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Characterization of *Plasmodium yoelii* Monoclonal Antibodies Directed against Stage-Specific Sporozoite Antigens

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A battery of monoclonal antibodies against *Plasmodium yoelii* sporozoites was produced. Five of these (NYS1 through NYS5) were selected for characterization. All five were positive in the indirect immunofluorescent antibody test with *P. yoelii* sporozoites; however, each showed a different immunofluorescence pattern. Although NYS1 (immunoglobulin G3 [IgG3]), NYS2 (IgG3), and NYS3 (IgM) were positive in the circumsporozoite precipitation test, only NYS1 and NYS2 were able to neutralize sporozoite infectivity in mice. NYS4 (IgM) and NYS5 (IgG1) were not positive in the precipitation test and did not protect mice from sporozoite infection. All except NYS4 were species as well as stage specific. NYS4 cross-reacted with sporozoites of *P. berghei*. Electrophoretic immunoblotting analysis showed that these monoclonal antibodies detected sporozoite antigens of various molecular weights. Inhibition enzyme-linked immunosorbent assays indicated that each recognized a different antigenic epitope. The differences in their immunochemical and biological reactivity make them useful for screening a variety of *P. yoelii* antigens in recombinant DNA libraries. These antigens will be used in an animal model system for vaccine development.

Yoshida and co-workers (18) were the first to prepare and characterize monoclonal antibodies (MAbs) directed against sporozoites of *Plasmodium berghei*. They reported that their MAb recognized the circumsporozoite (CS) protein, a stage-specific antigen on the surface of sporozoites, which they named Pb44. Subsequent work established that the Pb44 antigen was potentially protective (14). The same approach was next applied to a monkey malaria parasite, *P. knowlesi* (4), and the MAbs generated were used to identify recombinant clones coding for the CS protein of this species (7). This pioneering work eventually led to sequencing of the gene encoding the CS antigen of *P. falciparum* (5) and to rapid development of recombinant (19) and synthetic (1, 20) sporozoite vaccines against *P. falciparum* presently being tested in humans. However, many aspects of vaccination with these preparations have yet to be defined, and many experimental requirements cannot be met in a model system with humans as the host. For this reason, the rodent malarias remain useful.

In this study we characterize MAbs directed against sporozoites of *Plasmodium yoelii*. These antibodies are being used to identify products of recombinant clones from genomic libraries of this species. Clone products will then be used to analyze the protective immune response to recombinant and synthetic vaccines in the mouse system in parallel with the ongoing trials in humans.

MATERIALS AND METHODS

Mice. Female BALB/c and CAF_1 mice 6 to 10 weeks of age (Jackson Laboratories, Bar Harbor, Maine) were used.

Parasites. *P. yoelii* 17X(NL) was used in the production of MAbs. Strains 7G8 of *P. falciparum*, NK of *P. vivax*, ANKA of *P. berghei*, and 8A of *P. gallinaceum* were used to determine the species or stage specificity of the MAbs.

Preparation of sporozoite extracts. Sporozoites were separated from infected *Anopheles stephensi* mosquitoes by the discontinuous gradient technique (13). Sporozoites were extracted in phosphate-buffered saline (PBS) containing 1% sodium dodecyl sulfate (SDS) and 0.2% bovine serum albumin (BSA) in the presence of protease inhibitors (0.34 mg of phenylmethylsulfonyl fluoride, 25 μg of antipain, and 25 μg of leupeptin per ml). All extractions were performed for 1 h at room temperature. Following extraction, the suspensions were centrifuged at 15,000 × g for 10 min, and the soluble extracts (supernatant fluids) were used immediately or stored at −70°C.

Production of MAbs. BALB/c mice were intravenously immunized via the tail vein at 2-week intervals with 3×10^4 to 5×10^4 irradiated (10,000 rads delivered over 12.55 min from a cesium source) sporozoites. Three days after the third immunization, spleens were removed from each mouse for fusion with the mouse myeloma cell line X63.Ag8.653, a non-immunoglobulin secretor (8), by the technique previously described (6). The culture supernatants of growing hybrids were screened for antisporozoite antibodies by the indirect immunofluorescent antibody (IFA) test. The positive hybrids were cloned by a limiting dilution technique to obtain a cell line derived from a single hybrid cell or clone.

Preparation of ascitic fluids. Hybridoma clones secreting MAbs were grown in large quantities in Dulbecco modified Eagle medium containing 20% heat-inactivated fetal bovine serum. The cultures were incubated at 37°C in 10% CO_2 in air. Cells were harvested and washed once in PBS, and 10^7 cells were inoculated intraperitoneally into each of several BALB/c mice inoculated intraperitoneally with 0.5 ml of pristane 10 days earlier. The ascitic fluids were collected and centrifuged at 10,000 × g for 10 min to remove cell debris. In the experiment to determine epitope specificity, ascitic fluid antibodies were conjugated to horseradish peroxidase (HRP) by the periodate oxidation method (11) before use in the assay as second antibodies.

IFA test. Sporozoites and blood-stage parasites were used as antigens. Sporozoites were suspended in medium 199 (Gibco, Grand Island, N.Y.) without serum. Twenty microliters of sporozoite suspension was distributed to each well

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of multispot IFA slides, air-dried, and stored at −70°C until the time of assay. Red-cell stages of plasmodial parasites were prepared from the blood of infected animals. The infected blood was washed three times in PBS, pH 7.2, and the parasitemia was adjusted to 0.01% in this buffer. Twenty microliters of the cell suspension was added to each well, air-dried, and stored at −70°C. Supernatant fluids from hybridoma cultures or cell lines derived from a single hybridoma clone were added to the wells of antigen slides. The slides were incubated in a moist chamber at 37°C for 30 min and washed once in PBS. Fluorescein-conjugated rabbit anti-mouse immunoglobulin (Miles Laboratories, Elkhart, Ind.) was added, and the mixtures were incubated for an additional 30 min. Slides were washed with PBS to remove excess conjugated antibody, mounted in 10% glycerol in PBS, and examined under an American Optical UV microscope. In some experiments, IFA assays were performed with viable sporozoites. Sporozoites were incubated with MAbs, followed by fluorescein-conjugated rabbit anti-mouse immunoglobulin at the same concentration as used on the dried sporozoite preparation, and then examined under a UV microscope.

Isotype determination. Classes and subclasses of antisporozoite MAbs were determined by the gel diffusion method (12) with 1.0% Noble agar (Difco Laboratories, Detroit, Mich.) in PBS. Purified class- and subclass-specific heavy-chain reagents (goat anti-mouse immunoglobulin M [IgM], IgG1, IgG2a, IgG2b, IgG3, and IgA; Miles Laboratories) were used for the determinations.

CSP reaction. MAbs were tested for CS precipitation (CSP) reactivity by a method described previously (16). Briefly, viable sporozoites were incubated with MAbs at 37°C for 30 min and examined by phase-contrast microscopy. CSP positivity was shown by precipitate formation at the surface of the sporozoite with tail formation in at least 2 of 25 sporozoites counted.

Species and stage specificity. The species and stage specificity of antisporozoite MAbs were determined by the IFA assay. To determine species specificity, MAbs were tested against sporozoites from five malaria species: *P. yoelii*, *P. berghei*, *P. falciparum*, *P. vivax*, and *P. gallinaceum*. Stage specificity was determined by reacting MAbs with sporozoites, blood stages, and 42-h liver stages of *P. yoelii*.

Epitope specificity. Epitope specificity was determined by an inhibition enzyme-linked immunosorbent assay (ELISA). Sporozoite antigen extract (2 μl, or 4,000 sporozoite equivalents) was dotted onto strips of nitrocellulose paper, air dried, and incubated overnight at 4°C in a blocking buffer (3% BSA in PBS). The nitrocellulose strips were washed three times in PBS containing 0.05% Tween 20 (PBS-Tween) and incubated separately for 2 h at room temperature with each unconjugated ascitic MAb (NYS1 through NYS5), diluted 1:100 in PBS containing 20% heat-inactivated fetal bovine serum (diluting buffer). The negative controls were antigen strips incubated with an ascitic fluid containing an unrelated MAb (anti-*P. falciparum* sporozoites) and an ascitic fluid from mice inoculated with myeloma cells. At the end of the incubation period, all strips were washed three times in PBS-Tween and then incubated for 2 h in an appropriate HRP-conjugated test MAb at a concentration of approximately 2 to 5 μg/ml. The strips were washed three times in Tris-buffered saline (pH 7.2) before the substrate solution (30 mg of 4-chloro-1-napthol in 10 ml of methyl alcohol plus 30 μl of H_2O_2 in 50 ml of Tris-buffered saline) was added. Color reaction was allowed to develop for 45 min at room temperature.

SDS-PAGE and immunodetection. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of sporozoite antigens was carried out as described by Laemmli (9) with an 8 to 12% polyacrylamide gradient gel and a 3% stacking gel. The antigens were transferred from the gel onto a sheet of nitrocellulose by the procedure of Towbin et al. (15). The electrophoretic transfer was carried out at 64 V for 12 to 16 h at 4°C in a Bio-Rad Trans-Blot cell. The nitrocellulose sheet was submerged in 3% BSA in PBS for 45 min with continuous agitation on a rocking platform, and the sheet was washed three times in PBS-Tween. The sheet was then cut into small strips, and each of them was incubated for 2 h at room temperature with a 1:20 dilution of each of the hybridoma culture supernatants or a 1:100 dilution of ascitic fluids in a diluting buffer. The strips were washed three more times in PBS-Tween and incubated with ^{125}I-labeled goat anti-mouse immunoglobulin, which has been shown to react with all mouse isotypes (New England Nuclear Corp.; specific activity, 9.0 μCi/μg of purified immunoglobulin), at a concentration of 5×10^4 cpm/ml. The excess labeled antibodies were removed by washing the nitrocellulose strips with PBS-Tween solution. The strips were air-dried and exposed to Kodak X-Omat AR films (Eastman Kodak) for 3 days at −70°C before the films were developed.

Sporozoite neutralization. Culture supernatants containing MAbs were concentrated and dialized against PBS, and the antibody concentration was determined by an ELISA (2). Viable sporozoites of *P. yoelii* were incubated in vitro for 45 min at room temperature in mixtures of MAbs at a final concentration of 0.1 mg/ml and 20% normal mouse serum. Control sporozoites were incubated either with a mixture of concentrated culture supernatant of X63.Ag8.653 and 20% normal mouse serum or with an unrelated MAb and 20% normal mouse serum. CAF_1 mice in groups of 10 were intravenously inoculated via the tail vein with 5×10^3 treated sporozoites per mouse to determine the inhibitory effect of MAbs on sporozoite infectivity. At 7, 14, and 21 days after sporozoite inoculation, blood samples were taken from the tail vein of each mouse and examined for the presence of parasites by both thick and thin blood films.

RESULTS

IFA reactivity of antisporozoite MAbs. Five MAbs designated NYS1 through NYS5 from three fusion experiments were selected for further characterization based on differences in their IFA patterns (Fig. 1). Three MAbs (NYS1, NYS2, and NYS3) reacted with the sporozoite surface, although there were slight differences in reactivity patterns. NYS1 gave a rough outline of the surface of the sporozoites, and NYS2 produced a smoother overall surface reactivity, while NYS3 produced a heavier fluorescent staining over the entire sporozoite surface. NYS4 produced a large patchy reactivity that appeared to be both internal and on the surface of the sporozoite. MAb NYS5 produced an IFA reactivity pattern that appeared to be on the surface but was restricted to polar regions. IFA tests were also performed on viable sporozoites, and only NYS1, NYS2, and NYS3 showed definite surface reactivity.

Characterization of MAbs. MAbs were further characterized for their immunoglobulin isotypes and CSP reactivity (Table 1). MAbs NYS1 and NYS2 were IgG3 isotypes, NYS3 and NYS4 were both IgM isotypes, and NYS5 was an IgG1. NYS1, NYS2, and NYS3 were CSP positive, while NYS4 and NYS5 were negative in the CSP test.

SDS-PAGE and immunodetection of sporozoite antigens. Sporozoite antigen extracts were subjected to SDS-PAGE,

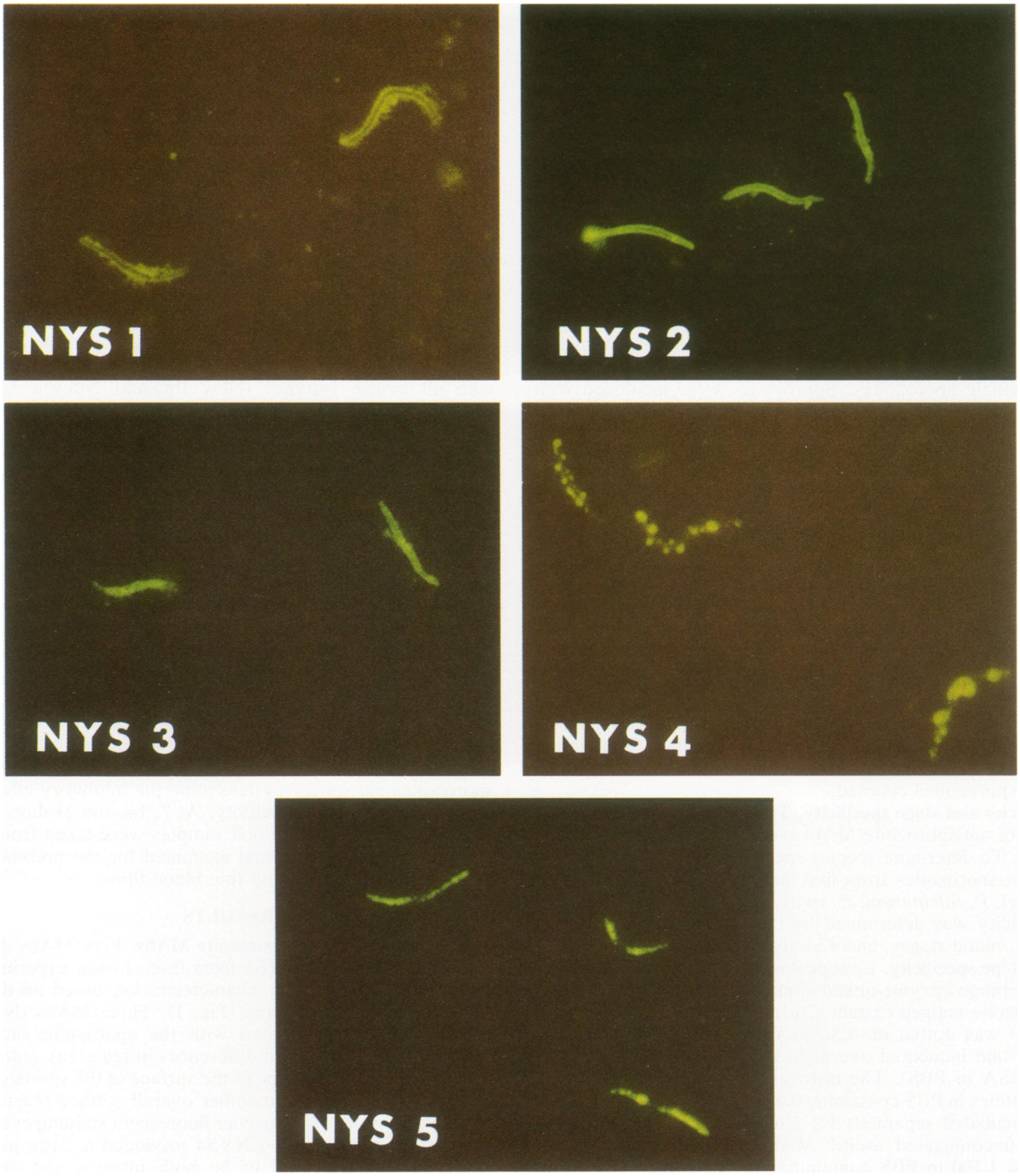

FIG. 1. IFA patterns of five MAbs directed against sporozoites of *P. yoelii*. MAb NYS1 produced a rough surface stain, NYS2 produced a smooth surface stain, and NYS3 produced heavy fluorescent staining over the entire sporozoite surface. NYS4 produced a large-patch pattern, and NYS5 produced an IFA pattern which appeared to be restricted to polar regions of the sporozoite.

and the separated bands of antigens were transferred onto nitrocellulose paper and detected by MAbs (Fig. 2). MAbs NYS1, NYS2, and NYS3 identified four bands with apparent molecular sizes of 56, 64, 75, and 84 kilodaltons (kDa). NYS2 also recognized an additional band of approximately 140 kDa. NYS3 recognized the four bands plus several minor bands of lower molecular size. NYS4 reacted with only a single band of 140 kDa. NYS5 failed to react with any of the antigen bands despite repeated attempts to detect it by varying the concentration of polyacrylamide in the gel.

Species and stage specificity. The species and stage specificity of the MAbs were determined by the indirect IFA assay. The species specificity results are shown in Table 1. four MAbs, NYS1, NYS2, NYS3, and NYS5, reacted with *P. yoelii* sporozoites but not with sporozoites of *P. berghei*. These MAbs were also tested against sporozoites of *P.*

TABLE 1. Characterization of anti-*P. yoelii* sporozoite MAbs

MAb	Immunoglobulin isotype	CSP reactivity (no. positive/no. tested)	IFA reactivity (antibody titer) to sporozoites of:	
			P. yoelii	*P. berghei*
NYS1	IgG3	+ (9/25)	+ (32)	−
NYS2	IgG3	+ (15/25)	+ (8)	−
NYS3	IgM	+ (9/25)	+ (64)	−
NYS4	IgM	− (0/25)	+ (64)	+ (256)
NYS5	IgG1	− (0/25)	+ (16)	−

falciparum, *P. vivax*, and *P. gallinaceum* and failed to react with them (not shown). NYS4, however, cross-reacted with *P. berghei* sporozoites, although the fluorescence pattern on *P. berghei* sporozoites with this MAb was markedly different from the pattern observed on sporozoites of *P. yoelii*. The NYS4 pattern on *P. berghei* more closely resembles the CS pattern of NYS1 reacting with sporozoites of *P. yoelii*. To determine stage specificity, the five MAbs were tested with sporozoites, blood stages, and liver stages of *P. yoelii*. Supernatants of all five MAbs (2 to 5 μg/ml) reacted only with the sporozoite stage of a parasite. However, NYS1 ascitic fluid (0.1 to 1.0 mg/ml) reacted with CS antigen present in the liver stage.

Epitope specificity. The inhibition ELISA results indicated that each MAb reacted with its own unique epitope. No cross-reactivity between any of these MAbs was observed. Positive controls consisting of tests in which the blocking MAb was followed by the same MAb conjugated to HRP always produced complete inhibition. Negative controls (antigen extract incubated with unrelated antibody or with ascitic fluid from mice inoculated with X63.Ag8.653 cells) showed no inhibition.

Sporozoite neutralization test. The results of the neutralization test are shown in Table 2. All MAbs were tested at a concentration of 0.1 mg/ml. Only NYS1 and NYS2 were able to neutralize sporozoite infectivity for 9 of 10 and 6 of 10 mice, respectively, giving a neutralization index of 90 and 60%, respectively. NYS3, NYS4, and NYS5 failed to protect in this assay. Parasitemia was observed in all control mice inoculated with sporozoites incubated either with an unrelated MAb (*P. falciparum* antisporozoite MAb) or with culture supernatant fluid of the X63.Ag8.653 myeloma cell line.

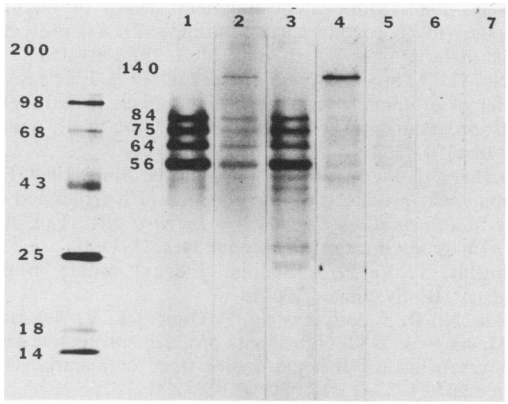

FIG. 2. Western blot analysis of sporozoite antigen extract of *P. yoelii* antisporozoite MAbs. MAb NYS1 (lane 1) detected four antigen bands with apparent molecular sizes of 56, 64, 75, and 84 kDa. NYS2 (lane 2) detected the same four antigen bands plus an additional band of 140 kDa. NYS3 (lane 3) recognized the same four antigen bands plus a few minor antigen bands of low molecular weight. NYS4 (lane 4) recognized only the 140-kDa band. NYS5 (lane 5) failed to detect any antigen on Western blotting. Control lanes are *P. yoelii* sporozoite antigen extract reacted with *P. falciparum* antisporozoite MAb (lane 6) and with ascitic fluid from mice inoculated with the X63.Ag8.653 myeloma cell line (lane 7).

DISCUSSION

MAbs from five hybridoma clones were selected for characterization based on the uniqueness of their immunochemical and biological properties. The belief that they were different from each other was supported by observed differences in IFA staining patterns, immunoglobulin isotypes, epitope specificity, biological reactivity, and molecular size of the antigens they recognize. The evidence also supports the conclusion that there is more than one sporozoite-specific antigen recognized by these MAbs. NYS1, NYS2, and NYS3 produced an immunofluorescent reaction on viable sporozoites covering the entire surface, and all three were CSP positive, which indicates that all three recognize the CS antigen of the sporozoite. NYS4 and NYS5, on the other hand, did not produce a typical surface pattern on IFA, nor were they positive in the CSP test, indicating that they react with antigens other than the CS protein.

The results of SDS-PAGE immunoblotting analysis agree with the conclusion that at least three sporozoite-specific antigens were recognized, corresponding to the three IFA reactivity patterns observed. The epitope recognized by NYS1 was present on four antigen bands with apparent molecular sizes of 56, 64, 75, and 84 kDa. The epitope recognized by NYS2 was present on the same four bands and on an additional band of approximately 140 kDa. In contrast, NYS3, while also reacting with epitopes on the bands of 56, 64, 75, and 84 kDa, appeared to detect a few minor bands of lower molecular size. NYS4, on the other hand, recognized an epitope present only on the 140-kDa band.

In contrast to the other four MAbs, NYS5 failed to detect any antigen band by immunoblotting even though it was positive in the IFA assay. It is possible that failure to detect an electrophoresed antigen from the sporozoite extract with MAb NYS5 is due to denaturation of the epitope by SDS. Denaturation of antigenic epitopes by SDS has been reported previously (3, 17). Another possibility may be that the molecular weight of this antigen is too small for the antigen to be retained on the gels.

The results of the inhibition ELISAs confirmed that the five MAbs recognized different antigenic epitopes. NYS2,

TABLE 2. In vitro neutralization of *P. yoelii* sporozoite infectivity[a]

MAb	Antibody concn (mg/ml)	No. of mice infected	% Neutralization
NYS1	0.1	1	90
NYS2	0.1	4	60
NYS3	0.1	10	0
NYS4	0.1	10	0
NYS5	0.1	10	0
Controls			
Anti-*P. falciparum* sporozoite MAb	0.1	10	0
X63.Ag8.653 supernatant	0	10	0

[a] Mice received 5×10^3 sporozoites each, and there were 10 mice inoculated per group.

which recognized the 140-kDa antigen in addition to the CS antigen, failed to inhibit labeled NYS4 in the dot ELISA, indicating that these two MAbs react with different epitopes on the 140-kDa antigen. This conclusion is further supported by the failure of NYS2 to react with *P. berghei* sporozoites by IFA, in contrast to NYS4, which gives a strong IFA reaction with sporozoites of *P. berghei* and detects a sporozoite antigen of M_r 44,000 (data not shown). The failure by NYS3 to neutralize sporozoite infectivity even though it produced a positive CSP reaction may indicate that there are protective and nonprotective epitopes on the sporozoite antigen. Alternatively, the results could reflect differences in affinity between NYS1, NYS2, and NYS3. We are unaware of any reason that the IgM should be less effective than IgG3 antibodies in producing protection. It has been shown in trypanosomes that IgM antibodies are more efficient than IgG in both in vitro reactivity and in vivo protection (10). The IFA test and CSP assay results indicate that NYS1, NYS2, and NYS3 react with antigens on the sporozoite surface; however, only NYS1 and NYS2 are protective. Our results indicate that there are at least two distinct protective epitopes on the CS protein, since both NYS1 and NYS2 reacted with this antigen and yet failed to inhibit each other in the inhibition ELISA. This indicates that there may be several protective epitopes on the antigen and that inclusion of critical areas of the molecule containing these may be required to obtain a satisfactory protective immune response.

Five MAbs used in this study have identified at least three sporozoite-specific antigens of *P. yoelii*; one is a CS antigen, and the other two are non-CS antigens. All three genes coding for these three antigens have now been cloned in a recombinant system (A. Wortman, Y. Charoenvit, A. McDermott, P. Rogers, M. F. Leef, M. Sedegah, and R. L. Beaudoin, submitted for publication). MAb NYS4 also recognized the CS antigen of *P. berghei*, a different plasmodial species. The differences in the immunochemical and biological properties of these MAbs make them useful for identifying gene products in recombinant DNA libraries and for analysis of the antigenic components of sporozoites. These antigens will be used in an animal model system for vaccine development.

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LITERATURE CITED

1. **Ballou, W. R., J. Rothbard, R. A. Wirtz, D. M. Gordon, J. S. Williams, R. W. Gore, I. Schneider, M. R. Hollingdale, R. L. Beaudoin, W. L. Maloy, L. H. Miller, and W. T. Hockmeyer.** 1985. Immunogenicity of synthetic peptides from circumsporozoite protein of *Plasmodium falciparum*. Science **228:** 996–999.
2. **Bosworth, J. M., Jr., A. A. Brimfield, J. A. Naylor, and K. W. Hunter, Jr.** 1983. Measurement of monoclonal antibody concentrations in hybridoma cultures: comparison of competitive inhibition and antigen capture enzyme immunoassays. J. Immunol. Methods **62:**331–336.
3. **Burnette, W. N.** 1981. Western blotting electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. **112:** 195–203.
4. **Cochrane, A. H., F. Santoro, V. Nussenzweig, R. W. Gwadz, and R. S. Nussenzweig.** 1982. Monoclonal antibodies identify the protective antigens of sporozoites of *Plasmodium knowlesi*. Proc. Natl. Acad. Sci. USA **79:**5651–5655.
5. **Dame, J. B., J. L. Williams, T. F. McCutchan, J. L. Weber, R. A. Wirtz, W. T. Hockmeyer, W. L. Maloy, J. D. Haynes, I. Schneider, D. Roberts, G. S. Sanders, E. Premkumar Reddy, C. L. Diggs, and L. H. Miller.** 1984. Structure of the gene encoding the immunodominant surface antigen on the sporozoite of the human malaria parasite *Plasmodium falciparum*. Science **225:** 593–599.
6. **Danforth, H. D., G. H. Campbell, M. F. Leef, and R. L. Beaudoin.** 1982. Production of monoclonal antibodies by hybridomas sensitized to sporozoites of *Plasmodium berghei*. J. Parasitol. **68:**1029–1033.
7. **Ellis, J., L. S. Ozaki, R. W. Gwadz, A. H. Cochrane, V. Nussenzweig, R. S. Nussenzweig, and N. Godson.** 1983. Cloning and expression in *E. coli* of malarial sporozoite surface antigen gene from *Plasmodium knowlesi*. Nature (London) **302:**536–538.
8. **Kearney, J. F., A. Radbruch, B. Liesegang, and K. Rajewsky.** 1979. A new mouse myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines. J. Immunol. **123:**1548–1550.
9. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
10. **Mansfield, J. M.** 1981. Immunology and immunopathology of African trypanosomiasis, p. 167–226. *In* J. M. Mansfield (ed.), Parasitic diseases, vol. 1: immunology. Marcel Dekker, Inc., New York.
11. **Nakane, P. K., and A. Kawaoi.** 1974. Peroxidase-labeled antibody: a new method of conjugation. J. Histochem. Cytochem. **22:**1084–1091.
12. **Ouchterlony, O.** 1958. Diffusion-in-gel methods for immunological analysis, p. 1–78. *In* P. Kallos (ed.), Progress in allergy, vol. 5. Karger, Basel.
13. **Pacheco, N. D., C. P. A. Strome, F. Mitchell, M. P. Bawden, and R. L. Beaudoin.** 1979. Rapid, large-scale isolation of *Plasmodium berghei* sporozoites from infected mosquitoes. J. Parasitol. **65:**414–417.
14. **Potocnjak, P., N. Yoshida, R. S. Nussenzweig, and V. Nussenzweig.** 1980. Monovalent fragments (Fab) of monoclonal antibodies to sporozoite surface antigen (Pb 44) protect mice against malarial infection. J. Exp. Med. **151:**1504–1513.
15. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
16. **Vanderberg, J. P., R. Nussenzweig, and H. Most.** 1969. Protective immunity produced by the injection of X-irradiated sporozoites of *Plasmodium berghei*. V. *In vitro* effects of immune serum on sporozoites. Milit. Med. **134:**1183–1190.
17. **Waehneldt, T. V.** 1975. Sodium dodecyl sulfate in protein chemistry. BioSystems **6:**176–187.
18. **Yoshida, N., R. S. Nussenzweig, P. Potocnjak, V. Nussenzweig, and M. Aikawa.** 1980. Hybridoma produces protective antibodies directed against the sporozoite stage of malaria parasite. Science **207:**71–73.
19. **Young, J. F., W. T. Hockmeyer, M. Gross, W. R. Ballou, R. A. Wirtz, J. H. Trosper, R. L. Beaudoin, M. R. Hollingdale, L. H. Miller, C. L. Diggs, and M. Rosenberg.** 1985. Expression of *Plasmodium falciparum* circumsporozoite proteins in *Escherichia coli* for potential use in a human malaria vaccine. Science **228:**958–962.
20. **Zavala, F., J. P. Tam, M. R. Hollingdale, A. H. Cochrane, I. Quakyi, R. S. Nussenzweig, and V. Nussenzweig.** 1985. Rationale for development of a synthetic vaccine against *Plasmodium falciparum* malaria. Science **228:**1436–1440.