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SAFETY AND IMMUNOGENICITY OF A PLASMODIUM VIVAX SPOROZOITE VACCINE

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Abstract. A recombinant DNA Plasmodium vivax sporozoite vaccine containing the repeating region of the Salvador I strain circumsporozoite (CS) protein was produced in Escherichia coli. This vaccine was tested in 13 naive volunteers at doses of 10–1,000 µg. No serious adverse reactions were noted. None of 4 volunteers receiving the 10 µg dose developed antibodies measurable by ELISA. Six of 9 volunteers in the other dose groups developed measurable antibodies: 5 of 5 volunteers receiving 100 µg and 1 of 4 receiving 1,000 µg. Antibody responses measured by immunofluorescence assays paralleled those seen by ELISA. None of the volunteers developed antisera that inhibited sporozoite invasion of human hepatoma cells in vitro. Lack of a classical anamnestic response and lack of a typical dose response to increasing amounts of antigen suggests the possible presence of an immunosuppressive epitope in the repetitive region of the CS protein.

The immunodominant protein covering the surface of malaria sporozoites, the circumsporozoite (CS) protein, continues to be a major target in the development of an effective antigenic protection because it may provide T cell epitopes important in the induction of cell mediated immunity. The NS1, V20 fusion protein, which is expressed at high levels and readily purified, has been shown to induce significant antibody responses in New Zealand White rabbits (G. Wasserman, personal communication) and Saimiri sciureus bolivensis.

Studies in our laboratory with a related fusion protein, R32NS1, (composed of 32 tetrapeptide repeats from the P. falciparum repeat region expressed as a fusion protein with NS1, (81 amino acids from the N-terminus of the nonstructural protein NS1 of influenza A virus). NS1, was chosen as part of the fusion protein with the P. vivax repeat region because it may provide T cell epitopes important in the induction of cell mediated immunity. The NS1, V20 fusion protein, which is expressed at high levels and readily purified, has been shown to induce significant antibody responses in New Zealand White rabbits (G. Wasserman, personal communication) and Saimiri sciureus bolivensis.

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and women, ages 18–50 years, underwent a medical history (with special attention to a previous history of malaria or splenectomy), physical examination, and routine standard laboratory tests, including complete blood count, serum biochemistries (serum creatinine, blood urea nitrogen, bilirubin, alanine aminotransferase, and aspartate aminotransferase), urinalysis, serum β-HCG for females, hepatitis B surface antigen, serologic test for HIV infection, 2 malaria antibody tests, sporozoite antibodies as measured by ELISA, and blood stage antibodies measured by an indirect fluorescent antibody (IFA) test using red blood cells infected with *P. cynomolgi* as antigen. All tests were performed within 2 weeks before immunization. Subjects were excluded from participation if they had been in a malarious area within 1 year of the start of the study, if they had previously had vivax malaria as determined by history, if the malarial antibody tests were positive, if they had had a splenectomy, or if they had had any cardiovascular, hepatic, renal, or immunological illness, or were taking any immunosuppressive medications. Thirteen volunteers, 7 males and 6 females, meeting the above criteria gave informed consent and entered this study.

Vaccine preparation

NS1*V 20* is a 26,692 dalton, single chain polypeptide of 265 amino acids with the following sequence:

```
MDPNTVSSFQVDCLVLWRKR
VADQELGDAPF
LDRLRDQKSLGRGSTLGL
DIETATRAGKQ
IVERILKEESDEALKMTMQIP
(GDRADGQPA)  (GDRAAGQPA) 
(GDRADGQPA)  (GDRAAGQPA) 
(GDRADGQPA)  (GDRAAGQPA) 
(GDRADGQPA)  (GDRAAGQPA) 
(GDRADGQPA)  (GDRAAGQPA).
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NS1*V 20* was produced by inserting a *Bst*NI-*MnII* restriction enzyme fragment encoding the repeat domain of the *P. vivax* Salvador I (Sal-1) strain, CS protein coding sequence into a vector which expresses 81 amino acids of the NS1 non-structural protein of A/PR/8/34 (H1N1) influenza virus, creating the plasmid pNS1*V 20*.

An *E. coli* expression strain, a derivative of the standard NIH *E. coli* K12 strain N99 (F‘sur-galK21acZ-thr–), was then transformed.

**Study subjects**

Volunteers were recruited under a protocol approved by The Army Surgeon General’s Human Subjects Research Review Board. Healthy men

![Graph A](image1.png)

**Figure 1.** Antibody response to PVSV-I as measured by ELISA. Results are reported in OD units, i.e., that serum dilution that would result in an absorbance of 1 at 414 nm in our standard ELISA. Doses of vaccine were administered at 0, 7, and 15 weeks.

![Graph B](image2.png)

![Graph C](image3.png)

R32NS1*V 20*, was safe, well tolerated, and able to induce partial protection against sporozoite challenge as manifested by a delay in the pre-patency period in individuals exposed to *P. falciparum* using a laboratory developed challenge model (J. E. Egan, WRAIR, personal communication).

Based on the above information, we considered NS1*V 20* to be a good candidate for a human *P. vivax* sporozoite vaccine. We now report the results of a study in human volunteers to evaluate the safety and immunogenicity of alum-adsorbed NS1*V 20*, referred to as *P. vivax* sporozoite vaccine 1 (PVSV-I).

**Materials and Methods**

**Study subjects**

Volunteers were recruited under a protocol approved by The Army Surgeon General’s Human Subjects Research Review Board. Healthy men
with pNS1\textsubscript{V}, V20 and used to produce the initial cell paste. Subsequently, crude NS1\textsubscript{V}, V20 was obtained, following the mechanical disruption of the cells, by a series of precipitation steps with polyethyleneimine, ammonium sulfate, and acidic pH conditions. Further purification of NS1\textsubscript{V}, V20 consisted of ion exchange chromatography on sulfoethyl-Sepharose, size exclusion chromatography on superose 12, and reversed-phase high performance liquid chromatography on Vydac C-4 packing. Heterogeneity of the final product was confirmed by immunoblot analysis with antisera directed against NS1 and a Mab specific for the \textit{P. vivax} repeat region, analytical size exclusion, and RP-HPLC.

NS1\textsubscript{V}, V20 was supplied by SmithKline Beecham Pharmaceuticals, Swedeland, PA, as a 4-vial multi-pack designated \textit{P. vivax} sporozoite vaccine 1 (PVSV-1). Vial no. 1 contained 0.4 ml of the NS1\textsubscript{V}, V20 antigen solution at 7 mg/ml in a 50 mM acetate buffer, pH 5.5. Vials nos. 2A and 2B contained the diluent (50 mM acetate buffer, pH 5.5) supplied in 2 sizes, 1.8 ml/vial (no. 2A) and 19.8 ml/vial (no. 2B). Vial no. 3 contained the adjuvant suspension consisting of 0.5 ml of 4.4 mg/ml aluminum oxide (2.2 mg/ml Al\textsuperscript{3+}) in 50 mM acetate buffer, pH 5.5, with 0.005% w/v thimerosal as a bactericide. The system is such that a 0.5 ml dose containing either 10, 100, or 1,000 \textmu{}g of NS1\textsubscript{V}, V20 and 2.2 mg of aluminum oxide could easily be made just prior to administration. All components of PVSV-1 were stored at 4\textdegree{}C prior to use.

\textbf{Immunization schedule}

Volunteers were randomly assigned to 1 of 3 groups and received either 10, 100, or 1,000 \textmu{}g of NS1\textsubscript{V}, V20 as PVSV-1 im at week 0, with identical booster doses being given at 7 and 15 weeks. Volunteers were observed for 20 min after each dose for immediate reactions and evaluated at 24 and 48 hr for symptoms of headache, fever, chills, malaise, local pain, fever, erythema, warmth, induration, lymphadenopathy, and other complaints. Prior to and again 2 days after each dose of vaccine, each volunteer had blood and urine collected for evaluation. Serum samples were obtained each week for 3 weeks after each dose. Whole blood was allowed to clot at room temperature, serum was separated and stored at -70\textdegree{}C until analyzed.

\textbf{Serological assays}

CS antibodies were measured using a standard ELISA technique\textsuperscript{12,13} except that a recombinant protein, referred to as VIVAX-1, which contained the entire repeat region of \textit{P. vivax} flanked by the 15 amino acids amino terminal and the 48 amino acids carboxy-terminal of the repeats, was used as the capture antigen.\textsuperscript{14} This molecule does not contain the NS1\textsubscript{V}, segment present in PVSV-1. Horseradish-peroxidase conjugated rabbit anti-human IgG (gamma chain specific) was used as the secondary antibody. Assays were run in triplicate and the mean absorbance and standard deviation was calculated for each dilution.

Immunofluorescence assays were performed as previously described.\textsuperscript{12} Hand dissected salivary-gland sporozoites from the Sal-1 strain of \textit{P. vivax} were suspended in Medium 199, containing 0.01% (v/v) bovine serum albumin, dotted onto multi-well immunofluorescence assay slides at 10,000 sporozoites/dot, air-dried at room temperature, and stored at -70\textdegree{}C until used. Antibodies reactive to sporozoites were detected with fluorescein-labeled anti-human IgG under ultraviolet light at 500 x magnification. Fluorescence was graded from 0 to +4, where 0 indicates no fluorescence detectable and +4 indicates intense fluorescence over the entire surface of the sporozoite.

Percent inhibition of sporozoite invasion of HepG2A-16 human hepatoma cells in vitro was calculated according to Hollingdale.\textsuperscript{15}

\textbf{RESULTS}

The 13 individuals (mean age 35 years, range 22-44 years), were randomly divided into 3 groups to receive PVSV-1. The vaccine was well-tolerated by all. The major side effect noted was minor discomfort at the injection site in all volunteers, which resolved within 24 hr. Two individuals in the 1,000 \textmu{}g dose group noted the onset of malaise and generalized myalgias 6-9 hr after the administration of the second dose of vaccine. These symptoms resolved after 48 hr. One of these individuals continued in the study, receiving the third dose of vaccine as scheduled, and experienced no untoward reaction, suggesting that the previous reaction was not causally related to the vaccine. There were no abnormalities in blood chemistry, hematology profiles,
TABLE 1

<table>
<thead>
<tr>
<th>Volunteer no.</th>
<th>Week 2</th>
<th>Week 9</th>
<th>Week 17</th>
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<tr>
<td>6</td>
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Results represent the highest serum dilution which produced a 2+ IFA reaction.

DISCUSSION

Mabs directed against the repeat region of CS proteins can passively transfer protection against sporozoite challenge. This fact provides compelling rationale for the development of anti-spore vaccine design to induce high levels of anti-CS antibody. Early studies with P. falciparum subunit vaccines supported this approach, although the degree of protection achieved was suboptimal.6,7 In an analogous manner, we have developed a recombinant P. vivax subunit vaccine designed to induce anti-

or urinalysis attributable to vaccine administration.

Six of the 9 volunteers who received either the 100 or 1,000 µg dose of PVSV-1 developed antibodies to the vivax repeat epitopes as measured by ELISA (Fig. 1). Four of the individuals receiving the 100 µg dose had an initial response after the first dose of vaccine, but failed to demonstrate any boosting with subsequent doses. Volunteer no. 8 demonstrated a minor response to the first dose of vaccine, which returned to baseline by week 4. This volunteer demonstrated a response after the second dose of vaccine, but not after the third dose of vaccine. Only 1 individual (no. 14) in the 1,000 µg dose group seroconverted. This volunteer showed an initial response to the vivax epitope, but antibody levels failed to rise after the second dose of vaccine. She withdrew from the study because of malaise and generalized myalgias after the second dose of vaccine.

Sera from week nine from volunteers in groups A and C were negative by IFA, while the IFA results from volunteers in Group B paralleled those measured by ELISA (Table 1). None of the volunteers developed antiserum capable of inhibiting sporozoite invasion of HepG2-A1G cells in vitro.

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