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Monoclonal Antibodies That Protect In Vivo Against *Plasmodium chabaudi* Recognize a 250,000-Dalton Parasite Polypeptide

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Twenty monoclonal antibodies have been prepared to the erythrocytes from CBA/Ca mice infected with the rodent malaria *Plasmodium chabaudi*. By immunofluorescence, 15 of these antibodies recognized parasite antigens expressed only during the development of mature trophozoites to schizonts and merozoites, 2 recognized parasite antigens that were expressed throughout most of the intraerythrocytic cycle, and 3 recognized the membranes of all infected and uninfected erythrocytes. By immunoprecipitation of [^{35}S]methionine-labeled, parasitized erythrocytes, parasite antigens recognized by all of the antiparasite antibodies were characterized. Eleven precipitated a 250,000-dalton parasite polypeptide which was synthesized and expressed late in the intraerythrocytic cell cycle and which appeared to be the major coat protein of the merozoites. In passive protection experiments, transfer of hyperimmune serum before infection with the parasite resulted in a delay in the rise of parasitemia, reduction in peak parasitemias, and a delay in the clearance of the parasitemia. Two monoclonal antibodies to the 250,000-dalton polypeptide had a similar but not as marked effect on parasitemia when given as a single dose before infection. When mixed and administered throughout the course of infection, their effects were greater. They had no influence on the course of *Plasmodium berghei* KSP11 parasitemia. Monoclonal antibodies to other parasite antigens and normal erythrocyte antigens failed to have a significant and reproducible effect on *P. chabaudi* parasitemia. The results suggest that this 250,000-dalton malaria parasite antigen may be important in the induction and expression of antibody-mediated immunity to malaria.

Monoclonal antibodies generated by the techniques originally described by Köhler and Milstein (15) provide powerful tools for the analysis of complex antigen mixtures and for the study of the role of antibody-mediated mechanisms in immunity to infections. With malaria, antibodies play a part in immunity to the intraerythrocytic stages, since passive transfer of immune serum or immunoglobulin provides some protection against rodent, simian, and human malarias (5, 7, 8, 19). The protective antibodies appear to be directed against parasite-coded proteins (and perhaps altered erythrocyte components) expressed on the surface of schizont-infected erythrocytes or on the surface of merozoites (2, 6, 12). However, the structural and antigenic complexity of the malaria parasite have made it difficult to identify parasite and erythrocyte antigens important in the induction and expression of antibody-mediated immunity to malaria.

Freeman et al. (12) have described monoclonal antibodies to the erythrocytic stages of the rodent malaria *Plasmodium yoelii*. Only those antibodies recognizing antigens exclusive to merozoites confer significant protection in passive transfer experiments. Perrin et al. (18) have developed monoclonal antibodies to *Plasmodium falciparum* schizonts and merozoites; three have an inhibitory effect on the in vitro growth of *P. falciparum*. These antibodies precipitate parasite polypeptides of 41,000 (two clones) and 96,000 and 36,000 (1 clone) daltons, which Perrin et al. suggest are present on the merozoite surface. Monoclonal antibodies to the surface of *Plasmodium knowlesi* merozoites recognizing a 250,000-dalton parasite polypeptide inhibit reinvasion of erythrocytes in vitro (9).

We have shown that the synthesis of many parasite polypeptides by the intraerythrocytic stages of the rodent malaria *Plasmodium cha-*

baudi is restricted to defined morphological stages of the parasite development (16). A 250,000-molecular-weight glycoprotein, synthesized late in the intraerythrocytic cycle, appeared to be expressed on the surface of the developing schizont or merozoite (17). To determine whether this parasite polypeptide is important in the induction and expression of antibody-mediated immunity to malaria, we have prepared monoclonal antibodies to the intraerythrocytic stages of *P. chabaudi*.

MATERIALS AND METHODS

Mice. CBA/Ca and CBA/Ca × BALB/c F1 mice and SD rats were supplied by the SPF Breeding Unit, National Institute for Medical Research.

Parasites. *P. chabaudi* 2722 AS was obtained as a recently mosquito-transmitted and cloned population from D. Walliker (University of Edinburgh, Edinburgh, Scotland). As described previously, the asexual intraerythrocytic development of *P. chabaudi* is a synchronous 24-h cycle (16). A line of *P. berghei* KSP11 which had been cloned was used (3).

Sera. Normal CBA/Ca, hyperimmune CBA/Ca anti-*P. chabaudi*, and rabbit anti-mouse immunoglobulin sera were prepared as previously described (16). Mouse immunoglobulin G (IgG) purified on protein A-Sepharose (10) was coupled to CNBr-activated Sepharose 4B and used as an affinity column to purify rabbit anti-mouse IgG antibodies. These purified antibodies were labeled with ^{125}I by using chloramine T (24), or with fluorescein isothiocyanate (23).

Production of monoclonal antibodies. Clone X63-Ag8.653, a subclone of the BALB/c mouse myeloma P3-X63-Ag8, which does not express immunoglobulin heavy or light chains, was used (14). CBA/Ca male mice which had recovered from a primary infection with 10^5 parasitized erythrocytes (over 30 days after infection) were rechallenged with 10^8 parasitized erythrocytes intraperitoneally 4 days before their spleens were used for fusion. Spleen and myeloma cells were fused with 42% polyethylene glycol 1000 and 8% dimethyl sulfoxide in RPMI 1640 without serum at cell ratios of 10:1, using an adaptation of techniques described by Köhler and Milstein (15). Clones were grown directly in 96-well tissue culture plates on feeder layers of normal mouse peritoneal exudate cells in RPMI 1640–10% fetal calf serum–HAT (hypoxanthine, aminopterin, thymidine) selective medium. Antibody-producing clones were subsequently cloned by limiting dilution on feeder layers. Clones were then grown as ascitic and solid tumors in CBA/Ca × BALB/c F1 mice pretreated with 0.5 ml of pristane (2,6,10,14-tetramethylpentadecane) and inoculated intraperitoneally with 10^6 to 10^7 hybrid cells. Sera and ascitic fluids were collected from these mice and stored at −70°C.

Antibody assays. Culture supernatants were screened for antibodies reacting with parasite, altered erythrocyte, or normal erythrocyte antigens expressed on parasitized or normal erythrocyte cell surfaces, by the techniques of Stocker and Heusser (21). Parasitized erythrocyte suspensions containing 10 to 20% schizonts (obtained by in vitro culture of mature trophozoites for 2 h, or harvested from mice at the time of peak schizogony) were fixed to the wells of plastic plates by using glutaraldehyde. ^{125}I-labeled rabbit anti-mouse IgG was used to detect monoclonal antibodies binding to these fixed schizont preparations.

Culture supernatants were also assayed by indirect immunofluorescence on methanol-fixed smears of washed parasitized erythrocytes, using fluorescein isothiocyanate-labeled rabbit anti-mouse IgG. Monoclonal antibodies recognizing only antigens expressed by late-stage parasites, i.e., schizonts and merozoites, were selected by immunofluorescence screening of culture supernatants on trophozoites collected at 09.00 h and on very late-stage trophozoites, schizonts, and merozoites collected at 14.00 h from mice maintained in the reversed light cycle (16).

The parasite species specificity of *P. chabaudi* monoclonal antibodies was determined by immunofluorescence staining on methanol-fixed smears of *P. berghei* KSP11 grown in CBA/Ca mice and in SD rats with phenyl hydrazine-induced reticulocytosis (3), *P. knowlesi* schizont-infected erythrocytes collected from rhesus monkeys (1), and *P. falciparum* cultured in vitro (20).

The intraerythrocytic stage specificity of the monoclonal antibodies to *P. chabaudi* was determined by immunofluorescence staining of smears prepared from infected mice at 4- to 6-h intervals throughout the 24-h synchronous intraerythrocytic cycle. Antibody titers of monoclonal antibodies to *P. chabaudi* were determined by immunofluorescence staining with twofold dilutions of sera or ascitic fluids on methanol-fixed preparations of *P. chabaudi* schizonts.

Immunoglobulin subclass of monoclonal antibodies. The mouse immunoglobulin subclass of the monoclonal antibodies was determined by gel diffusion tests against goat anti-mouse immunoglobulin subclass-specific antisera (Meloy Laboratories). The IgG subclass was confirmed and the immunoglobulin concentration in ascitic fluids and sera was determined by binding to and elution from protein A-Sepharose 4B (10).

Immunoprecipitation of [^{35}S]methionine-labeled parasite proteins. Parasite antigens recognized by the monoclonal antibodies were characterized by immunoprecipitation of [^{35}S]methionine-labeled parasite proteins with the lysate immunoprecipitation technique as previously described (17). The immunoprecipitates were analyzed by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography (16).

Protection tests in vivo. Sera and ascitic fluids were clarified by centrifugation at 30,000 × *g* and 10°C for 30 min before use. CBA/Ca male mice at 24 to 26 g were inoculated intraperitoneally or intravenously with predetermined doses of parasitized erythrocytes, diluted in 0.2% glucose in Krebs saline plus 20% normal mouse serum. Five or six mice per test group were treated before or after infection with sera or ascitic fluid collected from tumor-bearing mice. To test for synergistic effects between monoclonal antibodies of like specificities or of different specificities, pools were prepared by mixing equal volumes of sera or ascitic fluids. Untreated groups and groups treated with serum and hyperimmune normal serum were used as control. Tail blood smears were prepared daily from mice, fixed with methanol, and stained with Giemsa stain. Parasitemia was determined by microscopic

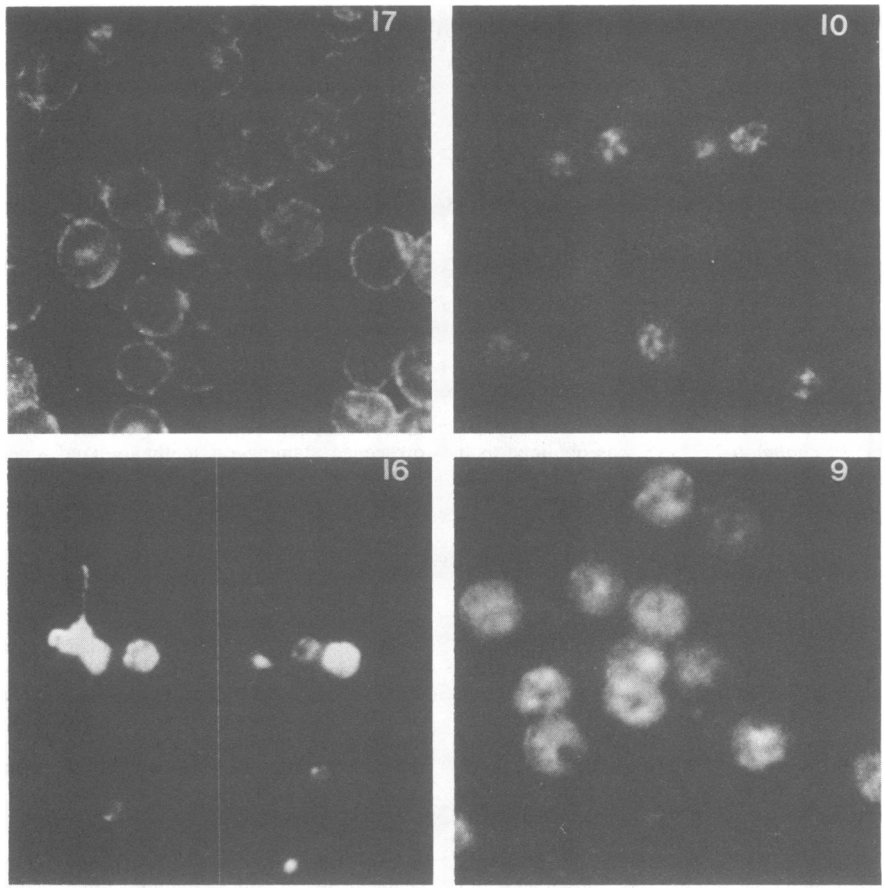

FIG. 1. Indirect immunofluorescence staining of *P. chabaudi* (very late stage trophozoites, schizonts, and merozoites) with monoclonal antibodies: clones 17, 10, 16, and 9. (Magnification, ×10,000).

counts, and groups were compared by using a sign test, as previously described (4). Geometric mean parasitemia is presented as $\log_{10}$ number of parasitized erythrocytes per 10^5 erythrocytes.

RESULTS

Preliminary experiments showed that high frequencies of antibody-producing clones could be generated in response to *P. chabaudi* antigens. Many hundreds of antibody-producing clones could be derived from the spleen of a single CBA/Ca mouse rechallenged once 4 days before the fusion. Four types of clones were recognized by immunofluorescence: clones producing antibodies to all erythrocyte membranes; to the membranes of parasitized erythrocytes; to most, if not all, stages of the parasite; and to specific stages of the parasite (Fig. 1). Twenty clones were recloned at least once, and sera and ascitic fluid were collected from mice bearing tumors (Table 1). Clones 1 to 6 from two independent fusions were originally screened by the surface radioimmunoassay and further characterized by immunofluorescence. Clones 7 to 20 were originally screened by immunofluorescence from a third fusion.

Characterization of monoclonal antibodies. The monoclonal antibodies were classified into seven specificities on the basis of immunofluorescence staining and immunoprecipitation of [^{35}S]methionine-labeled *P. chabaudi* proteins (Table 1, Fig. 2). Three monoclonal antibodies (specificity type G, see Table 1) stained the membranes of all erythrocytes in smears from infected and uninfected mice (clone 17, Fig. 1). All were IgM subclass and failed to immunoprecipitate any [^{35}S]methionine-labeled *P. chabaudi* parasite antigens (Fig. 2). All three cross-reacted to some extent on rat reticulocytes, but only one, clone 19, reacted on rhesus monkey erythrocytes, and none reacted on human erythrocytes.

Of the antiparasite specificities, four (types A, B, C, and D) recognized antigens expressed and

TABLE 1. Characterization of monoclonal antibodies to *P. chabaudi*

Specificity type	No. of clones	Clone identification	Specificity by immunofluorescence	Immunoglobulin subclasses	Antigens recognized (mol wt × 10^3)
A	11	1, 2, 3, 4, 8, 12, 14, 15[a], 16, 18, 20	Very late stage trophozoites, schizonts, and merozoites	M, G_1, G_{2a}, G_{2b}	250
B	2	5, 10	Very late stage trophozoites, schizonts, and merozoites	G_1, G_{2a}	155, 170
C	1	11	Very late stage trophozoites, schizonts, and merozoites	G_1	76
D	1	13	Very late stage trophozoites, schizonts, and merozoites (and membranes of parasitized erythrocytes)	G_1	92
E	1	7	All parasite stages	G_{2b}	15.5, 17
F	1	9	Membranes of parasitized erythrocytes; parasites (all stages)	M	105
G	3	6, 17, 19	All erythrocytes, infected and uninfected	M	

[a] Clones 14 and 15 are subclones from the same original clone.

synthesized only during the development of mature trophozoites to schizonts and merozoites. The late-stage synthesis of these polypeptides was confirmed by the fact that the monoclonal antibodies failed to immunoprecipitate parasite antigens from [^{35}S]methionine-labeled midstage trophozoites (Fig. 2).

Eleven monoclonal antibodies (specificity type A) recognized a 250,000-dalton parasite polypeptide (Fig. 2), which was only expressed and synthesized during the development of mature trophozoites to schizonts and merozoites. It was lost during merozoite reinvasion, as the antibodies failed to stain ring-stage parasites in the immunofluorescence assay. From the intensity of the immunofluorescence and from [^{35}S]methionine incorporation, this polypeptide was a major antigen synthesized during schizogony. By immunofluorescence staining of fixed, parasitized erythrocytes this antigen appeared to be located on the developing merozoite membrane within the schizont and on the surface of merozoites released from schizonts (Fig. 1, clone 16). Additional evidence presented elsewhere (17) shows that this polypeptide is accessible to antibody in undisrupted schizont and merozoite preparations, suggesting that it may be the coat protein of the merozoite.

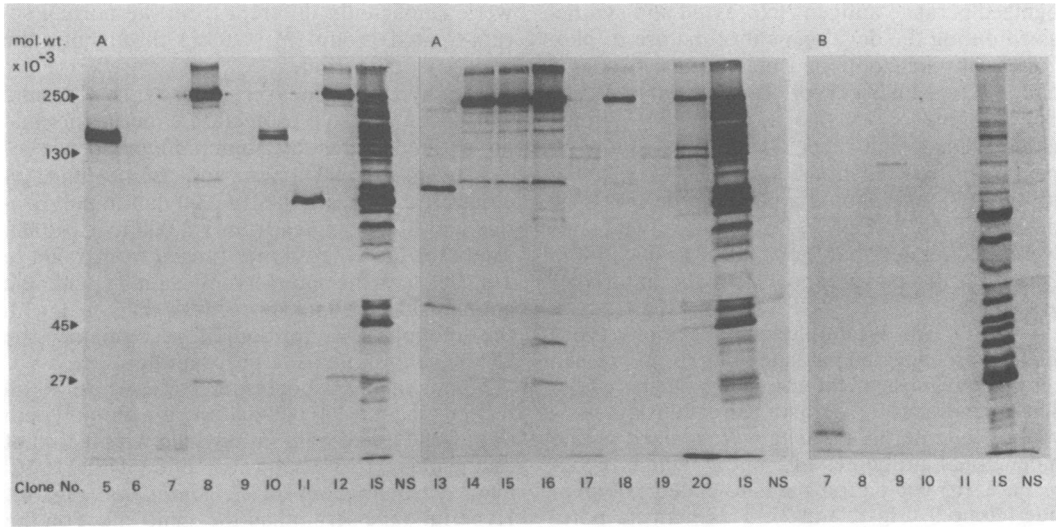

FIG. 2. Immunoprecipitation of [^{35}S]methionine-labeled *P. chabaudi* antigens with monoclonal antibodies. (A) Very late stage trophozoites, schizonts, and merozoites; (B) midstage trophozoites. IS, immune serum; NS, normal serum.

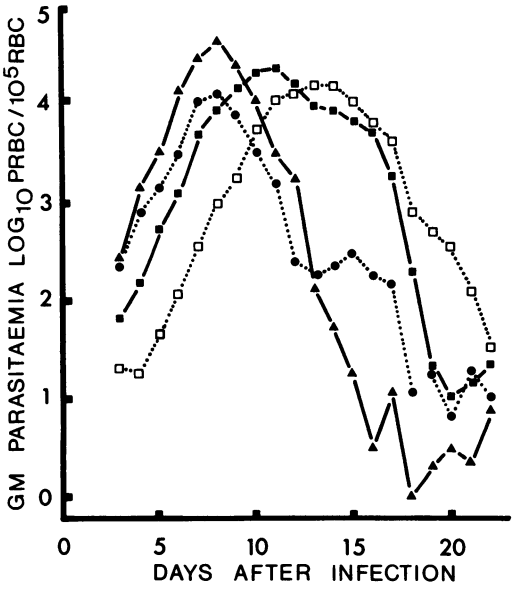

FIG. 3. Passive protection test with hyperimmune serum to *P. chabaudi*. Serum (0.5 ml per mouse) was given intravenously 4 days before (■), the same day (□), or 4 days after (●) the mice (24 to 26 g, CBA/Ca males) were each infected intravenously with 2×10^5 parasitized erythrocytes. Control mice received normal serum (▲) intravenously on the same day as the parasite.

The intensity of immunofluorescent staining with monoclonal antibodies of specificity types B, C, and D was much less than with the antibodies of specificity type A (Fig. 1, clone 10). On the basis of immunofluorescence and immunoprecipitation, all of these antibodies recognized parasite antigens expressed and synthesized during the development of mature trophozoites into schizonts and merozoites. Clones 5 and 10 (specificity type B) precipitated two polypeptides of 150,000 and 170,000 daltons, clone 11 (specificity type C) precipitated a polypeptide of 76,000 daltons, and clone 13 (specificity type D) precipitated a polypeptide of 92,000 daltons (Table 1, Fig. 2). For clone 5, two bands were precipitated, but they appear as one in Fig. 2 as a result of overexposure of the autoradiograph.

Clones 7 and 9 (antiparasite specificity types E and F) recognized parasite antigens expressed throughout most of the intraerythrocytic cycle. The membranes of parasitized erythrocytes as well as the internal parasite were stained by the monoclonal antibodies of clone 9, which recognized a 105,000-dalton parasite-coded polypeptide (clone 9, Fig. 1 and 2). This parasite polypeptide appeared to be synthesized to a maximum extent by midstage trophozoites; however, it may have been synthesized to some extent earlier, since the membranes of ring-stage-infected erythrocytes were also stained faintly. Clone 7 recognized two low-molecular-weight polypeptides (17,000 and 15,500) which were synthesized to some extent by both midstage trophozoites and late-stage parasites (Fig. 2). These antigens were expressed by all stages of the intraerythrocytic cycle, as determined by immunofluorescence staining.

None of the antiparasite antibodies cross-reacted with *P. berghei* grown in mice or rats, when tested by immunofluorescence. Clones 9, 19, and 20 cross-reacted on *P. knowlesi* and clone 9 cross-reacted on *P. falciparum*; however, the pattern of staining was similar in all cases and different from that observed on *P. chabaudi*, suggesting that the staining may have been nonspecific. All these antibodies were of the IgM subclass.

Passive protection tests with hyperimmune serum and monoclonal antibodies. To determine the extent to which passively transferred antibodies might influence the course of *P. chabaudi* parasitemia, mice were treated with hyperimmune serum (0.5 ml per mouse) 4 days before, a few hours before, or 4 days after infection with 2×10^5 parasitized erythrocytes (Fig. 3). Hyperimmune serum given 4 days or a few hours before infection produced a marked delay in the rise of parasitemia, a reduction in peak parasitemia, and a significant delay in the clearance of the parasitemia. If given on day 4 after infection, when the parasitemia had reached approximately 1%, the immune serum reduced the rate of increase in parasitemia, decreased peak parasitemia, but did not have as marked an effect on the rate of clearance of the parasitemia. All groups were significantly different from the normal serum-treated group ($P < 0.01$) throughout the experimental period.

The hyperimmune serum used in this and subsequent passive protection experiments had an antibody titer by immunofluorescence of 1:8,000 to 1:16,000. Sera and ascitic fluids of clones reacting with the 250,000-dalton polypeptide had titers ranging from 1:8,000 to 1:64,000. Titers for the other clones ranged from 1:500 to 1:8,000, and the intensity of staining with the antiparasite antibodies was less than that with the monoclonal antibodies recognizing the 250,000-dalton parasite polypeptide.

Monoclonal antibodies of clones 1 to 4, all recognizing the 250,000-dalton parasite polypeptide, were tested by treating mice with 0.5 ml of ascitic fluid 2 to 3 h before infection with 2×10^5 mature trophozoites of *P. chabaudi* (Fig. 4). Hyperimmune serum had the same effect on the parasitemia as before. Clones 2 and 4 had no significant effect on the course of the infection. The parasitemias were the same as in animals

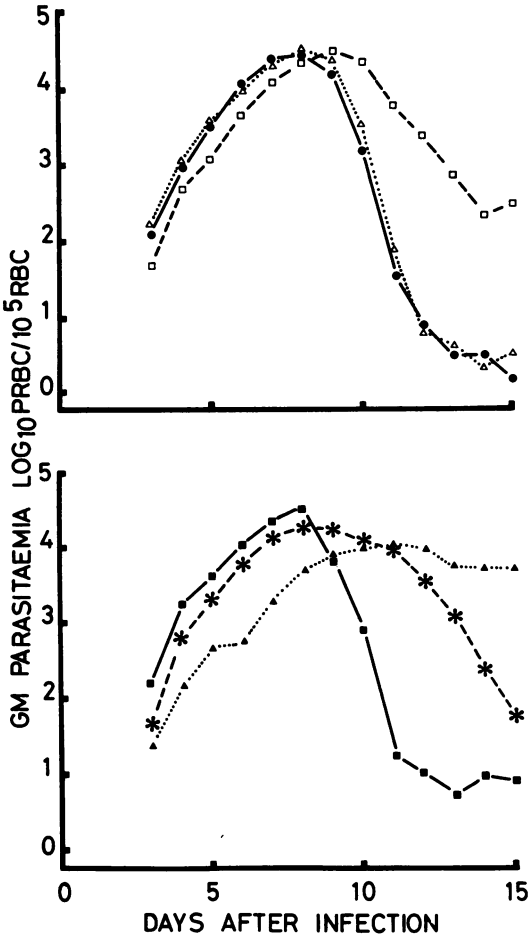

FIG. 4. Passive protection test with monoclonal antibodies to *P. chabaudi*. Doses (0.5 ml) of serum or ascitic fluid were given intraperitoneally to mice 2 to 3 h before intraperitoneal infection with 2×10^5 mature trophozoites. CBA/Ca male mice (24 to 26 g) were maintained on the reversed light cycle. Ascitic fluid of clones 1 (*), 2 (△), 3 (□), and 4 (●); normal serum (■); hyperimmune serum (▲).

treated with normal serum. Clones 1 and 3 had a significant effect ($P < 0.05$) on the parasitemia; clone 1 delayed the rise of parasitemia, reduced the peak parasitemia, and delayed the clearance of the parasitemia. Clone 3 had a similar effect, but the peak parasitemias were not significantly reduced. There appeared to be no relationship between immunoglobulin subclasses and the effect on parasitemia since clones 1 and 4 and clones 2 and 3 were IgG_{2a} and IgG_1, respectively (Table 2).

In four experiments, for which the data are not presented, ascitic fluids or sera of clones 7, 8, 9, 10, 11, and 13 alone, clones 5 and 10, and the antierythrocyte clones 6, 17, and 19 in pools were tested by treatment of mice either before or after infection. The mice were infected either intravenously or intraperitoneally and treated by the opposite route with the monoclonal antibodies. None of the antibodies had significant effects on the course of *P. chabaudi* parasitemia. In these same experiments, clones 1 and 3, alone or in pools with the other clones recognizing the 250,000-dalton parasite polypeptide, produced small but significant and reproducible effects on the course of the parasitemia. When given 1 day after infection or when the parasitemia had reached 0.05 to 0.1%, they slowed the rate of increase in parasitemia and reduced the peak percentage of parasitemia by one-third to one-half compared to peaks reached in normal serum-treated controls.

In a final experiment, mice infected with 1.7×10^5 *P. chabaudi*-parasitized erythrocytes were treated on days −1, 0, 1, 3, 5, 7, and 9 with pools of ascitic fluid of clones 1 and 3, clones 2 and 4, normal serum, or hyperimmune serum (0.25 ml per mouse per day). The immunoglobulin concentrations of the ascitic fluids and sera and the antibody titers by immunofluorescence are summarized in Table 2. To control for the possibility that the ascitic fluids contained nonspecific factors toxic for plasmodia, the pool of clones 1 and 3 and the normal serum were tested with the same treatment protocol in mice infected with 10^5 *P. berghei*-parasitized erythrocytes.

As compared with normal serum treatment, the pool of ascitic fluids of clones 1 and 3 had no significant effect on the course of *P. berghei* parasitemia (Fig. 5A). The apparent enhance-

TABLE 2. Passive protection test with monoclonal antibodies: immunoglobulin concentrations and antibody titers of ascitic fluids and sera

Serum or ascitic fluid	IgG subclass	Antibody titer by immunofluorescence	IgG concentration (mg/ml)	Dose per mouse per day (mg)
Clone 1	IgG_{2a}	8,000	4.9	0.6
Clone 2	IgG_1	16,000	7.6	0.95
Clone 3	IgG_1	8,000	3.8	0.48
Clone 4	IgG_{2a}	32,000	8.5	1.1
Hyperimmune	IgG	16,000	15.0	3.3
Normal	IgG	<100	3.3	0.83

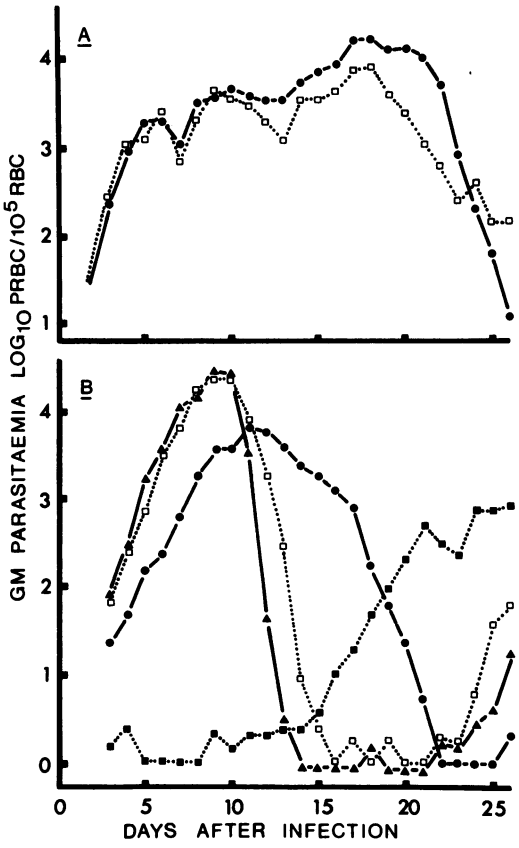

FIG. 5. Passive protection test with monoclonal antibodies to *P. chabaudi*. CBA/Ca mice (24 to 26 g) were infected with (A) *P. berghei* (10^5 parasitized erythrocytes) or (B) *P. chabaudi* (1.7×10^5 parasitized erythrocytes) intravenously. Mice received intraperitoneally, on days −1, 0, 1, 3, 5, 7, and 9, 0.25 ml of ascitic fluid of clones 1 + 3 (●) or 2 + 4 (▲), normal serum (□), or hyperimmune serum (■).

ment of parasitemia in the monoclonal antibody-treated group after day 15 was not significant since there was a very large spread of parasitemia values in the individual animals after this time.

The parasitemia in mice infected with *P. chabaudi* and treated with the pool of clones 2 and 4 was not significantly different from that in normal serum-treated mice (Fig. 5B). Continuous administration of hyperimmune serum had a very significant effect on the development of the parasitemia, with most mice having subpatent parasitemia throughout the period of administration of the serum. Nevertheless, the mice failed to clear the parasites during this time. When the administration of the hyperimmune serum was discontinued on day 9, all mice developed parasitemia by days 15 to 20. Although clones 1 and 3 had IgG concentrations that were half those of clones 2 and 4, and the antibody titers were lower (Table 2), the pool of clones 1 and 3 had a highly significant effect ($P < 0.001$) on the course of parasitemia, delaying the development of parasitemia by 3 to 4 days and reducing the peak geometric mean parasitemia from 23 and 29% in normal serum and clone 2- and 4-treated groups to 7% in the group treated with clones 1 and 3 (Fig 5A). There was also a significant delay in the clearance of the parasitemia similar to that seen when hyperimmune serum was given as a single dose before infection (Fig. 3).

DISCUSSION

With this rodent malaria model it was easy to generate high frequencies of monoclonal antibodies to parasite and erythrocyte antigens. The problem was to select clones having useful or interesting biological activities. We have shown that polypeptide synthesis by the intraerythrocytic stages of *P. chabaudi* is stage specific and that a 250,000-dalton glycoprotein, synthesized only during schizogony, is exposed on the surface of schizonts and merozoites (16, 17). For this reason we selected clones binding to the surface of glutaraldehyde-fixed schizonts and merozoite preparations and clones which by immunofluorescence recognized antigens expressed and synthesized during the late stages of the intraerythrocytic development. The immunofluorescence assay on methanol-fixed, parasitized erythrocytes provided some information about the structural location of antigens recognized by the monoclonal antibodies; however, it did not show unequivocally whether these antigens were exposed at the surface of schizonts or merozoites.

The high proportion of monoclonal antibodies (11/20) recognizing the 250,000-dalton polypeptide is a reflection of the selection made for clones recognizing antigens expressed and synthesized only during schizogony. It is a major polypeptide synthesized at this time (16). A polypeptide of the same molecular weight is also synthesized by *P. berghei* (17) and by *P. knowlesi* (9; Boyle, unpublished data). We have not fully characterized the structural location of this antigen. However, from our observation that it is accessible to antibody on undisrupted preparations of schizonts and merozoites (17), and from its immunofluorescence staining pattern with the monoclonal antibodies (i.e., it appeared to be on the surface of budding merozoites in schizonts and on the surface of merozoites released from schizonts), we suggest that it is the major coat protein of the merozoite. This is in agreement with the results of Epstein et al. (9), who have shown that monoclonal antibodies to the surface of *P. knowlesi* merozoites recognize a 250,000-dalton parasite polypeptide.

The other monoclonal antibodies provide some interesting information about the basic biology of the erythrocyte-parasite relationship. Antibodies of clone 9 recognized a parasite-coded polypeptide of 105,000 daltons which by immunofluorescence staining appeared to be associated with the membrane of infected erythrocytes. We have not determined whether this polypeptide is exposed on the surface of infected erythrocytes. It may play a role in the changes observed in erythrocyte membrane transport which occur in *Plasmodia*-infected erythrocytes (B. C. Elford, personal communication). Possible functional activities for the polypeptides recognized by the other monoclonal antibodies are not clear. Several appear, on the basis of immunofluorescence staining, to be internal parasite components. Recognition of more than one polypeptide by some clones (e.g., specificity type B) may indicate processing of polypeptides after synthesis. Confirmation of the exact location of the antigens recognized by these monoclonal antibodies awaits immune electron microscope studies.

The high degree of species specificity with these monoclonal antibodies to *P. chabaudi* contrasts with those prepared by Taylor et al. (22) against *P. yoelii*, many of which cross-react with a wide range of *Plasmodia* species. Perrin et al. (18) also observed some cross-reaction between monoclonal antibodies to *P. berghei* and *P. falciparum*, one of which, raised against *P. berghei*, inhibits *P. falciparum* growth in vitro. By immunoprecipitation with hyperimmune sera we have shown that the 250,000-dalton parasite polypeptide has a large species-specific component, with minor cross-reacting determinants when *P. chabaudi* is compared with *P. berghei* (17). Cross-reactive determinants on this and other parasite antigens appear to be of little importance in antibody-mediated immunity, since there is little or no cross-protection between *P. chabaudi* and *P. berghei* in passive serum transfer experiments (W. Jarra, personal communication). The monoclonal antibodies to the 250,000-dalton *P. chabaudi* polypeptide confirm this species specificity; none cross-reacted on the other *Plasmodia* species.

The ability of passively transferred hyperimmune serum to alter the course of malaria parasite infections depends very much upon the host-parasite system under study. With the rodent malaria model, *P. yoelii* 17X in BALB/c mice, continuous administration of single-infection immune serum or a single dose of hyperimmune serum is able to prevent the development of an infection, or to eliminate an established infection (11). On the other hand, with *P. berghei* (8, 19) and in the data presented here with *P. chabaudi*, a single dose of hyperimmune serum or purified immunoglobulins significantly delays the onset of patent parasitemia, but fails to prevent its development. Even when administered throughout the infection, hyperimmune serum to *P. chabaudi* failed to prevent the development of parasitemia when the administration of the serum was discontinued.

The development of parasitemia in mice treated with hyperimmune serum or monoclonal antibodies could be explained by antigenic variation or mutation giving rise to a parasite population resistant to the transferred antibodies. We have not shown, however, whether these recrudescent populations are antigenically different from the inoculated population. Both the hyperimmune serum and the protective monoclonal antibodies produced a significant delay in parasite clearance, perhaps as a result of modulation or suppression of the developing immune response by the high levels of administered IgG, although there may be other possible explanations.

Two monoclonal antibodies recognizing a 250,000-dalton parasite polypeptide had significant and reproducible effects on the course of *P. chabaudi* parasitemia. When these antibodies were mixed and administered throughout the course of the infection, their effects were greater than those produced when they were given as a single dose near the time of infection. These effects cannot be attributed to nonspecific factors in the ascitic fluids toxic for plasmodia, since these clones failed to stain *P. berghei* by immunofluorescence and the ascitic fluids failed to have any effect on the course of *P. berghei* infections. Furthermore, we tested several different batches of ascitic fluids and sera from mice bearing tumors of these two clones; they consistently produced an effect on parasitemia, whereas different batches of ascitic fluids of the other clones failed to produce any effects.

The protection provided by these two clones did not appear to be related to immunoglobulin subclass since two other clones identifying the same antigen and of the same immunoglobulin subclasses failed to have any effects on the parasitemia. There may be functionally important regions on this 250,000-dalton parasite polypeptide, since two monoclonal antibodies had a significant effect in vivo whereas two others did not. These monoclonal antibodies will enable us to explore this possibility.

Epstein et al. (9) have reported that monoclonal antibodies to a 250,000-dalton polypeptide on the surface of *P. knowlesi* merozoites inhibit reinvasion in vitro. This polypeptide appears to be equivalent to that reported here and elsewhere with *P. chabaudi* and *P. berghei* (16, 17). Here we have shown that monoclonal antibodies to the merozoite coat protein have a significant effect in vivo on the course of parasitemia.

Monoclonal antibodies protecting in vivo against *P. yoelii* recognize similar but lower-molecular-weight polypeptides. When purified, these antigens provide significant protection in immunization experiments (13). That other parasite antigens may be of importance cannot be excluded since we have tested monoclonal antibodies to only six parasite antigens. Perrin et al. (18) have shown that monoclonal antibodies to other parasite antigens of *P. falciparum* inhibit growth in vitro.

Our results reported here and elsewhere suggest that this 250,000-dalton parasite-coded polypeptide, which appears to be the surface coat protein of the merozoite, is important in the induction and expression of antibody-mediated immunity to the intraerythrocytic stages of malaria.

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