Partial Protection against *Plasmodium vivax* Blood-Stage Infection in *Saimiri* Monkeys by Immunization with a Recombinant C-Terminal Fragment of Merozoite Surface Protein 1 in Block Copolymer Adjuvant

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Received 26 May 1998/Returned for modification 24 July 1998/Accepted 1 October 1998

Merozoite surface protein 1 is a candidate for blood-stage vaccines against malaria parasites. We report here an immunization study of *Saimiri* monkeys with a yeast-expressed recombinant protein containing the C terminus of *Plasmodium vivax* merozoite surface protein 1 and two T-helper epitopes of tetanus toxin (YP30Pv20019), formulated in aluminum hydroxide (alum) and block copolymer P1005. Monkeys immunized three times with YP30Pv20019 in block copolymer P1005 had significantly higher prechallenge titers of immunoglobulin G (IgG) antibodies against the immunogen and asexual blood-stage parasites than those immunized with YP30Pv20019 in alum, antigen alone, or phosphate-buffered saline (PBS) (*P* < 0.05). Their peripheral blood mononuclear cell proliferative responses to immunogen stimulation 4 weeks after the second immunization were also significantly higher than those from the PBS control group (*P* < 0.05). Upon challenge with 100,000 asexual blood-stage parasites 5 weeks after the last immunization, monkeys immunized with YP30Pv20019 in block copolymer P1005 had prepatent periods longer than those for the control alone group (*P* > 0.05). Three of the five animals in this group had low parasitemia (peak parasitemia, ≤20 parasites/μl of blood). Partially protected monkeys had significantly higher levels of prechallenge antibodies against the immunogen than those unprotected (*P* < 0.05). There was also a positive correlation between the prepatent period and titers of IgG antibodies against the immunogen and asexual blood-stage parasites and a negative correlation between accumulated parasitemia and titers of IgG antibodies against the immunogen (*P* < 0.05). These results indicate that when combined with block copolymer and potent T-helper epitopes, the yeast-expressed P30Pv20019 recombinant protein may offer some protection against malaria.

*Plasmodium vivax* is one of the most widely distributed human malaria parasites, prevalent in South America, Asia, and Oceania (27). With the appearance of resistance to current antimalarial drugs (9), an effective vaccine against the parasite is urgently needed. Several antigens expressed at different stages of the parasite life cycle have been characterized and found to have the potential for use in a subunit vaccine against *P. vivax* (2, 15, 32, 37). One of these antigens, merozoite surface protein 1 (MSP-1), is considered a leading candidate for a vaccine against *P. vivax* infections (2, 15, 32, 37). MSP-1 is a glycoprotein synthesized during schizogony and proteolytically processed into a complex of polypeptides (18). The C-terminal 19-kDa fragment derived from a second processing step remains on the merozoite surface during the invasion of a new erythrocyte. Two epidermal growth factor-like domains have been identified in the cysteine-rich region of the fragment (3, 4). Comparison of the MSP-1 amino acid sequences of two monkey-adapted *P. vivax* stains with those of *P. falciparum* MAD 20 and *P. yoelii* YM has revealed that the C-terminal 19-kDa fragment, especially the cysteine residues responsible for the formation of the two cysteine-rich repeats, is conserved among these plasmodial species. This finding indicates that the C-terminal 19-kDa fragment of MSP-1 may be involved in important biological functions during invasion.

In vitro and in vivo studies with *P. falciparum* (3, 7, 8, 10, 20, 26, 33) and *P. yoelii* (6, 11, 28, 30, 34, 42) have shown that immunoglobulin G (IgG) antibodies or monoclonal antibodies directed against the C-terminal 19-kDa fragment can inhibit the invasion of parasites into erythrocytes or protect mice or monkeys against live parasite challenges. Field studies have also shown that production of IgG antibodies against the 19-kDa fragment of *P. falciparum* correlates with the development of clinical immunity against falciparum malaria (17, 35, 36). Taken together, these findings suggest that the C-terminal 19-kDa fragment of MSP-1 is a vaccine candidate antigen against asexual blood-stages of malaria parasites.

Both humoral and cellular immune responses are necessary for an effective malaria vaccine against blood-stage parasites (29, 40). One way to influence the host immune responses to an antigen is by the use of adjuvants (1, 19, 23, 25, 45). Immunization studies with C-terminal fragments of *P. falciparum* MSP-1 in primate malaria models showed that no protection was induced when an *Escherichia coli*-expressed 19-kDa antigen was formulated in aluminum hydroxide (alum) and liposomes (5). However, protection was achieved when a baculovirus-expressed 42-kDa or yeast-expressed 19-kDa antigen
mixed with the Freund’s complete adjuvant (7, 26). A recent report has also shown that rhesus monkeys immunized with baculovirus-expressed 42- or 19-kDa antigen of *P. cynomolgi* MSP-1 in Freund’s complete or incomplete adjuvants were protected (32). Thus, adjuvants affect the efficacy of recombinant MSP-1 vaccines. Unfortunately, Freund’s adjuvant is too toxic for human use, and alum is currently the only approved human-usable adjuvant.

One adjuvant currently under development for use in humans is the nonionic block copolymer, which is a simple linear chain of the hydrophobic polyoxypropylene (22). Antigens bind to the hydrophobic surface of copolymers by hydrophobic and hydrogen bond interactions. Our studies with different formulations of nonionic block copolymers P1004 and P1005 with *Saimiri* monkeys have shown that they can modulate both humoral and cellular immune responses, resulting in different outcomes of challenge infections (21, 41, 46, 47).

In contrast to the intensive studies done on *P. falciparum* and *P. yoelii* MSP-1, little is known about *P. vivax* MSP-1. Our previous study of mice with a yeast-expressed 19-kDa antigen of *P. vivax* MSP-1 formulated in nonionic block copolymer P1005 showed that this formulation was highly immunogenic. Mice produced high antibody and proliferative responses comparable to those induced by using Freund’s complete adjuvant (46). In this study, we further evaluated the immunogenicity of this yeast-expressed *P. vivax* MSP-1 19-kDa fragment in *Saimiri* monkeys and assessed the protective effect of immunizations with this recombinant protein in the human-usable adjuvant and a potentially useful adjuvant block copolymer.

**MATERIALS AND METHODS**

**Antigen.** The antigen was a yeast-expressed recombinant protein, yP2P30Pv20019, consisting of the C terminus (amino acids Asn162 to Ser172) of the MSP-1 of *P. vivax* Sal1 (16). In addition, two universal T-helper epitopes (P2 and P6) of tetanus toxin (31, 44) and six histidine residues for purification purposes were attached to the N and C termini, respectively. The expression, purification, and characterization of this antigen are described in detail elsewhere (24). The purity of the recombinant protein was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). No aggregates were present in the recombinant antigen preparation used in this study.

**Adjuvants.** The two adjuvants used were the nonionic block copolymer P1005 (provided by Vascel Corp., Norcross, Ga.) and alum (Reheisort, Intergen, Purchase, N.Y.). For the nonionic block copolymer P1005, a water-in-oil emulsion was prepared by mixing a 20% oil phase (10% Span 80 in isopropyl) and a 80% aqueous phase (200 μg of yP2P30Pv20019, and 5 mg of P1005 in phosphate-buffered saline (PBS) per immunization) (39). For the adsorption of yP2P30Pv20019 onto aluminum hydroxide, the agarose-bound antigen was used. Briefly, acid washed Reheisort was mixed with yP2P30Pv20019 in a ratio of 1 μg of antigen to 2 μg of alum. The antigen-alum mixture was incubated for 1 h at room temperature. The adsorbed alum-antigen complex was then centrifuged and resuspended in PBS. The adsorption efficiency was monitored by measuring the remaining protein in the supernatant after centrifugation.

**Animals and immunization.** Twenty *Saimiri boliviensis boliviensis* monkeys of Bolivian origin (8 males and 12 females, average body weight of about 700 g) were used. Before the study began, the animals were quarantined and acclimated for 6 weeks. During this period, they were weighed, physically examined by a staff veterinarian, and temperature was monitored to ensure the development of parasitemia, starting at day 6 after the blood-stage parasite challenge and ending at day 42.

**Antibody assays.** (i) ELISA. To monitor humoral immune responses to immunizations, titers of antibodies against immunogen yP2P30Pv20019 were determined by a standard enzyme-linked immunosorbent assay (ELISA) procedure (47). Briefly, 96-well microtiter plates (Immobilon 2, Dynatech Laboratories, Inc., McLean, Va.) were coated with yP2P30Pv20019 (100 μl/well, 0.2 μg/ml in borate-buffered saline [167 mM boric acid, 134 mM NaCl (pH 8.0)] at 4°C overnight. The plates were washed with PBS containing 0.05% Tween 20 (PBS-T), blocked with 5% fat-free dry milk in borate-buffered saline for 1 h at room temperature, and rinsed again with PBS-T to remove unbound recombinant proteins. Serial dilutions of serum samples were added to the wells in triplicate, starting with a 1:100 dilution followed by twofold dilutions. After incubation at room temperature for 1 h, plates were washed three times with PBS-T plus 0.1% Tween 20 and then incubated with peroxidase-conjugated goat anti-*Saimiri* IgG for 1 h at 37°C. The bound antibodies were detected by incubation of wells with 100 μl of tetramethylbenzidine-peroxidase substrate (Kirkgaard & Perry Laboratories, Gaithersburg, Md.). The reaction was stopped by the addition of 50 μl of 1 M H2PO4 and read at 450 nm with a microplate reader. Titers were based on the highest dilution of the sample that generated an optical density greater than the mean of preimmunization sera plus 2 standard deviations (cut-off optical density = 0.805).

(ii) Protection against the blood-stage parasites. One monkey each from the controls and received PBS. Immunization consisted of three subcutaneous immunizations, 50- or 100-μl doses of the immunization formulations were injected into four sites on the back of each animal. The code for antigen administration was revealed only after the completion of parasitemia determination and cellular and humoral immune response analysis.

**Assay for blood-stage parasite challenge.** Five weeks after the third immunization (week 0), each of the animals was intravenously challenged with 100,000 *P. vivax* Sal1 axenic blood-stage parasites from a infected donor *Saimiri* monkey. Seven or eight days following the blood-stage parasite challenge, monkeys were sacrificed to allow higher parasitemia. Animals were monitored for the development of parasitemia, starting at day 6 after the blood-stage parasite challenge and ending at day 42.

**RESULTS**

**Prepatent period and parasitemia.** Monkey were immunized three times at 4-week intervals with yP2P30Pv20019 recombinant protein in two different adjuvants: nonionic block copolymer P1005 and alum. No adverse reactions to the immunizations were detected in monkeys immunized with yP2P30Pv20019 or yP2P30Pv20019 in alum during the experimental period. Animals immunized with yP2P30Pv20019 in P1005, however, developed skin sores at the injection sites after the second immunization. One monkey each from the
alum and PBS control groups died from causes unrelated to the study after the second and third immunizations, respectively. All remaining animals were challenged intravenously with 100,000 *P. vivax* Sal I asexual blood-stage parasites. Parasitemia developed in all four animals in the PBS control group (Fig. 1). Of the four animals, three had high parasitemia (accumulated parasite counts of 54,587, 68,536, and 185,916 parasites/μl) and one had a low parasite count (100 parasites/μl) (Table 1). Three of the five animals immunized with yP2P30Pv20019 in block copolymer P1005 developed low parasitemia (25, 30, and 120 Parasites/μl) and thus were considered to be partially protected; the other two monkeys developed a high parasite counts (93,399 and 291,391 parasites/μl). Among the animals immunized with yP2P30Pv20019 in alum or antigen alone, one animal from each group had a low parasite count (<2,000 parasites/μl), and the remaining animals all developed moderate to high parasitemia (11,703 to 485,947 parasites/μl). In addition, monkeys immunized with yP2P30Pv20019 in block copolymer P1005 had slightly longer prepatent periods than control monkeys (geometric mean, 12.3 days versus 9.9 days; *P* >0.05) Table 1.

**IgG antibodies against the yP2P30Pv20019 recombinant protein.** Levels of total IgG antibodies against the immunogen yP2P30Pv20019 were measured by ELISA at 2-week intervals, starting 2 weeks after the first immunization (~11 weeks) until the end of the study (6 weeks). Monkeys from the PBS control group had no detectable antibodies after the third immunization (Fig. 2). Animals immunized with the yP2P30Pv20019 alone also had no detectable antibodies after the first immunization. Although antibody titers in monkeys immunized with yP2P30Pv20019 in block copolymer P1005 or in alum were lower after the first immunization, monkeys immunized with block copolymer P1005 as adjuvant had antibody titers significantly higher than those from all of the three other groups (*P* < 0.05). Antibody titers increased in animals immunized with yP2P30Pv20019 in block copolymer P1005 or in alum after the second immunization and reached the maximal levels after the third immunization (*P* < 0.05 between the P1005 or alum group and the antigen-alone or PBS group).

Challenge of monkeys with 100,000 asexual blood stage-parasites failed to further boost the titers of antibodies against the immunogen in monkeys immunized with yP2P30Pv20019 in block copolymer P1005 or in alum, although antibody levels 4 weeks after the challenge were higher than at challenge (week 0). Titers of antibodies against the immunogen from animals immunized with yP2P30Pv20019 alone or PBS were moderately increased after the challenge and reached higher levels 6 weeks after the challenge. However, antibody levels from animals immunized with yP2P30Pv20019 in block copolymer P1005 or alum were still significantly higher than in those immunized with antigen alone or PBS at challenge and at 2, 4, and 6 weeks after the challenge (*P* < 0.05).

**IgG antibodies against asexual blood-stage parasites.** Titers of IgG antibodies against air-dried *P. vivax* asexual blood-stage parasites were determined by IFA 1 week after the third immunization, at challenge, and 2 and 4 weeks after the challenge (Fig. 3). One week after the third immunization or at challenge (5 weeks after the third immunization), animals immunized with yP2P30Pv20019 in block copolymer P1005 or in alum had higher antibody levels than those immunized with antigen alone or PBS. The difference between the block copolymer P1005 group and the three other groups was significant (*P* < 0.05). Challenge with 100,000 *P. vivax* asexual blood-stage parasites boosted antibody titers in all groups, with the block copolymer P1005 group having the highest titers. The difference between the block copolymer P1005 or alum group and

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**FIG. 1.** Parasitemia (parasites per microliter) of control monkeys (PBS) and monkeys immunized with yP2P30Pv20019 (Ag [antigen] only), yP2P30Pv20019 in block copolymer (Ag + P1005), or yP2P30Pv20019 in alum (Ag + Alum) three times at 4-week intervals (weeks −13, −9, and −5) and challenged at week 0.
the antigen-alone or PBS group was significant \((P < 0.05)\). This boosting in IFA titers after parasite challenge might be due to specific antibodies or antibodies of other parasite specificities.

**PBMC proliferative responses to the yP\(_{30}\)P\(_{200}\) recombinant protein.** To evaluate T-cell responses in the yP\(_{30}\)P\(_{200}\) recombinant protein, PBMC proliferation assays were performed with monkey PBMCs collected at preimmunization, 4 weeks after the first and second immunization, and 4 weeks after the asexual blood-stage parasite challenge. As a control, PBMCs were simultaneously stimulated with the mitogen PHA. PBMCs from all monkeys responded to PHA stimulation at SI values ranging from 5 to 45, with unstimulated PBMCs having around 2,500 cpm (data not shown). All monkeys (except one from the antigen-alone group) had no proliferative responses to yP\(_{30}\)P\(_{200}\) \((\text{SI values ranges from 0.62 to 1.80} ; P > 0.05 \text{ among all groups})\) at preimmunization (Fig. 4A). Four weeks after the first immunization, PBMC proliferative responses yP\(_{30}\)P\(_{200}\) started to increase in monkeys immunized with yP\(_{30}\)P\(_{200}\) in block copolymer P1005 or alum compared with those in the antigen-alone or PBS group. The difference in SI values between the block copolymer P1005 group and alum, antigen alone, or PBS control group significantly \((P < 0.05)\) (Fig. 4B). The SI values of monkeys immunized with yP\(_{30}\)P\(_{200}\) in P1005 or alum continued to increase 4 weeks after the second immunization, with the block copolymer P1005 group having the highest proliferative responses \((\text{SI ranging from 4.0 to 11.47})\) (Fig. 4C and Table 1). The difference between the block copolymer P1005 group and the other three groups was significant \((P < 0.05)\). Challenge of monkeys with 100,000 asexual blood-stage parasites failed to boost PBMC proliferative responses to the immunogen (Fig. 4D). Although the P1005 and alum groups had significantly higher SI values than the control group, the proliferative response in the P1005 group was actually lower than before the challenge.

**Splenocyte proliferative responses to the yP\(_{30}\)P\(_{200}\) recombinant protein.** Splenocyte proliferative responses to the immunogen were also conducted 1 week after the asexual blood-stage parasite challenge. Splenocytes from PBS control monkeys had no responses to the stimulation of yP\(_{30}\)P\(_{200}\) recombinant protein \((\text{mean SI of 0.55 to 1.07, with counts per})\). **TABLE 1.** Prepatent periods, peak parasitemia, accumulate parasitemia, PBMC proliferative responses, and titers of IgG antibodies against the immunogen and blood-stage parasites in *Saimiri* monkeys after immunization with three doses of yP\(_{30}\)P\(_{200}\) and challenged 5 weeks after the last immunization with 100,000 *P. vivax* asexual blood-stage parasites

<table>
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<th>Immunogen</th>
<th>Monkey</th>
<th>Prepatent period (day)</th>
<th>Parasitemia (parasites/μl of blood) peak</th>
<th>SI (^{a})</th>
<th>Antibody titer at challenge</th>
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<td></td>
<td></td>
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<td>ELISA</td>
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\(^a\) Sum of parasitemia for 42 days.

\(^b\) Determined 4 weeks after the second immunization. ND, not done.

![FIG. 2.](http://iai.asm.org/) Levels of IgG antibodies against the yP\(_{30}\)P\(_{200}\) recombinant protein in sera from control monkeys (PBS) and monkeys immunized with yP\(_{30}\)P\(_{200}\) (Ag [antigen] only), yP\(_{30}\)P\(_{200}\) in block copolymer (P1005), or yP\(_{30}\)P\(_{200}\) in alum (Alum) three times at 4-week intervals (weeks \(-13, -9, \text{ and } -5\)) and challenged at week 0. Group geometric means are shown.
minute ranging from 769 to 1,439) (Fig. 5). In contrast, splenocytes from monkeys immunized with yP2P30Pv20019 in P1005 or alum responded to the immunogen stimulation with average SI values of 4.19 and 4.38, respectively. The difference between the block copolymer or alum groups and the PBS control group was significant ($P < 0.05$).

**Association of partial protection with IgG antibody production.** To assess the role of IgG antibodies in protection, the association between partial protection and IgG antibody levels measured by ELISA and IFA was analyzed. Monkeys (SI-1024, -2126, -2053, -1021, -1069, and -2142) partially protected (with peak parasitemia lower than 1,000/m$^3$) from the asexual blood-stage parasite challenge had significantly higher prechallenge IgG antibody levels than those unprotected when IgG antibody titers were determined by ELISA (geometric mean of 147,327.7 versus 2,775.1; $P < 0.05$). Likewise, there was also a significant positive correlation between the prepatent period and prechallenge IgG antibody levels by both ELISA and IFA ($r = 0.64$ and 0.63, respectively; $P < 0.05$) (Fig. 6) and a significant negative correlation between accumulated parasitemia and prechallenge IgG antibody titers ($r = -0.59$ for ELISA and $-0.48$ for IFA; $P < 0.05$).

**DISCUSSION**

We have assessed the immunogenicity of a yeast-expressed recombinant protein containing the C-terminal 19-kDa fragment of *P. vivax* MSP-1 and two T-helper epitopes of tetanus.

![FIG. 3. Levels of IgG antibodies against asexual blood-stage parasite antigens in sera from control monkeys (PBS) and monkeys immunized yP2P30Pv20019 (Ag[antigen]), yP2P30Pv20019 in block copolymer (P1005), or yP2P30Pv20019 in alum (Alum). Monkey sera were tested by IFA for total IgG antibodies against air-dried *P. vivax* blood-stage-infected *Saimiri* monkey erythrocytes 1 week after the third immunization (■), on the day of challenge (□), and 2 (△) and 4 (■) weeks after challenge. Group geometric means are shown.](http://iai.asm.org/)

![FIG. 4. Proliferative responses (SI) of PMBCs) from control monkeys (PBS) and monkeys immunized with yP2P30Pv20019 (Ag [antigen]), yP2P30Pv20019 in block copolymer (P1005), or yP2P30Pv20019 in alum (Alum) after stimulation with 2 μg of yP2P30Pv20019/well: (A) preimmunization; (B and C) 4 weeks after the first and second immunizations; (D) 4 weeks after asexual blood-stage parasite challenge. Individual SI values are shown. Asterisks indicate that the group mean is significantly different from that of the immunized control monkeys ($P < 0.05$).](http://iai.asm.org/)
toxin and have evaluated this recombinant protein formulated in human usable and potentially human-usable adjuvants, alum and block copolymer P1005, for the ability to induce protective immune response in a nonhuman primate malaria model system. Our results showed that Saimiri monkeys immunized three times with the C-terminal 19-kDa recombinant protein formulated in block copolymer P1005 produced titers of IgG antibody response against both the immunogen and asexual blood-stage parasite antigens higher than those immunized with the C-terminal 19-kDa antigen in alum or antigen alone. These monkeys also had significantly higher PBMC proliferative response than PBS control animals when stimulated with the immunogen 4 weeks after the second immunization. Upon challenge with 100,000 asexual blood-stage parasites, monkeys immunized with the C-terminal 19-kDa recombinant antigen in block copolymer P1005 had somewhat longer prepatent periods than control monkeys. Three of the five monkeys in this group developed very low blood-stage parasite infections and thus were partially protected.

Both humoral and cellular immune responses were induced by vaccination with the recombinant 19-kDa protein. Monkeys immunized with the recombinant antigen in P1005 or alum had high titers against the immunogen as well as proliferative responses. Antibody production and proliferative responses were the highest in animals immunized with the 19-kDa antigen in P1005, probably as a result of the potent stimulatory effect of P1005 to both humoral and cellular immunity (43). The protective immunity induced by vaccination, nevertheless, appears to be mediated in part by antibodies. Monkeys with higher IgG antibody titers at the challenge had longer prepatent periods and lower peak and accumulated parasite counts than those with lower IgG antibody titers. Studies with rodent malaria P. yoelii also showed that protection against blood-stage infection after immunization of mice with the C-terminal domain of P. yoelii MSP-1 was partially mediated by antibodies since passive transfer of immune sera or purified IgG protected some naive mice against lethal disease (28). Factors other than antibodies probably also played a role in protection against malaria, because one partially protected monkey (SI-1069) had not titers of antimalaria antibodies.

Adjuvants appear to play a role in protective immunity induced by MSP-1. Previous studies with the C-terminal 42- or 19-kDa fragment of P. falciparum and P. cynomolgi MSP-1 showed that monkeys immunized with the C-terminal fragment were partially protected against blood-stage parasite challenge when the immunogen was formulated in Freund’s complete adjuvant (7, 26, 32). However, when liposome and alum were used as adjuvants, monkeys were not protected against blood-stage parasite challenge (5). In agreement with previous studies (5, 12), results of this study with the 19-kDa fragment of P. vivax showed that alum, the only approved human-usable adjuvant, failed to induce a protective immune response against blood-stage parasite challenge following immunizations, al-

FIG. 5. Proliferative responses (SI) to yP3P19Pv200p of splenocytes from control monkeys (PBS) and monkeys immunized with yP3P19Pv200p (Ag [antigen]), yP3P19Pv200p in block copolymer (P1005), or yP3P19Pv200p in alum (Alum) 1 week after blood-stage parasite challenge. Individual SI values are shown. Asterisks indicate that the group mean is significantly different from that of the unimmunized control monkeys (P < 0.05).

FIG. 6. Correlation between titers of prechallenge IgG antibodies against the immunogen (A) and blood-stage parasite (B) and the prepatent period in monkeys immunized with yP3P19Pv200p.
though it induced moderate levels of IgG antibodies. In contrast, when the potentially human-useful adjuvant block copolymer P1005 was used as adjuvant, the recombinant 19-kDa MSP-1 antigen induced a protective immune response in a nonhuman primate malaria model. Our previous studies showed that mice immunized with the C terminus of \textit{P. vivax} MSP-1 antigen in Freund's adjuvant. This difference was obtained by immunization with baculovirus-expressed MSP-1 antigen (32). In the latter, close to complete protection was shown that mice immunized with the C terminus of MSP-1 antigen of \textit{P. vivax} kilodalton MSP-1 antigen of \textit{Saimiri} monkeys observed in this study is lower than that recently achieved by immunization with baculovirus-expressed \textit{P. cynomolgi} MSP-1 antigens in Freund's adjuvant. This difference is likely the result of differences in model systems and/or adjuvants. Development of immunity against \textit{P. cynomolgi} in toque monkeys is rapid; thus, animals recovered from primary infection appear refractory to subsequent infections. In contrast, development of immunity against \textit{P. vivax} in \textit{Saimiri} monkeys is a gradual process; animals that recover from primary infection are not protected against subsequent infections.

Further investigations, however, are needed to validate the efficacy of this vaccine. These studies should include adjuvant controls as well as large number of animals per group to minimize the effect of variations in parasitemia and the prepatent period. Subsequent studies should also investigate the protective efficacy of this vaccine in intact rather splec- torized animals. Notwithstanding these issues, results of this preliminary study suggest that the C terminus of MSP-1 is highly immunogenic and may offer some protection against \textit{P. vivax} blood-stage parasites.

**ACKNOWLEDGMENTS**


