POLYMORPHISM OF A HIGH MOLECULAR WEIGHT SCHIZONT ANTIGEN OF THE HUMAN MALARIA PARASITE *PLASMODIUM FALCIPARUM*

BY JANA S. MCBRIDE, CHRIS I. NEWBOLD,* AND RITA ANAND

*From the Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JT, Scotland; and the *Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Headington, Oxford, England*

Clinical manifestations of malaria are associated with repeated cycles of multiplication of the parasite in the blood, and any prospective immunoprophylaxis needs effectively to control the blood phase of the infection. Present attempts (1–10) to identify parasite antigens of potential value in vaccination against asexual blood stages of the human parasite *Plasmodium falciparum* have implicated a number of different parasite polypeptides. However, experimentally induced immunity against the blood forms of *P. falciparum* is known to have a significant isolate-specific component (11–16), indicating intraspecies antigenic diversity in one or several of the relevant protective antigen(s). Furthermore, in endemic areas, individuals naturally exposed to the infection suffer repeated parasitemias and clinical attacks of the disease before any degree of immunity is acquired (17, 18). The reasons for such a slow and gradual development of immunity are unknown but the pattern is consistent with the existence of a variety of antigenically distinct parasite strains to which a human host may need to be exposed before he acquires cumulative immunity against the majority of the diverse strains present in his environment. It is therefore crucial to research aimed at the development of widely applicable vaccine that the degree of antigenic diversity in defined parasite antigens is elucidated. Moreover, most immunological research on malaria has been carried out with a limited number of antigenically poorly defined *P. falciparum* isolates and it is not known how far such isolates are representative of wild parasite populations in endemic areas. Thus there is a need for a system of immunological classifications of the parasite in order to understand how experimental results obtained in the laboratory may relate to the situation in the field.

Recently, we have used a panel of strain-specific monoclonal antibodies (mAb)[1] to demonstrate that the species *P. falciparum* consists of a number of antigenically diverse strains. Some of these strains may have a world-wide distribution (19,

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; DMSO, dimethyl sulfoxide; IFA, indirect immunofluorescence assay; mAb, monoclonal antibody; NP-40, Nonidet P-40; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; PPO, 2,5,-diphenyloxazole; PSA, polymorphic schizont antigen; SDS-PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; TCLK, tosyl-L-lysine chloromethyl ketone.

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20), while substantial antigenic diversity occurs even within parasite populations present in a relatively small geographical area (21). In the present study we use some of the strain-specific antibodies to identify a family of structurally and antigenically polymorphic proteins of M_r 190,000–200,000. We show that these proteins are essentially the same as a putative protective antigen of *P. falciparum* that is synthesized in schizonts and then processed into several discrete fragments, some of which are associated with the surface of merozoites (2–4). We also show that this family of proteins is responsible for much of antigenic diversity in *P. falciparum* and propose that it may be possible to devise a system for serological classification of the parasite based on the antigenic polymorphism of these proteins.

Materials and Methods

Culture Media and Chemicals. RPMI 1640 tissue culture medium was supplied by Flow Laboratories Ltd., Ayrshire, Scotland. Methionine-free RPMI 1640, Hepes, sodium glutamate, sodium bicarbonate, and antibiotics were obtained from Gibco Europe Laboratories, Paisley, Scotland, and fetal calf serum from Seralab Laboratories, Sussex, England.

Aminopterin, hypoxanthine, thymidine, saponin, tosyl-L-lysine chloromethyl ketone (TLCK), phenylmethylsulfonyl fluoride (PMSF), dimethyl sulfoxide (DMSO), 2,5-diphenyloxazole (PPO), 2-mercaptoethanol, Tris, bovine serum albumin (BSA), *Staphylococcus aureus* V8 protease, and *S. aureus* protein A–agarose were purchased from Sigma Chemical Co., St. Louis, MO. BDH Pharmaceuticals Ltd., London, England supplied the following: polyethylene glycol 1500, Nonidet P-40 (NP-40), sodium dodecyl sulfate (SDS), iodoacetamide, acrylamide, *N,N'*-methylenebisacrylamide, ammonium persulfate, EDTA, EGTA, glycine, bromophenol blue, sorbitol, and glycerol. Radiolabeled L-[^{35}S]methionine (sp act, 800–1,200 Ci/mmol) was purchased from Amersham International Ltd., Amersham, England.

Immunoadsorbent-purified rabbit anti-mouse immunoglobulin conjugated with fluorescein isothiocyanate was obtained from Miles-Yeda Ltd., Rehovot, Israel. Rabbit antisera specific for heavy chain isotypes of mouse immunoglobulin were purchased from Litton Bionetics, Inc., Kensington, MD. Precipitating sheep anti-mouse IgG and goat anti-human IgG antisera were obtained from Scottish Antibody Production Unit, Carluke, Scotland.

Antimalaria Hybridomas, mAb, and Sera. The procedure for preparation of mouse hybridomas secreting *P. falciparum*–specific mAb was as described (19). Briefly, female BALB/c mice were preimmunized with two to four doses of $4–6 \times 10^6$ schizonts (or 100-200 μg of protein) obtained from asynchronous parasite cultures lysed by treatment with 0.15% (vol/vol) saponin and were injected intraperitoneally with incomplete Freund's adjuvant. The mice were then challenged with similar doses of antigen in saline intravenously and 3 d later their spleen cells were fused with the P3/NS-1/1-Ag4-1 (NS-1) myeloma (22) in the presence of polyethylene glycol (23). The cells were grown in 2-ml volumes of hypoxanthine-aminopterin-thymidine selective medium, and, after 10–14 d, hybridoma cultures producing antiplasmodial antibodies were identified by indirect immunofluorescence assay (IFA) on acetone-fixed schizonts. Selected hybridomas were purified by limiting dilution cloning in the presence of BALB/c thymocytes as feeders, and the cloned cells were grown as ascites tumors in BALB/c mice, yielding ascitic fluids that contained mAb. Isotypes of the antibodies were determined by Ouchterlony double diffusion in agar, by reacting 20-fold concentrated culture supernatants against rabbit antisera specific for mouse heavy chain classes and subclasses.

Hybridoma lines were given the prefix EUZ (Edinburgh University, Zoology Department) and a numerical code consisting of the sequential number of fusion experiment followed by the number given to a cell line derived from an independent fusion event within that experiment. Thus, EUZ 6.1 indicates the cell line No. 1 obtained from fusion No. 6. Additional numbers were given to lines derived from such primary hybridomas by

cloning and recloning; e.g., EUZ 6.1-1-3 indicates clone No. 3 derived by recloning of the cloned line EUZ 6.1-1. mAb were given the same basic numerical codes as the producer hybridoma lines but without the prefix and, for simplicity, without the numbers identifying the clones and subclones, since these were all found to secrete antibodies of the same characteristics, such as isotype, and parasite stage and strain specificities etc.

Antibodies selected for the present experiments were raised in several fusions against four different isolates of *P. falciparum*. Antibodies designated 6.1, 7.3, 7.5, and 7.6 were produced against isolate K1; antibodies designated 9.2, 9.5, 9.7, 9.8, and 10.3 were against isolate PB1; and antibodies 12.1, 12.2, and 12.4 against isolate T9. These isolates originated from Thailand. Reference antibody 89.1 was raised against West African Wellcome isolate and was kindly provided by Drs. R. R. Freeman and A. A. Holder who described its properties earlier (2–4). Immune human serum was a pool of five sera from adult residents of a malaria hyperendemic area, and was kindly supplied by Dr. A. Adjukiewicz of Medical Research Laboratories, The Gambia, West Africa.

Indirect Immunofluorescence Assays (IFA). The tests were performed at pH 7.3 as described (19, 26–27) on acetone-fixed films of parasitized blood or on unfixed suspensions of parasites released from red cells lysed by glycerol treatment (see below). Specificities of mAb for different developmental stages of the blood cycle were determined using parasites derived from synchronous cultures (28, 29), as well as synchronous parasites obtained from malaria patients and matured in vitro (20). Gametocytes of *P. falciparum* were obtained from human carriers and were kindly provided by Dr. L. Panton of the MRC Laboratories, The Gambia, West Africa. Strain specificities of the mAb were determined using schizonts prepared from a panel of *P. falciparum* isolates originating from several endemic areas (see below).

Isolates[2] *and Clones of P. falciparum.* mAb were raised against uncloned isolates designated K1, PB1, and T9, all of which originated from Thailand (24–26). From the isolate T9 a number of cloned parasite lines were derived using the limiting dilution technique (25, 26); four of the clones, designated Nos. 94, 96, 101 and 102, were chosen for use in the present study since an earlier investigation had shown them to be phenotypically homogeneous and distinct from each other by several criteria, including enzyme, protein, and antigen variant markers (26). These isolates and clones as well as a selection of another 42 culture-adapted lines of *P. falciparum* originating from Asia, Africa, Papua New Guinea, and Central and South America, were obtained from the World Health Organisation Reference Bank of Malaria Strains held in the Department of Genetics, University of Edinburgh.

In Vitro Cultivation of P. falciparum. The parasites were maintained using the petri dish method of continuous cultivation as described by Trager and Jensen (28). 2–4 ml cultures were started at 0.5% parasitemia in human group O erythrocytes added to a hematocrit of 5% (vol/vol) into RPMI 1640 medium supplemented with 25 mM Hepes, 22 mM $NaHCO_3$, 50 μg/ml gentamycin, and 10% (vol/vol) human group A serum. The culture medium was changed daily and fresh erythrocytes were used to dilute the cultures whenever the parasitemia reached 10–15%, usually every 4 d. These cultures were asynchronous and contained all stages of the asexual blood cycle (rings, trophozoites, schizonts, merozoites). For some experiments, cultures at 8–10% parasitemia were synchronized by the method of Lambros and Vanderberg (29) using one or two cycles of treatment with sorbitol as described (2)

Release of Parasites from Infected Erythrocytes. Red cells containing synchronous parasites at the schizont stage were lysed by osmotic shock using treatment with glycerol: 1 vol of packed cells was resuspended in 25 vol of 7% (vol/vol) glycerol in RPMI 1640 medium for 30 min at room temperature, the cells repacked by centrifugation at 500 *g* for 5 min,

[2] "Isolate" is used here to refer to a sample of parasites, not necessarily phenotypically or genetically homogeneous, collected from a naturally infected human host on a single occasion. The present isolates had all been adapted to growth in vitro and were maintained in the laboratory for periods ranging from a few months to several years before this study; it is not known whether their composition was the same as the original samples obtained from the patients. "Clone" refers to genetically identical organisms derived from a single parasite by asexual division.

the supernatant removed, and the cells lysed by *rapid* resuspension in 25 vol RPMI 1640. This procedure resulted in preparations containing free parasites, red cell ghosts (some with parasites trapped inside them), and small numbers of unlysed red cells. For immunizations of mice, parasites were obtained from erythrocytes lysed with 0.15% (vol/vol) saponin. Total parasites and multinuclear schizonts were counted using aliquots of the above preparations stained with ethidium bromide (0.001% wt/vol in phosphate-buffered saline [PBS]), with a hemocytometer and a fluorescence microscope.

[^{35}S]Methionine Biosynthetic Labeling of P. falciparum. The parasites were labeled essentially as described (2). Erythrocytes containing 2–4 × 10^8 parasites, either from asynchronous cultures (all stages including trophozoites and schizonts) or from cultures harvested 33 hs after synchronization (mostly early schizonts), were washed twice in methionine-free RPMI 1640 prewarmed to 37°C and were then resuspended in 6 ml of the same medium, containing 10% (vol/vol) human serum and supplemented with 400 μCi of [^{35}S]methionine. The cells in petri dishes were returned to a gassed culture jar and were incubated for various periods from 30 min to 6 h. After labeling, the parasitized red cells were washed twice in cold PBS, pH 7.2 and were stored as frozen pellets at −70°C before extraction.

Cell Extraction and Immunoprecipitation. Cell pellets were thawed and extracted on ice for 1 h in 5 ml of 1% (vol/vol) NP-40 in buffer containing 50 mM Tris-HCl, pH 8.0, 5 mM EDTA, 5 mM EGTA, 5 mM iodoacetamide, 1 mM PMSF and 0.1 mM TLCK (2). Insoluble material was removed by centrifugation at 100,000 *g* for 30 min at 4°C. Total and protein-bound (insoluble in 5% wt/vol TCA) radioactivity of the extracts was determined by scintillation counting in the ^{14}C channel of a Packard 2407 counter (Packard Instrument Co., Downers Grove, IL). Typically, 40–50% of the incorporated label was solubilized by the above extraction procedure and >90% of this was present as TCA-precipitable material.

Aliquots of the labeled extracts containing 1–2 × 10^5 cpm were mixed with 20 μl of mouse ascitic fluids containing mAb or with 20 μl of human antisera and the mixtures were incubated at room temperature for 1 h. Soluble immune complexes were precipitated using 300 μl of sheep anti-mouse IgG or goat anti-human IgG at 4°C overnight. Antibody 9.5 (IgG2b) was precipitated using 20 μl of protein A–agarose instead of the sheep antiserum. The immunoprecipitates were washed three times in 50 mM Tris-HCl, pH 8.0, 5 mM EDTA, 0.5% NP-40, containing 0.5 M NaCl and 1 mg/ml BSA, then twice in the same buffer without NaCl and BSA (2). For each wash, 2.5 ml of the buffer was used, followed by centrifugation at 200 *g* for 5 min, resuspension of the precipitates by vortexing, and addition of the next wash. The washed complexes were dissolved in 60 μl of 125 mM Tris-HCl, pH 6.8, 10% SDS, 5% 2-mercaptoethanol, 2 mM EDTA, 20% glycerol, and 0.25% bromophenol blue, by boiling for 5 min (31).

SDS–Polyacrylamide Gel Electrophoresis (SDS-PAGE). Immune complexes containing [^{35}S]methionine-labeled parasite polypeptides were analyzed by SDS-PAGE (30) using 7.5% acrylamide slab gels as described (31). Molecular weight markers were human spectrin heterodimer (240,000 and 220,000 mol wt, a gift from Dr. A. Maddy, Zoology Department, University of Edinburgh), phosphorylase *b* (94,000 mol wt), albumin (67,000 mol wt), catalase (60,000 mol wt), ovalbumin (43,000 mol wt), lactate dehydrogenase (36,000 mol wt), carbonic anhydrase (30,000 mol wt), and trypsin inhibitor (20,100 mol wt), supplied by Pharmacia Fine Chemicals AB, Uppsala, Sweden. After electrophoresis, the gels were stained with Coomassie Blue in fixative and treated with 22% (wt/vol) PPO in DMSO before drying. Radiolabeled bands were detected by fluorography at −70°C (32) using preflashed Kodak X-Omat S film.

V8 Protease Peptide Mapping. Washed mAb precipitates containing 2.5 × 10^4 cpm of [^{35}S]methionine-labeled parasite products were resuspended in 300 μl of 50 mM Tris-HCl, pH 7.5, 0.5% SDS, 10% glycerol, and were then digested with staphylococcal V8 protease at 60 μg/ml and 37°C as described (33). 50-μl samples were removed at intervals and the digestion was halted by addition of 50 μl double strength sample buffer (2% SDS, 100 mM Tris-HCl, pH 7.5, 2 mM EDTA, 10% 2-mercaptoethanol, 4 mM PMSF, 10% glycerol) and boiling for 5 min. The digestion products were analyzed by SDS-PAGE in

12.5% wt/vol acrylamide gels, and peptides containing [^{35}S]methionine were visualized by fluorography.

Results

Hybridomas and mAb. In 12 fusions, >280 hybridomas were generated that reacted with different erythrocytic stages of *P. falciparum.* Preliminary work (19, 27, 33 and unpublished data) indicated that some of these antibodies react with a class of mutually related antigens, which were in many respects similar to a schizont protein recently described by other workers (2). From this preliminary information, mAb produced by 13 different cloned hybridoma lines were selected for the present comparative study. Properties of the antibodies are summarized in Table I.

Location and Stage Specificity of Antigenic Determinants[3] *Recognized by mAb.* When tested by IFA on acetone-fixed films of schizonts and merozoites of the homologous isolates, all of the antibodies produced an indistinguishable staining pattern in reaction with antigen(s) that appeared to be associated with the plasma membrane of these stages (Fig. 1). Furthermore, the mAb reacted with the surface and caused indirect agglutination of free, unfixed schizonts and, where present, of merozoites released from erythrocytes lysed by osmotic shock. The antibodies also reacted with pigment-containing particles without a nucleus, which were presumed to be residual bodies remaining after rupture of mature

TABLE I

Summary of Anti–P. falciparum Hybridomas and mAb

Hybridoma		Antibody		Reactivity in indirect immunofluorescence							
				Acetone-fixed blood stages*					Unfixed parasites‡		
Code	Immunizing isolate	Code	Mouse isotype	S	M	R	T	G	S	M	Isolate distribution§
EUZ 6.1-1-3	Thai K1	6.1	IgG1	++	+	−	−	−	++	NT	16
EUZ 7.3-7	K1	7.3	IgG2a	++	+	−	−	−	++	++	16
EUZ 7.5-1	K1	7.5	IgG1	++	++	++	+	−	++	NT	46 (17)
EUZ 7.6-2	K1	7.6	IgG1	++	+	−	−	−	++	NT	16
EUZ 9.2-6-2	Thai PB1	9.2	IgG1	++	+	−	−	−	++	++	30
EUZ 9.5-1-5	PB1	9.5	IgG2b	++	+	−	−	−	++	+	15
EUZ 9.7-1	PB1	9.7	IgG1	++	+	−	−	−	+	NT	30
EUZ 9.8-4-4-1	PB1	9.8	IgG1	++	+	−	−	−	++	++	46
EUZ 10.3-2	PB1	10.3	IgG1	++	+	−	−	−	++	NT	30
EUZ 12.1-5-4	Thai T9	12.1	IgG1	++	+	−	−	−	++	NT	17
EUZ 12.2-1-1	T9	12.2	IgG1	++	+	−	−	−	++	+	14 (7)
EUZ 12.4-3-4	T9	12.4	IgG1	++	+	−	−	−	++	NT	46
WIC 89.1‖	West African Wellcome	89.1	IgG1	++	+	−	−	−	NT	NT	46

* Reactivity with acetone-fixed films of erythrocytic stages: (++, +) relative intensity of positive staining; (−) negative reaction. *R*, ring forms without pigment; *T*, mononuclear trophozoites containing dispersed pigment; *S*, schizonts with four or more nuclei and aggregated pigment; *M*, merozoites; *G*, gametocytes.

‡ Schizonts released from erythrocytes lysed by glycerol treatment as described in Materials and Methods; some preparations also contained small mononuclear parasites assumed to be merozoites released from the most advanced schizonts. NT, not tested.

§ Number of *P. falciparum* isolates positively reactive out of 46 tested (see Table III for details). Numbers in brackets indicate the number of positive isolates which gave consistently only subnormal dull reactions.

‖ See reference 2 for details of hybridoma WIC 89.1 and antibody 89.1.

[3] "Antigenic site", "determinant", and "specificity" are used here interchangeably to refer to a region or structure on the antigen that is involved in antibody binding; cross-reactivity of more than one antigen with the same monoclonal antibody indicates similarity but does not prove identity of chemical composition between sites expressed on separate molecules.

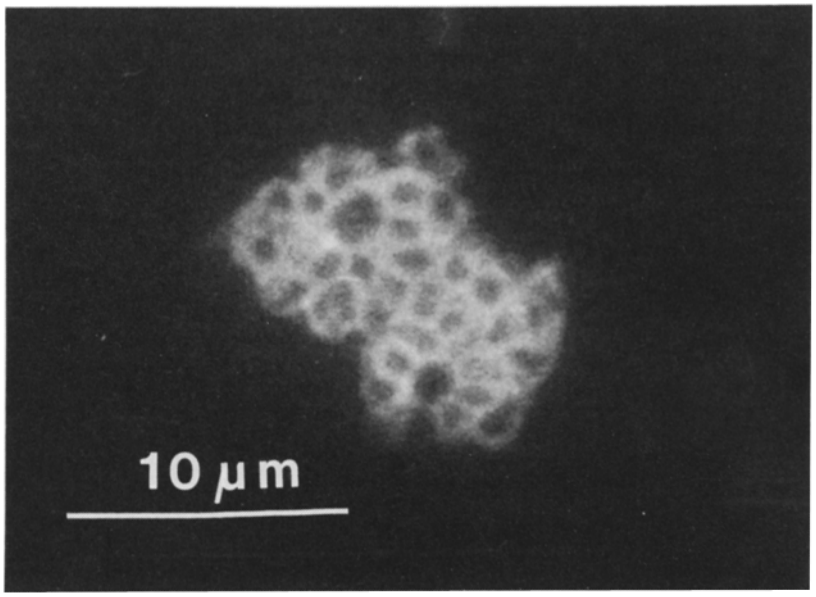


FIGURE 1. Indirect immunofluorescence staining pattern produced on acetone-fixed films of *P. falciparum* schizonts by all of the mAb used in this present study. The photo shows two segmented schizonts of clone 94, stained with antibody 7.6.

schizonts. None of the mAb reacted with gametocytes or with the surface of erythrocytes infected by any of the developmental stages of the parasite.

All but one antibody reacted specifically with schizonts and merozoites, but did not react with either the preceding (trophozoite) nor the subsequent (ring) developmental stages of the asexual blood cycle. This indicated that the antigenic sites recognized by these mAb were formed only during schizogony and were subsequently lost or degraded around the time of invasion of new red cells by merozoites.

The one exception was a determinant recognized by antibody 7.5, which reacted strongly with all asexual blood forms. In schizonts and merozoites, this determinant seemed to be membrane associated, similar to those recognized by the other antibodies, but in rings and trophozoites it appeared to be present also in the parasite's cytoplasm.

Differential IFA Reactivities of mAb with Clones of P. falciparum. Four clones of *P. falciparum*, designated 94, 96, 101, and 102, which were all derived from a single isolate of the parasite, were used to test the mAb for their capacity to differentiate between schizonts of the different clones. The IFA tests were performed on $\sim 10^4$ acetone-fixed schizonts using serial 10-fold dilutions of ascitic fluids containing the mAb. We examined the reaction at 520× magnification, recorded antibody titer (the highest dilution still giving positive reaction), and compared the intensity of staining between schizonts of positively reactive clones obtained at 1:10 and 1:100 dilutions.

As summarized in Table II, three of the tested mAb (9.8, 12.4, and 89.1) reacted equally well with schizonts of all four clones, indicating that the clones shared common antigenic determinants operationally defined by these reagents.

TABLE II

Summary of Reactivities of Four Clones of P. falciparum with mAb Directed Against Different Determinants of the High Molecular Weight Schizont Antigen

Antibody[§]	Immunofluorescence $\log_{10}$ titer*				Immunoprecipitation[‡]			
	Parasite clone				Parasite clone			
	94	96	101	102	94	96	101	102
89.1 (1)	4	4	4	4	++	++	++	++
9.8 (2)	5	5	5	5	++	++	++	++
12.4 (3)	4	4	4	4	++	++	+	+
7.5 (4)	4	*3*	*3*	*3*	++	±	−	−
6.1 (5)	4	−	−	−	++	−	−	−
7.3 (6)	6	−	−	−	++	±	−	−
7.6 (7)	5	−	−	−	++	−	−	−
9.2 (8)	−	5	5	5	−	++	++	++
9.7 (9)	−	5	5	5	−	++	++	+
10.3 (10)	−	5	5	5	−	++	++	++
9.5 (11)	−	−	5	−	−	−	++	−
12.2 (12)	−	6	−	*3*	−	++	−	±
12.1 (13)	−	5	−	5	−	++	−	++

* IFA titers are expressed as logs of the reciprocal of the highest dilution giving a positive reaction using ~10^4 schizonts per test; e.g., 1:10 = 1; 1:100 = 2. Negative reactions at 1:10 and all other dilutions are shown as (−). Positive but subnormal dull reactions of some antibodies are italicized.

‡ Reactivity with high molecular weight polypeptides (195,000–200,000) was determined for each clone and antibody combination in at least three separate experiments. (+ and ++) Consistently positive results; (±) weak reactions in some but not in all experiments. See Fig. 2 for representative examples of SDS-PAGE analysis.

§ Numbers in parentheses correspond to the coding of lanes in Fig. 2, which shows an analysis of precipitates obtained with the mAb.

The other 10 antibodies clearly revealed the existence of specificites that were expressed in some but not all parasite clones, and such determinants will be termed "restricted" in this study. The ability to differentiate between the parasites was most striking for antibodies 6.1, 7.3, 7.6, 9.2, 9.5, 9.7, 10.3, 12.1, and 12.2, which reacted with certain clone(s) up to at least a 1:10,000 dilution but failed to give any detectable reaction with schizonts of other clone(s). We interpret these different reactions in terms of the presence and absence, respectively, of antigenic determinants recognized by these mAb. In addition, antibodies 12.2 and 7.5 produced positive reactions that differed in strength between certain clones. For example, mAb 12.2, which stained schizonts of clone 96 brightly and titrated to 1:1,000,000 with these parasites, had to be used in at least 1,000-fold higher concentration to produce a detectable, but weak, reaction with mature schizonts of clone 102. Such different reactivities are assumed to reflect differences in avidity of binding of these mAb to similar but not identical antigens expressed by schizonts of the different clones.

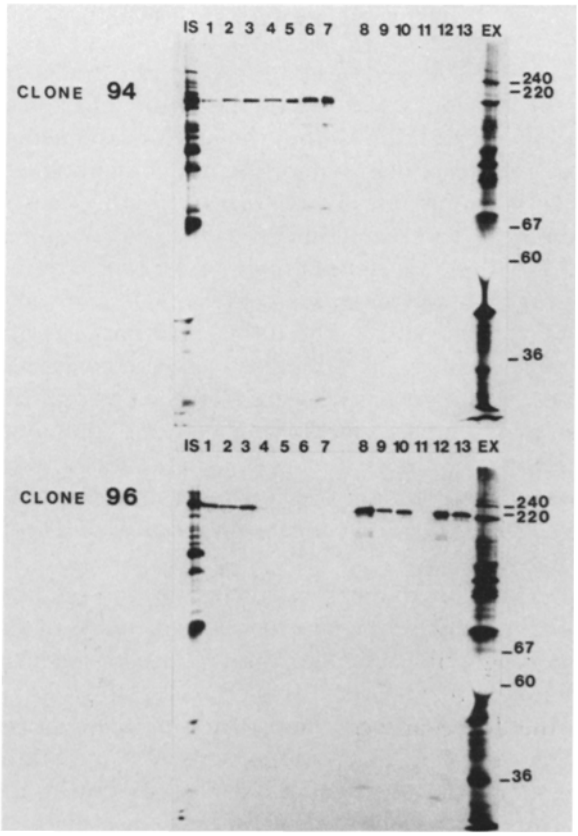
In Vitro Stability of Antigenic Phenotypes of Cloned P. falciparum. Each of the parasite clones could be distinguished (Table II) from the others by its characteristic combination of mAb-defined specificities or antigenic serotype. Stability of the clonal serotypes was tested by repeated IFA typing on schizonts sequentially harvested from long-term continuous cultures, as follows: clone 94 was tested 35 times over a period of 39 mo in vitro; clone 96 was typed 29 times during 20

mo; and clones 101 and 102 were tested four and nine times, respectively, in 17 mo. Reactivities of all clones with all of the antibodies remained unchanged during these periods. In addition, within the limits of the technique (19, 26), all of the clones appeared to remain homogeneous, containing either positively or negatively reactive schizonts but not mixtures of both. Thus we conclude that the variant serotypes, as determined in IFA by the mAb are phenotypically stable characteristics of *P. falciparum* clones grown under standard in vitro conditions.

Differential Immunoprecipitation by mAb of High Molecular Weight Antigens Produced by Clones of P. falciparum. The differential patterns of reactivity of the antibodies with the clones 94, 96, 101, and 102 were confirmed, and molecules carrying the respective antigenic sites were identified by indirect immunoprecipitation. For these experiments, synchronous cultures containing early schizonts were biosynthetically pulse-labeled with [^{35}S]methionine, and labeled antigens were then extracted in NP-40 and precipitated with the mAb. The precipitates were analyzed by SDS-PAGE and the labeled parasite polypeptides were visualized by fluorography.

Fig. 2 shows precipitation of antigens labeled for 150 min; comparable results were obtained after a pulse of 30 min (not shown). Each of the parasite clones synthesized a major high molecular weight polypeptide at (195,000–200,000 M_r) that was recognized by several mAb (Fig. 2, lanes *1–13*) as well as by human immune serum (lane IS). Although the products of different clones were immunologically related, as shown by cross-reactions of some antibodies with all of them (Fig. 2, lanes *1–3*), they were not antigenically identical and each presented a characteristic pattern of reactivities with antibodies directed against the restricted determinants (lanes *4–13*). Thus, the antigen produced by clone 94 was precipitated by antibodies 7.5, 6.1, 7.3, and 7.6 (Fig. 2, lanes *4–7*) but not by antibodies 9.2, 9.7, 10.3, 9.5, 12.2, and 12.1 (lanes *8–13*, respectively). In contrast, the specificities that characterized the product of clone 94 were recognized poorly (mAb 7.5, Fig. 2, lane *4*) or were not detectable on the products of clones 96, 101, and 102. Instead, the antigens of all the later clones expressed specificities defined by antibodies 9.2, 9.7, and 10.3 (Fig. 2, lanes *8–10*), and could be distinguished from one another by the presence of determinants defined by antibodies 9.5 (lane *11*, clone 101), or 12.2 (lane *12*, clone 96) and/or 12.1 (lane *13*, clones 96 and 102). The immunoprecipitation results are summarized and compared with those of the immunofluorescence studies in Table II. The results are in good agreement and strongly indicate that, although immunologically related, the high molecular weight schizont polypeptides display antigenic polymorphism between clones of *P. falciparum*. They will therefore be termed polymorphic schizont antigens (PSA).

Identity of Polypeptides Precipitated by mAb of Different Specificities from the Same Parasite Clone. In the above experiments all of the antibodies that reacted positively with a given parasite clone appeared to react with the same high molecular polypeptide also recognized by mAb 89.1 (Fig. 2, lane *1*). This antibody had been previously used (2) to identify an antigen that is posttranslationally processed into a series of well-defined smaller fragments. Since the processing is a characteristic feature of this antigen, we ascertained whether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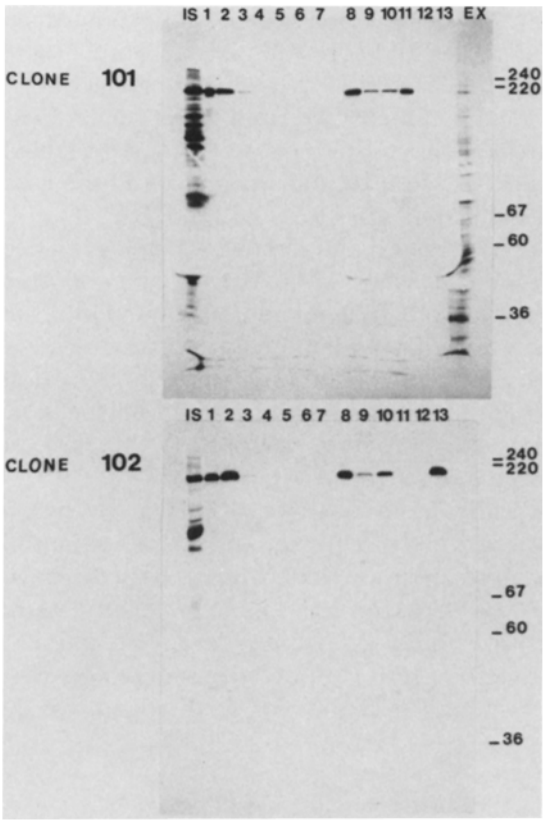
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CLONE 101
240
220
67
60
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IS 1 2 3 4 5 6 7 8 9 10 11 12 13
CLONE 102
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antibodies directed against the different stage-restricted and/or parasite clone–restricted determinants also recognized the processed polypeptides.

Fig. 3 illustrates that, from extracts of asynchronous cultures, identical patterns of multiple bands were precipitated by antibodies recognizing strain-common (lane *3*) and strain-restricted (lane *4*) specificities limited to schizonts, or those present throughout the asexual cycle (lane *2*). The banding pattern obtained, reproducibly, from extracts of clone 94 in six experiments included the major band at M_r 195,000, a doublet or triplet at M_r 156,000, a single band at M_r 76,000, and a band at about M_r 32,000 (the smallest band seemed to be displaced by the large amounts of immunoglobulin light chains that migrated just ahead of it). In some experiments a weak band at M_r 110,000 was also present (not shown). From clone 94, this characteristic pattern of polypeptides was identically precipitated by all antibodies known to react positively with this clone, i.e., mAb 6.1, 7.3, 7.5, 7.6, 9.8, 12.4 and, with the exception of the smallest (M_r 32,000) species, also by mAb 89.1, strongly indicating that all of these antibodies recognized the same antigen and its processed products. In contrast, none of the polypeptides were recognized in extracts of clone 94 by antibodies against specificities absent from this clone (e.g., mAb 9.2, Fig. 3, lane *5*; see Table II for the others), or by control mAb directed against a variety of other parasite and nonparasite antigens, nor by nonimmune mouse and human sera (not shown).

Similar results (not shown) were obtained using restricted mAb 9.2, 9.7, 10.3, 12.1, 12.2 and the three anti-common mAb in immunoprecipitations from extracts of clones 96 and 101, and uncloned isolate PB1. The combined results can be summarized as follows: (*a*) From any given extract, the same pattern of bands was precipitated by those antibodies that reacted positively with the parasite line tested, and these bands were not precipitated by the control, negatively reactive reagents. The apparently identical M_r of the major as well as of the minor bands precipitated from extracts of a single parasite line, by antibodies directed against different determinants of PSA, indicated that the common and restricted sites were present on the same polypeptides. (*b*) The banding patterns consisted of the large antigen at M_r 190,000–200,000 and, usually, of additional polypeptides at approximately M_r 155,000–165,000, 75,000–85,000, and 30,000–33,000. There was some variation in the apparent sizes of the bands, depending on the different parasite lines from which they originated (see below and Fig. 5). (*c*) Antibody 89.1, which cross-reacted with the products of all parasite lines tested, recognized, with the exception of the smallest (M_r 30,000–33,000) peptides, the same banding patterns as the other antibodies used in the

FIGURE 2. Parasite clone–specific patterns of immunoprecipitation of high molecular weight schizont antigens by mAb directed against strain-common (lanes *1–3*) and strain-restricted (*4–13*) antigenic determinants. Synchronous cultures of *P. falciparum* clones 94, 96, 101, or 102 contained mostly schizonts and were labeled with [^{35}S]methionine for 150 min. Immunoprecipitates by mAb were compared with peptides precipitated by human immune serum (*IS*) from NP-40-soluble parasite extracts (*EX*). Lanes 1–13 were loaded with equal volumes (30 μl) of dissolved precipitates obtained with mAb 89.1, 9.8, 12.4, 7.5, 6.1, 7.3, 7.6, 9.2, 9.7, 10.3, 9.5, 12.2, and 12.1, respectively. SDS-PAGE analysis in 7.5% acrylamide gels. Molecular weight markers are indicated on the right: human spectrin heterodimer (240,000 and 220,000 mol wt), BSA (67,000), catalase (60,000), and lactate dehydrogenase (36,000). (Weak bands visible at ~55,000 mol wt, particularly on gels of clones 101 and 102, are considered to be due to material nonspecifically co-migrating with the large amounts of the heavy chains of gamma-globulin present in the immunoprecipitates.)

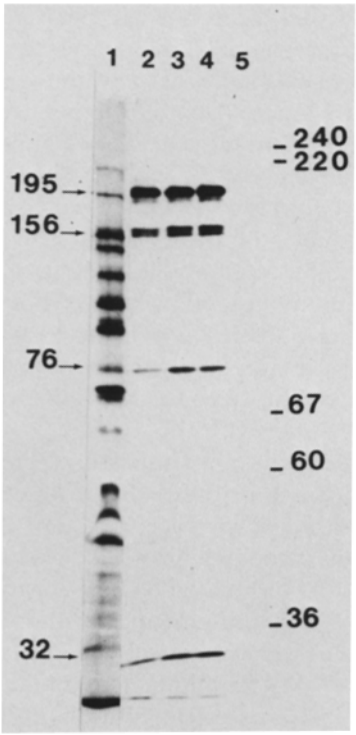

FIGURE 3. [^{35}S]methionine-labeled polypeptides immunoprecipitated by antibodies of different stage and strain specificities (*lanes 2–5*) from the NP-40-soluble fraction of clone 94 (*lane 1*). (*Lane 2*) Products precipitated by the antibody 7.5, which reacts with all asexual blood stages (schizonts, merozoites, rings, trophozoites). (*Lanes 3* and *4*) Precipitates obtained with two antibodies, mAb 12.4 and 7.6, respectively, which react only with schizonts and merozoites; while the former mAb reacts with a determinant present on all strains of *P. falciparum*, the latter defines a strain-restricted specificity. In contrast, the antibody 9.2, which recognizes a different strain-restricted antigenic determinant absent from clone 94, precipitates no antigen from this parasite and is used here as a negative control (*lane 5*). SDS-PAGE analysis on a 7.5% gel and fluorography of polypeptides labeled in asynchronous culture during a period of 6 h. Molecular weight markers are indicated on the right: human spectrin heterodimer (240,000 and 220,000 mol wt) BSA (67,000), catalase (60,000), lactate dehydrogenase (36,000). The estimated relative molecular weights ($\times 10^3$) of polypeptides specifically precipitated by the mAb are indicated on the left.

present study. As shown previously (2), the peptides at approximately M_r 155,000 and 80,000, recognized by mAb 89.1, were probably derived from the M_r 195,000–200,000 precursor, but the identity of the smallest species remains to be elucidated.

An additional experiment established that, as indicated above, common and restricted variant antigenic sites were indeed present on the same polypeptide. In this experiment, the [^{35}S]methionine-labeled, M_r 200,000 product of clone 102 was precipitated by either mAb 9.8 or 9.2, which recognize common and restricted epitopes, respectively. The precipitates were digested with staphylococcal V8 protease for various periods, and the digestion peptides obtained from the two precipitates were analyzed in parallel by SDS-PAGE (Fig. 4). Although the two antibodies must have reacted with separate sites on the antigen, the

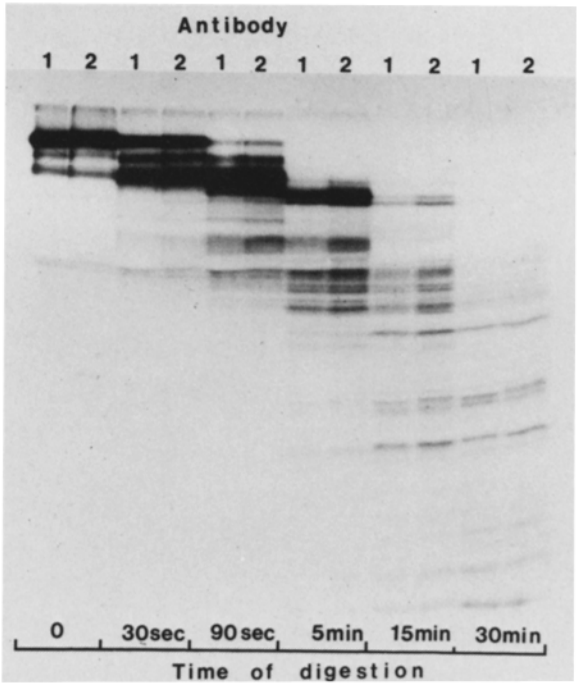

FIGURE 4. Peptide mapping of antigens precipitated from the same single parasite clone (No. 102) with antibodies against a strain-common (mAb 9.8, lane *1*) and a strain-restricted (mAb 9.2, lane *2*) determinants. The M_r 200,000 antigen of clone 102 was labeled with [^{35}S]methionine for 150 min and was precipitated from NP-40 extract by either antibody. The precipitates were digested with staphylococcal V8 protease at 60 μg/ml for various times as indicated, and digestion peptides were analyzed in one dimension by SDS-PAGE on a 12.5% gel. Labeled products were detected by fluorography. Patterns of the digestion peptides from the two precipitates were identical, showing that both antibodies reacted with the same antigen.

digestion of both precipitates progressed identically, yielding an indistinguishable pattern of fragments. This finding is consistent with both the common and variant determinants being located on the same molecule.

Differences in the Relative Molecular Weight and Peptide Composition of the Polymorphic Schizont Antigens Produced by Different Clones of P. falciparum. The apparent molecular weights of PSAs produced by synchronous schizonts of clones 94 or 96 during a 4-h labeling with [^{35}S]methionine were directly compared by parallel SDS-PAGE analysis (Fig. 5). Both parasite clones contained the large polypeptide and also several additional bands that were precipitated by the antibody 9.8 cross-reactive with both clones (Fig. 5, lanes *3,4*), by antibodies against epitopes restricted to clone 94 or 96, respectively (lanes *2* or *5*), and by antibodies present in human immune serum (lanes *1* and *6*). The relative molecular weights of the polypeptides precipitated from clone 94 were estimated at M_r 195,000 (major), 156,000 (weak doublet), 76,000 (weak), and ~32,000, while the analogous polypeptides precipitated from clone 96 were M_r 200,000, 165,000, 80,000, and 33,000. (The intermediate peptides, while visible on examination of the autoradiograph, are not readily detectable on the printed figures.)

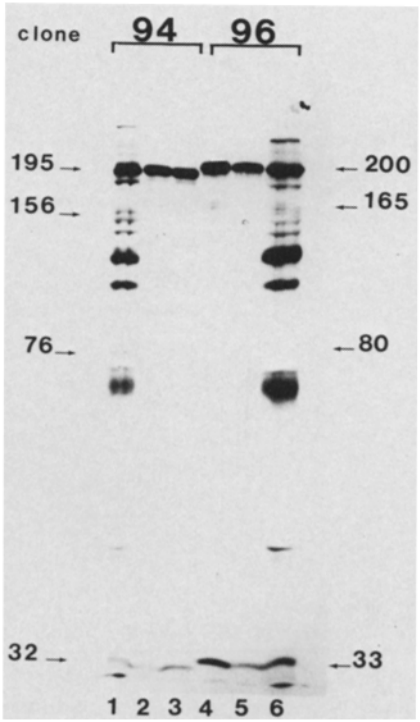

FIGURE 5. Comparison of relative molecular weights of polypeptides immunoprecipitated from *P. falciparum* clones 94 or 96 with schizont-specific mAb and with human immune serum (lanes *1,6*). (Lanes 2 and *5*) Precipitates from extracts of clone 94 or 96 by strain-restricted mAb 7.3 or 9.2, respectively. (Lanes *3* and *4*) Precipitates with mAb 9.8 against a PSA specificity common to both clones. SDS-PAGE analysis in 7.5% gel and fluorography of NP-40-soluble peptides labeled with [^{35}S]methionine during a period of 4 h in synchronous cultures. The relative molecular weights of bands precipitated by the mAb from clone 94 were 195,000 (major), 156,000 (weak doublet), 76,000 (weak), and 32,000, while the analogous bands in clone 96 were M_r 200,000, 165,000, 80,000, and 33,000. Peptides of these relative sizes were also precipitated by immune serum (lanes *1,6*). (The intermediate peptides were detectable upon examination of the autoradiograph but are not readily detectable in the printed figure.)

The major M_r 195,000 and 200,000 products of the parasite clones 94 and 96, respectively, were compared further by examination of one-dimensional maps of fragments obtained after their digestion with staphylococcal V8 protease. To avoid the complicating presence of the natural processed fragments, the antigens produced by synchronous immature schizonts were pulse-labeled with [^{35}S]methionine for 30 min. The cross-reactive antibody 9.8 was used to precipitate both antigens, the precipitates digested for various times with staphylococcal V8 protease, and the digestion peptides derived from the two clonal products were then analyzed in parallel by SDS-PAGE (Fig. 6). Although some digestion peptides of similar mobility were obtained from both antigens, suggesting a common conserved structure, many of the bands were different for the two antigens, indicating a significant degree of structural diversity between analogous products of the two *P. falciparum* clones, both derived from the same single isolate. A comparable degree of diversity of V8 protease digestion fragments was

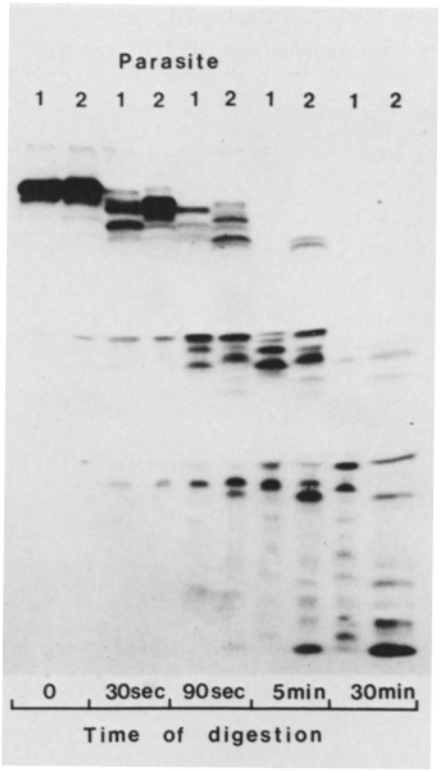

FIGURE 6. Comparison by peptide mapping of the high molecular weight antigens from parasite clones 94 and 96. The clones were grown, synchronized, and then labeled in parallel, when most parasites were at the schizont stage, with [^{35}S]methionine for 30 min. Antibody 9.8, which reacts with both parasites, was used to precipitate their PSA from NP-40 extracts. The precipitates were digested with staphylococcal V8 protease at 60 μg/ml for various times and the digestion products were analyzed by SDS-PAGE on a 12.5% gel. Labeled bands were visualized by fluorography. Lanes are alternately for digests from clone 94 (*1*) and clone 96 (*2*) obtained at 0, 30 s, 90 s, 5 min, and 30 min. The patterns of digestion peptides derived from the cross-reactive antigens produced by different parasites are not identical.

also found between the serologically distinct M_r 200,000 (indistinguishable by size) polypeptides produced by the clones 96 and 101, also both derived from the same isolate (results not shown). (Note that in control experiments comparing V8 protease peptides derived from the same clonal product precipitated by different antibodies, the digestion profiles were identical; e.g., Fig. 4.)

Indirect Immunofluorescence Typing with Anti-PSA mAb and PSA Serotypes of Different Isolates of P. falciparum. To confirm and extend the findings obtained with the four clones derived from isolate T9, the distributions of anti-PSA specificities on a panel of 37 uncloned isolates and five additional clones (derived from T9 as well as from other isolates) were determined by IFA. The results are presented in Table III.

The antibodies 9.8, 12.4, and 89.1 reacted positively with all isolates and within each isolate appeared to react with most if not all multinuclear schizonts, thus confirming the existence of conserved determinants on all PSAs. Antibodies 9.8 and 12.4 were thereafter used to estimate the numbers of mature parasites

TABLE III

Indirect Immunofluorescence Reactivity of Anti-PSA mAb with P. falciparum Isolates

Monoclonal antibody	K1, T9/94, T9/105, K31, K34, T22, SL3, NF58, IM2, It, H1, M23, FCB-1 Wellcome, BW, RFCR-3	T9/101, PB1, PB1/1, SK15, SK16, SK17 K28, G1, T9	K29, MAD13, MAD20, MAD22, MAD27, FCQ27	NF54/C, LE5/P9	T9/96, S3, MAD21, TZ, NF7	Palo Alto, S2	T9/102, PB1/3, SK18, SK19 T21, K36
89.1	+++	+++	+++	+++	+++	+++	+++
9.8	+++	+++	+++	+++	+++	+++	+++
12.4	+++	+++	+++	+++	+++	+++	+++
7.5	+++	+/+++	+/+++	+++	+	+	+/+++
6.1	+++	−	−	−	−	−	−
7.3	+++	−	−	−	−	−	−
7.6	+++	−	−	−	−	−	−
9.2	−	+++	+++	+++	+++	+++	+++
9.7	−	+++	+++	+++	+++	+++	+++
10.3	−	+++	+++	+++	+++	+++	+++
9.5	−	+++	+++	−	−	−	−
12.2	−/+	−	−	+++	+++	+	−/+
12.1	−	−	+++	−	+++	−	+++
PSA serotype	I	II	III	IV	V	VI	VII

Isolates and clones are shown as isolate/clone. Absence or presence of reaction is indicated by − or +++, respectively; (+) positive but consistently subnormal weak reactivity of some antibodies with certain isolates (see text and Table II); (−/+) some isolates within the group were negative while others weakly cross-reacted. Most isolates were tested at least three times using schizonts harvested from continuous culture at weekly intervals; the Wellcome isolate and clones NF54/C and LE5/P9 were each tested once using acetone-fixed antigens provided by Drs. R. R. Freeman, J. H. Meuwissen, and R. Carter, respectively. In every test, 10^4 schizonts were screened for reactivity with each antibody. A majority of the isolates appeared to be homogeneous, i.e., on reaction with any one antibody, most parasites reacted either positively or not at all. The uncloned isolates NF58, T9, PB1, SK16, T21, and K36, however, were not homogeneous and, as detected by at least one antibody in each case, consisted of mixtures of a majority of nonreactive organisms and a minority (<10%) of positively reactive parasites. These six isolates are grouped according to the antigenic phenotype of the predominant parasite population.

containing PSA and served as positive controls to compare with reactions of antibodies differentiating between restricted variant epitopes. Most isolates appeared to consist of homogeneous populations containing schizonts that reacted well with the control and some restricted antibodies but which lacked specificities detectable by other reagents. However, six isolates were heterogeneous, containing a minority of schizonts reacting positively with at least one restricted antibody among a majority of nonreactive ones; these isolates were considered (in the cases of isolates T9 and PB1, shown formally by cloning) to be mixtures of more than one antigenically distinct clone. Such mixed isolates are grouped in Table III according to serotypes of the predominant subpopulations. With this qualification, each isolate could be allocated to one of seven groups, termed PSA serotypes, which were defined operationally as distinct combinations of restricted specificities recognized by nine mAb. Thus, serotype I was characterized by positive reactivity with mAb 6.1, 7.3, and 7.6 and the absence of detectable reactions with mAb 9.2, 9.7, 10.3, 9.5, 12.2, and 12.1. Serotypes II–VII all lacked the marker specificities of type I and shared the determinants defined by mAb 9.2, 9.7, and 10.3. Serotypes II and III both reacted with prototype antibody 9.5 and lacked specificity 12.2, but could be differentiated by negative or positive reactions, respectively, with mAb 12.1. Conversely, serotypes IV and V both carried the 12.2 marker and lacked reactivity with mAb 9.5, and could be distinguished from each other by reactivity with antibody 12.1. Serotypes VI and VII were negative or positive, respectively, with mAb 12.1, did not express

the marker defined by mAb 9.5, and reacted weakly or not at all with mAb 12.2. This classification of PSA has proved useful for identification of laboratory and field isolates of *P. falciparum* (manuscript in preparation) but is not considered final and will doubtless be expanded and more extensively defined in the future by the use of additional anti-PSA antibodies on larger panels of *P. falciparum* isolates.

Discussion

In previous reports, we have demonstrated that mAb specific for schizonts and merozoites of the human malaria parasite *P. falciparum* recognize considerable antigenic diversity among culture-adapted (19) and clinical (20, 21) isolates of the parasite. Our aim has been to identify and characterize the molecules responsible for the diversity, and here we describe a family of polymorphic schizont antigens (PSA) of M_r 190,000–200,000 that significantly contribute to antigenic heterogeneity within the causative pathogen of malignant malaria.

Since uncloned isolates of *P. falciparum* frequently contain mixtures of genetically diverse organisms (see ref. 34 for review), in the present analysis we have preferred to use genetically distinct (26) and, at least with respect to the antigens studied, phenotypically homogeneous and stable clones of the parasite. Using four such clones as a source of [^{35}S]methionine-labeled proteins, we show that schizonts of each clone synthesize an analogous antigen that may vary moderately in size between the clones, and which is recognized by pooled human immune serum and, more selectively, by 13 mouse mAb.

The serological reactivities of clonal PSA, as demonstrated by comparative immunoprecipitation and immunofluorescence, have several implications. Firstly, PSA of all four clones originating from Thailand share determinants recognized by some monoclonal reagents, including the antibody 89.1, which had been used previously to identify an M_r 195,000 antigen of a West African isolate (2). The shared specificities were detected by IFA in every culture-adapted isolate studied, regardless of its geographical origin (Table III; 19) as well as in >200 clinical isolates from Africa and Brazil (McBride and Walliker, unpublished results). These findings strongly indicate that PSA of all clones of *P. falciparum* have related or identical common regions in their structure, as is also indicated by certain limited similarities between their proteolytic digestion profiles (Fig. 6; 33). Furthermore, since in living parasites PSA seem to be specifically processed into a series of discrete fragments, similar in number and related in size between clones (2; Fig. 3), it seems likely that they all share structure(s) susceptible to cleavage by specific parasite protease(s). The shared antigenic determinants recognized by the present mAb were species specific, since none of the antibodies cross-reacted in IFA with schizonts of *P. vivax* or the rodent parasites *P. yoelii*, *P. berghei*, *P. chabaudi*, *P. killicki*, or *P. vinckei* (results not shown). Thus, these species-specific determinants are additional to those shown to be shared between a M_r 195,000 PSA of *P. falciparum* and analogous antigens of *P. yoelii* and *P. chabaudi* (35).

Secondly, PSA expressed by different clones of *P. falciparum* are not identical and display antigenic (Fig. 2; Tables II, III) as well as structural (Fig. 6; 33) polymorphism. Thus in addition to the common structure(s), the PSA of each

clone carries, and can be distinguished by, antigenic determinants that are not detectable on PSA of certain other clones. Distributions of reactivities of antibodies recognizing the polymorphic part(s) of PSA of a panel of parasite isolates (Table III) indicate that the mAb used in this study recognize a minimum of five different specificities, and that these specificities probably belong to three non-overlapping segregating sites. None of the specificities are unique to a single isolate, but their segregation does not appear to be random, suggesting that certain combinations of determinants are mutually exclusive and may represent markers of alternative, possibly allelic forms of PSA. In the present study we have identified seven antigenically distinct forms of PSA. Our preliminary results with an extended panel of mAb against restricted determinants indicate that some 10–20 immunologically distinct PSA exist. This is a minimum estimate and the full extent of the polymorphism is unknown. It seems likely that a serotyping system based on the antigenic composition of PSA could be developed to help identify *P. falciparum* 'strains' for epidemiological, genetic, and immunological use.

The biological and immunological significance of PSA and their polymorphism remains to be determined. In living schizonts they are processed into fragments, of which three (M_r 83,000, 42,000, and 19,000) have been shown to be present as the major components on the surface of merozoites (3, 4). The antigenic composition of these fragments may not be identical and it seems likely that each can play a different biological and/or immunological role. The fragments at M_r 75,000–85,000 reacted with all antibodies used in this work, indicating that they carried both common and polymorphic specificities. With the exception of antibody 89.1 (a common determinant), the reagents also precipitated a peptide at M_r 30,000–33,000 from extracts of our *P. falciparum* lines. Since the mAb 89.1 also failed to react with an M_r 42,000 fragment of the antigen from another parasite (4), it is possible that this fragment and our smallest molecular weight species are analogous, the apparent difference in size being due to variation between isolates. Alternatively, the small peptide could be an as yet unreported fragment of PSA or an unrelated parasite protein specifically associated and thus coprecipitated with PSA.

As discussed elsewhere (2, 35), the PSA of *P. falciparum* shares a number of biological and immunological similarities, including antigenic polymorphism (33) with products of several species of animal malaria parasites that have been implicated as protective antigens of these species. It was suggested that, by analogy, it may be possible to immunize against human malaria using the *P. falciparum* antigen (2) described here as PSA. This expectation seems to be supported by the results of a study (36) in which *Saimiri* monkeys were partly protected by immunization with a purified *P. falciparum* antigen corresponding to the description of PSA. If these results are confirmed, it will be essential to determine the relative importance of immune responses against the common vs. polymorphic sites of PSA, in order to assess the feasibility of developing a vaccine based on this antigen. In addition, such assessment should logically include investigations on the antigenic structure of PSA in different populations of *P. falciparum* in the main endemic areas (20, 21).

A final comment should be made on the use of 'homologous' and 'heterolo-

gous' isolates of *P. falciparum* in immunological and particularly in protective or vaccination experiments. Such studies have often yielded inconsistent results, providing apparent evidence for strain specificity of protection in some combinations of parasite isolates but not in others (11–16, 37–41). In these studies the terms homologous and heterologous have been assumed to imply antigenic identity and probable nonidentity, respectively, between parasites used for immunization and subsequent challenge. Since a single isolate can contain antigenically different clones (Table II), it is likely that such a mixed population may change in composition, e.g., under the selective pressure of immune response, and thus even a so-called homologous challenge may not be antigenically identical to the immunizing inoculum. Conversely, since some antigenically distinct parasites have wide geographical distribution (Table III; 19) it is not unlikely that by chance alone, an isolate chosen as heterologous will have a similar antigenic composition as the homologous one. With the progress made and the potential for immunological classification of *P. falciparum*, it seems no longer justified to select parasite material for comparative immunological studies by ad hoc criteria such as origin, rather than by the at least partly known antigenic composition of defined parasite products, such as PSA or other, so far less extensively investigated, variant molecules (8, 19, 42, 43).

Summary

Intraspecies antigenic diversity in the blood stages of the human malaria parasite *Plasmodium falciparum* was investigated using a collection of murine monoclonal antibodies and clones of the parasite. The results were as follows: (*a*) The schizont and merozoite stages of the parasite express on their surface clonally restricted antigens detectable by strain-specific antibodies in indirect immunofluorescence tests. (*b*) These restricted antigens are phenotypically stable characteristics of clones grown in vitro. (*c*) The molecules carrying the specific antigens were isolated by immunoprecipitation and were found to be parasite proteins ranging in size from M_r 190,000 to 200,000 between clones. (*d*) Comparative immunoprecipitation and peptide mapping of these molecules showed that each parasite clone expresses a protein that is antigenically and structurally distinct from the equivalent products of several other clones. (*e*) The different clonal products are, however, immunologically interrelated, since they possess determinants in common with all tested isolates of the parasite. (*f*) These polymorphic molecules are closely related to a previously described schizont protein of *P. falciparum* that is posttranslationally cleaved into fragments located on the merozoite surface.

These findings show the existence of a family of related polymorphic schizont antigens (PSA) of *P. falciparum*, whose expression is clonally restricted, and indicate that these proteins have regions of constant and variable antigenicity. We propose that a system of immunological classification of the parasite can be developed based on the polymorphism of these proteins.

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