PRELIMINARY OBSERVATIONS ON THE EFFICACY OF A RECOMBINANT MULTISTAGE *PLASMODIUM FALCIPARUM* VACCINE IN *AOTUS NANCYMAI* MONKEYS

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Abstract. A vaccine trial was conducted to determine the efficacy of a multicomponent candidate vaccine, FALVAC-1, against *Plasmodium falciparum* in *Aotus nancymai* monkeys. After two immunizations, animals were challenged intravenously with parasites of the Vietnam Oak Knoll (FVO) strain of *P. falciparum*. The primary outcome was to determine the protective response of the monkeys to immunization with the FALVAC-1 antigen produced in baculovirus when combined with different adjuvants (alum, QS-21, ASO2a, CRL1005/oil, and CRL1005/saline) as compared with FALVAC-1 with FCA/FIA and antigen alone. When compared with the monkeys immunized with FALVAC-1 alone, FALVAC-1 with FCA/FIA reduced the mean parasite count (to Day 11), reduced the mean accumulated parasitemia (through Day 11), and extended the number of days to treatment. None of the other 5 antigen-adjuvant combinations were able to provide discernable levels of protection based on log(parasitemia) and log(cumulative parasitemia) to Day 11.

INTRODUCTION

Based on our studies on the infectivity of different strains of *Plasmodium falciparum* and *Plasmodium vivax* to New World monkeys, different combinations of parasites and species of *Aotus* and *Saimiri* monkeys have been selected as suitable models for biological, immunologic, and chemotherapeutic investigations.1–40 We have used these surrogate models to conduct vaccine trials in New World monkeys to determine the immunogenicity and efficacy of candidate vaccines against *P. falciparum*11–40 and *P. vivax*.51–58 One of our standard combinations to test blood-stage vaccines is the *Aotus nancymai* monkey and the Vietnam Oak Knoll (FVO) strain of *P. falciparum*.

Shi and others59 reported on the construction of a multistage *P. falciparum* candidate vaccine believed to have potential for further development. The 41-kDa antigen originally referred to as CDC/NII MAL VAC-1 as modified here is referred to as FALVAC-1. Small animal studies indicated that the construct could induce a strong immune response; in vitro studies indicated that these antibodies inhibited the growth of blood-stage parasites in the presence of monocytes. An immunization and challenge trial was designed to determine the protective immune response in *A. nancyamai* monkeys immunized with the FALVAC-1 antigen in combination with various adjuvants; some of these adjuvants are believed to have potential for use in humans. At this preliminary stage in the developmental process, the standard for protection was those animals immunized with FALVAC-1 combined with Freund’s adjuvant. No attempts were made to include adjuvant control groups or to select immunization dosages or regimens. At the end of the immunization period, all animals were challenged with the FVO strain of *P. falciparum* to determine if one or more of the antigen/adjuvant combinations would provide protection comparable to that of animals immunized with FALVAC-1 combined with Freund’s adjuvant.

Here, we report the results of this preliminary study to test the ability of FALVAC-1 to induce protective immunity in *A. nancymai* monkeys. FALVAC-1 was combined with 1) QS-21 an adjuvant active saponin obtained from *Quillaya saponaria*, 2) ASO2a, 3) the nonionic block copolymer CRL1005 in saline, 4) CRL1005 in oil, 5) alum, and Freund’s complete (FCA) and Freund’s incomplete (FIA) adjuvants.

MATERIALS AND METHODS

**Antigen.** The characteristics of FALVAC-1 vaccine has been described previously.59,60 Briefly, the construct includes 12 B cell and 9 T-cell epitopes derived from 9 stage-specific vaccine candidate antigens of *P. falciparum*. One universal T-cell epitope from tetanus toxoid61 was also incorporated. The modification from the previous CDC/NII MAL VAC-1 preparation involved the exclusion of the his-tag from the N-terminus region of the expressed recombinant protein. The baculovirus-expressed FALVAC-1 was produced by Protein Sciences Corporation (Meriden, CT). The vaccine product was 90% to 85% pure and free from endotoxin contamination.

**Vaccine formulation.** FALVAC-1 vaccine alone was prepared by diluting the antigen in PBS (pH 7.4; Gibco, Grand Island, NY) to a final concentration of 100 µg/dose and was administered in a final volume of 500 µL/monkey.

For vaccine containing Freund’s adjuvants (Sigma, St. Louis, MO), the antigen in PBS (pH 7.4) and the adjuvant were emulsified by connecting two separate syringes with a Luer fitting, and passing the contents back and forth for approximately 15 minutes. The stability of the emulsion was tested using the water-drop method. The first dose was given in Freund’s complete adjuvant (FCA); subsequent dose was given in Freund’s incomplete adjuvant (FIA).

The FALVAC-1 with alum formulation was prepared by the procedure developed by de Oliveira and others,62 with slight modifications. Briefly, aluminum hydroxide (2% rehydrox, Intergen, Purchase, NY) was washed in acetate

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Animals were given 2 immuniza-
tion trials. Before this trial, stud-
ies had shown that the Vietnam Oak Knoll (FVO) strain of P. falciparum would be expected to induce a high-density, life-
threatening parasitemia following the inoculation of 10,000 parasitized erythrocytes. A frozen sample was used to induce a high-density parasitemia in a donor A. nancymai monkey. Blood was drawn via venipuncture, diluted in RPMI 1640 culture medium, and 10,000 parasitized erythrocytes passed into each of the 53 monkeys remaining in the immunization trial. For rechallenge, a donor animal was again infected with parasites after being stored frozen; blood was drawn, diluted in RPMI 1640, and 50,000 parasitized erythrocytes injected intravenously into each of the remaining test animals.

**Preparation of challenge inoculum.** Before this trial, studies had shown that the Vietnam Oak Knoll (FVO) strain of P. falciparum would be expected to induce a high-density, life-threatening parasitemia following the inoculation of 10,000 parasitized erythrocytes. A frozen sample was used to induce a high-density parasitemia in a donor A. nancymai monkey. Blood was drawn via venipuncture, diluted in RPMI 1640 culture medium, and 10,000 parasitized erythrocytes passed into each of the 53 monkeys remaining in the immunization trial. For rechallenge, a donor animal was again infected with parasites after being stored frozen; blood was drawn, diluted in RPMI 1640, and 50,000 parasitized erythrocytes injected intravenously into each of the remaining test animals.

**Immunization schedule.** Animals were given 2 immunization trials at 8-week intervals (also identified as Days 0 and 56). Day 112 (Week 16) was the day of the first challenge. Animals receiving FCA followed by FIA were immunized subcutaneously in 4 sites in the back to facilitate drainage if an ulcer developed. All other animals were immunized intramuscularly in the quadriceps muscles of the hind legs. The injection sites were shaved to allow visual monitoring for any adverse reactions. Animals were examined weekly, and reactions at the site of immunization were graded 1+ (erythema), 2+ (erythema and induration), 3+ (erythema and induration), or 4+ (ulceration).

The trial was conducted in a blind format. Persons responsible for the reading of blood smears, and making parasite count determinations, and the persons responsible for collection of specimens, and physical examinations did not know

**Animals.** Fifty-four A. nancymai (37 female and 17 male) monkeys were included in the vaccine trial. Twenty-two monkeys were laboratory-born (7 female and 15 male); 32 were feral animals imported from Peru (30 female and 2 male). All feral animals were quarantined upon arrival at the facility for as little as possible. All procedures involving the animals were under the direction of the resident clinical veterinarian. Recorded observations on local and/or systemic reactions (e.g., lymphadenopathy, cellulitis, abscesses, necrotizing lesions, arthritis, anorexia, and weight loss) to the candidate vaccines were made minimally once a week at the time of blood collection. In the event of a reaction, additional observations were made daily, and supportive treatment instituted.

**Experimental protocol.** A schematic of the trial: 1) immunization at 0 and 8 weeks, 2) first challenge at week 16, 3) treatment of all animals by week 24. Animals were assigned to 8 groups, taking into account sex and weight, using a table of random numbers. These groups received the following vaccinations. Group 1: 100 μg FALVAC-1 in PBS at weeks 0 and 8. Group 2: 100 μg FALVAC-1 combined with Freund’s complete adjuvant at week 0; a second immunization with 100 μg FALVAC-1 combined with Freund’s incomplete adjuvant at week 8. Group 3: 100 μg FALVAC-1 with alum at weeks 0 and 8. Group 4: 100 μg FALVAC-1 combined with QS-21 at weeks 0 and 8. Group 5: 100 μg FALVAC-1 combined with ASO2a at weeks 0 and 8. Group 6: 100 μg FALVAC-1 combined with CRL1005 in saline at weeks 0 and 8. Group 7: 100 μg FALVAC-1 combined with CRL1005-1 in oil at weeks 0 and 8. Group 8: phosphate-buffered saline (PBS) at weeks 0 and 8 (PBS control).

Four criteria of protection were specified in the approved protocol: 1) decrease in the initial growth rate of parasites; 2) increase in the time to reach a level requiring treatment (> 200,000/μL); 3) an increase in the proportion not requiring treatment; 4) decrease in the maximum parasite count in the animals not requiring treatment. Note that these criteria applied to the experimental groups, not individual animals.

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The trial was conducted in a blind format. Persons responsible for the reading of blood smears, and making parasite count determinations, and the persons responsible for collection of specimens, and physical examinations did not know
the experimental group to which the monkeys had been assigned. To achieve and maintain this level of masking, a person having none of the responsibilities listed above prepared a code book listing each animal by its tattooed identification number, its group assignment, and the corresponding treatment group to which each animal had been assigned. The same person was responsible for directing the administration of the immunizations.

**Challenge.** Eight weeks after the last immunization (Day 112–Week 16), each animal was injected with 10,000 parasitized erythrocytes of the FVO strain of *P. falciparum* taken from a donor animal, diluted in RPMI 1640 culture medium, and injected intravenously into the femoral vein of each animal.

**Rechallenge.** After termination of primary infection, animals were rechallenged with the homologous parasite to determine if there was persistence of immunity. For the rechallenge (Day 238–Week 34), each animal was injected with 50,000 erythrocytes parasitized with the same strain of *P. falciparum* from an infected donor animal.

**Parasite monitoring.** After challenge, thick and thin blood films were collected daily and stained with Giemsa by the method of Earle and Perez. Parasite counts were recorded per microliter of blood. After the parasitized erythrocytic challenge, blood films were made and examined from days 1 through 56, unless the animal had been previously cured of its infection. After the second challenge, blood films were examined from Days 239 to 280 (42 days after rechallenge).

When parasite counts exceeded 200,000/µL (approximately 5% of the erythrocytes infected), animals were treated orally with 20 mg mefloquine and 50 mg of quinine, which cured their infections. Fifty-six days after injection or 42 days after reinfection, all untreated infections in animals remaining in the trial were cured by treatment with 20 mg mefloquine. A parasite count of ≥ 200,000/µL was selected for treatment of several reasons. Death of a primate cannot be an end point in any of our vaccine trials. Our previous experience with infections in New World monkeys has been that some animals may die if their parasitemia is allowed to exceed 10%. A parasite count of 200,000/µL (approximately 5% of erythrocytes infected) was considered an acceptable level that would allow most animals to survive if treated adequately.

**Hematology.** Biweekly collections of < 10% of each monkey’s estimated total blood volume (based on a total blood volume calculation of 40 mL blood per kilogram body weight), were made via venipuncture from the femoral vein. A complete blood count was done: 1) erythrocytes/µL; 2) leukocytes/µL; 3) hematoctrit; 4) hemoglobin concentration; 5) platelets/µL; 6) mean corpuscular hemoglobin; 7) mean corpuscular volume; and 8) mean corpuscular hemoglobin concentration. The remaining blood was centrifuged and the plasma stored frozen for serologic studies.

**Statistical analysis.** Kaplan-Meier product moment estimators were used to estimate survivor functions describing the first day the parasite count was ≥ 100, initial treatment day, and first day negative. The Wilcoxon test was used to test for differences in survivor factor functions between each treatment group. Adjuvant differences in parasite counts were tested by Tukey’s HSD multiple comparison test. Geometric means were computed by taking the log of each parasite count, computing the mean of the log values, then exponentiating the log mean back to the original scale. Statistical significance was set at alpha = 0.05. SAS version 8.2 was used to analyze the data.

**RESULTS**

**Parasitemia after challenge.** FALVAC-1 alone, Group 1: Seven monkeys (T-0683, T-1750, T-0484, T-0752, AI-1758, AI-1742, and AI-1761) were immunized and challenged (Table 1; Figures 1 and 2). Prepatent periods were 7, 7, 6, 6, 6, and 6 days. All monkeys had maximum parasite counts ≥ 200,000/µL and were treated on Days 14, 17, 12, 11, 12, and 12, respectively.

FALVAC-1 with FCA/FIA adjuvants, Group 2: Six animals (T-0517, T-0708, T-0444, AI-1740, T-0767, and AI-1753) completed immunization and were challenged (Table 1; Figures 1 and 2). Prepatent periods were 7, 7, 5, 7, 7, and 7 days. Three monkeys (T-0517, T-0767, and AI-1753) had maximum parasite counts ≥ 200,000/µL and were treated on Days 17, 19, and 14, respectively. Three monkeys (T-0708, T-0444, and AI-1740) had maximum parasite counts of 127,624, 1,530, and 108,000/µL, respectively. Monkey T-0708 died on Day 14; monkey AI-1740 was treated on Day 22, and T-0444 was treated on Day 54.

**Table 1**

Descriptive statistics regarding first-day parasite count ≥ 100, treatment day, and Day 11 parasite counts for *Aotus nancymai* monkeys immunized with FALVAC-1 vaccine combined with different adjuvants and challenged with the Vietnam Oak Knoll strain of *Plasmodium falciparum*.

<table>
<thead>
<tr>
<th>Group</th>
<th>FALVAC-1, None</th>
<th>FALVAC-1, FCA/FIA</th>
<th>FALVAC-1, alum</th>
<th>FALVAC-1, QS-21</th>
<th>FALVAC-1, ASO2a</th>
<th>FALVAC-1, CRL1005Sal</th>
<th>FALVAC-1, CRL1005OIl</th>
<th>None</th>
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<tr>
<td>No.</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>4</td>
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<tr>
<td>Mean</td>
<td>7.86</td>
<td>8.17</td>
<td>7.14</td>
<td>7.00</td>
<td>7.14</td>
<td>7.42</td>
<td>7.14</td>
<td>7.25</td>
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<tr>
<td>Median</td>
<td>7.0</td>
<td>8.5</td>
<td>7.0</td>
<td>7.0</td>
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<td>7.0</td>
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<tr>
<td>SD</td>
<td>1.2</td>
<td>0.98</td>
<td>0.38</td>
<td>0.37</td>
<td>0.37</td>
<td>1.13</td>
<td>0.38</td>
<td>0.50</td>
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<tr>
<td>1st day parasite count</td>
<td>12.86</td>
<td>18.0*</td>
<td>12.14</td>
<td>12.57</td>
<td>12.57</td>
<td>13.71</td>
<td>12.17</td>
<td>12.01</td>
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<tr>
<td>Geo. mean</td>
<td>55.073</td>
<td>8,721†</td>
<td>92.969</td>
<td>107,634</td>
<td>62.279</td>
<td>63,806</td>
<td>112,182</td>
<td>99.805</td>
</tr>
<tr>
<td>Median</td>
<td>871,460</td>
<td>13,473†</td>
<td>93,142</td>
<td>117,625</td>
<td>57,960</td>
<td>77,329</td>
<td>117,900</td>
<td>86,940</td>
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<tr>
<td>Geo. mean</td>
<td>80,644</td>
<td>30,257</td>
<td>132,044</td>
<td>170,191</td>
<td>115,498</td>
<td>88,766</td>
<td>170,665</td>
<td>151,318</td>
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<tr>
<td>Accum. para. count</td>
<td>143,446</td>
<td>199,862</td>
<td>149,862</td>
<td>207,082</td>
<td>105,180</td>
<td>129,055</td>
<td>188,280</td>
<td>137,932</td>
</tr>
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</table>

*Wilcoxon test, P < 0.05. FCA/FIA significantly different when compared with other adjuvants. No other significant differences between adjuvant pairs were detected.
†Tukey’s multiple comparison HSD test, P < 0.05, when FCA/FIA compared with adjuvants alum, QS-21, CRL1005Sal, and none. No other significant differences between adjuvant pairs were detected.
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monkeys with the FVO strain of Plasmodium falciparum. Four measures of initial growth rate were examined: 1. Parasite counts 11 days after challenge of Aotus nancymai (T-0822, T-0827, T-0828, T-0755, AI-1765, T-0818, and AI-1748) were immunized with FALVAC with QS-21 (Table 1; Figures 1 and 2). Prepatent periods were 6, 7, 6, 7, and 6 days. All monkeys developed maximum parasite counts \( \geq 200,000/\mu L \) and were treated on Days 14, 13, 12, 12, 12, and 11, respectively.

FALVAC-1 with alum adjuvant, Group 3: Seven monkeys (T-0811, AI-1763, T-0510, AI-1747, T-0483, AI-1738, and T-0815) were immunized and challenged (Table 1; Figures 1 and 2). Prepatent periods were 6, 7, 6, 7, and 6 days. All monkeys developed maximum parasite counts \( \geq 200,000/\mu L \) and were treated on Days 14, 13, 12, 11, 12, 12, and 11, respectively.

FALVAC-1 with QS-21 adjuvant, Group 4: Seven monkeys (T-0822, T-0827, T-0828, T-0755, AI-1765, T-0818, and AI-1748) were immunized with FALVAC with QS-21 (Table 1; Figures 1 and 2). Prepatent periods were 6, 7, 6, 7, and 6 days. All monkeys developed maximum parasite counts \( \geq 200,000/\mu L \) and were treated on Days 14, 13, 12, 12, 12, and 11, respectively.

Comparison of different measures of efficacy. Initial growth rate. Four measures of initial growth rate were examined: 1) Prepatent period, day on which the parasitemia first exceeded 100/\( \mu L \). 2) Parasitemia on Day 11 (the day the first monkey was treated), 3) cumulative parasitemia up to and including Day 11, and 4) day to required treatment. The prepatent period was relatively short with 3 of 53 monkeys patent on Day 5, 28 on Day 6, 20 on Day 7, and 1 on Day 9. The average time to \( > 100/\mu L \) for animals immunized with various combinations was Day 7.4 with 40 of the 52 being Day 7.

Inspection of the daily parasitemia figures indicated that there was some initial synchrony, with parasite density decreasing on every other day. Thus, 1) a plot of log(parasitemia) on the day when the first animals required treatment, and 2) the log(cumulative parasitemia) was used in the comparison of the different immunization groups. The groups of monkeys can only be compared by this method up to the time of the first treatment on Day 11. An examination of the parasite counts on Day 11 (Table 1; Figure 2) indicated that 15 of the 48 immunized monkeys had parasite counts \(< 50,000/\mu L \): 2 of 7 in Group 1; 6 of 6 in Group 2; 2 of 7 in Group 3; 1 of 7 in Group 4; 3 of 7 in Group 5; 1 of 7 in Group 6; and 0 of 7 in Group 7.

**Figure 1.** Parasite counts 11 days after challenge of Aotus nancymai monkeys with the FVO strain of Plasmodium falciparum. Group 1 was immunized with FALVAC-1 alone, Group 2 with FALVAC-1 and FCA/FIA, Group 3 with FALVAC-1 and QS-21, Group 4 with FALVAC-1 and ASO2a, Group 5 with FALVAC-1 and alum, Group 6 with ASO2a adjuvant, Group 5: Seven monkeys (T-1004, T-1760, T-0207, T-0527, AI-1754, T-0826, and T-0543) were immunized and challenged. Prepatent periods were 6, 7, 7, 7, 7, 7, and 5 days, respectively (Table 1; Figures 1 and 2). All monkeys had maximum parasite counts \( \geq 200,000/\mu L \) and were treated on Days 12, 15, 14, 12, 11, 13, and 11, respectively.

FALVAC-1 with CRL1005 adjuvant in saline, Group 6: Seven monkeys (AI-1752, T-0499, T-0800, AI-1741, T-0763, T-0533, and T-0768) were immunized and challenged (Table 1; Figures 1 and 2). Prepatent periods were 6, 6, 6, 9, 7, 7, and 6 days. All monkeys developed parasite counts \( \geq 200,000/\mu L \) and were treated on Days 18, 12, 12, 18, 11, and 12, respectively.

FALVAC-1 with CRL1005 adjuvant with oil, Group 7: Seven monkeys (T-0474, AI-1756, AI-1743, AI-1749, AI-1739, T-0694, and AI-1755) were immunized and challenged (Table 1; Figures 1 and 2). Prepatent periods were 5, 6, 6, 6, 7, 7, and 7 days. Monkey AI-1756 had a maximum parasite count of 136,000/\( \mu L \) on Day 14 and was treated on Day 56. The other animals all had maximum parasite counts \( \geq 200,000/\mu L \) and were treated on Days 14, 12, 12, 12, and 11, respectively.

PBS control, Group 8: Five monkeys (T-0805, T-1052, AI-1757, T-0762, and T-0698) were immunized with saline (PBS control) (Table 1; Figures 1 and 2). For some undetermined reason, the rise in parasitemia in monkey T-0805 was abnormally delayed. The parasite count eventually reached 244,000/\( \mu L \) and the animal was treated. Because of this, it was not considered in the analysis. Prepatent periods for the 4 remaining animals were 7, 6, 7, and 7 days, respectively. All of the animals developed parasite counts \( > 200,000/\mu L \) and were treated on Days 11, 12, 12, and 13, respectively, with quinine and mefloquine.

**Figure 2.** Accumulated parasite counts to 11 days after challenge of Aotus nancymai monkeys with the FVO strain of Plasmodium falciparum. Group 1 was immunized with FALVAC-1 alone, Group 2 with FALVAC-1 and FCA/FIA, Group 3 with FALVAC-1 and QS-21, Group 4 with FALVAC-1 and ASO2a, Group 5 with FALVAC-1 and alum, Group 6 with FALVAC-1 and CRL1005/saline, Group 7 with FALVAC-1 and CRL1005/oil, Group 8 with PBS. Geometric mean accumulated parasite counts are shown for each group.
We chose to examine log(cumulative parasitemia), as this is less affected by the 48 hour periodicity in some monkeys. Figure 2 shows the cumulative parasitemia to Day 11, plotted on a log scale for the monkeys in each group, together with the geometric mean cumulative parasitemia. Nineteen of the 48 immunized animals had accumulated parasitemias through Day 11; <100,000/μL (Figure 2). There were 2 of 7 in Group 1; 6 of 6 in Group 2; 2 of 7 in Group 3; 2 of 7 in Group 4, 3 of 7 in Group 5, 3 of 7 in Group 6, and 1 of 7 in Group 7. It was readily apparent that those animals immunized with Falvac-1 with the Freund’s adjuvants were better protected when compared with the other immunized groups of monkeys.

The results suggested that these groups contained responder and non-responder animals with respect to decreased initial parasite growth.

Proportion of animals requiring treatment. With the exception of Group 2 (Falvac-1 plus Freund’s adjuvants), only monkey AI-1756 from Group 7 had a maximum parasite count < 200,000/μL after challenge and did not require treatment.

Parasitemia after rechallenge. Falvac-1 alone, Group 1: Six remaining monkeys (T-0683 had died) were rechallenged; prepatent periods ranged from 9 to 12 days (Table 2). Monkey T-0484 developed a maximum parasite count of 14,310/μL on Day 18 and died on Day 20. The remaining animals had maximum parasite counts from 4,454 to 180,000/μL and were treated 42 days after rechallenge.

Falvac-1 with FCA/FIA adjuvants, Group 2: Monkeys T-0444, AI-1740, T-0767, and AI-1753 were rechallenged. Monkey T-0444 failed to develop detectable parasitemia. Prepatent periods for the remaining animals were 14, 14, and 12 days, respectively. Monkeys AI-1740 and T-0767 had maximum parasite counts of 2,700 and 10,260/μL on Day 18 and died on Day 20. The remaining animals had maximum parasite counts from 4,454 to 180,000/μL and were treated 42 days after rechallenge.

Falvac-1 with alum adjuvant, Group 3: All 7 animals were rechallenged. Prepatent periods ranged from 6 to 14 days. Maximum parasite counts ranged from 0 to 140,000/μL. All infections were treated 42 days after rechallenge.

Falvac-1 with Qs-21 adjuvant, Group 4: Five monkeys were rechallenged with the FVO strain of Plasmodium falciparum. Prepatent periods ranged from 9 to 14 days. Monkey AI-1848 had a maximum parasite count of 244,000/μL on Day 23 and was treated. The remaining monkeys had maximum parasite counts from 720 to 47,160/μL.

Falvac-1 with ASO2a adjuvant, Group 5: All 7 monkeys were rechallenged. Monkey T-1004 failed to develop detectable parasitemia. Prepatent periods ranged from 9 to 14 days. Monkey T-0543 developed a maximum parasite count of 1,120,000/μL on Day 20 and died. The remaining animals had maximum parasite counts ranging from 150 to 38,160/μL, and were treated 42 days after rechallenge.

Falvac-1 with CRL1005 adjuvant in saline, Group 6: Maximum parasite counts ranged from 1,260 to 25,740/μL, and monkeys were treated 42 days after rechallenge.

Falvac-1 with CRL1005 adjuvant with oil, Group 7: Six of the 7 remaining animals were rechallenged (monkey T-0474 was not because of poor health). Monkey T-1756 failed to develop detectable parasitemia. Prepatent periods ranged from 10 to 12 days. Maximum parasite counts ranged from 0 to 27,000/μL.

PBS control, Group 8: Three of the 5 monkeys were rechallenged with the homologous FVO strain of P. falciparum. Maximum parasite counts for AI-1757, T-0762, and T-0698 were 17,640, 25,200, and 65,240/μL, respectively.

Protection against rechallenge with homologous parasite. Only 3 animals, AI-1748 from Group 4, T-0543 from Group 5, and AI-1753 from Group 2 were unable to control their infections after rechallenge (Table 2). Four monkeys (T-1004, AI-1740, AI-1763, and AI-1756), one each from Groups 5, 2, 3, and 7, failed to support detectable parasite counts during the 42 days of observation. Thirteen animals had maximum parasite counts < 10,000/μL; 5 of these had accumulated parasitemias < 10,000.

Adverse responses. Adverse responses at the immunization sites were recorded during the trial. Groups 1, 3, 4, 5, 6, and 8 had no discernable reactions. Of the 6 animals receiving Falvac-1 with FCA/FIC, all animals had 3+ or 4+ reactions through the challenge period (3+ = erythema and induration; 4+ = ulceration). One monkey in Group 7 had 3+ responses after the second immunization; reactions in the other animals were of a low intensity and transient.

An unexpected observation during this trial was that some animals in all groups had marked dehydration that required treatment with subcutaneous fluids. Because this was seen in all groups, including the animals receiving PBS only, the exact cause of this was not determined. Such widespread dehydration has not been observed in previous trials or in another trial ongoing at the same time, although infected animals have occasionally required rehydration during infection.

Hematology. The hematocrits for the animals were determined every 2 weeks during the trial. At the beginning of the

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>First-day parasitemia ± 100</th>
<th>Maximum Parasite count</th>
<th>First-day treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Falvac-1, none</td>
<td>6</td>
<td>12.3</td>
<td>12.0</td>
<td>1.0</td>
</tr>
<tr>
<td>2. Falvac-1, FCA/FIA</td>
<td>3</td>
<td>13.3</td>
<td>14.0</td>
<td>1.1</td>
</tr>
<tr>
<td>3. Falvac-1, alum</td>
<td>6</td>
<td>14.0</td>
<td>14.0</td>
<td>1.7</td>
</tr>
<tr>
<td>4. Falvac-1, Qs-21</td>
<td>5</td>
<td>13.8</td>
<td>14.0</td>
<td>1.5</td>
</tr>
<tr>
<td>5. Falvac-1, ASO2a</td>
<td>6</td>
<td>13.3</td>
<td>12.5</td>
<td>3.0</td>
</tr>
<tr>
<td>6. Falvac-1, CRL1005Sal</td>
<td>4</td>
<td>11.7</td>
<td>12.5</td>
<td>2.6</td>
</tr>
<tr>
<td>7. Falvac-1, CRL1005Oil</td>
<td>5</td>
<td>13.8</td>
<td>14.0</td>
<td>2.4</td>
</tr>
<tr>
<td>8. None</td>
<td>3</td>
<td>13.3</td>
<td>13.0</td>
<td>.57</td>
</tr>
</tbody>
</table>

* No significant differences between any adjuvant pairs were detected.
immunizations, the mean hematocrit value for all animals was 51.5%. At the end of the challenge period, the mean value was 50.3%. There was a marked drop during and immediately after infection. During the period after the second immunization, mean hematocrit values rose, reflective of the marked dehydration observed in the animals.

**DISCUSSION**

The *A. nancymai* monkey and the FVO strain of *P. falciparum* has been used in previous immunization and challenge trials to test candidate antigens as potential components of an antimalarial vaccine. The present trial was designed to test the potential efficacy of the multi-stage FALVAC-1 vaccine using this model system. FCA/FIA was the standard adjuvant for comparison, even though it was realized that further development of a candidate vaccine would require adjuvants with potential for use in humans.

Immunization and challenge trials in *Aotus* and *Saimiri* monkeys have been used repeatedly in efforts to find an alternative to the primary testing of candidate antimalarial vaccines in human volunteers. The question of appropriate criteria of protection in nonhuman primates, and their influence on vaccine development continues to remain unclear. Is a strong serologic response in a nonhuman primate to the different components of a vaccine sufficient to proceed in the developmental process? If efficacy trials with monkeys are included in the critical pathway for vaccine development, what levels of protection are significant?

Few of the vaccine efficacy trials we have conducted in New World monkeys have provided evidence of solid protection after primary challenge. Nonetheless, New World monkeys are the only surrogate hosts that can be infected with *P. falciparum* to measure efficacy. Many of our studies have shown that these hosts are capable of developing high-level immune responses to infection and reinfection. Thus, we believe that demonstration of efficacy as well as immunogenicity would further support the continued development of candidate vaccines. A candidate vaccine that is highly immunogenic and highly protective in monkeys would appear to be a prime candidate for development in humans.

The planning and conduct of vaccine trials in nonhuman primates involves commitment of animals and many months of time before decisions can be made for movement to the next step in the development of a candidate vaccine. Trials have involved the testing of candidate antigens, usually combined with Freund’s adjuvant or with alum. Here, a novel candidate vaccine was used to test the potential of different adjuvants for the development of protective immunity in *A. nancymai* monkeys against the FVO strain of *P. falciparum*. When compared with the monkeys immunized with FALVAC-1 alone, FALVAC-1 with FCA/FIA produced statistically significant parasitologic differences noted between the immunization or the previous parasitemia resulted in the control groups, particularly for Freund’s makes assessment of the contribution of FALVAC-1 difficult.

Obviously, we expected the FALVAC-1 construct to be more protective than was shown in this preliminary trial. Otherwise, fewer adjuvants and more controls would have been included. A new vaccine construct, FALVAC-1A has been produced and is in preliminary stages of testing. Further studies with adequate controls to further the potential efficacy of these various adjuvants when combined with the candidate vaccine will be needed. After rechallenge indeed, some animals failed to demonstrate detectable parasite counts and others had very low levels of parasitemia. However, whether the immunization or the previous parasitemia resulted in the protection shown against the homologous strain of *P. falciparum* upon rechallenge is not clear. There were similar statistically significant parasitologic differences noted between the various adjuvant groups or control groups upon homologous rechallenge. Whether protection would be shown against heterologous strains of the parasite remains to be determined. It may be determined that a vaccine directed against the erythrocytic stage of the parasite may have its greatest efficacy and field application after rechallenge and that the *Aotus* monkey model system may be sufficient to test this.

As expected, immunization with FALVAC-1 combined with FCA/FIA resulted in serious adverse responses at the site of immunization. The appearance of dehydration, primarily after the second immunizations and primary parasitemia was baffling. Frequency of this condition had not been encountered previously in our vaccine trials. Only occasionally do monkeys in vaccine trials require dehydration. The exact cause of this has not been determined, but the fact that the condition was scattered across all the groups suggests some factor not associated with the recombiant product or adjuvant.

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