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Use of a Two-Sited Monoclonal Antibody Assay to Detect a Heat-Stable Malarial Antigen in the Sera of Mice Infected with *Plasmodium yoelii*

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Antigens, circulating in the blood during malarial infections, have been implicated in immune protection, immunosuppression, and immune-complex formation. We used a monoclonal antibody (MAb 7H8) to identify an antigen (Ag-7H8) in the sera of mice infected with *Plasmodium yoelii*. The major form of the antigen has a molecular weight of ~120,000 in *P. yoelii*, with minor components of 220,000; 65,000 to 75,000; and 45,000. Ag-7H8 remains antigenic after boiling for 5 min. A two-sited assay was developed with MAb 7H8 that demonstrated that the Ag-7H8 has at least two similar epitopes per molecule. The two-sited assay was used to follow Ag-7H8 in the blood of mice during lethal (strain 17XL) and nonlethal (strain 17XNL) *P. yoelii* infections. Ag-7H8 appeared on days 6 and 7 after infection with 10^6 and 10^4 17XL *P. yoelii* parasites, respectively, and remained until the animals died. It was in plasma samples between days 6 and 14 after 17XNL *P. yoelii* injections in several inbred strains of mice, regardless of the course of parasitemia. Thus, the kinetics of antigenemia correspond with early stages of infection and not with the number of circulating parasites. Indirect immunofluorescence assays demonstrated that MAb 7H8 detects a cross-reactive antigen in other malarial parasites, including *Plasmodium berghei* and *Plasmodium falciparum*. Thus, this two-sited assay may have general application for the serodiagnosis of malaria and may be beneficial in determining the relationship of circulating antigens to malarial immunity.

Malarial antigens appear in the serum of humans infected with *Plasmodium falciparum* (9). The antigens may be secreted from infected erythrocytes or released during erythrocyte rupture and reinvasion (17). Some circulating malarial antigens are biochemically stable to heating in boiling water for 5 min and are termed heat-stable (S) antigens (16). S antigens from various isolates of *P. falciparum* show substantial antigenic diversity (1, 3, 15). The immunologic importance of S antigens is unclear, but they have been implicated in both immune protection (2, 9, 10; A. Saul, J. Cooper, L. Ingram, R. F. Anders, and G. V. Brown, Parasite Immunol., in press) and immune suppression (8, 16). In addition, circulating antigens may form immune complexes and may thus play an important role in the pathogenesis of malaria, especially glomerulonephritis (5). The role of S antigens is currently being studied by numerous investigators.

The presence and importance of circulating antigens similar to those in *P. falciparum* infections have not been studied extensively for rodent malarias. In this study we identify and characterize an antigen of *Plasmodium yoelii* that has several characteristics of S antigens (i.e., presence in sera and thermostability). Using a monoclonal antibody (MAb) to this antigen, we developed a two-sited assay for detecting it in the sera of infected mice and then followed the kinetics of antigenemia during lethal and nonlethal *P. yoelii* infections. Results showed that the antigen is present during the initial stages of infection regardless of the course of parasitemia.

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MATERIALS AND METHODS

Strains of mice and parasites. In the majority of the studies, 6- to 8-week-old BALB/c mice (Cumberland View Farms, Clinton, Tenn.) were injected intraperitoneally (i.p.) with specified numbers of either the 17XL (lethal) or 17XNL (nonlethal) strain of *P. yoelii*. Parasitemias were monitored every other day from Giemsa-stained tail blood smears. In studies comparing the kinetics of parasitemia and antigenemia, mice of the following strains were injected i.p. with 10^6 17XNL *P. yoelii* parasites: AKR/J (Jackson Laboratory, Bar Harbor, Maine), C57BL/6Boy and C57BL/6 ($H\text{-}2^k$) (NIAID breeding contract; Bioqual Inc., Rockville, Md.). Parasites used in indirect immunofluorescence (IIF) studies included *Plasmodium berghei* (NYU/2) and *P. falciparum* (Malayan Camp strain) from *Aotus* monkeys.

Production of MAbs. Details of the procedures for the production of anti-*P. yoelii* MAbs have been described previously (11). The hybridoma reported here was produced by infecting BALB/c mice with 10^4 17 XL *P. yoelii* parasites. When the parasitemias reached ~54% 9 days later, mice were killed, and a suspension of spleen cells was prepared and fused with the P3-X63-NS/1 cell line. Hybrids were selected in a hypoxanthine-aminopterin-thymidine medium and screened by IIF with acetone-fixed smears of *P. yoelii* (14) and by radioimmunoassay as previously described (12) with extracts of 17XL and 17XNL parasites. The hybrid cell line (7H8) used in this study was cloned twice by limiting dilution. Ascites was produced in pristane-primed mice. The isotype of the hybrid was determined by polyethylene glycol Ouchterlony analysis, immunoelectrophoresis, and an isotype-specific radioimmunoassay as previously described (12).

Molecular weight of Ag-7H8. *P. yoelii* parasites of the

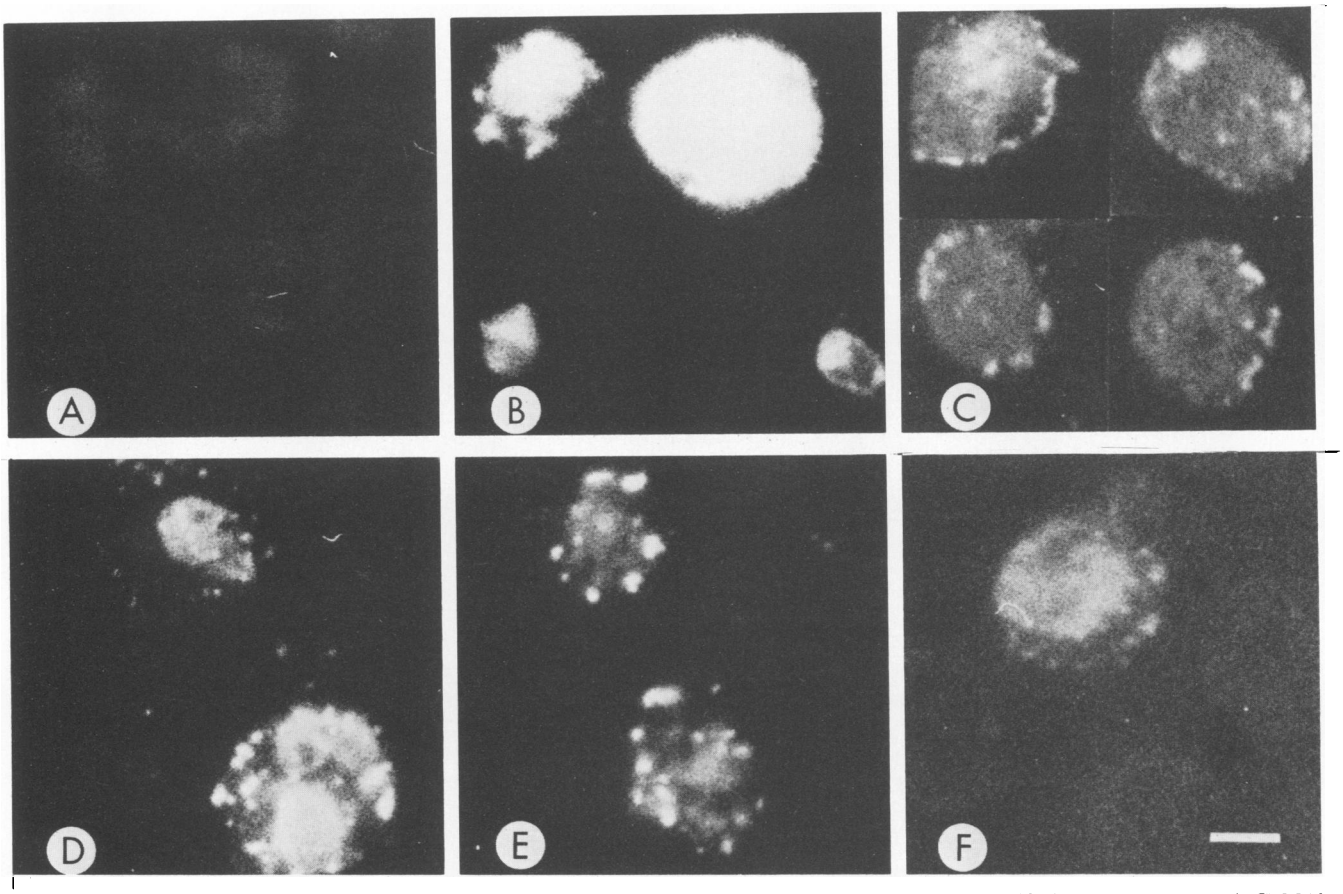

FIG. 1. IIF patterns. *P. yoelii* (17XL) parasites were treated with (A) normal mouse serum, (B) polyspecific immune serum, and (C) MAb 7H8. MAb 7H8 produced a similar pattern of fluorescence when assayed on (D) 17XNL *P. yoelii*, (E) *P. berghei*, and (F) *P. falciparum*. Bar, 3 μm.

17XL strain were cultured in vitro for 14 h in Selectamine (GIBCO Laboratories, Grand Island, N.Y.) in the presence of 1 μCi of [^{35}S]methionine as previously described (7). Cells were harvested, washed three times with saline, and solubilized with 0.5% Nonidet P-40 in NET buffer (0.15 M NaCl, 2 mM EDTA, 0.5 M Tris; pH 7.4). Aliquots of labeled parasite proteins were adsorbed with protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, N.J.) and then incubated for 30 min with 5 μl of (i) normal mouse serum; (ii) immune mouse serum; (iii) ascites from the MAb 8B11, known to react with a protein of 230,000 M_r in *P. yoelii*; and (iv) MAb 7H8. Next, 50 μl of a 1:20 dilution of rabbit anti-mouse mu serum was added and incubated for 30 min at room temperature. Finally, 50 μl of a 50% slurry of protein A-Sepharose in NET buffer was added and the mixture incubated for 45 min. Antigen-antibody-protein-A-Sepharose complexes were washed extensively, solubilized in sodium dodecyl sulfate (SDS)-sample buffer (nonreducing), and electrophoresed on a 10% slab SDS-polyacryl-amide gel (SDS-PAG) by the method of Laemmli (6). Gels were treated with En-^{3}Hance (New England Nuclear Corp., Boston, Mass.), dried, and processed for autoradiography with X-Omat R film (Eastman Kodak Co., Rochester, N.Y.).

Molecular weight determinations were also made by Western blot analysis as previously described (R. J. Howard, S. B. Aley, J. H. Leech, A. M. Lew, T. E. Wellems, K. Marsh, S. Uni, M. Aikawa, J. Rener, and D. W. Taylor, J. Cell Biol., in press). Extracts of 17XL *P. yoelii*, prepared by freeze-thawing Percoll-enriched, *P. yoelii*-infected erythrocytes, were incubated for 10 min at 37°C in 5% SDS–5% 2-mercaptoethanol and then electrophoresed on a 5 to 15% SDS-PAG according to Laemmli (6). Proteins were transblotted onto nitrocellulose paper (13) overnight in a Hoefer apparatus (Hoefer Scientific Instruments, San Francisco, Calif.) at 30 V with Tris-glycine buffer plus 20% methanol. Nitrocellulose strips were then incubated successively with 2 ml of 0.3% Tween 20 plus 0.3% bovine serum albumin (BSA) in NET buffer, a 1:100 dilution of MAbs, a 1:1,000 dilution of rabbit anti-mouse immunoglobulin M (IgM), and ^{125}I-protein A (~ 30,000 cpm/ml; ICN Pharmaceuticals Inc., Irvine, Calif.). Strips were dried on gel bond and autoradiographed as described above. High- and low-molecular-weight standards (Bio-Rad Laboratories, Richmond, Calif.), were used throughout.

Antigen detection assay. A two-sited assay was developed for detecting Ag-7H8 in the sera of infected mice. This assay consists of four major steps: (i) binding of partially purified MAb 7H8 to microtiter wells, (ii) adding either parasite extract or plasma samples containing soluble antigens, (iii) adding alkaline-phosphatase-labeled MAb 7H8, and finally (iv) adding a substrate and recording the subsequent amount of color development. Initially, MAb 7H8 was partially purified by a 50% ammonium sulfate precipitation. Then, 1 mg was coupled to 1,000 U of alkaline phosphate (Sigma Chemical Co., St. Louis, Mo.) by incubation with 0.25% glutaraldehyde for 2 h at room temperature, followed by extensive dialysis against phosphate-buffered saline (PBS), pH 7.4. In the two-sited assay, 100 μl of partially purified

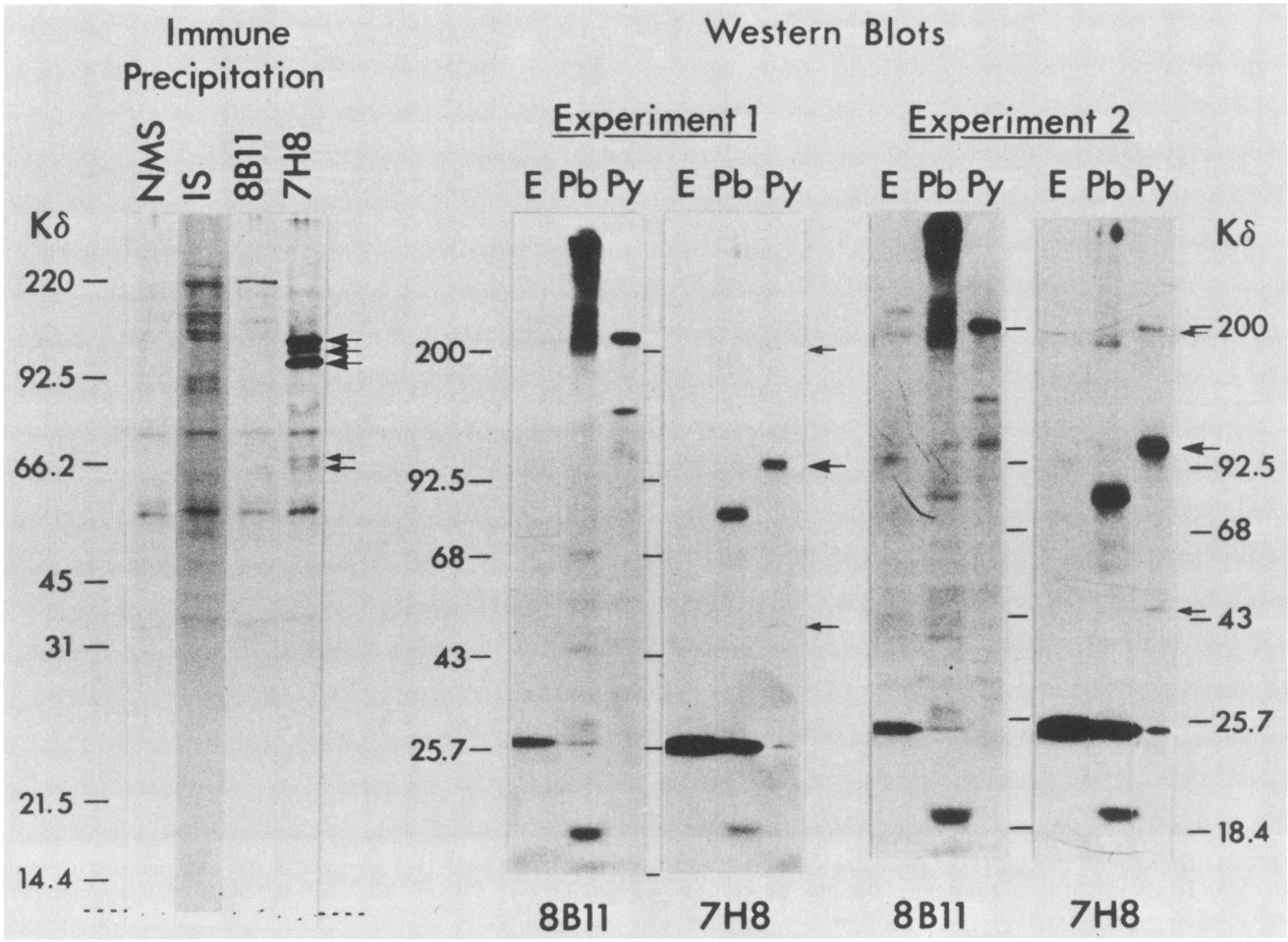

FIG. 2. Molecular weight determinations for antigens bound by MAb 7H8. Immune precipitation results were obtained when an extract of [^{35}S]methionine-labeled 17XL *P. yoelii* parasites was immune precipitated with normal mouse serum (NMS) (lane 1), immune serum (IS) (lane 2), MAb 8B11 to a *P. yoelii* antigen of ~230 kDa (lane 3), and MAb 7H8 (lane 4). Immune precipitates were assayed on a 10% SDS-PAG under nonreducing conditions. Western blot analysis: extracts of either normal mouse erythrocytes (E), *P. berghei* (Pb)-, or *P. yoelii* (Py)-infected erythrocytes were separated on a 5 to 15% SDS-PAG under reducing conditions and then electrophoresed onto nitrocellulose paper. Samples were treated with 8B11, an IgM MAb to a 230,000-kDa protein of *P. yoelii* known to cross-react with *P. berghei* and MAb 7H8.

MAb 7H8 in PBS, at 100 μg/ml unless otherwise specified, was applied to the wells of flat-bottom polystyrene microtiter plates (Immunolon I; Dynatech Industries, Inc., McLean, Va.), and control wells were treated with 3% BSA (radioimmunoassay grade) in PBS. Plates were incubated overnight at 4°C. Wells were washed, treated with 200 μl of 3% BSA in PBS for 2 h at 37°C, and washed five times with PBS. Then, 50 μl of *P. yoelii* extract, plasma samples diluted in 3% BSA in PBS, or PBS (used as a negative control for blanking the microplate reader) was added to triplicate wells and incubated overnight. After being washed five times with PBS, 100 μl of alkaline-phosphatase-labeled 7H8 MAb (at a 1:500 dilution unless otherwise stated) was added. Plates were incubated for 2 h at 37°C and washed three times with PBS, and 200 μl of 1 mg of *p*-nitrophenol substrate (Sigma P104) per ml of diethanolamine buffer was added. Color development was determined in about 30 min at 405 nm with an EL310 microplate reader (Bio-Tek Instruments, Inc., Burlington, Vt.).

The assay was initially developed with extracts of *P. yoelii* parasites. During preparation of the extracts, blood samples from BALB/c mice with high 17XL *P. yoelii* parasitemias were fractionated on a Percoll gradient to enrich for parasitized erythrocytes. Parasitized cells were freeze-thawed three times and centrifuged at 10,000 × *g* for 30 min. The supernatant, designated as a water-soluble cell cytosol, was removed, and the remaining pellet was washed twice with saline and extracted with 1% Triton X-100. The supernatant, containing proteins released from the erythrocyte and parasite membranes, and the Triton-X-100-insoluble pellet, containing erythrocyte and parasite cytoskeletons, were saved. The protein concentrations of the three extracts were determined by a protein determining reagent (Bio-Rad).

In assays using boiled extracts or plasma, samples were placed in a rapidly boiling water bath for 5 min. The aggregated proteins were centrifuged at 1,000 × *g* for 10 min, and the clear, colorless supernatant was carefully removed and assayed immediately.

RESULTS

Description of MAb 7H8. MAb 7H8 has an IgM isotype. The antibody produced a characteristic pattern in an IIF assay, using acetone-fixed smears of infected erythrocytes (Fig. 1). Erythrocytes containing ring stage parasites were

negative. However, a characteristic set of fluorescent spots can be seen in trophozoite- and schizont-containing erythrocytes (Fig. 1C through F). The fluorescent spots appear to be external to the parasitophorous vacuole membrane but internal to the erythrocyte membrane. Occasionally, the erythrocyte membrane ruptured during preparation of the smear, and discrete spots of fluorescence can be seen as released intact granules. Similar patterns of fluorescence were produced in erythrocytes infected with *P. berghei* and *P. falciparum* (Fig. 1E and F, respectively). In addition to fluorescence spots that appeared outside the parasite, there was strong fluorescence from the 17XNL *P. yoelii* and *P. falciparum* parasites (trophozoites and schizonts) themselves (Fig. 1D and F). In contrast, 17XL *P. yoelii* and *P. berghei* parasites were negative or only marginally positive (Fig. 1C and E). IIF studies with intact erythrocytes and mechanically released intact parasites demonstrated that the antigen is not expressed on these membrane surfaces.

Biochemical studies. Immune precipitation studies with [^{35}S]methionine-labeled parasites were conducted to determine the molecular weight of the antigen bound by MAb 7H8 (Fig. 2). Under nonreducing conditions, the antigen appeared as a doublet or triplet at ~120, 135, and 148 kilodaltons (kDa) (large arrows), with several lesser bands between 65,000 and 75,000 (small arrows). Similar results were obtained by Western blot analysis under reducing conditions (Fig. 2). A predominant protein band of ~117,000 to 120,000 (large arrows) was present with lesser bands at molecular weights of 200,000 and 45,000 (small arrows). MAb 7H8 also identified a protein in *P. berghei* with the significantly lower molecular weight of ~90,000.

Development of a two-sited assay for detecting Ag-7H8. Initially, a direct-binding assay was used to titrate the preparation of alkaline-phosphatase-labeled MAb 7H8 and to determine if the antigen was water soluble. In this assay, microtiter wells were treated with 100 μl of approximately 1 mg of *P. yoelii* antigen extracts of either parasite cytosol, Triton-X-100-extracted membrane proteins, or Triton-X-100-insoluble cytoskeletons per ml. The results demonstrated that the labeled antigen bound preferentially to an antigen in the parasite cytosol (Fig. 3). In later studies, the conjugate was used routinely at a 1:500 dilution.

Next, a two-sited assay was attempted. In step 1 of this initial assay, wells were coated with 100 μl of partially purified MAb 7H8 at a 1-mg/ml concentration or with 3% BSA. Extracts of *P. yoelii* or plasma samples from acutely infected mice were used as the source of the antigen in step 2, and a 1:500 dilution of alkaline-phosphatase-labeled MAb 7H8 was employed in step 3. The *P. yoelii* antigen was usually captured by MAb-coated wells but not by wells coated with BSA (Fig. 4, upper left portion). However, when large amounts of Ag-7H8 were used (e.g., as in parasite extract 2), a substantial amount of reactivity was observed in BSA-coated wells. Thus, in the presence of high antigen concentrations, both captured and nonspecifically bound antigens were detected. These results demonstrated that *P. yoelii* cytosol contains an antigen with at least two similar epitopes and that the antigen appears in acute-phase plasma.

Aliquots from four parasite extracts and plasma samples from mice acutely infected with 17XL and 17XNL *P. yoelii* were heated in a boiling water bath for 5 min to determine if the antigen detected in the two-sited assay is heat stable. Paired samples of unboiled extract or plasma and the colorless supernatant from boiled samples were compared. Ag-7H8 could easily be detected after boiling (Fig. 4, lower half). However, the amount of reactivity was frequently diminished after boiling the samples, especially the sera. Finally, we determined the optimal amount of partially purified MAb to apply to the plate (step 1 of the assay). Accordingly, wells were coated with various amounts of MAb 7H8 (Fig. 5). When wells were coated with less than 10 μg/ml (i.e., 1 μg per well), they were unable to capture sufficient amounts of the antigen. Thus, in all subsequent experiments wells were coated with 100 μg of MAb 7H8 per ml.

In the development of the assay, ascites from two different clones of MAb 7H8 were used. Similar results were obtained for kinetics of reactivity and titration curves.

Detection of Ag-7H8 in plasma collected during *P. yoelii* infection. Plasma samples collected during the 17XL *P. yoelii* infection in BALB/c mice were diluted 1:1 in 3% BSA in PBS and assayed for Ag-7H8. The results demonstrated that the antigen could be detected in plasma on days 6 and 7 after injection of 10^4 and 10^6 parasites, respectively (Fig. 6).

Various inbred strains of mice were infected with 10^6

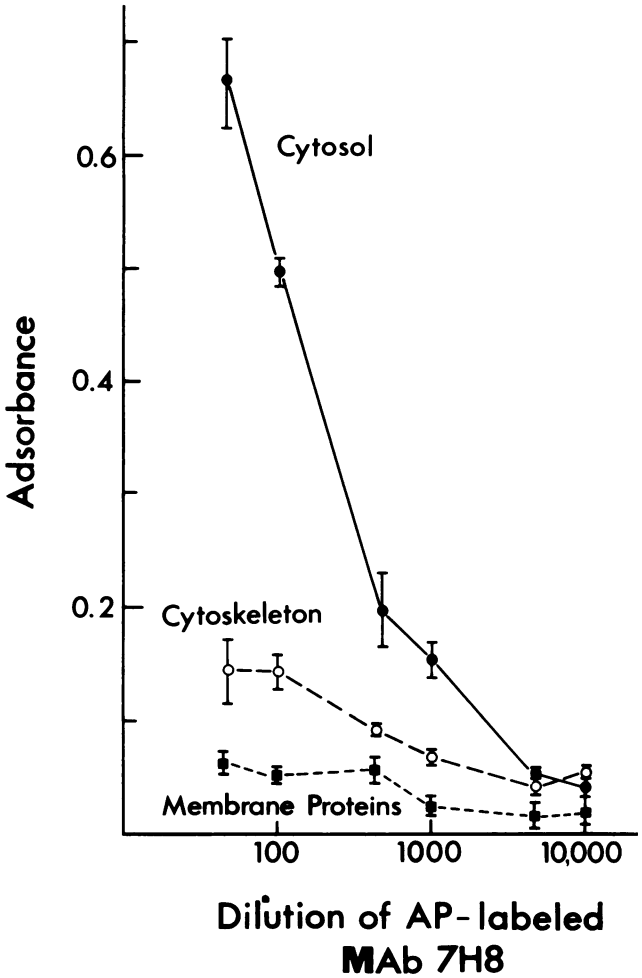

FIG. 3. Direct binding of alkaline-phosphatase (AP)-labeled MAb 7H8 to three extracts of *P. yoelii*. Wells were coated with 100 μl of ~1 mg of water-soluble parasite cytosol, Triton-X-100-extracted membrane proteins, or Triton-X-100-insoluble erythrocyte-parasite cytoskeletons per ml (the suspension was prepared in a tissue homogenizer before use). Antigen-coated wells treated with PBS served as the blank. In subsequent studies, the alkaline-phosphatase-labeled conjugate was used at a 1:500 dilution.

17XNL *P. yoelii* parasites. Blood samples, collected from three mice on the days indicated, were pooled and assayed. Results demonstrated the presence of Ag-7H8 in the plasma samples of BALB/c, AKR/J, C57BL/6, and C57BL/6 (H-2^k) mice between days 6 and 14 of infection, with amounts peaking at day 11 (Fig. 7). The appearance of Ag-7H8 in plasma correlated with the onset and early stages of infection and did not parallel the course of parasitemia.

DISCUSSION

In this study we identified an antigen (7H8) that circulates in the peripheral blood during the early stages of *P. yoelii* infection. This antigen is produced by the parasite and has a major high-molecular-weight component of ~117,000 to 120,000 as determined by both immune precipitation of [^{35}S]methionine-labeled proteins and by Western blot analysis. In addition to the major band detected by the two assays, several lesser bands can be identified, including bands of 200,000; 65,000 to 75,000; and 45,000. Thus, it appears that the epitope is on molecules of diverse sizes. The identification of the same epitope on molecules of different sizes is not unusual. For example, the dominant protein on the surface of malarial merozoites in *P. yoelii* has a major band of 230,000 plus lesser bands of 197,000, 160,000, 151,000, 126,000, 90,000, 56,000, and 28,000 (4). MAb 8B11 (Fig. 2) identifies this protein. It is not yet clear if the lesser components recognized by MAb 7H8 are aggregates, breakdown products, or true alternative forms of the antigen.

Ag-7H8 can be detected in cytosol extracts of parasites and acute-phase plasma samples after boiling for 5 min (Fig. 4). However, the amount of activity is reduced after boiling, especially when plasma samples from acutely infected mice are used. The reason for diminished activity is not clear but could be due to: (i) a portion of Ag-7H8 becoming trapped in the protein aggregate formed during boiling (which is considerably greater in plasma than in extracts); (ii) some of the molecules (i.e., bands) that possess epitope 7H8 being thermostable, whereas others are not; or (iii) the basic structure of the molecule being altered by heating but the epitope recognized by MAb 7H8 being only moderately affected. The last possibility could result in a lowered-affinity interaction between the MAb and the epitope. We are now attempting to make this distinction by identifying the protein moieties in plasma samples after boiling. However, the data shown in Fig. 4 support the conclusion that Ag-7H8 in parasite extracts and a portion of the antigen in plasma samples are stable to boiling for 5 min.

Should Ag-7H8 be classified as an S antigen? Currently, there appear to be several heat-stable antigens in the sera of *P. falciparum*-infected individuals, but only one of these antigens fits the complete definition for S antigens. This

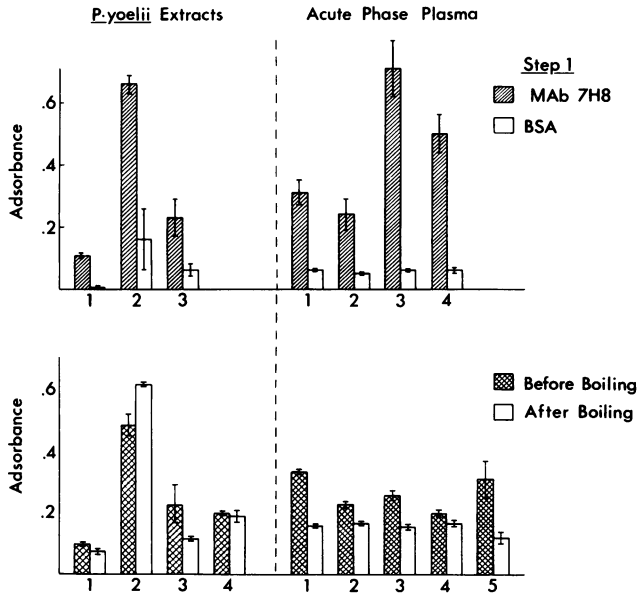

FIG. 4. Use of a two-sited assay for the detection of Ag-7H8 in extracts of *P. yoelii* cytosol and plasma samples from mice with acute malaria. The upper portion shows that, in general, Ag-7H8 was captured by wells coated with MAb 7H8 but not with BSA. The exception was *P. yoelii* extract 2. The lower portion demonstrates the heat-stable nature of Ag-7H8. The four antigen extracts on the left contained cytosol isolated from approximately (1) 10^7, (2) 2.5×10^7, (3) 10^6, and (4) 10^6 Percoll-isolated, infected erythrocytes. The acute-phase plasma samples on the right were collected on days 5 (samples 1 and 5) and 6 (samples 2 and 3) after infection with 17XL *P. yoelii* and on day 7 (sample 4) after inoculation with 17XNL *P. yoelii*. Samples contained plasma pooled from 20 to 50 BALB/c mice.

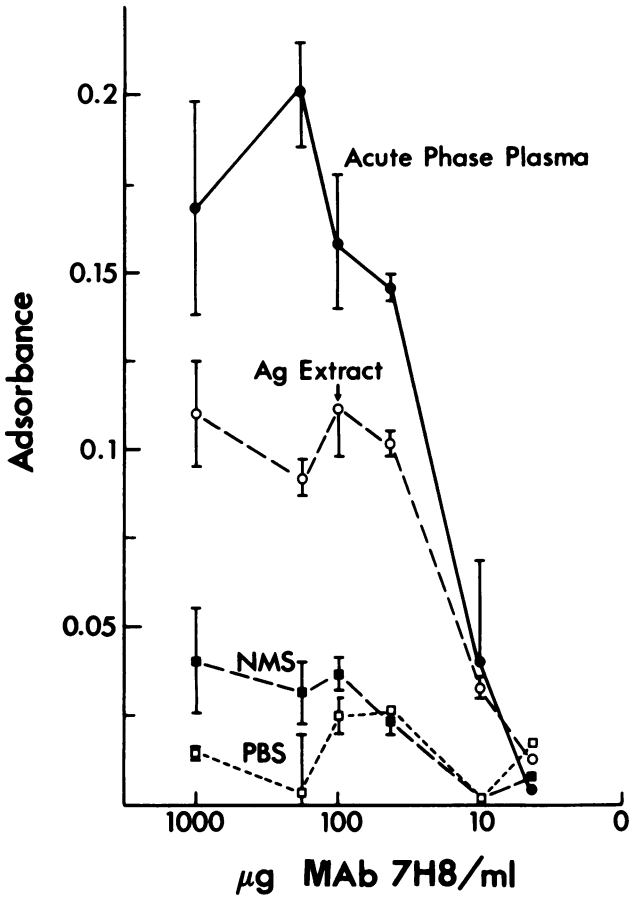

FIG. 5. Determination of the optimal amount of partially purified MAb 7H8 to apply to microtiter wells. In step 1 microtiter wells were coated with various amounts from 1 to 1,000 μg of partially purified MAb 7H8 per ml. PBS, normal mouse serum (NMS), acute-phase plasma samples from mice with ~30% parasitemia, and *P. yoelii* cytosol extract (Ag) (~1 mg/ml) were diluted 1:1 with 3% BSA in PBS and used in step 2. A 1:500 dilution of alkaline-phosphatase-labeled MAb 7H8 was used in step 3. Results are expressed as means ± 1 standard deviation for triplicate samples.

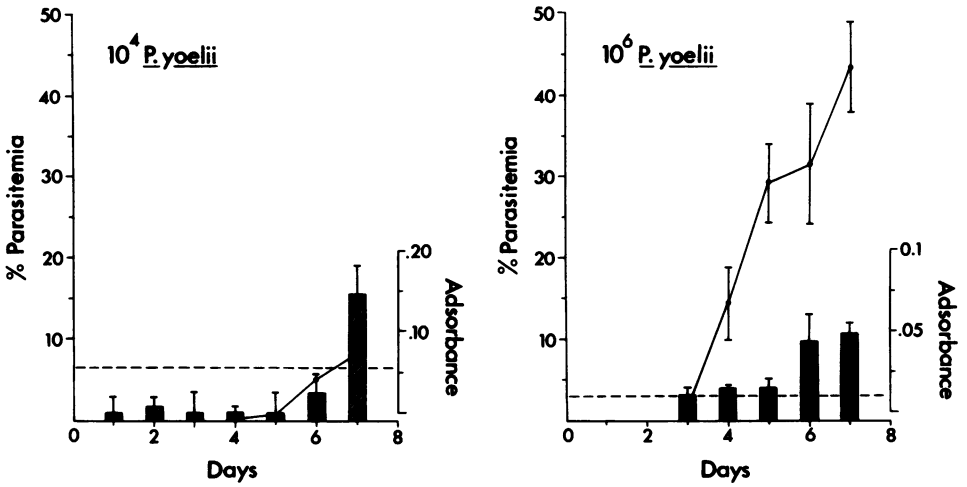

FIG. 6. Detection of Ag-7H8 in the serum of mice during 17XL *P. yoelii* infection. Groups of 21 and 15 BALB/c mice were infected with 10^4 and 10^6 17XL *P. yoelii* parasites, respectively. Three mice in each group were bled out daily, and plasma samples from individual mice were pooled and examined in the two-sited assay. The left axis and ascending curve show the course of parasitemia (the mean ± the standard error of the mean for three mice). The right axis and bars show results of the two-sited assay. Results are expressed as means ± 1 standard deviation for triplicate samples. Normal BALB/c plasma samples (- - - - -) were used as a control.

antigen is in the plasma of infected individuals, produces a band in double-diffusion Ouchterlony analysis against a set of well-characterized immune sera, maintains antigenicity as determined by Ouchterlony analysis after boiling the antigen for 5 min, and has antigenic diversity among isolates (1–3). Recently, the amino acid sequence of the S antigen from two isolates has been determined (2, 3). Accordingly, it will be necessary to determine the sequence of Ag-7H8 and to compare it with the above S antigen before we can be certain if it is a true S antigen. Theoretically, Ag-7H8 could be the S antigen described above, as Ag-7H8 is in *P. falciparum* (Fig. 1) as well as in *P. yoelii*, but it is more likely that Ag-7H8 is an antigen that has yet to be described.

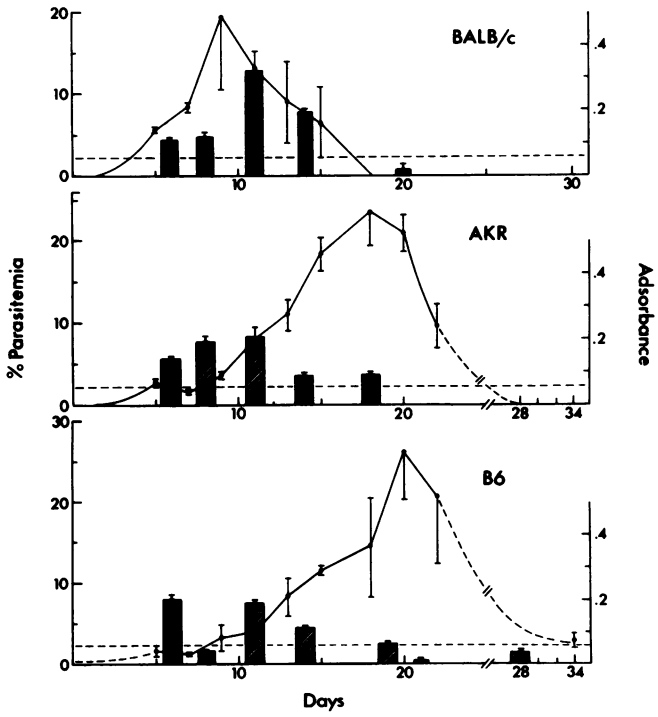

FIG. 7. Detection of Ag-7H8 in the plasma samples of various strains of mice infected with the 17XNL strain of *P. yoelii*. Groups of nine mice each were infected with 10^6 17XNL *P. yoelii* parasites. Parasitemias (the left axis and ascending curves) are the mean for six to nine animals. Blood samples, collected from three mice on the days indicated, were pooled and assayed in the two-sited assay. Results (the right axis and bars) are expressed as means ± 1 standard deviation for triplicate samples. Results for all the C57B1/6 (B6) congenics were virtually identical, so only one is shown.

Ag-7H8 appeared in plasma samples 6 to 7 days after infection (Fig. 6 and 7), regardless of the inoculation dose in both lethal and nonlethal *P. yoelii* infections. At that time, the antigens detected were probably in the free state and not in immune complexes, as only low levels of antibodies were being produced (data not shown). As the infection progressed, Ag-7H8 was detected up to day 14. It is possible that during this time antibodies were produced against it, resulting in the clearance of the antigen from the circulation. The kinetics of antigenemia were similar in BALB/c, AKR, and C57BL/6 mice, although the courses of parasitemia differed greatly in these inbred strains (Fig. 7). Thus, the kinetics of antigenemia correlate with the initial stages of infection and not the level of parasitemia.

The above explanation of the clearance of Ag-7H8 is based on the assumption that antibodies are produced against it during the early stages of infection. This, however, may not be true. In the last several years we have infected mice with the 17XL and 17XNL strains of *P. yoelii*, removed their spleens at various times after infection, and used these tissues for hybridoma production. Only 1 (7H8) of ~200 MAbs produces the pattern of fluorescence described here, suggesting that Ag-7H8 might be a weak immunogen. In addition, careful examination of SDS-PAG results (Fig. 2) shows that approximately equal amounts of the upper 150- and 120-kDa bands are immune precipitated by MAb 7H8, but sera from immune mice contain antibodies to the upper two proteins of the triplet (150 and 136 kDa) but not to the third (120 kDa) or the smaller bands. This result has been

observed in other immune precipitation studies with hyperimmune serum and sera collected on day 11 of the primary infection (data not shown). That is, antibodies to the lower band (120 kDa and the smaller proteins with this epitope) are rarely, if ever, found in immune serum. Thus, this antigen may have low immunogenicity.

Is Ag-7H8 secreted into sera, released during erythrocyte rupture and parasite reinvasion, or produced by degenerating parasites? The pattern of fluorescence produced by MAb 7H8 is consistent with that of a secreted protein and appears similar to that reported for the S antigen of *P. falciparum* (2, 9). Ag-7H8 is absent in ring stage parasites and appears during early trophozoite formation (Fig. 1). The antigen appears to be located primarily in the space external to the parasite and internal to the erythrocyte membrane. The finding that the antigen remains in discrete granules when the erythrocyte membrane is ruptured but when the parasite membrane remains intact supports the idea that the antigen is transported packaged in vesicles through the erythrocyte and then either stored within the erythrocyte cytoplasm or actively secreted into the serum. Attempts to make this distinction have not been successful.

Circulating antigens in sera could have a role in immunosuppression, immune protection, and immune-complex formation. Recently, Saul et al. (10; Saul et al., in press) showed that a MAb against *P. falciparum* S antigens could block parasite growth in vitro. The mechanism of this MAb is unclear. Accordingly, Ag-7H8 could play several immunologic roles. The results from 17XNL *P. yoelii* studies suggest that Ag-7H8 may be involved in immune protection or immune-complex formation (Fig. 7). Studies are in progress to elucidate the role of this antigen.

A two-sited assay was developed for detecting Ag-7H8. The sensitivity of the assay was difficult to ascertain, but antigens could be detected circulating in the blood when the parasitemias were <2% (Fig. 7). Technical difficulties encountered with the two-sited assay for detecting Ag-7H8 were minimal. However, as the amount of Ag-7H8 in extracts of malarial parasites increased, the amount of color development in wells not coated with MAb 7H8 (i.e., the BSA used in step 1) increased. This suggests that during the overnight incubation period, some of the antigens bound directly to the plate. It appears that in samples containing large amounts of Ag-7H8, both captured and nonspecifically bound antigens were detected. In addition, it is possible that Ag-7H8 was not detected beyond day 14 because antibodies produced by the host interfered with the assay.

Since Ag-7H8 is in *P. yoelii*, *P. berghei*, and *P. falciparum* parasites, it is likely that this assay has general application for the detection of this circulating malarial antigen. Preliminary studies show that Ag-7H8 can be detected in plasma samples of individuals living in The Gambia and Nigeria with acute *P. falciparum* infections.

Thus, identification of a *P. yoelii* antigen that shares many characteristics with S antigens in *P. falciparum* may provide us with an animal model system for determining if this family of high-molecular-weight, water-soluble, heat-stable antigens found in sera during acute malaria plays a role in immune protection, immunosuppression, or immune complex formation.

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