INFECTION AND IMMUNITY, Feb. 2002, p. 820–825 Vol. 70, No. 2
0019-9567/02/$04.00+0 DOI: 10.1128/IAI.70.2.820–825.2002

Protective Immune Responses to the 42-Kilodalton (kDa) Region of *Plasmodium yoelii* Merozoite Surface Protein 1 Are Induced by the C-Terminal 19-kDa Region but Not by the Adjacent 33-kDa Region

Niklas Ahlborg,[1]† Irene T. Ling,[2] Wendy Howard,[1] Anthony A. Holder,[2] and Eleanor M. Riley[1]*

Institute of Cell, Animal and Population Biology, Edinburgh University, Edinburgh EH9 3JT,[1] and National Institute for Medical Research, Mill Hill, London NW7 1AA,[2] United Kingdom

Received 18 June 2001/Returned for modification 17 October 2001/Accepted 5 November 2001

Vaccination of mice with the 42-kDa region of *Plasmodium yoelii* merozoite surface protein 1 ($MSP1_{42}$) or its 19-kDa C-terminal processing product ($MSP1_{19}$) can elicit protective antibody responses in mice. To investigate if the 33-kDa N-terminal fragment ($MSP1_{33}$) of $MSP1_{42}$ also induces protection, the gene segment encoding $MSP1_{33}$ was expressed as a glutathione *S*-transferase (GST) fusion protein. C57BL/6 and BALB/c mice were immunized with GST-$MSP1_{33}$ and subsequently challenged with the lethal *P. yoelii* YM blood stage parasite. GST-$MSP1_{33}$ failed to induce protection, and all mice developed patent parasitemia at a level similar to that in naive or control (GST-immunized) mice; mice immunized with GST-$MSP1_{19}$ were protected, as has been shown previously. Specific prechallenge immunoglobulin G (IgG) antibody responses to MSP1 were analyzed by enzyme-linked immunosorbent assay and immunofluorescence. Despite being unprotected, several mice immunized with $MSP1_{33}$ had antibody titers (of all IgG subclasses) that were comparable to or higher than those in mice that were protected following immunization with $MSP1_{19}$. The finding that *P. yoelii* $MSP1_{33}$ elicits strong but nonprotective antibody responses may have implications for the design of vaccines for humans based on *Plasmodium falciparum* or *Plasmodium vivax* $MSP1_{42}$.

The *Plasmodium falciparum* merozoite surface protein 1 (MSP1) is one of the major surface proteins of the merozoite, and immunization with full-length, native MSP1 protein can protect monkeys from otherwise lethal infection with parasites (29). MSP1 is processed into a complex of polypeptides on the merozoite surface, including N-terminal and central regions of 82, 30, and 38 kDa, as well as the C-terminal region of 42 kDa (18, 24). At the time of invasion of red blood cells (RBC), $MSP1_{42}$ is further processed by proteolytic cleavage into a 33-kDa fragment ($MSP1_{33}$), which is shed with the rest of the complex, and a C-terminal 19-kDa fragment ($MSP1_{19}$) (3). Only the C-terminal $MSP1_{19}$ remains on the merozoite surface and is carried into parasitized RBC (pRBC) (2). In monkeys, immunization with recombinant $MSP1_{42}$ and $MSP1_{19}$ has been shown to elicit various degrees of protection against *P. falciparum* challenge (6, 11, 19). The importance of the *P. falciparum* $MSP1_{33}$ region for induction of protective immunity is not known.

MSP1 from the rodent malaria parasite *Plasmodium yoelii* appears to be processed in a manner similar to that of *P. falciparum* MSP1 (17), and recombinant *P. yoelii* $MSP1_{19}$ can elicit protective immunity in mice (8, 15, 21). The immunity induced by $MSP1_{19}$ in mice is antibody dependent (1, 7, 15, 22) and may, to a large extent, be mediated by antibodies that act directly on the parasite (21, 27). A possible mechanism could be interference with the processing of $MSP1_{42}$, resulting in inhibition of merozoite invasion; this has been shown in vitro with antibodies to *P. falciparum* $MSP1_{19}$ (4). While immunization with recombinant *P. yoelii* $MSP1_{42}$, which contains both $MSP1_{33}$ and $MSP1_{19}$, protects mice, immunization with recombinant proteins corresponding to N-terminal or central regions of MSP1 has little if any protective effect (34).

P. yoelii $MSP1_{33}$ has not previously been evaluated for its ability to elicit protection. Antibodies to $MSP1_{33}$ could be potentially protective—for example, if they interfere with processing of $MSP1_{42}$, as has been shown for antibodies to *P. falciparum* $MSP1_{19}$. Alternatively, antibodies to $MSP1_{33}$ may hinder the binding of protective antibodies induced by $MSP1_{19}$ and thus compromise immunity induced by $MSP1_{19}$ alone (14).

In the present study, mice were immunized with *P. yoelii* $MSP1_{33}$ and challenged with a virulent clone of *P. yoelii* (YM) to assess the protective capacity of $MSP1_{33}$. Although $MSP1_{33}$ was found to be highly immunogenic and elicited levels of antibodies to native MSP1 that were comparable to the levels induced in mice immunized with $MSP1_{19}$, no protection of $MSP1_{33}$-immunized mice was observed, suggesting that antibody responses to recombinant *P. yoelii* $MSP1_{33}$ are not protective.

MATERIALS AND METHODS

Cloning of *P. yoelii* $MSP1_{33}$ and expression of recombinant GST fusion proteins. In order to express *P. yoelii* $MSP1_{33}$ as a glutathione *S*-transferase (GST) fusion protein, genomic DNA from *P. yoelii* YM was purified using Instagene according to the manufacturer's instructions (Bio-Rad, Hemel Hempstead, United Kingdom). DNA encoding residues 1394 to 1655 of *P. yoelii* MSP1

* Corresponding author. Present address: Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel St., London WC1E 7HT, United Kingdom. Phone: 0207 927 2706. Fax: 0207 837 4314. E-mail: e.riley@lshtm.ac.uk.

† Present address: Mabtech, SE-131 37 Nacka, Sweden.

(PyMSP1) (20) was PCR amplified using the sense primer 5′-GCG GAT CCA ATC CGA AGA TGC ACC AGA A-3′ and the antisense primer 5′-GCG AAT TCT AGC TGG AAG AAC TAC AGA A-3′ (VH BIO, Newcastle, United Kingdom). The PCR product was purified from a 1% agarose gel using Prep-a-gene (Bio-Rad), restricted with *Bam*HI and *Eco*RI (Promega, Madison, Wis.), and then purified using a High Pure PCR kit (Boehringer GmbH, Mannheim, Germany).

The PCR product was ligated into linearized pGEX-3X (Pharmacia, Uppsala, Sweden), digested with the same restriction enzymes, using T4 DNA ligase (Promega). After transformation of *Escherichia coli* strain DH5α with ligated plasmid, positive clones were screened for protein expression after induction with isopropyl-β-D-thiogalactopyranoside (IPTG) (Sigma, Poole, United Kingdom). The plasmid from a clone expressing a protein of the expected size was purified using Wizard miniprep columns (Promega), and the complete insert was confirmed by DNA sequencing.

For production of recombinant GST fusion proteins, *E. coli* DH5α was transformed with plasmids encoding either GST-$MSP1_{33}$, GST-$MSP1_{19}$ (residues 1649 to 1754 [21]), or GST alone. The proteins were produced as described previously (30) with some modifications (1). Briefly, overnight cultures of transformed bacteria were induced with 1 mM IPTG. The cells were harvested and lysed in phosphate-buffered saline (PBS). The lysate was centrifuged and the supernatant was recovered. Recombinant proteins were purified by affinity chromatography on glutathione-agarose (Sigma) using 10 mM reduced glutathione (Sigma) for elution. Free glutathione was removed by dialysis against PBS.

Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) indicated that GST-$MSP1_{19}$, GST-$MSP1_{33}$, and GST were predominantly full length and had relative molecular masses of 42, 65, and 26 kDa, respectively. The concentrations of purified proteins were determined by multiplying the absorbance at 280 nm with factors calculated for each protein based on size and content of aromatic amino acids (13).

For production of GST-free $MSP1_{19}$ and $MSP1_{33}$, glutathione-agarose was incubated with supernatants from bacterial culture (21). After extensive washing, the agarose beads were treated with factor Xa (Sigma) for 12 h at 22°C. Factor Xa was removed from the solution containing the GST-free proteins by incubation with *p*-aminobenzamidine bound to agarose (Sigma). Simultaneous incubation with glutathione-agarose ensured that any contaminating GST was removed. The proteins were dialyzed against PBS and analyzed by SDS-PAGE, and the concentrations were determined as above. Only one band was observed by SDS-PAGE for purified GST-free $MSP1_{19}$ as well as $MSP1_{33}$ and corresponded to relative molecular masses of 20 and 34 kDa, respectively.

***P. yoelii* parasites.** *P. yoelii* parasites of the lethal strain YM (38), kindly provided by David Walliker, Edinburgh University, were kept frozen at −70°C or by regular passage in mice. Infected mice were given drinking water supplemented with 2.5 g of *para*-benzoaminoic acid/liter (25).

Immunization and challenge of mice. Female C57BL/6 and BALB/c mice (Harlan, Oxford, United Kingdom) were used at 7 to 10 weeks of age. Groups of five mice were injected intraperitoneally with 40 μg of GST-$MSP1_{19}$, GST-$MSP1_{33}$, or GST in complete Freund's adjuvant (Sigma). Booster injections were given 3 and 6 weeks later using the same amount of antigen but in incomplete Freund's adjuvant (Sigma). Prechallenge sera were drawn 2 weeks after the last booster injection. Three weeks after the last immunization, mice were inoculated intravenously with 10^4 pRBC. Giemsa-stained blood smears were made daily from day 3 of infection onwards. Parasitemia was assessed by counting the proportion of pRBC by light microscopy.

ELISA. GST-free $MSP1_{19}$ or $MSP1_{33}$ was adsorbed to Immunolon 4 plates (Dynatech Laboratories Inc., Billingshurst, United Kingdom) at a concentration of 2 μg/ml in PBS overnight at 8°C. The wells were blocked with 1% bovine serum albumin in PBS for 1 h at room temperature, and then diluted sera were added and incubated for 1 h at 37°C. Bound antibodies were detected by subsequent incubation with goat anti-mouse immunoglobulin G1 (IgG1), IgG2b, or IgG3 (Southern Biotechnology, Birmingham, Ala.) or sheep anti-mouse IgG2a (The Binding Site, Birmingham, United Kingdom) conjugated to horseradish peroxidase. As the substrate, *o*-phenylenediamine (Sigma) was used. Endpoint serum titers were estimated by analyzing sera at consecutive twofold dilutions, starting at 1:64, using an absorbance value of 0.3 as the cutoff for positive sera. The mean + 2 standard deviations absorbance for normal mouse serum, diluted 1:64, was lower than the cutoff for all antigens analyzed. A high-titer serum pool was included in all assays as an internal standard.

IFAT. For the indirect immunofluorescent antibody test (IFAT), blood was drawn from *P. yoelii* YM-infected mice into heparinized tubes. The blood was washed and diluted 1:200 in phosphate-saline buffer containing 0.2% glucose. Then 10 μl of cell suspension was added to wells of 15-well multitest slides (ICN Biomedicals Inc., Aurora, Ohio) that were air dried before storage at −20°C. Prior to staining, the slides were fixed with acetone for 2 min and blocked in PBS with 1% bovine serum albumin for 30 min at room temperature. The wells were incubated for 1 h at 37°C with serum in consecutive twofold dilutions in PBS. Specific antibodies were detected by incubation for 1 h at 37°C with fluorescein isothiocyanate-conjugated goat anti-mouse IgG1, IgG2a, IgG2b, or IgG3 (Southern Biotechnology). Between incubations, the slides were washed in PBS. Titers were determined as the reciprocal of the last positive dilution when analyzed by UV microscopy.

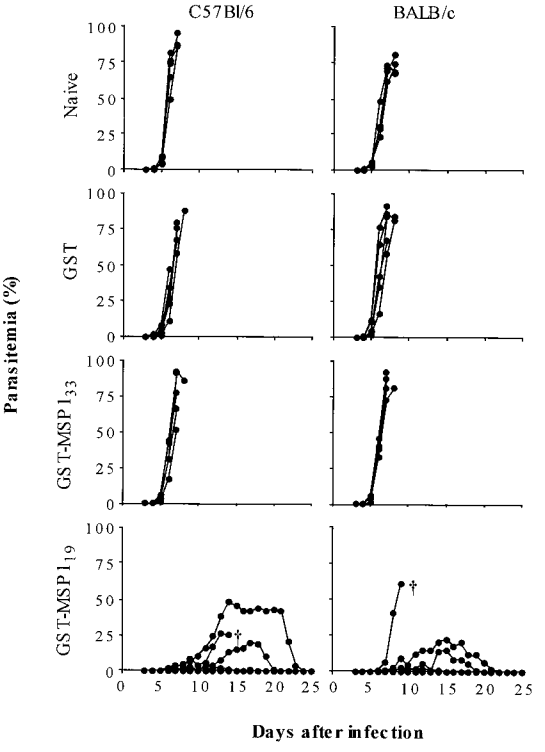

FIG. 1. Challenge infection with *P. yoelii* YM of mice immunized with $MSP1_{33}$ or $MSP1_{19}$. Three weeks after the third immunization with GST-$MSP1_{33}$ or -$MSP1_{19}$, groups of four or five C57BL/6 and BALB/c mice were inoculated intravenously with 10^4 *P. yoelii* YM. Parasitemia was monitored daily from day 3. Control groups consisted of mice immunized with GST alone and naive mice. Only mice in the GST-$MSP1_{19}$ groups were able to survive the challenge; mice that did not survive are indicated by †.

RESULTS

Challenge of immunized mice with *P. yoelii* parasites. BALB/c and C57BL/6 mice were immunized three times with GST-$MSP1_{19}$, GST-$MSP1_{33}$, or GST. Following immunization, the mice were challenged with 10^4 parasites of the lethal strain *P. yoelii* YM; naive mice were included for comparison (Fig. 1). In naive mice and GST-immunized control mice, the course of infection was rapid. All mice died between days 7 and 9 with parasitemias above 60%. In BALB/c and C57BL/6 mice immunized with GST-$MSP1_{19}$, protection was observed in four of five mice in each group, with one mouse (BALB/c) being fully protected (i.e., not having any detectable parasitemia). One BALB/c mouse died with fulminant infection at day 10 and thus showed only a minor delay in the course of infection. One C57BL/6 mouse died despite a delayed course of infection (day 15) and a maximal parasitemia of 26%. In mice immunized with GST-$MSP1_{33}$, no protection was observed; all mice died

TABLE 1. IgG subclass responses to GST-$MSP1_{19}$ and GST-$MSP1_{33}$ in C57BL/6 and BALB/c mice

Mouse strain	Immunogen	Mean ELISA titer[a] (10^3) ± SD			
		IgG1	IgG2a	IgG2b	IgG3
C57BL/6	GST-$MSP1_{19}$	120 ± 29	0.18 ± 0.070	21 ± 11	0.076 ± 0.028
	GST-$MSP1_{33}$	41 ± 25	0.74 ± 0.81	56 ± 46	0.44 ± 0.36
BALB/c	GST-$MSP1_{19}$	260 ± 160	2.8 ± 1.8	15 ± 11	0.47 ± 0.88
	GST-$MSP1_{33}$	147 ± 82	4.4 ± 2.9	25 ± 9.5	0.45 ± 0.38

[a] For specific detection of antibodies to MSP1 proteins, sera from mice immunized with GST-$MSP1_{19}$ and GST-$MSP1_{33}$ were tested in ELISA for reactivity with GST-free $MSP1_{19}$ and $MSP1_{33}$, respectively.

at day 8 or 9, and there was no difference between the two mouse strains.

Antibody responses to GST-MSP1 fusion proteins as measured by ELISA. To evaluate the predictive value of prechallenge IgG antibody levels on protection, sera from all mice immunized with GST-$MSP1_{19}$ and GST-$MSP1_{33}$ were analyzed for IgG subclass reactivity by ELISA and by IFAT. Direct comparison of ELISA IgG titers to $MSP1_{19}$ and $MSP1_{33}$ is not possible because different coating antigens were used in the ELISAs. Nevertheless, the results suggest that BALB/c mice responded with roughly comparable antibody titers to $MSP1_{19}$ and $MSP1_{33}$, although there was some difference in the subclass of the antibodies, with mean titers in the GST-$MSP1_{19}$ group being higher for IgG1 and lower for IgG2a and IgG2b compared with the GST-$MSP1_{33}$ group (Table 1). A similar result was observed for the C57BL/6 mice, although the differences between the groups were more pronounced, with mean IgG1 titers being twice as high for $MSP1_{19}$ as for $MSP1_{33}$ and mean IgG2a titers being threefold higher for $MSP1_{33}$ than for $MSP1_{19}$ (Table 1).

Antibody responses to GST-MSP1 fusion proteins as measured by IFAT. To compare antibody responses to native MSP1 in mice immunized with GST-$MSP1_{19}$ or GST-$MSP1_{33}$, individual sera from mice in each group were tested by IFAT to determine the endpoint antibody titer and antibody subclass by using acetone-fixed blood from *P. yoelii*-infected naive mice. Although absolute titers determined by IFAT were lower than those measured by ELISA, IFAT titers correlated well with the ELISA data; the individual IFAT titers for each group of mice are shown in Fig. 2.

Despite the small number of mice included in the study, there was a clear trend towards an association between antibody responses and protection in the GST-$MSP1_{19}$-vaccinated mice, as described previously (1). The fully protected BALB/c mouse had an extremely high IgG1 response in comparison with the other mice, whereas the BALB/c mouse that did not survive had the lowest antibody titer. Also, the C57BL/6 mice with the lowest maximal parasitemias had the highest overall antibody titers in that group. However, the C57BL/6 mouse that died on day 15 with a parasitemia of 26% did not stand out as a low responder in that group.

The mice immunized with GST-$MSP1_{33}$ had, as also observed by ELISA, lower mean titers of IgG1 but higher titers of IgG2a and IgG2b than mice immunized with GST-$MSP1_{19}$. In BALB/c mice, the IgG3 response to GST-$MSP1_{33}$ was also higher than that to GST-$MSP1_{19}$.

Despite the fact that IgG1 responses to GST-$MSP1_{33}$ were generally lower than those to GST-$MSP1_{19}$, some of the nonprotected GST-$MSP1_{33}$-immunized mice had antibody levels that were comparable to or higher than those of several mice that were protected by immunization with GST-$MSP1_{19}$. Two BALB/c mice immunized with GST-$MSP1_{33}$ had IgG1 levels that were comparable to those of three of the protected mice in the GST-$MSP1_{19}$-immunized group. These GST-$MSP1_{33}$-immunized mice also had comparable or higher titers of the other IgG subclasses. Of the C57BL/6 mice immunized with GST-$MSP1_{33}$, one mouse had comparable IgG1 and higher IgG2a and IgG2b titers than two of the mice protected by immunization with GST-$MSP1_{19}$.

It was clear from the IFAT analysis that the specificity of antibodies raised by GST-$MSP1_{33}$ differed from the specificity of antibodies to GST-$MSP1_{19}$. Antisera to GST-$MSP1_{19}$ stained both pigmented schizonts and ring stages, whereas antisera to GST-$MSP1_{33}$ only stained pigmented schizonts.

DISCUSSION

Immunization of BALB/c mice with *P. yoelii* $MSP1_{42}$, which comprises both $MSP1_{33}$ and $MSP1_{19}$ polypeptides, elicits protective immune responses (34), but similar levels of protection can be achieved in both BALB/c and C57BL/6 mice by immunization with the $MSP1_{19}$ polypeptide alone (8, 15, 21, 34). The extent to which the $MSP1_{33}$ region contributes to the protective immune response in $MSP1_{42}$-immunized mice is thus rather unclear. The data presented here show that epitopes contained entirely within the $MSP1_{33}$ polypeptide do not mediate protection. This finding is in agreement with the finding that a monoclonal antibody to *P. yoelii* $MSP1_{33}$ is unable to passively protect mice; this monoclonal antibody binds to an epitope located some distance from the cleavage site within $MSP1_{42}$ (31).

However, another monoclonal antibody, D3, which does passively protect mice against infection, recognizes an epitope in $MSP1_{42}$ that is not present in either $MSP1_{19}$ or $MSP1_{33}$ (31) and may therefore recognize an epitope formed by the physical interaction of the two polypeptides. As the protective capacity of antibodies against *P. falciparum* $MSP1_{19}$ ($PfMSP1_{19}$) is, at least in part, related to the ability of the antibodies to inhibit proteolytic processing of $PfMSP1_{42}$ (4, 14), antibodies binding to an epitope formed by sequences from both $MSP1_{33}$ and $MSP1_{19}$ and lying close to the cleavage site could inhibit processing and thus contribute to protection. A monoclonal antibody that inhibits *P. falciparum* invasion may also bind to epitopes formed from both $PfMSP1_{33}$ and $MSP1_{19}$ (37).

In order to examine the protective effect of $MSP1_{33}$, it was expressed as a GST fusion protein (GST-$MSP1_{33}$) and used, in parallel with GST-$MSP1_{19}$, to immunize BALB/c and C57BL/6 mice. The results confirmed the protective effects of vaccination with GST-$MSP1_{19}$; in contrast, no protection was observed in mice immunized with $MSP1_{33}$. Protective immunity in mice immunized with recombinant *P. yoelii* $MSP1_{19}$ is largely dependent on antibodies; passive transfer of antiserum to $MSP1_{19}$ can confer partial protection on naive recipients, whereas immunoglobulin μ-chain-deficient mice cannot be immunized with $MSP1_{19}$ (7, 15, 22, 23). We therefore investigated whether the lack of protection following immunization with $MSP1_{33}$ was due to an insufficient antibody response.

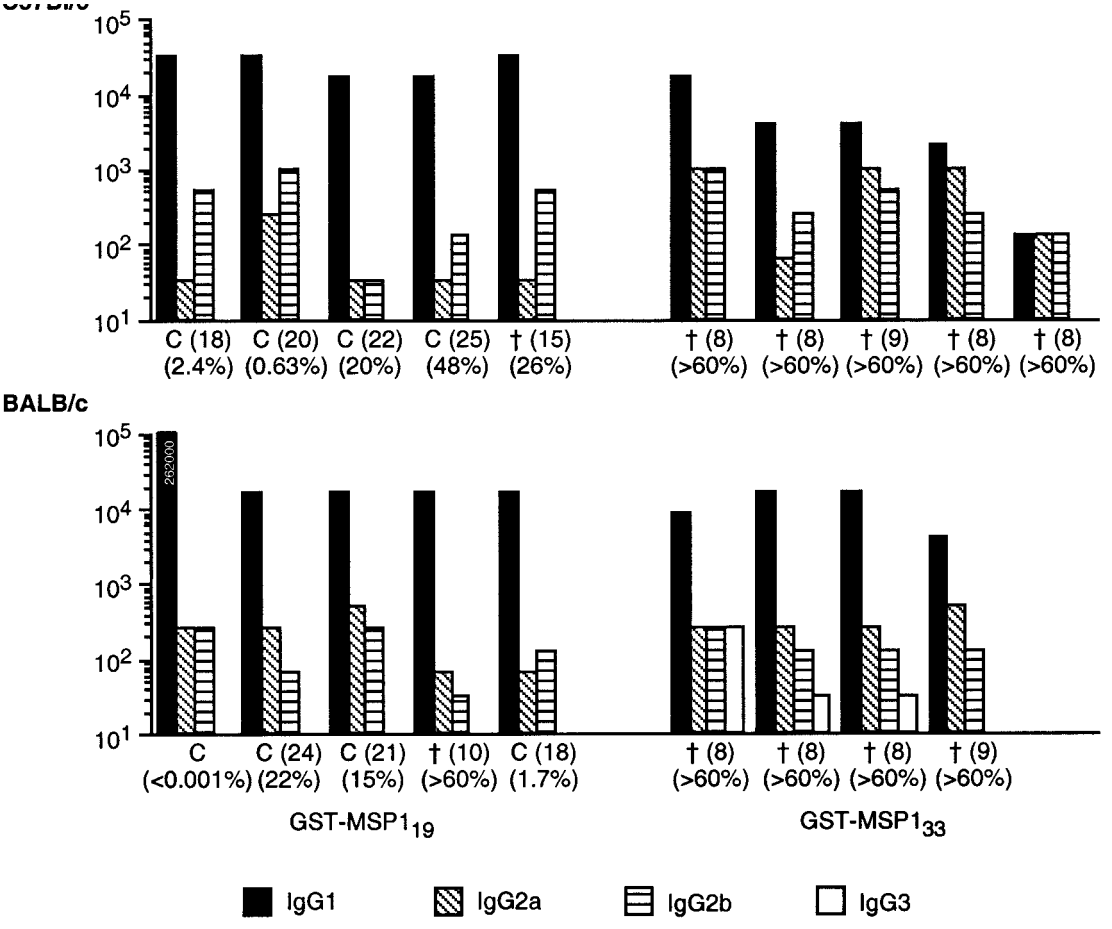

FIG. 2. IgG subclass of antibodies to native *P. yoelii* MSP1 in mice immunized with GST-MSP1$_{33}$ or -MSP1$_{19}$, as measured by IFAT. Sera from C57BL/6 and BALB/c mice immunized three times with either GST-MSP1$_{33}$ or -MSP1$_{19}$ were analyzed by IFAT for reactivity with mouse RBC infected with *P. yoelii* YM. IFAT reactivity for all four IgG subclasses was measured and is shown as titers, with different bar patterns representing different subclasses. One of the GST-MSP1$_{19}$-immunized BALB/c mice had an IgG1 titer in excess of 100,000 (262,000). IgG3 was only detected in BALB/c mice immunized with GST-MSP1$_{33}$. C indicates mice that cleared their infection; the number in parentheses indicates the day on which the infection was cleared. † indicates mice that died or were humanely killed, with the day of death in parentheses. Percentages indicate the highest parasitemia detected during the course of infection.

Although the antibody response to MSP1$_{33}$ was, on average, lower than the response to MSP1$_{19}$, several mice immunized with MSP1$_{33}$ developed an anti-*P. yoelii* MSP1 antibody response comparable to or higher than the response in mice that were well protected following immunization with GST-MSP1$_{19}$. We therefore conclude that the lack of a sufficient antibody titer is, in itself, not responsible for the failure of GST-MSP1$_{33}$ to induce protection, and we assume that antibodies elicited by recombinant MSP1$_{33}$ are of a specificity that fails to inhibit the growth of parasites.

It has been shown that folding of the MSP1$_{19}$ recombinant protein into the correct three-dimensional conformation is important for its ability to elicit protective antibodies (21). The importance of correct folding of MSP1$_{19}$ is probably related to the high number of cysteine residues, which form multiple disulfide bonds; this is less likely to present problems for expression of MSP1$_{33}$ because it does not contain any disulfide bonds (20). Indeed, antibodies induced by GST-MSP1$_{33}$ reacted strongly with native, parasite-derived antigen in IFAT. It is thus likely that the antibodies to MSP1$_{33}$ are specific for nonprotective epitopes.

A relevant question is whether or not a vaccine based on MSP1$_{19}$ is affected by the inclusion of MSP1$_{33}$. Comparisons of the immunogenicity and protective capacity of *P. yoelii* MSP1$_{19}$ and MSP1$_{42}$ in BALB/c and A/J mice did not show any difference between the constructs (34). However, it is possible that in some mouse strains which respond less well to MSP1$_{19}$ (33), the addition of MSP1$_{33}$ could provide additional T-cell epitopes to enhance the antibody response to MSP1$_{19}$. T-cell responses are known to contribute to protection induced by MSP1$_{19}$ vaccination and are essential for immune memory (16). The linkage of exogenous T-cell epitopes to MSP1$_{19}$ has been shown to improve protective antibody responses in mouse strains that are poorly protected by immunization with MSP1$_{19}$ alone (1).

The importance of increasing the number of T-helper-cell epitopes in immunogens based on *P. falciparum* MSP1$_{19}$, intended for use in humans, is not known. Individuals from areas

where malaria is endemic who have been immunized by natural infection with *P. falciparum* are frequently seronegative for antibodies to $MSP1_{19}$ (9, 12, 28, 35), and T-cell proliferative responses to peptides derived from $PfMSP1_{19}$ tend to be infrequent and relatively weak (10, 26, 36). Importantly, however, T-cell proliferative and cytokine responses to recombinant proteins, including the 33-kDa region of PfMSP1, have been associated with protective immunity to malaria in epidemiological studies (26).

Vaccination of nonhuman primates with MSP_{19} or MSP_{42} has produced inconsistent and sometimes contradictory results. In one primate immunization study, vaccination with bacterium-derived $MSP1_{19}$ induced only relatively low-titer antibody responses which were not protective (5), whereas in a similar study using a different adjuvant, vaccination with baculovirus-derived $PfMSP1_{42}$ induced strong lymphoproliferative responses and high antibody titers to $PfMSP1_{19}$; vaccine-induced antibodies effectively inhibited parasite growth in vitro, and all immunized animals were protected against challenge infection (6).

In a direct comparison of the efficacy of $PfMSP1_{42}$ and $PfMSP1_{19}$ as immunogens, monkeys immunized with $PfMSP1_{19}$ appeared to be better protected than those immunized with $PfMSP1_{42}$ (19), but the number of animals tested was small and the two antigens were produced in different expression systems (*Saccharomyces cerevisiae* and *E. coli*, respectively), which may have contributed to differences in immunogenicity.

In another study, monkeys vaccinated with yeast-derived $PfMSP1_{19}$ were significantly less well protected than monkeys immunized with baculovirus-derived $PfMSP1_{42}$ (32). Inclusion of exogenous T-cell epitopes in the $MSP1_{19}$ construct does not necessarily enhance protection to immediate challenge, although such vaccines seem to induce strong memory responses (11). Further studies are therefore justified to determine the importance of $MSP1_{33}$-derived T helper epitopes in contributing to natural and vaccine-induced immunity to MSP1 in animal models and in human vaccination studies.

ACKNOWLEDGMENTS

This work was funded by a Research Training Grant to N. Ahlborg from the Life Sciences and Technologies Directorate of the European Commission (contract number BMH4-CT97-5037) and by the Wellcome Trust.

We thank S. Haley for technical support and D. Cavanagh and S. Franks for technical advice.

REFERENCES

1. **Ahlborg, N., I. T. Ling, A. A. Holder, and E. M. Riley.** 2000. Linkage of universal T-cell epitopes to the 19-kilodalton region of *Plasmodium yoelii* merozoite surface protein 1 ($MSP\text{-}1_{19}$) can enhance protective immunity against malaria and modulates the immunoglobulin subclass response to $MSP\text{-}1_{19}$. Infect. Immun. **68:**2102–2109.
2. **Blackman, M. J., H.-G. Heidrich, S. Donachie, J. S. McBride, and A. A. Holder.** 1990. A single fragment of a malaria merozoite surface protein remains on the parasite during red cell invasion and is the target of invasion-blocking antibodies. J. Exp. Med. **172:**379–382.
3. **Blackman, M. J., and A. A. Holder.** 1992. Secondary processing of the *Plasmodium falciparum* merozoite surface protein-1 (MSP1) by a calcium-dependent membrane-bound serine protease: shedding of $MSP1_{33}$ as a non-covalently associated complex with other fragments of the MSP1. Mol. Biochem. Parasitol. **50:**307–316.
4. **Blackman, M. J., T. J. Scott-Finnigan, S. Shai, and A. A. Holder.** 1994. Antibodies inhibit the protease-mediated processing of a malaria merozoite surface protein. J. Exp. Med. **180:**389–393.
5. **Burghaus, P. A., B. T. Wellde, T. Hall, R. L. Richards, A. F. Egan, E. M. Riley, W. R. Ballou, and A. A. Holder.** 1996. Immunization of *Aotus nancymai* with recombinant C terminus of *Plasmodium falciparum* merozoite surface protein 1 in liposomes and alum adjuvant does not induce protection against a challenge infection. Infect. Immun. **64:**3614–3619.
6. **Chang, S. P., S. E. Case, W. L. Gosnell, A. Hashimoto, K. J. Kramer, L. Q. Tam, C. Q. Hashiro, C. M. Nikaido, H. L. Gibson, C. T. Lee-Ng, P. J. Barr, B. T. Yokota, and G. S. N. Hui.** 1996. A recombinant baculovirus 42-kilodalton C-terminal fragment of *Plasmodium falciparum* merozoite surface protein 1 protects *Aotus* monkeys against malaria. Infect. Immun. **64:**253–261.
7. **Daly, T. M., and C. A. Long.** 1995. Humoral response to a carboxyl-terminal region of the merozoite surface protein-1 plays a predominant role in controlling blood-stage infection in rodent malaria. J. Immunol. **155:**236–243.
8. **Daly, T. M., and C. A. Long.** 1993. A recombinant 15-kilodalton carboxyl-terminal fragment of *Plasmodium yoelii yoelii* 17XL merozoite surface protein 1 induces a protective immune response in mice. Infect. Immun. **61:**2462–2467.
9. **Dodoo, D., T. G. Theander, J. A. L. Kurtzhals, K. Koram, E. Riley, B. D. Akanmori, F. K. Nkrumah, and L. Hviid.** 1999. Antibody levels to conserved parts of *Plasmodium falciparum* merozoite surface protein 1 (PfMSP1) in Ghanaian children are not associated with protection from clinical malaria. Infect. Immun. **67:**2131–2137.
10. **Egan, A., M. Waterfall, M. Pinder, A. Holder, and E. Riley.** 1997. Characterization of human T- and B-cell epitopes in the C terminus of *Plasmodium falciparum* merozoite surface protein 1: evidence for poor T-cell recognition of polypeptides with numerous disulfide bonds. Infect. Immun. **65:**3024–3031.
11. **Egan, A. F., M. J. Blackman, and D. C. Kaslow.** 2000. Vaccine efficacy of recombinant *Plasmodium falciparum* merozoite surface protein 1 in malaria-naive, -exposed and/or -rechallenged *Aotus vociferans* monkeys. Infect. Immun. **68:**1418–1427.
12. **Egan, A. F., J. A. Chappel, P. A. Burghaus, J. S. Morris, J. S. McBride, A. A. Holder, D. C. Kaslow, and E. M. Riley.** 1995. Serum antibodies from malaria-exposed people recognize conserved epitopes formed by the two epidermal growth factor motifs of $MSP1_{19}$, the carboxy-terminal fragment of the major merozoite surface protein of *Plasmodium falciparum.* Infect. Immun. **63:**456–466.
13. **Gill, S. C., and P. H. von Hippel.** 1989. Calculation of protein extinction coefficients from amino acid sequence data. Anal. Biochem. **182:**319–326. (Erratum, **189:**283.)
14. **Guevara Patino, J. A., A. A. Holder, J. S. McBride, and M. J. Blackman.** 1997. Antibodies that inhibit malaria merozoite surface protein-1 processing and erythrocyte invasion are blocked by naturally acquired human antibodies. J. Exp. Med. **186:**1689–1699.
15. **Hirunpetcharat, C., J. H. Tian, D. C. Kaslow, N. van Rooijen, S. Kumar, J. A. Berzofsky, L. H. Miller, and M. F. Good.** 1997. Complete protective immunity induced in mice by immunization with the 19-kilodalton carboxyl-terminal fragment of the merozoite surface protein-1 (MSP1[19]) of *Plasmodium yoelii* expressed in *Saccharomyces cerevisiae*: correlation of protection with antigen-specific antibody titer, but not with effector CD4+ T cells. J. Immunol. **159:**3400–3411.
16. **Hirunpetcharat, C., P. Vukovic, X. Liu, D. Kaslow, L. Miller, and M. Good.** 1999. Absolute requirement for an active immune response involving B cells and Th cells in immunity to *Plasmodium yoelii* passively acquired with antibodies to the 19-kDa carboxyl-terminal fragment of merozoite surface protein-1. J. Immunol. **162:**7309–7314.
17. **Holder, A. A., and R. R. Freeman.** 1984. Characterization of a high molecular weight protective antigen of *Plasmodium yoelii.* Parasitology **88:**211–219.
18. **Holder, A. A., J. S. Sandhu, Y. Hillman, L. S. Davey, S. C. Nicholls, H. Cooper, and M. J. Lockyer.** 1987. Processing of the precursor to the major merozoite surface antigens of *Plasmodium falciparum.* Parasitology **94:**199–208.
19. **Kumar, S., A. Yadava, D. Keister, J. Tian, M. Ohl, K. Perdue-Greenfield, L. Miller, and D. Kaslow.** 1995. Immunogenicity and in vivo efficacy of recombinant *Plasmodium falciparum* merozoite surface protein-1 in Aotus monkeys. Mol. Med. **1:**325–332.
20. **Lewis, A. P.** 1989. Cloning and analysis of the gene encoding the 230-kilodalton merozoite surface antigen of *Plasmodium yoelii.* Mol. Biochem. Parasitol. **36:**271–282.
21. **Ling, I. T., S. A. Ogun, and A. A. Holder.** 1994. Immunization against malaria with a recombinant protein. Parasite Immunol. **16:**63–67.
22. **Ling, I. T., S. A. Ogun, P. Momin, R. L. Richards, N. Garcon, J. Cohen, W. R. Ballou, and A. A. Holder.** 1997. Immunization against the murine malaria parasite *Plasmodium yoelii* using a recombinant protein with adjuvants developed for clinical use. Vaccine **15:**1562–1567.
23. **Majarian, W. R., T. M. Daly, W. P. Weidanz, and C. A. Long.** 1984. Passive immunization against murine malaria with an IgG3 monoclonal antibody. J. Immunol. **132:**3131–3137.
24. **McBride, J. S., and H.-G. Heidrich.** 1987. Fragments of the polymorphic Mr185,000 glycoprotein from the surface of isolated *Plasmodium falciparum* merozoites form an antigenic complex. Mol. Biochem. Parasitol. **23:**71–84.
25. **Peters, W.** 1967. Chemotherapy of *Plasmodium chabaudi* infection in albino mice. Ann. Trop. Med. Parasitol. **61:**52–56.

26. **Riley, E. M., S. Morris-Jones, M. J. Blackman, B. M. Greenwood, and A. A. Holder.** 1993. A longitudinal study of naturally acquired cellular and humoral immune responses to a merozoite surface protein (MSP1) of *Plasmodium falciparum* in an area of seasonal malaria transmission. Parasite Immunol. **15:**513–524.
27. **Rotman, H. L., T. M. Daly, R. Clynes, and C. A. Long.** 1998. Fc receptors are not required for antibody-mediated protection against lethal malaria challenge in a mouse model. J. Immunol. **161:**1908–1912.
28. **Shi, Y., U. Sayed, S. Qari, J. Roberts, V. Udhayakumar, A. Oloo, W. Hawley, D. Kaslow, B. Nahlen, and A. Lal.** 1996. Natural immune response to the C-terminal 19-kilodalton domain of *Plasmodium falciparum* merozoite surface protein 1. Infect. Immun. **64:**2716–2723.
29. **Siddiqui, W. A., L. Q. Tam, K. J. Kramer, G. S. N. Hui, S. E. Case, K. M. Yamaga, S. P. Chang, E. B. T. Chan, and S.-C. Kan.** 1987. Merozoite surface coat precursor protein completely protects Aotus monkeys against *Plasmodium falciparum* malaria. Proc. Nat. Acad. Sci. USA **84:**3014–3018.
30. **Smith, D. B., and K. S. Johnson.** 1988. Single step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. Gene **67:**31–40.
31. **Spencer Valero, L. M., S. A. Ogun, S. L. Fleck, I. T. Ling, T. J. Scott-Finnigan, M. J. Blackman, and A. A. Holder.** 1998. Passive immunization with antibodies against three distinct epitopes on *Plasmodium yoelii* merozoite surface protein 1 suppresses parasitemia. Infect. Immun. **66:**3925–3930.
32. **Stowers, A., V. Cioce, R. Shimp, M. Lawson, G. Hui, O. Muratova, D. Kaslow, R. Robinson, C. Long, and L. Miller.** 2001. Efficacy of two alternate vaccines based on *Plasmodium falciparum* merozoite surface protein 1 in an Aotus challenge trial. Infect. Immun. **69:**1536–1546.
33. **Tian, J., L. Miller, D. Kaslow, J. Ahlers, M. Good, D. Alling, J. Berzofsky, and S. Kumar.** 1996. Genetic regulation of protective immune response in congenic strains of mice vaccinated with a subunit malaria vaccine. J. Immunol. **157:**1176–1183.
34. **Tian, J.-H., S. Kumar, D. C. Kaslow, and L. H. Miller.** 1997. Comparison of protection induced by immunization with recombinant proteins from different regions of merozoite surface protein 1 of *Plasmodium yoelii.* Infect. Immun. **65:**3032–3036.
35. **Tolle, R., K. Fruh, O. Doumbo, O. Koita, M. N′diaye, A. Fischer, K. Dietz, and H. A. Bujard.** 1993. Prospective study of the association between the human humoral immune response to *Plasmodium falciparum* blood stage antigen gp190 and control of malaria infections. Infect. Immun. **61:**40–47.
36. **Udhayakumar, V., D. Anyona, S. Kariuki, Y. P. Shi, P. B. Bloland, O. H. Branch, W. Weiss, B. L. Nahlen, D. C. Kaslow, and A. A. Lal.** 1995. Identification of T and B cell epitopes recognized by humans in the C-terminal 42-kDa domain of the *Plasmodium falciparum* merozoite surface protein (MSP)-1. J. Immunol. **154:**6022–6030.
37. **Uthaipibull, C., B. Aufiero, S. E. H. Syed, B. Hansen, J. A. Guevara Patiño, E. Angov, I. T. Ling, K. Fegeding, W. D. Morgan, C. Ockenhouse, B. Birdsall, J. Feeney, J. A. Lyon, and A. A. Holder.** 2001. Inhibitory and blocking monoclonal antibody epitopes on merozoite surface protein 1 of the malaria parasite *Plasmodium falciparum*. J. Mol. Biol. **307:**1381–1394.
38. **Yoeli, M., B. Hargreaves, R. Carter, and D. Walliker.** 1975. Sudden increase in virulence in a strain of *Plasmodium berghei yoelii.* Ann. Trop. Med. Parasitol. **69:**173–178.

Editor: R. N. Moore