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A Recombinant 15-Kilodalton Carboxyl-Terminal Fragment of *Plasmodium yoelii yoelii* 17XL Merozoite Surface Protein 1 Induces a Protective Immune Response in Mice

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Since the developmental stages of malarial parasites which replicate within erythrocytes are responsible for the morbidity and mortality associated with this disease, antigens produced by these stages have been proposed as candidates for a vaccine. One surface protein of merozoites (MSP-1) has been shown to immunize both rodents and primates against virulent challenge infection in experimental systems. However, little is known of relevant epitopes on the molecule, and attempts to obtain recombinant MSP-1 polypeptides in a native configuration have proven difficult. We have found that the cysteine-rich, carboxyl-terminal region of the MSP-1 protein from the rodent malarial parasite *Plasmodium yoelii yoelii* can be expressed in a native configuration as a fusion protein in *Escherichia coli*. This recombinant polypeptide containing 15 kDa of the predicted 197-kDa protein elicits antibodies in mice which recognize the native parasite MSP-1. Most significantly, both inbred and outbred mice immunized with the fusion protein in Ribi adjuvant are partially and in some cases completely protected against challenge infection with an otherwise lethal parasite strain. This is the first observation of such significant protection obtained with a small portion of the MSP-1 produced in recombinant systems.

In the final phase of malarial parasite maturation within the erythrocyte, a large plasmodial protein which becomes a significant surface protein of merozoites (MSP-1) is synthesized (12). In both primate and rodent models of plasmodial infection, MSP-1 obtained by affinity purification from parasites has been shown to induce immune responses which can partially or completely protect the host against challenge with viable parasites (7–9, 13, 24, 26). This antigen has therefore emerged as a significant candidate for a vaccine directed toward the erythrocytic stages. However, the nature of this immunity and the regions of the MSP-1 protein to which it is directed have not been identified. The latter question is particularly significant in view of the known antigenic variability of MSP-1 obtained from different strains of *Plasmodium falciparum*, the most virulent human malarial parasite (21, 28).

During merozoite maturation and release from erythrocytes, this protein, which ranges in apparent molecular size from 185 to 250 kDa, depending on the plasmodial species, is cleaved into a series of smaller fragments which have been best characterized in the case of *P. falciparum* MSP-1. One of these fragments, a carboxyl-terminal 19-kDa polypeptide, is derived from a larger fragment of 42 kDa and is retained on the merozoite after erythrocyte invasion (1). Interestingly, in all species of plasmodia examined, the carboxyl terminus of the MSP-1 polypeptide contains a series of cysteine residues which are extremely conserved (6), and it has been suggested that these cysteines are arranged in two epidermal growth factor-like domains (2).

Several lines of evidence have pointed to the importance of the carboxyl-terminal portion of the MSP-1 protein in the immunological and biological functions of this molecule. We have previously shown that a monoclonal antibody (MAb 302) which protects mice against challenge infection in passive transfer experiments recognizes a discontinuous, disulfide-dependent epitope in the carboxyl terminus of the MSP-1 protein of the rodent malarial parasite *Plasmodium yoelii yoelii* (3, 4, 20). Both MAbs and polyclonal antibodies directed to reduction-sensitive epitopes in the carboxyl-terminal region of the MSP-1 from the human malarial parasite *P. falciparum* have been shown to inhibit invasion of erythrocytes in vitro (1, 5, 25). A number of investigators have sought to express this portion of the molecule in recombinant systems in order to facilitate immunological and biological studies. Expression of the carboxyl-terminal 42-kDa region of the *P. falciparum* MSP-1 in both bacteria and yeasts produced recombinant polypeptide which could induce only a minimal, humoral reactivity with native protein (5, 14), presumably due in part to the large number of cysteine residues. However, synthesis of this region in the eukaryotic baculovirus expression system produced a recombinant protein with a conformation similar to that of the native *P. falciparum* MSP-1 (5, 22). While extensive serological analyses have been performed with this material, no protection data have been presented.

Here we show that a 15-kDa portion of the carboxyl terminus of *P. yoelii yoelii* MSP-1 can be expressed as a fusion protein in *Escherichia coli* and that the product retains the disulfide-dependent, discontinuous epitope defined by the protective MAb 302. Moreover, when this fusion protein is used to immunize mice, the antibodies elicited recognize the native MSP-1 molecule. Of greatest interest is the finding that the immunized mice are partially or in some cases completely protected against a normally lethal challenge infection with the homologous parasite.

MATERIALS AND METHODS

Experimental animals and parasites. Six- to 8-week-old male BALB/cByJ mice were purchased from Jackson Laboratories and housed in our American Association for the Accreditation of Laboratory Animal Care-approved animal

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facility. Swiss Webster/Br mice of the same age and sex were purchased from Taconic Farms, Inc. The 17XL lethal variant of *P. yoelii yoelii* was originally obtained from John Finerty (National Institutes of Health) and maintained as a cryopreserved stabilate (20).

Expression construct and fusion protein isolation. A 417-bp region of the *P. yoelii yoelii* MSP-1 gene which encodes the C terminus (nucleotides 1579 to 1995 [3] and nucleotides 5044 to 5460 of *P. yoelii yoelii* YM [17]) was amplified by the polymerase chain reaction from *P. yoelii yoelii* 17XL genomic DNA, isolated as previously described (3). The oligonucleotide sequences used for amplification were 5'CCCGAATTCACATAGCCTCAATAGCT (+) and 5'CCC GAATTCTCCCATAAAGCTGGAAG (−). The amplified product was inserted into the *Eco*RI site of the pGEX/2T expression vector, resulting in an in-frame joining of the *Schistosoma japonicum* glutathione *S*-transferase (GST) gene (27) and the *P. yoelii yoelii* MSP-1 3' region. Following transformation of *E. coli* cells (JM 101), the recombinant construct was induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and clones expressing the fusion protein (designated GST-PYC1) were identified by Western blot (immunoblot) analysis with MAb 302 (4). To provide sufficient quantities of the recombinant protein for immunological studies, 1-liter cultures of recombinant bacteria were grown, and the GST-PYC1 fusion protein was then isolated from bacterial lysates by affinity chromatography on glutathione-agarose columns (27). GST alone was isolated as described above from *E. coli* cells transformed with the pGEX/2T vector containing no inserted DNA.

Antibodies. Polyclonal mouse anti-GST antibody was generated by repeated intraperitoneal injection of 50 μg of GST suspended in phosphate-buffered saline (PBS) with Ribi adjuvant (MPL + TDM; Ribi Immunochemical Research Laboratories, Inc.). Serum was taken 2 weeks after the third inoculation. Polyclonal anti-*P. yoelii yoelii* serum (PyHIS) was obtained from mice repeatedly infected with *P. yoelii yoelii* (3). MAb 302 (20) was used directly from hybridoma supernatant culture fluid.

Metabolic radiolabeling of fusion protein and parasites. *E. coli* cells expressing the GST-PYC1 fusion protein were grown in medium containing 100 μCi of [^{35}S]methionine and [^{35}S]cysteine (Tran-^{35}S-Label; ICN) per ml, and synthesis was induced by 1 mM IPTG. After isolation of the cells, labeled proteins were released by mild sonication and cell debris was removed by centrifugation at 12,000 × *g* (27). *P. yoelii yoelii* 17XL-infected erythrocytes were briefly incubated in medium containing [^{35}S]methionine and [^{35}S]cysteine as described previously (20). Labeled parasite proteins were released from cells by the addition of detergents in the presence of proteinase inhibitors, and cell debris was removed by centrifugation at 100,000 × *g* (20). Labeled preparations were stored at −70°C.

Immunoprecipitation and SDS-polyacrylamide gel electrophoresis. Equal volumes of radiolabeled bacterial lysates or parasite proteins were used with 1 μl of each serum sample or 25 μl of MAb 302 culture supernatant fluid. Immunoprecipitations were carried out, and immune complexes were isolated with *Staphylococcus aureus* cells as described previously (20). Labeled proteins were denatured in sodium dodecyl sulfate (SDS) sample buffer (16), resolved on either 7.5 or 10% continuous polyacrylamide gels and detected by fluorography.

PYC1 enzyme-linked immunosorbent assay (ELISA). GST-PYC1 recombinant protein, isolated by affinity chromatography as described above, was cleaved with the proteolytic enzyme thrombin (27). Free GST and uncleaved fusion protein were removed with glutathione-agarose, leaving free PYC1. Protein concentrations were determined by the method of Lowry et al. (18). On the basis of molar percent, equivalent amounts of PYC1, either free or joined to GST, were used to coat wells of microtiter plates. Wells were blocked with 0.5% Tween 20 in Tris-buffered saline, pH 8.0. Twofold serial dilutions of normal mouse serum (NMS) and mouse PyHIS sera were made in 0.1% Tween 20 in Tris-buffered saline, pH 8.0, while fivefold dilutions of MAb 302 culture supernatant fluid were made in culture medium. Antibody dilutions were added to the appropriate wells followed by biotinylated protein A (Zymed), avidin-alkaline phosphatase (Zymed) and *p*-nitrophenyl phosphate (Sigma) substrate (19). Optical density was measured at 405 nm.

Immunization and challenge infection. Eight- to 10-week-old BALB/cByJ and Swiss Webster/Br mice were immunized by subcutaneous injection of 50 μg of GST or 60 μg of GST-PYC1 fusion protein (10 μg of PYC1) administered in 200 μl of Ribi adjuvant suspended in PBS. These mice were subsequently boosted with similar material at 3 weeks (subcutaneously) and at 7 weeks (intraperitoneally) after the initial immunization. Two weeks after the final inoculation, all mice were challenged with the erythrocytic stages of the lethal *P. yoelii yoelii* 17XL parasite. Parasitized erythrocytes were isolated from a donor mouse of the appropriate strain which had been infected with cryopreserved malarial parasites. Following enumeration, 10^4 cells infected with viable *P. yoelii yoelii* 17XL parasites were injected intravenously into each mouse. The course of infection was monitored by microscopic examination of stained-blood films (20).

RESULTS

A 417-bp segment of the *P. yoelii yoelii* MSP-1 gene was amplified by the polymerase chain reaction from *P. yoelii yoelii* 17XL genomic DNA. The amplified region encodes the entire cysteine-rich domain, as well as the 44 amino acids which are amino terminal to the first cysteine, but lacks the hydrophobic anchor sequence (3). This region was previously shown to include the epitope recognized by MAb 302 (3, 4). The amplified fragment was then inserted into the *Eco*RI site of the expression plasmid pGEX-2T (27) to allow synthesis of a fusion protein (designated GST-PYC1) whose amino terminus is composed of the GST protein of *S. japonicum*. Following transformation, recombinant *E. coli* clones were selected by Western blot analysis with MAb 302 (data not shown). A clone which expressed the fusion protein was grown in the presence of [^{35}S]methionine and [^{35}S]cysteine and induced with IPTG to produce radiolabeled GST-PYC1. Recombinant bacteria were lysed by mild sonication, and the lysates were tested by immunoprecipitation for radiolabeled polypeptides which could be recognized by antibodies directed to native *P. yoelii yoelii* MSP-1. Figure 1A demonstrates that antibodies to GST immunoprecipitate a protein of 42 kDa, which is the predicted size of the GST-PYC1 fusion protein. Moreover, the same protein is immunoprecipitated by MAb 302 and by polyclonal antisera (PyHIS) obtained from mice repeatedly infected with *P. yoelii yoelii*. Further studies have demonstrated that when the PYC1 polypeptide is cleaved from the GST by thrombin, it retains the capacity to be recognized by antibodies specific for the *P. yoelii yoelii* MSP-1 carboxyl terminus. In a direct-binding ELISA, serial dilutions of both MAb 302 and PyHIS demonstrated no difference in titer with equivalent

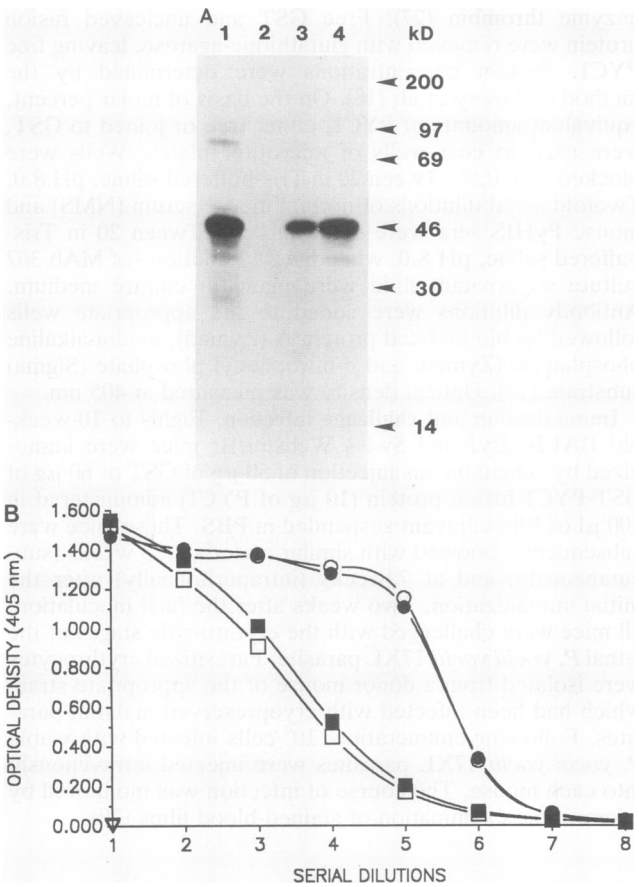

FIG. 1. (A) Immunoprecipitation of radiolabeled GST-PYC1 fusion protein. A 417-bp fragment from the gene encoding the *P. yoelii yoelii* MSP-1 C terminus was inserted in frame with the *S. japonicum* GST gene of the expression vector pGEX/2T. *E. coli* cells transformed with this construct were induced with IPTG in medium containing [^{35}S]methionine and [^{35}S]cysteine, and following release of the labeled GST-PYC1 fusion protein, equal volumes of lysate were used for each precipitation with 1 μl of mouse anti-GST (lane 1), normal mouse serum (NMS) (lane 2), mouse PyHIS (lane 3), and MAb 302 (25 μl of hybridoma culture fluid) (lane 4). Labeled proteins were dissociated with SDS sample buffer and resolved on 10% polyacrylamide gels. (B) Direct-binding ELISA of antibodies specific for the *P. yoelii yoelii* MSP-1 C terminus with PYC1. PYC1 was isolated free of GST by affinity chromatography and subsequent cleavage of the fusion protein with thrombin. Serial dilutions of MAb 302 with equivalent amounts of PYC1 either free (○) or bound to GST (●), and serial dilutions of PyHIS with PYC1 (□) or GST-PYC1 (■) showed no difference in titer. NMS (▽) showed no reactivity to either form of PYC1.

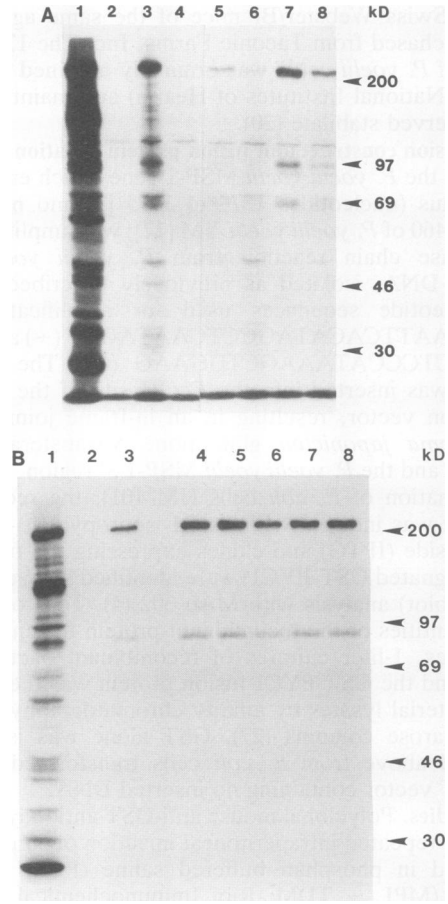

FIG. 2. Immunoprecipitation of radiolabeled *P. yoelii yoelii* proteins with sera from mice immunized with GST-PYC1 fusion protein. *P. yoelii yoelii* 17XL parasites were metabolically labeled with [^{35}S]methionine and [^{35}S]cysteine and solubilized with detergents in the presence of proteinase inhibitors. (A) Precipitations were done with equal aliquots of the labeled proteins and 1 μl of prechallenge sera from four individual BALB/c mice immunized with the fusion protein (lanes 5 to 8). Mouse PyHIS (1 μl; lane 1) and MAb 302 (25 μl of hybridoma culture fluid; lane 3) served as positive control precipitations, while NMS (1 μl; lane 2) and serum from mice immunized with GST (1 μl; lane 4) served as negative controls. Immune complexes were dissociated with SDS sample buffer, and labeled proteins were resolved on 7.5% polyacrylamide gels. (B) Sera from five individual Swiss Webster mice immunized with the fusion protein were used in immunoprecipitations (1 μl; lanes 4 to 8) as described above. Similar controls were done (lanes 1 to 3), with the exception of mouse anti-GST. Labeled proteins were resolved as described above.

amounts of PYC1, either free or bound to GST, as shown in Fig. 1B.

To generate sufficient amounts of recombinant protein for immunologic experiments, larger quantities of bacteria were grown and processed, and the GST-PYC1 fusion protein was then isolated from bacterial lysates by affinity chromatography on glutathione-agarose columns (27). Despite the addition of proteinase inhibitors during isolation, some breakdown of the fusion protein occurred so that free GST was also present in the product. The GST-PYC1 fusion protein was then tested for its capacity to induce antibodies which recognize native malarial antigen. Groups of inbred BALB/cByJ and outbred Swiss Webster/BR mice were immunized three times with the GST-PYC1 fusion protein (equivalent of 10 μg of PYC1) or the corresponding amount of GST, both administered in Ribi adjuvant. Serum was taken from immunized animals 1 week after the third inoculation and was assessed for the presence of antibody which could immunoprecipitate metabolically radiolabeled *P. yoelii yoelii* proteins. Although variable in intensity, the precipitation pattern shown in Fig. 2A demonstrates that all four BALB/c mice immunized with the GST-PYC1 fusion protein have generated antibody specific for a labeled protein with an apparent molecular mass of 230 kDa (lanes 5 to 8). A pool of sera from BALB/c mice immunized with GST did not demonstrate any specific reactivity with labeled proteins

(lane 4). As seen in Fig. 2B, sera from all five Swiss Webster mice immunized with the fusion protein also precipitated a labeled protein with an apparent molecular mass of 230 kDa (lanes 4 to 8) and gave a more uniform pattern of band intensity. Sera from Swiss Webster mice immunized with GST showed no specific reactivity with labeled proteins (data not shown). A labeled protein with an apparent molecular mass of 230 kDa was precipitated by both PyHIS and MAb 302, which served as positive controls (Fig. 2A, lanes 1 and 3, and 2B), while NMS served as a negative control (Fig. 2A and B, lanes 2).

After the serological assessment described above, the same groups of BALB/c and Swiss Webster mice were given a virulent challenge of 10^4 *P. yoelii yoelii* 17XL-parasitized erythrocytes. As shown in Fig. 3A, control BALB/c mice given GST in Ribi adjuvant all developed fulminating infections and died within 10 days. In contrast, three of four BALB/c mice which received the fusion protein were completely or partially protected, as seen in Fig. 3B. One mouse did not exhibit patent parasites when monitored by blood smears for 14 days. To ensure that the animal had been appropriately infected, it was rechallenged with another lethal dose and thereafter showed a very low-grade infection for 4 days which did not exceed 0.1% parasitemia (data not shown). Two other mice displayed intermediate levels of protection, with significant delay in the onset of patent parasitemia. In these animals, the parasitemias peaked at 11 and 14%, and they subsequently resolved these infections with no evidence of recrudescence. One animal displayed little protection and was overcome by the infection, dying by day 11. This pattern of responses was repeated in a larger group of BALB/c mice, with the exception that the group exhibiting intermediate protection displayed higher parasitemias. Five mice in a group of seven were completely or partially protected. Two mice showed no patent parasitemia, which was confirmed on rechallenge. Three mice displayed an intermediate level of protection, and two mice could not control the infection and died. As in the previous experiment, all control animals succumbed to the infection by day 10.

As shown in Fig. 4A, challenge infections in Swiss Webster mice immunized with GST in Ribi adjuvant or adjuvant alone displayed virulence similar to that seen in the BALB/c control mice, with all animals dying by day 9. However, among the Swiss Webster mice immunized with GST-PYC1, four of five were completely or partially protected, as seen in Fig. 4B. Two mice showed no parasites in thorough examination of daily blood smears for 15 days; they were rechallenged with viable parasites and again showed no parasitemia when examined for 17 additional days after rechallenge. Two other animals developed low-grade infections with peak parasitemias of 3 and 6%, and they subsequently resolved these infections. Parasitemia in one mouse was delayed in onset, but the animal eventually succumbed to the infection. Immunization of Swiss Webster mice with twice as much fusion protein (20 μg of PYC1) did not decrease the variability of protection (data not shown).

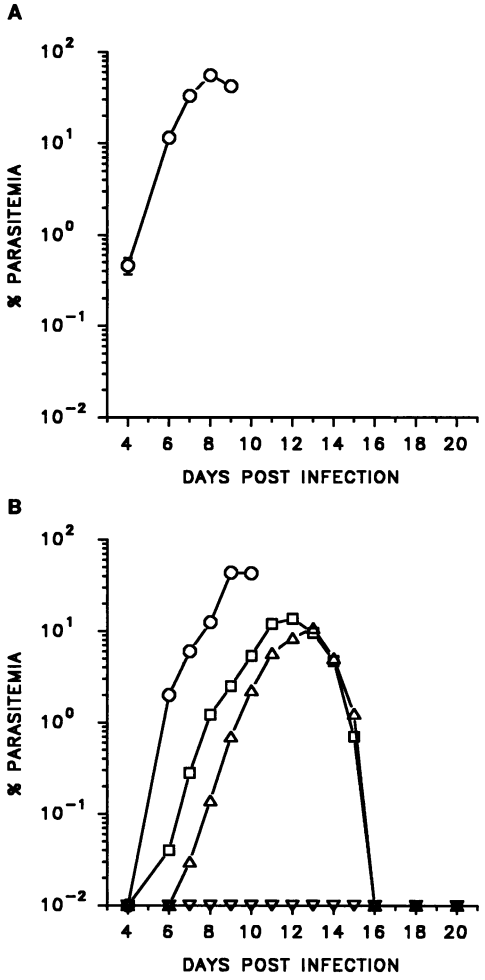

FIG. 3. *P. yoelii yoelii* 17XL challenge infection of BALB/c mice immunized with either GST or the GST-PYC1 fusion protein. BALB/cByJ mice were immunized three times with GST or GST-PYC1 fusion protein (10 μg of PYC1) in Ribi adjuvant suspended in PBS. Following the serological assessment shown in Fig. 2A, all mice were challenged by intravenous injection of 10^4 erythrocytes infected with viable *P. yoelii yoelii* 17XL parasites 2 weeks after the third inoculation. The course of infection was monitored by microscopic examination of stained-blood films. (A) Parasitemia data from the group of mice immunized with GST. All mice in this group died by day 10 following challenge infection. (B) Parasitemia data for four individual mice immunized with the GST-PYC1 fusion protein. The one mouse which exhibited no patent parasitemia was rechallenged, as described above, 14 days after the initial infection. This animal developed a low-grade parasitemia which was patent for 4 days and peaked at 0.1% (data not shown). No period of recrudescence was observed in any of the surviving mice during the 21 days following resolution of infection.

DISCUSSION

These results show that the carboxyl-terminal region of the MSP-1 molecule can be expressed in a prokaryotic system in a manner which allows the formation of a discontinuous, disulfide-dependent epitope recognized by a protective MAb. While other investigators have reported synthesis of the carboxyl-terminal 42-kDa portion of the MSP-1 from *P. falciparum*, attainment of native configuration was best achieved with the eukaryotic baculovirus expression system (5, 22). Clearly, effective expression in prokaryotes provides significant advantages in terms of the relative simplicity of the techniques involved and the costs of producing recombinant protein.

The availability of recombinant material will facilitate both structural and functional analyses of the carboxyl-terminal region of MSP-1. With regard to structure, it has been suggested that the disulfide arrangement in this region may

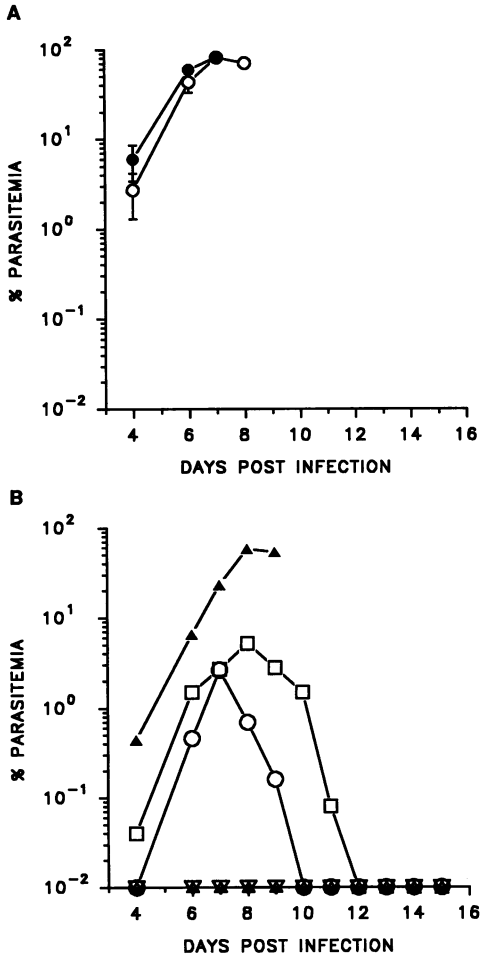

FIG. 4. *P. yoelii yoelii* 17XL challenge infection of Swiss Webster mice immunized with the GST-PYC1 fusion protein. Outbred Swiss Webster/Br mice were immunized, serologically assessed (Fig. 2B), and challenged with *P. yoelii yoelii* 17XL-parasitized erythrocytes as described in the legend for Fig. 3. (A) Parasitemia data from animals immunized with GST in Ribi adjuvant (○) or Ribi adjuvant alone (●). All animals in these groups died by day 9 following challenge infection. (B) Parasitemia data from five individual mice immunized with the GST-PYC1 fusion protein. The two mice exhibiting no patent parasitemia were rechallenged 14 days after the initial infection as described. Neither of these mice developed a patent infection during the ensuing 15 days.

be similar to that of two epidermal growth factor-like domains (2), although the first epidermal growth factor-like domain in the *P. yoelii yoelii* MSP-1 lacks two of the six cysteine residues (2, 6). Sufficient recombinant material can now be generated to examine this suggestion by nuclear magnetic resonance. In the case of *P. falciparum*, this cysteine-rich region has been shown to be part of a proteolytic cleavage fragment with an apparent molecular mass of 19 kDa. This fragment is retained on the merozoite after invasion of the next erythrocyte (1). Such data as well as the capacity of antisera to this region to inhibit invasion in vitro have led to suggestions that the MSP-1 protein is involved in erythrocyte binding and penetration. This concept has been supported by observations of direct binding of *P. falciparum* MSP-1 to erythrocytes (23), and additional functional studies are now possible.

Of greatest interest is the ability of the polypeptide representing only 15 kDa of the 197-kDa *P. yoelii yoelii* MSP-1 protein (on the basis of predicted molecular mass from genomic sequencing [17]) to elicit a significant host protective response. Moreover, mice were immunized with the fusion protein administered in Ribi adjuvant, and protection did not require complete Freund's adjuvant. This contrasts with previous studies by other investigators in which complete Freund's adjuvant has been used to protect *Aotus* monkeys immunized with parasite-derived MSP-1 (7, 9, 24, 26). The antibodies elicited to the GST-PYC1 fusion protein were shown to recognize the native MSP-1 molecule. Since the malarial portion of the antigen alone (PYC1) did not elicit a serological response or protect mice, the GST was required for protection, perhaps because it provided T-cell epitopes. Challenge with malaria parasites would clearly not boost T-cell responses to GST in hosts immunized with GST-PYC1, so that it would be desirable to include selected MSP-1 T-cell epitopes in future constructs.

The most striking observation was that between 70 and 80% of the animals immunized with GST-PYC1 protein appeared to be completely or partially protected. This is the first observation of such significant protection obtained with a small portion of the MSP-1 produced in recombinant systems. Previous attempts in our laboratory to protect mice with portions of the *P. yoelii yoelii* MSP-1 carboxyl terminus produced in recombinant prokaryotes were completely unsuccessful (unpublished results). Other investigators have reported partial protection of primates immunized with recombinant polypeptides derived from several regions of the *P. falciparum* MSP-1 protein, but all the monkeys tested developed patent infections even though the antigen was administered in complete Freund's adjuvant (7, 10, 11, 14). As in the primate studies, the protection seen in both inbred BALB/c and outbred Swiss Webster/Br mice was not complete, since in all experiments some animals developed some level of parasitemia which was subsequently resolved, and a few succumbed to the infection. However, it should be recalled that the challenge infection is extremely virulent, with all control animals developing fulminating infections and dying within 10 days. The level of protection could not be predicted from the immunoprecipitation profiles, in that the animals whose serum produced the most intense bands in immunoprecipitation assays did not necessarily demonstrate the highest degree of protection. Such variability in serological responses could reflect differences in idiotype, isotype, affinity, or concentration of the antibodies induced. Further studies will be required to determine the role of these antibodies in protection in vivo and to establish the mechanism of immunity induced by PYC1. Other investigators have reported that BALB/c mice immunized with affinity-purified *P. yoelii* 230,000-molecular-weight antigen (MSP-1) developed high-titer antibodies specific for this protein and demonstrated a significant degree of protection against homologous challenge infection (8). However, passive transfer of high-titer serum from these resistant animals had no effect on the course of infection in naive recipients. This result led them to conclude that the protective response they observed was cell mediated, although no definite mechanism of immunity was determined.

The carboxyl-terminal, cysteine-rich region of the MSP-1 protein is highly conserved among isolates of both rodent and primate plasmodia (6). Moreover, primates immunized with one allele of the *P. falciparum* MSP-1 are protected against challenge with the other allele, suggesting that protective responses are not strain specific (7). Antibodies

induced to the *P. falciparum* MSP-1 have also been reported to inhibit erythrocyte invasion by *P. falciparum* merozoites of both major MSP-1 alleles (15). Our data indicate that prokaryotic expression systems can produce the carboxyl terminus in an appropriate fashion and that significant protective responses can be induced in both inbred and outbred strains of mice. Given the conservation of this region, such responses may not be specific for particular isolates of *P. falciparum*, and this region of the molecule is therefore an important candidate for inclusion in a human vaccine against the erythrocytic stages of malaria.

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REFERENCES

1. **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-inhibiting antibodies. J. Exp. Med. **172**:379–382.
2. **Blackman, M. J., I. T. Ling, S. C. Nicholls, and A. A. Holder.** 1991. Processing of the *Plasmodium falciparum* merozoite surface protein-1 produces a membrane-bound fragment containing two epidermal growth factor-like domains. Mol. Biochem. Parasitol. **49**:29–34.
3. **Burns, J. M., Jr., T. M. Daly, A. B. Vaidya, and C. A. Long.** 1988. The 3′ portion of the gene for a *Plasmodium yoelii* merozoite surface antigen encodes the epitope recognized by a protective monoclonal antibody. Proc. Natl. Acad. Sci. USA **85**:602–606.
4. **Burns, J. M., Jr., W. R. Majarian, J. F. Young, T. M. Daly, and C. A. Long.** 1989. A protective monoclonal antibody recognizes an epitope in the carboxy-terminal cysteine-rich domain in the precursor of the major merozoite surface antigen of the rodent malarial parasite, *Plasmodium yoelii*. J. Immunol. **143**:2670–2676.
5. **Chang, S. P., H. L. Gibson, C. T. Lee-Ng, P. J. Barr, and G. S. N. Hui.** 1992. A carboxy-terminal fragment of *Plasmodium falciparum* gp195 expressed by a recombinant baculovirus induces antibodies that completely inhibit parasite growth. J. Immunol. **149**:548–555.
6. **Daly, T. M., J. M. Burns, Jr., and C. A. Long.** 1992. Comparison of the carboxy-terminal, cysteine-rich domain of the merozoite surface protein-1 from several strains of *Plasmodium yoelii*. Mol. Biochem. Parasitol. **52**:279–282.
7. **Etlinger, H. M., P. Caspers, H. Matile, H.-J. Schoenfeld, D. Stueber, and B. Takacs.** 1991. Ability of recombinant or native proteins to protect monkeys against heterologous challenge with *Plasmodium falciparum*. Infect. Immun. **59**:3498–3503.
8. **Freeman, R. R., and A. A. Holder.** 1983. Characteristics of the protective response of BALB/c mice immunized with a purified *Plasmodium yoelii* schizont antigen. Clin. Exp. Immunol. **54**: 609–616.
9. **Hall, R., J. Hyde, M. Goman, D. Simmons, I. Hope, M. Mackay, J. Scaife, B. Merkli, R. Richle, and J. Stocker.** 1984. Major surface antigen gene of a human malaria parasite cloned and expressed in bacteria. Nature (London) **311**:379–382.
10. **Herrera, M. A., F. Rosero, S. Herrera, P. Caspers, D. Rotmann, F. Sinigaglia, and U. Certa.** 1992. Protection against malaria in *Aotus* monkeys immunized with a recombinant blood-stage antigen fused to a universal T-cell epitope: correlation of serum gamma interferon levels with protection. Infect. Immun. **60**: 154–158.
11. **Herrera, S., A. Herrera, B. L. Perlaza, Y. Burki, P. Caspers, H. Doebeli, D. Rotmann, and U. Certa.** 1990. Immunization of Aotus monkeys with *Plasmodium falciparum* blood stage recombinant proteins. Proc. Natl. Acad. Sci. USA **87**:4017–4021.
12. **Holder, A. A.** 1988. The precursor to the major merozoite surface antigens: structure and role in immunity. Prog. Allergy **41**:72–97.
13. **Holder, A. A., and R. R. Freeman.** 1981. Immunization against blood-stage rodent malaria using purified parasite antigens. Nature (London) **294**:361–364.
14. **Holder, A. A., R. R. Freeman, and S. C. Nicholls.** 1988. Immunization against *Plasmodium falciparum* with recombinant polypeptides produced in *Escherichia coli*. Parasite Immunol. **10**:607–617.
15. **Hui, G. S., A. Hashimoto, and S. P. Chang.** 1992. Roles of conserved and allelic regions of the major merozoite surface protein (gp195) in immunity against *Plasmodium falciparum*. Infect. Immun. **60**:1422–1433.
16. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
17. **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.
18. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
19. **Majarian, W. M., T. M. Daly, J. M. Burns, Jr., and C. A. Long.** 1988. *Plasmodium yoelii*: characterization of a protective idiotype during malarial infection in mice. Exp. Parasitol. **67**:227–237.
20. **Majarian, W. R., T. M. Daly, W. P. Weidanz, and C. A. Long.** 1984. Passive protection against murine malaria with an IgG3 monoclonal antibody. J. Immunol. **132**:3131–3137.
21. **McBride, J. S., C. I. Newbold, and R. Anand.** 1985. Polymorphism of a high molecular weight schizont antigen of the human malaria parasite *Plasmodium falciparum*. J. Exp. Med. **161**:160–180.
22. **Murphy, V. F., W. C. Rowan, M. J. Page, and A. A. Holder.** 1990. Expression of hybrid malaria antigens in insect cells and their engineering for correct folding and secretion. Parasitology **100**:177–183.
23. **Perkins, M. E., and L. J. Rocco.** 1988. Sialic acid-dependent binding of *Plasmodium falciparum* merozoite surface antigen, Pf200, to human erythrocytes. J. Immunol. **141**:3190–3196.
24. **Perrin, L. H., M. Loche, J.-P. Dedet, C. Roussilhon, and T. Fandeur.** 1984. Immunization against *Plasmodium falciparum* asexual blood stages using soluble antigens. Clin. Exp. Immunol. **56**:67–72.
25. **Pirson, P., and M. Perkins.** 1985. Characterization with monoclonal antibodies of a surface antigen of *Plasmodium falciparum* merozoites. J. Immunol. **134**:1946–1951.
26. **Siddiqui, W. A., L. Q. Tam, K. J. Kramer, G. S. Hui, K. M. Yamaga, S. P. Chang, E. B. Chan, and S. C. Kan.** 1987. Merozoite surface coat precursor completely protects Aotus monkeys against *Plasmodium falciparum* malaria. Proc. Natl. Acad. Sci. USA **84**:3014–3018.
27. **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.
28. **Tanabe, K., M. Mackay, M. Goman, and J. G. Scaife.** 1987. Allelic dimorphism in a surface antigen gene of the malaria parasite *Plasmodium falciparum*. J. Mol. Biol. **195**:273–287.