Passive Immunization with Antibodies against Three Distinct Epitopes on Plasmodium yoelii Merozoite Surface Protein 1 Suppresses Parasitemia

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We have produced monoclonal antibodies against Plasmodium yoelii merozoite surface protein 1 (MSP-1) and have assessed their ability to suppress blood stage parasitemia by passive immunization. Six immunoglobulin G antibodies were characterized in detail: three (B6, D3, and F5) were effective in suppressing a lethal blood stage challenge infection, two (B10 and G3) were partially effective, and one (B4) was ineffective. MSP-1 is the precursor to a complex of polypeptides on the merozoite surface; all of the antibodies bound to this precursor and to an ~42-kDa fragment (MSP-142) that is derived from the C terminus of MSP-1. MSP-142 is further cleaved to an N-terminal ~33-kDa polypeptide (MSP-133) and a C-terminal ~19-kDa polypeptide (MSP-119) comprised of two epidermal growth factor (EGF)-like modules. D3 reacted with MSP-142, but not with either of the constituents MSP-133 and MSP-119, B4 recognized an epitope within the N terminus of MSP-133, and B6, B10, F5, and G3 bound to MSP-119, B10 and G3 bound to epitopes that required both C-terminal EGF-like modules for their formation, whereas B6 and F5 bound to epitopes in the first EGF-like module. These results indicate that at least three distinct epitopes on P. yoelii MSP-1 are recognized by antibodies that suppress parasitemia in vivo.

Malaria is a disease caused by parasites of the genus Plasmodium, which have a complex life cycle with several distinct stages in vertebrates. A number of antigens that may be targets of protective host immune responses have been identified. One attractive target is a soluble recombinant protein synthesized as a precursor during schizogony and then expressed in Escherichia coli as glutathione S-transferase (GST) fusion proteins (23, 24) and purified by binding to glutathione-agarose (30). Recombinant MSP-119 (rMSP-119) was produced by cleavage of GST-MSP-119 with the protease factor Xa (Boehringer) and separated from GST by gel filtration (24). Three recombinant GST fusion proteins, corresponding to most of the sequence of MSP-133 and to the N- and C-terminal parts, were produced. The oligonucleotides 5′-CTAGGATCTGCACAAAAAAAGTTGGTGAAG (primer 1), 5′-CCAGATATTATATTTCGAGTTGTG (primer 2), 5′-CTAGGATCTGCACAAAAAAAGTTGGTGAAG (primer 3), and 5′-CCAGATATTATATTTCGAGTTGTG (primer 4) were used to amplify DNA by PCR from the clone PyM4.3 (kindly provided by Alan Lewis), corresponding to amino acid residues Thr1415 to Lys1513 (primers 1 and 2; PyMSP1 33B), Ala1527 to His1619 (primers 3 and 4; PyMSP1 33C), and Thr1415 to His1619 (primers 1 and 4; PyMSP1 33A). The DNA was restricted with BamHI and EcoRI (sites in the primers above are underlined) and then cloned into the corresponding sites of the plasmid pGEXXX. The fusion proteins were purified as described above.

MATERIALS AND METHODS

Expression and purification of recombinant proteins. Both the C-terminal fragment of MSP-1 containing both EGF-like modules (MSP-119 residues 1640 to 1754 in the amino acid sequence [22]) and the two individual modules were expressed in Escherichia coli as glutathione S-transferase (GST) fusion proteins (23, 24) and purified by binding to glutathione-agarose (30). Recombinant MSP-119 (rMSP-119) was produced by cleavage of GST-MSP-119 with the protease factor Xa (Boehringer) and separated from GST by gel filtration (24). Three recombinant GST fusion proteins, corresponding to most of the sequence of MSP-133 and to the N- and C-terminal parts, were produced. The oligonucleotides 5′-CTAGGATCTGCACAAAAAAAGTTGGTGAAG (primer 1), 5′-CCAGATATTATATTTCGAGTTGTG (primer 2), 5′-CTAGGATCTGCACAAAAAAAGTTGGTGAAG (primer 3), and 5′-CCAGATATTATATTTCGAGTTGTG (primer 4) were used to amplify DNA by PCR from the clone PyM4.3 (kindly provided by Alan Lewis), corresponding to amino acid residues Thr1415 to Lys1513 (primers 1 and 2; PyMSP1 33B), Ala1527 to His1619 (primers 3 and 4; PyMSP1 33C), and Thr1415 to His1619 (primers 1 and 4; PyMSP1 33A). The DNA was restricted with BamHI and EcoRI (sites in the primers above are underlined) and then cloned into the corresponding sites of the plasmid pGEXXX. The fusion proteins were purified as described above.

Production and characterization of Mabs. Female BALB/c mice were immunized intraperitoneally with the recombinant protein GST-MSP-119 by using Freund’s adjuvant (23). Alternatively, the mice were immunized by injection following passive immunization with an inhibitory Mab derived from the first fusion: mice that had been challenged with 5 × 10⁷ parasites after passive immunization with MAb B10 (see below) were inoculated with a further 5 × 10⁷ parasitized erythrocytes on three occasions. Spleen cells were fused with SP2/0-Ag14 mouse myeloma cells in the presence of 50% polyethylene glycol, using 8 × 10⁶ spleen cells and 2 × 10⁶ myeloma cells. The cells were dispensed into 96-well culture plates in 100 μl of hypoxanthine-aminopterin-thymidine selective medium. After 10 days, the supernatants were tested for specific antibody by indirect immunofluorescence on acetone-fixed preparations of erythrocytes infected with P. yoelii YM and by enzyme-linked immunosorbent assay (ELISA) with rMSP-119. The hybridomas producing antibody considered positive in one or both of the assays were cloned by three rounds of limiting dilution in hypoxanthine-thymidine medium, and selected hybridomas were expanded by growth in RPMI 1640 medium containing 10% (vol/vol) fetal calf serum.

The antibodies secreted by the hybridomas B4, B6, B10, D3, F5, and G3 were affinity purified from culture supernatant on columns of protein G-Sepharose 4 Fast Flow (Pharmacia) (1) according to the manufacturer’s recommendations and purity was monitored by sodium dodecyl sulfate-polyacrylamide gel electro-
phoresis (SDS-PAGE). Antibody isotypes were determined by double diffusion and capture ELISA with a kit (Sigma) according to the manufacturer's recommendations. Antibody titers were determined by indirect immunofluorescence and by ELISA with rMSP-119, using twofold serial dilutions of antibody, essentially as described previously (24).

Passive immunization and parasite challenge. Female BALB/c mice from 8 weeks of age and bred under specific-pathogen-free conditions were used in groups of 10 animals. The purified MAbs (a total of 1.5 mg/mouse) were administered by intraperitoneal injection on three occasions, i.e., 1 day before, 1 day after and on the day of challenge infection. The parasite used for the challenge was the lethal YM strain of *P. yoelii*; the parasite stock was stored at −195°C and passaged once before use in the experiment. The challenge was administered by intravenous injection into the lateral tail vein with 5 × 10^3 parasitized erythrocytes per mouse, at least 1 h after administration of the MAb. Parasitemia was assessed daily on smears made from tail blood and stained with Giemsa's reagent.

Immunoprecipitation, immunoblotting, and immunofluorescence. Blood was harvested from *P. yoelii*-infected mice, passed through a cellulose powder (CF-11) column to eliminate the leukocytes, and eluted with Krebs glucose saline. The parasites were then washed with RPMI 1640 medium (methionine and cysteine free) containing 2 mM glutamine. Trophozoites and schizonts were isolated by centrifugation through a Percoll gradient (60 to 80%) and metabolically radiolabelled with 5 MBq of Tran35S-label (70% L-[35S]methionine, 15% L-[35S]cysteine) (ICN) per ml at 37°C for 2 to 3 h. The preparation of detergent extract and the immunoprecipitation and analysis of labelled polypeptides by SDS-PAGE and fluorography were carried out essentially as described previously (24). For immunoblotting, recombinant proteins or parasite extract was denatured in SDS sample buffer, fractionated by SDS-PAGE, and electrophoretically transferred to nitrocellulose (Schleicher and Schuell; 0.2-μm pore size). The detection of proteins on the blot with specific antibody was carried out as described previously (24). MAb 25.1 (18), normal mouse serum, and a polyclonal antiserum raised by immunization with GST–MSP-119 (24) were used as controls.

For immunofluorescence studies, erythrocytes infected with *P. yoelii* YM were

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### TABLE 1. Properties of MSP-1-specific MAbs

<table>
<thead>
<tr>
<th>MAb</th>
<th>Subclass</th>
<th>Reaction with MSP-1</th>
<th>Binding to recombinant GST fusion proteina</th>
<th>MSP1EGF1</th>
<th>MSP1EGF2</th>
<th>MSP-119</th>
<th>MSP-133</th>
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<tr>
<td>B4</td>
<td>IgG1</td>
<td>+</td>
<td>+</td>
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<tr>
<td>B6</td>
<td>IgG3</td>
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<td>B10</td>
<td>IgG2b</td>
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a The reaction with MSP-1 was determined by immunofluorescence, immunoprecipitation, and Western blotting.

b The binding of the MAbs to the different recombinant proteins was determined by Western blotting and ELISA. MSP1EGF1 is the first EGF-like module of *P. yoelii* MSP-119, MSP1EGF2 is the second EGF-like module of MSP-119, MSP-119 is the two combined EGF-like modules, and MSP-133 is the N-terminal part of MSP-122; all of the recombinant proteins were expressed as GST fusion proteins in *E. coli*.

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FIG. 1. Course of *P. yoelii* YM infection in groups of 10 BALB/c mice injected intraperitoneally with solutions of MAbs, followed by intravenous challenge with 5 × 10^3 parasitized erythrocytes per mouse. Mice in each group received 0.5 mg of IgG in PBS intraperitoneally on days −1, 0, and +1 relative to the day of parasite challenge (day 0). B6 ([]) and G3 (○) substantially modified the course of the infection; B10 (●) and G3 (○) were less effective. The parasitemias in mice treated with B4 (■) and in mice treated with an irrelevant MAb (data not shown) were indistinguishable from that in the group that received PBS (▲). All of the mice cleared the infection, except for those that received B4 and PBS, which died on day 7, and 40% of the mice treated with G3, which died on days 8 and 9. Each point represents the geometric mean parasitemia of mice in each group at the time after parasite challenge, and the vertical bars indicate the standard errors.
washed, aliquoted onto multwell slides, and fixed in methanol-acetone (1:1, vol/vol) for 10 min. MAbs were diluted 1:100 and incubated on the slide for 30 min at room temperature. After washing, the slides were incubated with fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G (IgG) (Sigma) for 30 min, washed, and dipped in 0.05% Evans blue-1 µg of DAPI (4',6-diamidino-2-phenylindole) per ml. They were then examined by fluorescence microscopy.

**Competition ELISA.** The wells of 96-well microplates (Dynatech Immunon-4) were coated with 1 µg of rMSP-119 per ml and then blocked with bovine serum albumin, as described previously (24). After incubation for 1 h at 37°C with the first MAb, the wells were washed three times with washing buffer and then incubated with the second biotinylated MAb (prepared by using sulfo-NHS-biotin [Pierce] according to the manufacturer’s recommendation) for 1 h at 37°C using a concentration that had been determined previously by titration. The wells were washed three times, and then 100 µl of horseradish peroxidase-conjugated streptavidin (10 µg/ml) was added and left for 1 h at 37°C. After a final three washes, 100 µl of o-phenylenediamine substrate was added. The color reaction was stopped by addition of 50 µl of 1 M sulfuric acid, and the absorbance was read at 492 nm as described previously (24). The ability of a MAb to inhibit the binding of the biotinylated second MAb to the antigen was analyzed by comparing the mean absorbance values in the presence of different competitor MAbs by the Kruskal-Wallis test.

**RESULTS**

**Passive immunization suppresses parasitemia.** Of 12 hybridoma cell lines that were cloned, 6 (B4, B6, B10, D3, F5, and G3) that secrete antibody specific for *P. yoelii* MSP-1 were characterized further, and the MAbs were analyzed in detail. Hybridomas B10 and G3 were produced after immunization with the recombinant protein GST–MSP-119, and the others were produced after passive immunization with B10 and infection with the parasite. The subclass of each antibody is shown in Table 1.

The ability of the MAbs to protect mice against a blood stage challenge infection with *P. yoelii* YM by passive immunization was evaluated. Purified IgG was inoculated into groups of mice at the time of parasite challenge, and the development of parasitemia was monitored (Fig. 1). One antibody (B4) had no effect on the course of parasitemia; all mice developed a fulminating infection similar to that in the group inoculated with phosphate-buffered saline (PBS) alone, and none survived beyond day 7. Two of the antibodies (B10 and G3) produced a partial suppression of parasite growth. However, the mean parasitemia in the group that received G3 exceeded 70% on day 14, and only 60% of the mice cleared the infection. In the group that received B10, the parasitemia exceeded 50%, although all of the mice cleared the infection and survived. Three of the antibodies (B6, D3, and F5) mediated a substantial reduction in parasitemia, and all of the mice in these groups cleared the infection. In the animals that received MAb D3, the parasites were largely restricted to reticulocytes.

**The MAbs react with MSP-1 and its fragments in parasite extracts.** As determined by indirect immunofluorescence, all of the MAbs produced a distinctive pattern with schizonts and merozoites, characteristic of an antigen present on the parasite surface (Fig. 2). In addition, B6 (Fig. 2a), B10, F5, and G3 also reacted with ring stage parasites. All MAbs immunoprecipitated the 230-kDa MSP-1 precursor from detergent lysates of schizonts (Fig. 3); this protein was also recognized by MAb 25.1 (18) and a polyclonal serum specific for MSP-1 (23) but not by antibodies in normal mouse serum. As determined by Western blotting, all of the MAbs recognized MSP-1, in parasite extract enriched for merozoites (Fig. 4A); this protein was also detected by polyclonal antibodies raised by immunization with GST–MSP-119. In addition, MAbs B6 (Fig. 4A, lane 2), B10 (lane 3), F5 (lane 5), and G3 (lane 6) detected MSP-119. In merozoite extracts incubated for 1 h to allow further MSP-1 processing and the formation of MSP-133, MAb B4 detected an additional species of ~33 kDa (Fig. 4B, lane 1) that was not detected by D3 (lane 2) or F5 (lane 3).

**Some of the MAbs react with recombinant proteins.** To locate more precisely the epitopes recognized by the MAbs, the ability of the antibodies to bind to recombinant proteins expressed from parts of the MSP-1 gene was investigated by Western blotting. The results of this analysis are summarized in Table 1. MAbs B6, B10, F5, and G3 bound to GST–MSP-119, indicating that they recognize epitopes in the C-terminal cysteine-rich region of MSP-1. None of the MAbs bound to the second EGF-like module fused to GST (GST-MSP1EGF2), but both B6 and F5 bound to the first EGF-like module fused to GST (GST-MSP1EGF1). This reactivity was abolished when the recombinant proteins were reduced and alkylated with iodoacetic acid before SDS-PAGE, a treatment that destroys epitopes that require disulfide bonds for their integrity (23, 24). MAbs B4 and D3 did not bind to GST–MSP-119. However, B4 reacted with the recombinant proteins GST–MSP-133A (Fig. 5, lane 1) and GST–MSP–133B (Fig. 5, lane 2) but not with GST–MSP–133C (Fig. 5, lane 3), indicating that it bound an epitope in the N-terminal half of MSP-133.

**Analysis of the antibodies by competition ELISA.** To study the epitopes on rMSP-119, recognized by the different MAbs, they were analyzed by competition ELISA (Table 2). MAbs G3 and B10 competed with each other for binding to GST–MSP-119 but not with any of the other antibodies. F5 competed with itself but not with B6, although B6 was able to compete effec-
As expected from the Western blot data, D3 and B4 did not bind to rMSP-119 (not shown) and did not compete with the other antibodies.

**DISCUSSION**

Previous studies have shown that immunization of mice with *P. yoelii* MSP-1 provides some protection against challenge infection with lethal strains of the parasite (15, 18). Immunization with recombinant protein corresponding to the C-terminal cysteine-rich region of *P. yoelii* MSP-1 has been found to be highly effective (9, 17, 23), and it has been suggested that antibody is important in mediating the protection observed (10, 17, 23, 25). In previous studies polyclonal antibodies raised by immunization with affinity-purified MSP-1 (15) or a MAb specific for an epitope at the N terminus of the protein (14) was not effective by passive immunization. In contrast, a MAb specific for the C-terminal cysteine-rich region of MSP-1 (26) and polyclonal antibodies raised by immunization with recombinant MSP-119 (10, 25) were at least partly effective in suppressing parasitemia. In this study we report six additional MSP-1-specific MAbs and define additional specificities for antibodies that are at least partially protective after passive immunization. One of these (D3) defines an epitope on the larger C-terminal fragment MSP-142.

Of the three MAbs that were most effective at suppressing the parasitemia after passive immunization, two of them (F5 and B6) are of the IgG3 subclass, and the other (D3) is an IgG2a. The subclass and specificity (see below) of F5 and B6 suggest that they are antibodies similar to MAb 302, the IgG3 reported by Majarian et al. (26). Two other MAbs, B10 and G3 (IgG2b and IgG1, respectively), were also able to modify the course of parasitemia, and some mice survived the challenge infection. MAb B4 was unable to modify the course of infection after passive immunization, and all mice developed a fulminating infection, a pattern similar to that obtained with a control MAb (data not shown). Determination of whether there is a correlation between subclass, as well as epitope specificity, and the ability of IgG to protect mice requires further investigation. The specific contributions of different IgG subclasses to protection mediated by immunization with by immunization with affinity-purified MSP-1 (15) or a MAB specific for an epitope at the N terminus of the protein (14) was not effective by passive immunization. In contrast, a MAB specific for the C-terminal cysteine-rich region of MSP-1 (26) and polyclonal antibodies raised by immunization with recombinant MSP-119 (10, 25) were at least partly effective in suppressing parasitemia. In this study we report six additional MSP-1-specific MAbs and define additional specificities for antibodies that are at least partially protective after passive immunization. One of these (D3) defines an epitope on the larger C-terminal fragment MSP-142.

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![FIG. 3. The MAbs immunoprecipitate MSP-1 from extracts of 35S-labelled *P. yoelii* YM-infected erythrocytes solubilized with buffer containing detergent. In this experiment the following MAbs were used: B6 (lane 1), D3 (lane 2), F5 (lane 3), and G3 (lane 4). The immunoprecipitates were analyzed by SDS-PAGE on a 7.5% polyacrylamide gel and detected by fluorography. The position of MSP-1 on the left and the mobilities of standard molecular mass markers (Pharmacia) (in kilodaltons) on the right are indicated. MSP-1 was also precipitated by B4, B10, 25.1, and a polyclonal antiserum to GST–MSP-119 but not by antibodies in normal mouse serum (not shown).](http://iai.asm.org/article-figures/3928_f3.jpg)

![FIG. 4. The MAbs react with fragments of MSP-1 in an extract of *P. yoelii* YM merozoites by Western blotting. (A) The samples were subjected to SDS-PAGE (without prior reduction) on a 5 to 15% polyacrylamide gradient gel, blotted onto nitrocellulose and then probed with MAb B4 (lane 1), B6 (lane 2), B10 (lane 3), D3 (lane 4), F5 (lane 5), or G3 (lane 6) or with polyclonal anti-GST–MSP-119 (lane 7) and normal mouse serum (lane 8). The positions of MSP-142, MSP-133, and MSP-119 on the left and the mobilities of standard molecular mass markers (in kilodaltons) on the right are indicated. (B) The merozoites were incubated in vitro to allow processing to continue before analysis. The samples were fractionated on a 12.5% polyacrylamide gel, blotted, and probed with MAbs B4 (lane 1), D3 (lane 2), and F5 (lane 3). The positions of MSP-142, MSP-133, and MSP-119 on the left and the mobilities of standard molecular mass markers (in kilodaltons) on the right are indicated.](http://iai.asm.org/article-figures/3928_f4.jpg)

![FIG. 5. MAb B4 binds to an epitope in the N-terminal end of MSP-119. Recombinant proteins consisting of MSP-119 sequences fused to GST were fractionated by SDS-PAGE on a 12.5% polyacrylamide gel under reducing conditions, transferred to nitrocellulose, and then probed with the MAb. Lane 1, PyMSP119-A (Thr1425 to His1619); lane 2, PyMSP119-B (Thr1425 to Lys1513); lane 3, PyMSP119-C (Ala1427 to His1619). The mobilities of molecular mass standards (New England Biolabs) (in kilodaltons) are shown. The antibody did not react with GST alone, none of the other MAbs reacted with GST–MSP-119, and each of the fusion proteins was detected with antibody specific for GST (data not shown).](http://iai.asm.org/article-figures/3928_f5.jpg)
recombinant proteins corresponding to the C terminus of MSP-1 have not been evaluated in detail, although correlations of protection with subclass have suggested that IgG1, IgG2a, and IgG2b are important (11, 13, 25, 31). Passive immunization with IgG fractions isolated from sera of mice immune to \textit{P. yoelii} infection suggested that IgG2a was of particular importance (33). The fact that MAbs against \textit{P. falciparum} MSP-1, that inhibit erythrocyte invasion in vitro appear to do so by inhibiting secondary processing of MSP-1\(_{122}\) suggests that their activity is based on a steric effect rather than one requiring a specific Fe-mediated function (4). It will be of interest to determine whether the \textit{P. yoelii} MSP-1-specific MAbs that are effective after passive immunization also inhibit \textit{P. yoelii} MSP-1 processing in vivo.

All of the antibodies react with the intact MSP-1 precursor as assessed by immunoprecipitation from schizont extracts and with the C-terminal MSP-1\(_{42}\) in merozoite extracts. The Western blotting results with the recombinant proteins indicate that B10 and G3 recognize epitopes in MSP-1\(_{19}\) that require both EGF-like modules to be present in the protein, whereas B6 and F5 recognize epitopes in the first EGF-like module. The specificity of B6 and F5 appears to be similar to that of MAb 302, described previously (6). The epitopes for all of these antibodies are constrained by disulfide bonds, since they do not recognize the reduced and alkylated GST–MSP-1\(_{19}\). These results are consistent with the results of the competition ELISA, which suggest that the epitopes for B10 and G3 overlap, may be identical, and are different from the epitopes for B6 and F5, which also appear to overlap each other but clearly are distinct epitopes. All of these antibodies recognize schizont and ring stage parasites as determined by indirect immunofluorescence, indicating that they react with the small C-terminal fragment of MSP-1 that results from secondary processing of MSP-1 at the time of invasion and is carried into newly invaded erythrocytes (2, 5, 28).

MAbs D3 and B4 did not recognize ring stage parasites as determined by immunofluorescence, suggesting that they do not bind directly to MSP-1\(_{19}\). The epitope for B4 was mapped to the N-terminal region of MSP-1\(_{42}\) by reaction with a recombinant protein containing amino acid sequences from this region. This antibody also reacted with an \(-33\)-kDa polypeptide present in merozoite preparations that had been incubated for 1 h to allow processing to proceed. This polypeptide is probably analogous to the soluble 33-kDa fragment (PIMSP-1\(_{19}\)) resulting from secondary processing of \textit{P. falciparum} MSP-1\(_{122}\) and shed from the merozoite surface at the time of erythrocyte invasion (4). This is the first description of a MAb to this part of \textit{P. yoelii} MSP-1, and it will be of use in studying the proteolytic processing of this molecule. MAb D3 reacts with MSP-1\(_{122}\) but not with either the N-terminal (MSP-1\(_{13}\) or C-terminal (MSP-1\(_{19}\) part alone, suggesting that it binds to the site of secondary processing or to an epitope that requires intact MSP-1\(_{122}\).

The results are consistent with the demonstration that immunization with the C terminus of \textit{P. yoelii} MSP-1 can protect mice against challenge infection with blood stage parasites and that this protection is mediated, at least in part, by antibody. Although two of the protective antibodies are directed against the first EGF-like module, immunization with GST-MSP1IEGF1 alone does not protect mice against blood stage challenge (7, 24). Immunization with a recombinant protein containing the two EGF-like modules (MSP-1\(_{19}\)) does protect against a blood stage parasite or sporozoite challenge (9, 17, 23, 31). However, the identification of a MAb that is effective on passive immunization and does not recognize just the C terminus of MSP-1 alone suggests that the optimal immunogen may contain sequence in addition to MSP-1\(_{19}\). Successful immunization with a GST–MSP-1\(_{122}\) recombinant protein was reported recently, but the protection observed was no better than that mediated by MSP-1\(_{19}\) alone (32). For development of a vaccine against \textit{P. falciparum}, both MSP-1\(_{19}\) and MSP-1\(_{122}\) are being considered (8, 21), but comparative studies of the two have not been performed so far. The development of effective vaccines based on MSP-1 may also be compromised by the presence of epitopes that induce blocking antibodies that abrogate the action of neutralizing antibodies (4, 16), so it will be important to define in detail the fine specificity of MSP-1-specific IgG with biological activity and to modify the antigen to remove epitopes that induce blocking antibodies without affecting those that induce protective antibodies.

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