups. Rates of seroprotection and seropositivity were high for all antigens ($>94\%$) (Table 3).

Before vaccination, the prevalence of mater-

nally transferred antibodies against EPI antigens was relatively high, except for *B. pertussis*; however, geometric mean titers were low. After the full vaccination course, geometric mean titers for EPI antigens (apart from antibodies to hepatitis B surface antigen) tended to be lower among infants receiving the RTS,S/AS02D vaccine than among those receiving the hepatitis B vaccine. In the intention-to-treat population, 14 infants (4.1%) — 4 receiving the RTS,S/AS02D vaccine and 10 receiving the hepatitis B vaccine — did not reach seroprotective concentrations for all EPI antigens. All these children were revaccinated with the respective antigens.

At baseline, 33 of 141 children (23.4%) receiving the RTS,S/AS02D vaccine and 39 of 152 children (25.7%) receiving the hepatitis B vaccine were seropositive for anticircumsporozoite antibodies (≥ 0.5 enzyme-linked immunosorbent assay [ELISA] units per milliliter) with low titers (Table 3). One month after administration of the third dose of a study vaccine, 141 of 143 of infants (98.6%) receiving the RTS,S/AS02D vaccine were seropositive for anticircumsporozoite antibodies (geometric mean titer, 69.5; 95% CI, 53.9 to 89.6), as compared with 2 infants receiving the hepatitis B vaccine (1.4%). All infants receiving the RTS,S/AS02D vaccine were seroprotected against hepatitis B, as compared with 94.3% of those receiving the hepatitis B vaccine.

EFFICACY

After the administration of the third dose of a study vaccine, 28 incident malaria infections involving the detection of any level of parasitemia were reported in the two study groups between day 14 and approximately 7 months (Table 4). During this period, the incidence of the first malaria infection was 0.12 per person-year in infants receiving the RTS,S/AS02D vaccine and 0.29 per person-year in those receiving the hepatitis B vaccine. The crude vaccine efficacy against infection was 60.6% (95% CI, 10.4 to 82.6; $P=0.03$), which increased to 65.2% (95% CI, 20.7 to 84.7; $P=0.01$) after adjustment for the subject's area of residence and distance from the health center. Figure 2 shows Kaplan–Meier curves of the cumulative incidence of first malaria infections in the two study groups.

The adjusted rates of vaccine efficacy were 58.6% (95% CI, -1.8 to 83.2) for the incidence of febrile malaria (the first or only episode of axil-

Table 3. Rates of Seropositivity or Seroprotection and Geometric Mean Titers for Key Antibodies at Baseline and after Dose 3 of a Study Vaccine Coadministered with DTPw/Hib Vaccines (Per-Protocol Population).*

Antibodies and Timing	Hepatitis B Vaccine			RTS,S/AS02D Vaccine			Difference in Rates of Seroprotection (95% CI)†
	No. of Subjects	Seropositivity or Seroprotection‡ no. (%)	Geometric Mean Titer (95% CI)	No. of Subjects	Seropositivity or Seroprotection‡ no. (%)	Geometric Mean Titer (95% CI)	
Diphtheria							
Baseline	165	27 (16.4)	0.1 (0.1 to 0.1)	162	24 (14.8)	0.1 (0.1 to 0.1)	
1 Mo after dose 3	151	148 (98.0)	1.3 (1.1 to 1.5)	149	148 (99.3)	1.1 (1.0 to 1.3)	1.3 (-1.9 to 5.1)
Tetanus							
Baseline	165	156 (94.5)	1.1 (0.9 to 1.4)	162	155 (95.7)	1.2 (1.0 to 1.6)	
1 Mo after dose 3	151	151 (100.0)	4.2 (3.6 to 4.8)	149	149 (100.0)	3.0 (2.6 to 3.4)	0 (-2.5 to 2.5)
Pertussis							
Baseline	165	2 (1.2)	7.6 (7.4 to 7.8)	162	1 (0.6)	7.6 (7.4 to 7.7)	
1 Mo after dose 3	144	142 (98.6)	101.4 (92.5 to 111.2)	148	148 (100)	82.3 (75.4 to 89.9)	0.8 (0.7 to 0.9)§
Hib							
Baseline	165	86 (52.1)	0.2 (0.2 to 0.2)	162	78 (48.1)	0.2 (0.2 to 0.2)	
1 Mo after dose 3	151	150 (99.3)	19.3 (15.6 to 24.0)	148	147 (99.3)	14.3 (11.5 to 17.9)	0 (-3.1 to 3.0)
Hepatitis B surface antigen							
Baseline	134	45 (33.6)	13 (9.8 to 17.2)	116	44 (37.9)	14.3 (10.8 to 19.0)	
1 Mo after dose 3	141	133 (94.3)	113.8 (91.3 to 141.8)	141	141 (100)	667.4 (533.8 to 834.4)	5.7 (2.9 to 10.8)
Plasmodium falciparum circumsporozoite¶							
Baseline	152	39 (25.7)	0.4 (0.3 to 0.4)	141	33 (23.4)	0.3 (0.3 to 0.4)	
1 Mo after dose 3	144	2 (1.4)	0.3 (0.2 to 0.3)	143	141 (98.6)	69.5 (53.9 to 89.6)	

* Cutoffs for antibody levels providing seroprotection were as follows: diphtheria and tetanus, ≥0.1 IU per milliliter; pertussis, ≥15 ELISA units per milliliter; Hib (*Haemophilus influenzae* type b), ≥0.15 µg per milliliter; and hepatitis B surface antigen, ≥10 mIU per milliliter; the seropositivity cutoff for *Plasmodium falciparum* circumsporozoite was ≥0.5 ELISA units per milliliter. DTPw/Hib denotes a vaccine containing diphtheria and tetanus toxoids, whole-cell pertussis vaccine, and conjugated *H. influenzae* type b vaccine.

† The value is the difference between the rate in the group receiving the RTS,S/AS02D vaccine and that in the group receiving the hepatitis B vaccine.

‡ Seropositivity is defined as an antibody concentration equal to or greater than the assay cutoff value. Seroprotection is defined as an antibody concentration above the established level providing protection.

§ The value is the ratio of the geometric mean titer in the group receiving the RTS,S/AS02D vaccine to that in the group receiving the hepatitis B vaccine.

¶ No differences in seroprotection rates are provided because the rates observed in recipients of the hepatitis B vaccine reflect background levels.

Table 4. Vaccine Efficacy during a Period from 14 Days to 7 Months after the Administration of Dose 3 of a Study Vaccine.*

Level of Parasitemia	Hepatitis B Vaccine			RTS,S/AS02D Vaccine			Adjusted Vaccine Efficacy		Crude Vaccine Efficacy			
	No. of Subjects	No. of Events	Person-Yr at Risk no.	Rate	No. of Subjects	No. of Events	Person-Yr at Risk no.	Rate	Value (95% CI) %	P Value	Value (95% CI) %	P Value
Any infection	151	20	69.4	0.29	146	8	69.2	0.12	65.2 (20.7 to 84.7)	0.01	60.6 (10.4 to 82.6)	0.03
Disease 1	151	15	70.7	0.21	146	7	69.3	0.10	58.6 (-1.8 to 83.2)	0.06	53.1 (-15.0 to 80.9)	0.10
Disease 2	151	11	71.3	0.15	146	7	69.3	0.10	43.2 (-47.1 to 78.0)	0.24	35.4 (-66.5 to 74.9)	0.36

* Any infection refers to the first or only episode of parasitemia; disease 1, the first or only episode of documented fever (axillary temperature, $\geq 37.5^{\circ}\text{C}$) or a history of fever and parasitemia with at least one organism per microliter; disease 2, the first or only episode of documented fever (axillary temperature, $\geq 37.5^{\circ}\text{C}$) and parasitemia with more than 500 organisms per microliter. Rates of events were calculated by dividing the number of events by the number of person-years at risk. Vaccine efficacy estimates were adjusted according to the village of residence and the distance from the health facility. The vaccine efficacy values and P values were assessed with the use of Cox regression models.

lary fever of $\geq 37.5^{\circ}\text{C}$ or a history of fever and any parasitemia) and 43.2% (95% CI, -47.1 to 78.0) for febrile malaria with a parasite-density threshold of more than 500 per microliter. Vaccine efficacy against febrile malaria (first or only episode of axillary fever of $>37.5^{\circ}\text{C}$ or a history of fever and any parasitemia) in the intention-to-treat population from first vaccination was 41.8% (95% CI, -32.9 to 74.6; $P=0.20$). Exploration of the relationship between antibody titers and the risk of infection showed that a doubling of anticircumsporozoite titers corresponded to a 16% reduction in the risk of infection and that there was a significant difference in titers between infants receiving the RTS,S/AS02D vaccine who were not infected and those who were infected (74.8 vs. 17.8 ELISA units per milliliter, $P=0.03$).

DISCUSSION

In our trial, the use of the RTS,S malaria vaccine, formulated in the AS02 adjuvant system, when coadministered to infants with other routinely delivered EPI immunizations, did not pose any obvious safety concerns and did not interfere with the immunogenicity of the multiple coadministered antigens. The results are in keeping with those of a trial in which the administration of the RTS,S/AS02D vaccine to Mozambican infants was staggered with EPI vaccinations.¹⁰ Low-grade fever was reported more frequently in infants receiving the RTS,S/AS02D vaccine (29.6%) than in the control group receiving the hepatitis B vaccine (13.6%). There were no cases of high-grade fever in infants receiving the RTS,S/AS02D vaccine, and there was no significant difference in the number of other solicited and unsolicited reports of adverse events between the two study groups. Since hospitalizations for pneumonia were more common in the control group, the malaria vaccine candidate may reduce important indirect consequences of malaria.

At the time of the first vaccination, about 25% of the infants were seropositive with low concentrations of anticircumsporozoite antibodies, which were probably acquired transplacentally. After 3 months, the anticircumsporozoite antibodies had almost disappeared in the control group but were higher in almost all RTS,S/AS02D vaccine recipients. The concentrations of the anticircumsporozoite titer in our study were lower than those observed in the staggered administration

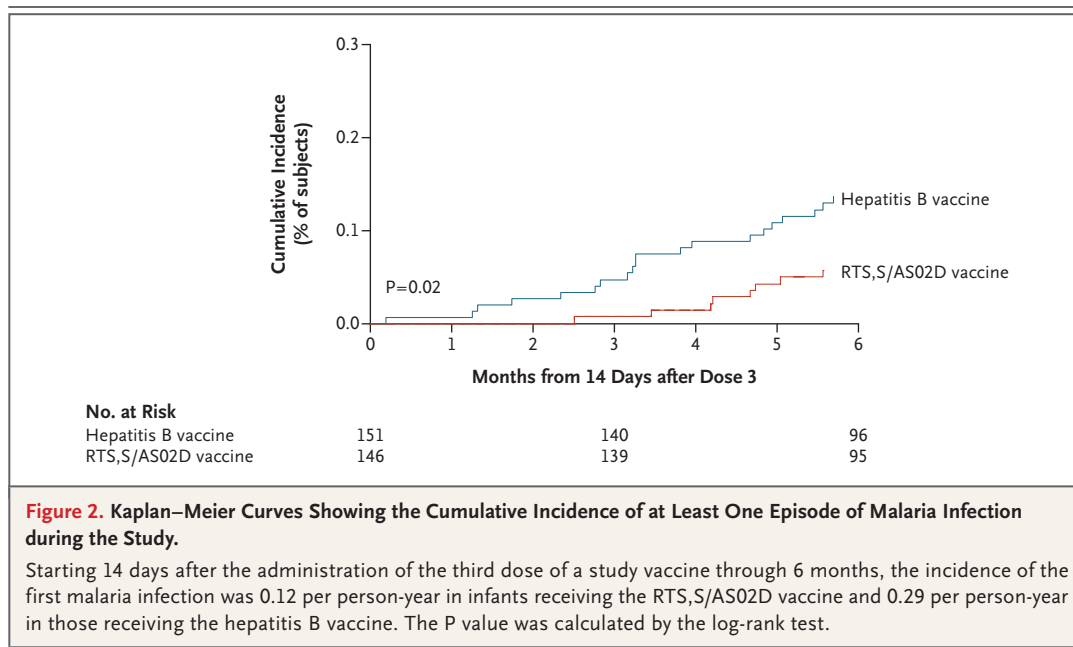


Figure 2. Kaplan–Meier Curves Showing the Cumulative Incidence of at Least One Episode of Malaria Infection during the Study.

Starting 14 days after the administration of the third dose of a study vaccine through 6 months, the incidence of the first malaria infection was 0.12 per person-year in infants receiving the RTS,S/AS02D vaccine and 0.29 per person-year in those receiving the hepatitis B vaccine. The P value was calculated by the log-rank test.

and among children between the ages of 1 and 4 years,^{6,10} which suggests that the EPI vaccines may have interfered with the anticircumsporozoite responses but had not prevented the conferring of protection. These data suggest an association between the level of anticircumsporozoite antibody titer and the risk of *P. falciparum* infection, in keeping with previous studies that have found a relationship between the level of anticircumsporozoite antibodies and efficacy against infection but not against clinical episodes.^{6,10} The response induced to hepatitis B surface antigen by the RTS,S/AS02D vaccine was higher than that of the licensed vaccine.

We also evaluated the feasibility of incorporating the RTS,S/AS02D vaccine into the standard EPI vaccination schedule in terms of immunogenicity. Our study followed a previous trial¹⁰ in which either the RTS,S/AS02D vaccine or the hepatitis B vaccine was given, staggered with the same DTPw/Hib vaccine that we used in our trial. Responses to antigens in the EPI vaccines were similar in the two comparator groups in which DTPw/Hib vaccine was administered either alone or in combination with hepatitis B vaccine. The geometric mean titers and seroprotection rates were broadly similar between a group receiving simultaneous administration of the EPI vaccines and a group receiving delayed (by 2 weeks) administration, with seroprotection rates of 100%

in the latter group for diphtheria, tetanus, pertussis, and Hib and of 98.5% for hepatitis B surface antigen.

Criteria of noninferiority for all EPI antigens were predefined in the protocol. Although responses that were induced to most components of the DTPw/Hib vaccine were slightly lower for infants receiving the RTS,S/AS02D vaccine than for those receiving the hepatitis B vaccine, seroprotective levels for both groups and all antigens were high, and all noninferiority criteria were met.

In an earlier trial of the RTS,S vaccine in children,⁶ efficacy against breakthrough infections was associated with reductions in both mild and severe malaria episodes. The rate of protection of 65% against new infections that we observed is encouraging and is similar to the observations in the earlier trial with staggered administration in Mozambican infants.¹⁰ However, rates of infection were substantially lower in our trial, and the finding of the previous trial with respect to efficacy against febrile episodes was not confirmed.

The low rate of detection of infection through active surveillance is likely to be a result of improved malaria control associated with distribution of bed nets, along with the close follow-up and improved clinical care of the infants in our study. Given that the infants in this trial had the best access to existing preventive tools and treat-

ment of malaria in Tanzania, these results indicate the added benefit of a malaria vaccine within an integrated approach for the control and elimination of malaria.

Further development of the RTS,S/AS vaccine in a large phase 3 trial is warranted. If licensed and recommended for inclusion in the EPI schedule, the RTS,S/AS vaccine could become an effective component of an integrated strategy to control malaria.

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