BIOSYNTHESIS AND PROCESSING OF A
PLASMODIUM FALCIPARUM SCHIZONT ANTIGEN
RECOGNIZED BY IMMUNE SERUM
AND A MONOCLONAL ANTIBODY

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Protective immunization of the vertebrate host against malaria can operate against either of two distinct stages of the parasite's life cycle. The sporozoite forms, which develop in the salivary glands of the infected mosquito, are immunogenic in the vertebrate host (1) and possess a major surface antigen against which a protective immune response can be elicited (2, 3). The asexual blood stage, which is responsible for the clinical symptoms of malaria as parasites invade and destroy the host's erythrocytes, is also susceptible to immune intervention. In man, acquired immunity against the blood stage of Plasmodium falciparum infection is mediated at least partly by antibody (4). Immunization studies using killed parasites have confirmed that protective antigens are associated with the asexual blood forms (5), and immunochemical analyses have shown that these forms are antigenically complex (6, 7). Monoclonal antibodies that agglutinate merozoites (8) or inhibit the growth of malaria parasites in vitro (9) or in vivo (10) have been used to define specific antigens against which the host may make a protective immune response.

We have recently shown (11) that two protein antigens, of 235,000 and 230,000 mol wt, associated with schizonts and merozoites of the rodent malaria parasite, Plasmodium yoelii, can be purified using monoclonal antibodies and used successfully to immunize mice against challenge infection. In the present study we have characterized a 195,000-mol wt protein antigen associated with schizonts and merozoites of P. falciparum, which is one of the major antigens recognized by human immune serum. Using a monoclonal antibody specific for this protein, we have investigated its biosynthesis during synchronous development of the parasite in vitro. This has revealed a specific processing of the protein that coincides with schizont maturation. On the basis of its localization, size, and specific processing, we suggest that this protein may be analogous to one of the protective antigens of P. yoelii.

Materials and Methods

Medium and Reagents. RPMI 1640 tissue-culture medium, Hapes buffer, and fetal calf serum were obtained from Flow Laboratories Ltd., Ayrshire, Scotland. Percoll was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Aminopterin, methionine, tosyl-L-lysine chloromethyl ketone (TLCK), phenylmethyl sulfonyl fluoride (PMSF), dithiothreitol (DTT), and chymotrypsin were purchased from Sigma Chemical Co., St. Louis, MO, and gentamycin was purchased from Peptide Institute, Inc., Osaka, Japan. Bovine serum albumin (BSA) was purchased from ICN, Costa Mesa, CA. Oligo(dT)_{30-35} was purchased from Pharmacia. Immunoaffinity chromatography was performed using Protein G-Sepharose (Pharmacia) and Staph A, Staphylococcus aureus, Cowan 1 strain. PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PMSF, phenylmethyl sulfonyl fluoride; TLCK, tosyl-L-lysine chloromethyl ketone.

Abbreviations used in this paper: BSA, bovine serum albumin; DTT, dithiothreitol; IIF, indirect immunofluorescence; NP-40, Nonidet P-40; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PMSF, phenylmethyl sulfonyl fluoride; Staph A, Staphylococcus aureus, Cowan 1 strain; SDS, sodium dodecyl sulfate; TCA, trichloroacetic acid; TLCK, tosyl-L-lysine chloromethyl ketone.
supplied by Schering Corp., Kennilworth, NJ. From BDH Ltd., Poole, England, the following chemicals were obtained: polyethylene glycol, thymidine, glucose, Nonidet P-40 (NP-40), iodoacetamide, acrylamide, N,N'-methylenebisacrylamide, sodium dodecyl sulfate (SDS), bromophenol blue, ammonium persulfate, N,N,N',N'-tetramethylenediamine, urea, and sorbitol. Rabbit antiserum against mouse IgG, conjugated with fluorescein isothiocyanate, was purchased from Miles Laboratories Ltd., Slough, England, as were rabbit antisera specific for mouse IgG1, IgG2a, IgG2b, IgG3, IgM, IgA, and IgG.

The radiolabeled compound 1-[35S]methionine (sp act 800–1,200 Ci/mmol) was obtained from Amersham International Ltd., Amersham, England.

High-titer P. falciparum-immune human sera were supplied by Dr. B. M. Greenwood, Medical Research Council Laboratories, The Gambia, West Africa. The P3-NS1/1-Ag4-1 mouse myeloma cell line was supplied by Dr. A. F. Williams, University of Oxford, England.

In Vitro Cultivation of P. falciparum. The technique for the continuous cultivation of P. falciparum was based on that described by Trager and Jensen (12). The West African Wellcome strain of P. falciparum was grown asynchronously in 75-cm² plastic tissue-culture flasks (Corning Glass Works, Corning, NY) containing 12 ml RPMI 1640 medium supplemented with 22 mM glucose, 92 μM hypoxanthine, 16 mM NaHCO₃, 35 mM Hepes, 20 μg/ml gentamycin, and 10% (vol/vol) human serum. Washed human A+ erythrocytes were added to the culture medium to a hematocrit of 10%. The flasks were gassed with a mixture containing 5% O₂, 2% CO₂, and 93% N₂, and were then sealed and incubated at 37°C, lying flat. The supernatant culture medium was aspirated and replaced daily. In cultures set up at a starting parasitemia of 0.1%, a 40-fold parasite multiplication was routinely achieved over a 4-d period.

For experiments requiring synchronous parasite growth, a modification of the method described by Lambros and Vanderberg (13) was used. Asynchronous cultures at 3–5% parasitemia were pooled and centrifuged at 500 g for 10 min. The pellet was resuspended in 5 vol of sterile 5% (wt/vol) aqueous sorbitol, prewarmed to 37°C, and left at room temperature for 5 min. After centrifugation at 150 g for 5 min and removal of the supernatant, the pellet was resuspended in 9 vol of culture medium. The cell suspension was distributed in 12-ml volumes into culture flasks, which were gassed, sealed, and returned to the incubator. Immediately after the sorbitol treatment, the cultures contained only ring-form parasites. The next day the cultures were monitored hourly by examination of Giemsa-stained smears. 4 h after the appearance of new ring forms (33 h after the first sorbitol treatment), the cultures were again pooled and treated with sorbitol as above. The cells were then redistributed into flasks to give synchronized cultures containing ring forms 0–4 h old at a parasitemia of 2–3%.

Production of Hybridoma WIC 89.1. Asynchronous P. falciparum culture suspension (5% parasitemia) was layered directly on to a 15-ml cushion of Percoll (54% vol/vol in unbuffered RPMI 1640) in 50-ml polycarbonate centrifuge tubes (Sorvall, Newton, CT) and centrifuged at 1,200 g for 15 min. The cells remaining at the interface were mainly schizonts at various stages of maturation. The schizont-enriched cells were washed in saline, and 10⁸ were injected intraperitoneally into female BALB/c mice. After 28 d a similar number of schizonts was injected intravenously. 3 d after the second immunization, the spleens were taken for fusion. As described previously (10), 2 × 10⁸ spleen cells were fused with 2 × 10⁷ P3-NS1/1-Ag4-1 myeloma cells in the presence of polyethylene glycol. The cells were dispersed into 144 tissue-culture wells in 2-ml volumes of hypoxanthine-aminopterin-thymidine-selective medium, and after 10 d the culture supernatants were tested for specific antibody by indirect immunofluorescence (IF) on acetone-fixed preparations of P. falciparum schizonts. Cloned hybridoma lines were obtained from some of the positive wells by limiting dilution on BALB/c thymus cell feeder layers. One of the cloned lines, WIC 89.1, was used in some of the experiments described in this study. WIC 89.1 was grown as an ascites tumor in BALB/c mice, yielding serum and ascitic fluid containing the monoclonal antibody 89.1. The isotype of antibody 89.1 was determined by double diffusion in agar, using 100-fold-concentrated, serum-free supernatant from a culture of the hybridoma WIC 89.1 and rabbit antisera specific for the isotypes of mouse immunoglobulin.

[35S]Methionine Biosynthetic Labeling of P. falciparum. For labeling of unsynchronized parasites, 5 × 10⁸ parasitized erythrocytes (including late trophozoites and early and late schizonts) were obtained by centrifugation on Percoll, as described above. The cells were washed twice in methionine-free RPMI 1640 supplemented with 10% human serum, then were resuspended in...
5 ml of the same medium to which was added \[^{[35S]}\text{methionine}\] at 200 \(\mu\text{Ci} / \text{ml}\). The cells were added to a culture flask which was gassed and incubated at 37°C for 150 min. After incubation, the cells were washed twice in cold phosphate-buffered saline (PBS) before solubilization.

Pulse labeling and pulse-chase labeling of synchronized cultures at regular intervals during the parasite's 48-h developmental cycle was performed as follows. A \(P. falciparum\) culture in 20 flasks was synchronized by two sorbitol treatments, spaced 33 h apart, as described above. The time of the second sorbitol treatment was defined as \(t = 0\) h, when all the parasites were 0-4 h old. At \(t = 0, 6, 12, 18, 24, 30, 36, 42, 45, \) and \(48\) h, the cells from two flasks were pooled, washed twice with methionine-free RPMI 1640 supplemented with 10% human serum, and incubated in a gassed flask in 8 ml of the same medium supplemented with 60 \(\mu\text{Ci} / \text{ml} \[^{[35S]}\text{methionine}\]. After 30 min at 37°C the cells were washed and resuspended in 15 ml of complete culture medium supplemented with 1.7 mM cold methionine. Immediately, 5 ml of the cell suspension was removed, and the cells were washed twice in cold PBS and frozen rapidly at \(-80°C\) as a pellet. The remaining 10 ml of culture was gassed and further incubated at 37°C.

After 60 min of cold chase a further 5 ml of culture was removed, and the cells were washed twice with PBS and frozen. The remaining cells were washed and frozen after 120 min of chase. At the end of the cycle the frozen cell pellets were solubilized as described below. At 3-h intervals throughout the cycle a smear of the cultured cells was prepared and stained with Giemsa's stain to monitor the parasite's development by light microscopy.

**Cell Solubilization and Immunoprecipitation.** Cells were extracted on ice in at least 5 vol of 50 mM Tris-HCl pH 8.0, 5 mM EDTA, 5 mM EGTA, 5 mM iodoacetamide, 1 mM PMSF, 0.1 mM TLCK, and 1% (vol/vol) NP-40. Insoluble material was removed by centrifugation at 100,000 \(\times\) g for 30 min at 4°C.

Specific sera were added to the labeled extracts, and precipitation of immune complexes was performed using formalin-fixed \(Staphylococcus aureus\) Cowan 1 strain cells (Staph A), essentially as described by Kessler (14). When precipitating with antibody 89.1, 20 \(\mu\text{g}\) of immunoabsorbent purified rabbit anti-mouse IgG antibody was added to each tube before addition of Staph A. The complexes 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 bovine serum albumin (BSA), then twice in the same buffer without NaCl and BSA.

**SDS-Polyacrylamide Gel Electrophoresis (PAGE).** Immunoprecipitated complexes were analyzed by SDS-PAGE (15) in slab gels using 3% polyacrylamide in the stacking gel and 7.5% polyacrylamide in the separation gel. After electrophoresis the slab gels were stained with Coomassie Blue in fixative. The gels were then treated with Enhance (New England Nuclear, Boston, MA) before drying. Radiolabeled polypeptides were visualized by fluorography at \(-70°C\), using X-omat S film (Eastman Kodak Co., Rochester, NY). Molecular-weight markers were human spectrin heterodimer (240,000 and 220,000 mol wt), \(\beta\)-galactosidase (120,000 mol wt), phosphorylase b (93,000 mol wt), BSA (68,000 mol wt), and aldolase (39,000 mol wt).

**Peptide Mapping.** Immune complexes containing \[^{[35S]}\text{methionine}-labeled\] polypeptides were dissolved in 40 \(\mu\)l 0.0625 M Tris, 2% SDS, 10% glycerol, 0.1 M DTT, and 0.005% bromophenol blue, and heated to 100°C for 2 min. After cooling, 16 \(\mu\)l of 0.5 M iodoacetamide and 5 \(\mu\)l of 1 M \(\text{NH}_4\) were added to carboxymidomethylate the reduced proteins. After 30 min the mixture was subjected to SDS-PAGE, stained with Coomassie Blue in fixative, and dried for autoradiography. Areas of the dried gel containing labeled polypeptides were cut out, and each was washed into 2 ml of 0.1 M \(\text{NH}_4\text{HCO}_3\), and washed for 2 h. The supernatant from each sample was discarded and replaced with 1 ml of 50 \(\mu\)g/ml chymotrypsin in 0.1 M \(\text{NH}_4\text{HCO}_3\). The digestion was allowed to proceed for 24 h at 37°C and was then repeated. The two supernatants from each sample were pooled, freeze-dried, and applied to a cellulose thin-layer plate for electrophoresis at pH 4.4 (pyridine:acetic acid:water:acetone = 2:4:79:15, vol/vol) and chromatography in the second dimension (butanol:acetic acid:water:pyridine = 15:3:12:10, vol/vol). Peptides containing \[^{[38S]}\text{methionine}\] were detected by autoradiography.

**Results**

**Characterization of Monoclonal Antibody 89.1.** The hybridoma line WIC 89.1 was generated by fusing P3-NS1/1-Ag4-1 mouse myeloma cells with spleen cells from a
BALB/c mouse immunized with *P. falciparum* schizonts, as described in Materials and Methods. The hybridoma secreted a monoclonal antibody of the murine IgG1 isotype as determined by agar double diffusion. By IIF using acetone-fixed *P. falciparum* smears, antibody 89.1 appeared to be specific for a parasite antigen associated with the plasma membrane of the developing intracellular schizont and with free merozoites (Fig. 1). Antibody 89.1 was also found to react with the surface of free merozoites in suspension. Pools of serum and ascitic fluid obtained from BALB/c mice carrying the hybridoma line WIC 89.1 had IIF titers of 1:80,000 and 1:20,000, respectively.

**Multiple *P. falciparum* Polypeptides Immunoprecipitated by Antibody 89.1.** An asynchronous population of cultured *P. falciparum* parasites was biosynthetically labeled with $^{[35S]}$methionine for 150 min. When the NP-40-soluble cell extract was used for immunoprecipitation with antibody 89.1, five polypeptides were detected by SDS-PAGE and fluorography (Fig. 2, track 1). The largest species was of 195,000 mol wt, and the molecular weights of the other species were 153,000, 150,000, 110,000, and 83,000. A 195,000-mol wt polypeptide was also a major component of the human immune serum immunoprecipitate (Fig. 2, track 2).

The five polypeptides reacting with antibody 89.1 were examined by two-dimensional mapping of their $^{[35S]}$methionine-labeled soluble chymotryptic peptides. The maps showed many features in common (Fig. 3) that indicated amino acid sequence relatedness between the polypeptides consistent with the lower molecular weight species being fragments all derived from the 195,000-mol wt species.

**Establishment of Synchronous *P. falciparum* Cultures and Pulse Labeling with $^{[35S]}$methionine.** To study the timing of synthesis of the 195,000-mol wt protein recognized by antibody 89.1 during the intraerythrocytic development of the parasite, it was

![Fig. 1. IIF staining pattern of antibody 89.1 on a schizont and a group of merozoites of *P. falciparum* in an acetone-fixed smear. The serum containing the antibody was diluted 1:1,000 with PBS. Scale bar = 10 μm.](image-url)
necessary to use a synchronized population of parasites. To achieve this, asynchronous cultures were treated with sorbitol on two occasions spaced 33 h apart, as described in Materials and Methods. For the first 12 h after the second sorbitol treatment, the parasites were all ring forms (Fig. 4A). Trophozoites appeared after this time and accounted for all parasites at 24 h. Schizogony began at 27 h: schizonts with 2-6 nuclei were observed until 33 h, and by 45 h most schizonts contained 10-20 nuclei. The appearance of new ring forms was first noted at 39 h, but the majority of schizonts did not release merozoites until after 42 h of culture. At 48 h, new ring forms accounted for 90% of parasitized erythrocytes.

At 6-h intervals after the initiation of synchronous parasite culture (with an extra time point at 45 h), one-tenth of the total culture was pulse labeled with [35S]methionine for 30 min. Parasite protein synthesis, assessed by TCA-precipitable incorporation of [35S]methionine, was at a maximum 36 h into the growth cycle (Fig. 4B). The NP-40-soluble *P. falciparum* polypeptides synthesized at the stated time points during the erythrocytic cycle were resolved by SDS-PAGE, as shown in Fig. 5A. Some parasite proteins were synthesized throughout the cycle, but most prominent polypeptides were made only at certain times during development, which indicates a strong stage specificity of protein synthesis associated with intracellular differentiation and development.

**Immunoprecipitation of Pulse-labeled *P. falciparum* Proteins Using Human Immune Serum.** The stage specificity of *P. falciparum* protein synthesis was shown clearly by immunoprecipitation from [35S]methionine pulse-labeled extracts of the synchronous culture, using human immune serum. In ring forms and trophozoites, only two parasite proteins, of 160,000 and 70,000 mol wt, were recognized by immune serum (Fig. 5B, tracks 1-5). These disappeared during schizogony and reappeared after reinvasion (tracks 6-10). Most of the polypeptides precipitated by immune serum
FIG. 3. Peptide mapping of the polypeptides immunoprecipitated by antibody 89.1. The polypeptides were separated by SDS-PAGE and were then digested with chymotrypsin within the gel. Peptides released by this treatment were separated in two dimensions on thin-layer plates, and the labeled peptides were detected by autoradiography. The molecular weights of the polypeptides analyzed in this way were: A, 195,000; B, 153,000; C, 150,000; D, 110,000; and E, 83,000.
were synthesized during schizogony, and many of these were of high molecular weight (the presence of faint high-molecular-weight bands in track 1 indicated that the initial culture contained a few viable schizonts that had apparently survived the sorbitol treatment). Of particular interest in the present study, a 195,000-mol wt \textit{P. falciparum} polypeptide was synthesized abundantly through schizogony and was precipitated by immune serum (Figs. 5 A and B, tracks 6–10). However, not all schizont-stage proteins were synthesized right through schizogony: for example, an 84,000-mol wt protein was made only by immature schizonts (tracks 6–8), and a 127,000-mol wt protein was synthesized only in mature schizonts (tracks 8 and 9).

\textbf{Biosynthesis and Processing of the 195,000-Mol Wt Protein Recognized by Antibody 89.1.} When the 30-min pulse-labeled samples taken during synchronous parasite development were used for immunoprecipitation with monoclonal antibody 89.1, it was found that radiolabel was associated predominantly with the 195,000-mol wt band, with minor representation of the 150,000-mol wt doublet (Fig. 6A). These polypeptides were not detected before 30 h of parasite development. The 83,000-mol wt fragment reactive with antibody 89.1 was apparent in samples from mature schizonts (>36 h) that had been chased with cold methionine for 60 or 120 min (Fig. 6B and C). At 30 h into the parasite's development cycle (immature schizonts), up to 120 min of chase resulted in no apparent shift in the radiolabel to the 83,000-mol wt band (Fig. 6A–C, tracks 1). At 36 h, the 83,000-mol wt band was barely detectable.
Fig. 5. SDS-PAGE analysis of *P. falciparum* polypeptides synthesized during an entire 48 h developmental cycle in vitro. A culture was synchronized at $t = 0$ h, and aliquots were pulse labeled with [$^{35}$S]methionine for 30 min at $t = 0, 6, 12, 18, 24, 30, 36, 42, 45$, and 48 h (tracks 1-10, respectively). Total labeled polypeptides in the NP-40 lysates (A) and those immunoprecipitated using human immune serum (B) were analyzed by SDS-PAGE and fluorography. Molecular-weight markers and the 195,000-mol wt *P. falciparum* protein are indicated.

After pulse and chase (tracks 2). However, after 42 h of development, the 83,000-mol wt fragment was detected after 60 min of chase (Fig. 6B, track 3) and was more strongly represented after 120 min of chase, at the expense of the 195,000-mol wt band (Fig. 6C, track 3). After 120 min of chase at 42 h, a novel polypeptide of 60,000-mol wt, reactive with antibody 89.1, was detected, and was clearly present after 120 min of chase at 45 h (Fig. 6C, track 4). At 48 h the 60,000-mol wt band was present even immediately after the pulse-labeling period (Fig. 6A, track 5) and was strongly represented after 120 min of chase, at which time the 195,000-mol wt band had
Fig. 6. Processing of the 195,000-mol wt *P. falciparum* polypeptide reactive with antibody 89.1. Aliquots of a synchronous *P. falciparum* culture were pulse labeled with [35S]methionine for 30 min at t = 0, 6, 12, 18, 24, 30, 36, 42, 45, and 48 h. Samples were taken immediately after labeling and after 60 min and 120 min of cold chase. The NP-40 soluble polypeptides immunoprecipitated from each sample using antibody 89.1 were analyzed by SDS-PAGE and fluorography. (A) Samples were taken immediately after pulse labeling at t = 30, 36, 42, 45, and 48 h (tracks 1–5, respectively). (B) Samples were taken after 60 min of cold chase following pulse labeling at t = 30, 36, 42, 45, and 48 h (tracks 1–5, respectively). (C) Samples were taken after 120 min of cold chase following pulse-labeling at t = 30, 36, 42, 45, and 48 h (tracks 1–5, respectively). The 195,000-mol wt polypeptide was not detected before t = 30 h. Some low-molecular-weight material was nonspecifically bound by IgG-Staph A complexes (tracks 1 and 2). The band at 75,000 mol wt (A, track 2) was considered to be artificial. The estimated molecular weights of the polypeptides are indicated.

almost disappeared. The appearance of the 83,000-mol wt polypeptide coincided with merozoite formation within mature schizonts, and the appearance of the 60,000-mol wt polypeptide was coincident with reinvasion of new host erythrocytes.

Discussion

Our aim has been to identify and characterize antigens associated with the blood stage of *P. falciparum* against which the host may effect a protective immune response. Using polyvalent human immune serum enables the range of candidate antigens to be identified, while individual antigens can be studied in detail using monoclonal antibodies. Using this approach we have studied the biosynthesis of *P. falciparum* proteins during synchronous parasite development in vitro. By pulse labeling synchronous cultures at 6 h intervals we found that the majority of proteins immunoprecipitated by human immune serum were made during schizogony. The parasite protein of 195,000-mol wt was a predominant antigen recognized by human immune serum and also by the murine monoclonal antibody 89.1.

From a lysate of an asynchronous population of parasites labeled with [35S]methionine, antibody 89.1 precipitated a series of smaller polypeptides in addition to the 195,000-mol wt species. The most dominant of these was of 83,000 mol wt. All of the polypeptides recognized by antibody 89.1 were shown to be related by peptide mapping. Pulse-chase labeling of synchronized schizonts and immunoprecipitation with antibody 89.1 suggested that the 195,000- and 83,000-mol wt polypeptides have a precursor-product relationship. The polypeptides of 153,000-, 150,000-, and 110,000-mol wt are presumably transient intermediates in the specific degradation process.
The results also indicated that processing of the 195,000-mol wt species down to the 83,000-mol wt species occurs in mature schizonts (i.e., segmenters), rather than in early schizonts. It is possible that the antigen detected on the merozoite surface by IIF using antibody 89.1 is the 83,000-mol wt species, and the results of preliminary surface labeling studies support this interpretation. The identity of the 60,000-mol wt polypeptide reactive with antibody 89.1 (Fig. 6) is less clear. It was not detected when asynchronous cultures of parasites were labeled under conditions where reinvasion did not take place (Fig. 2) and may be a cleavage product of the 83,000-mol wt polypeptide associated with merozoite invasion of new host erythrocytes.

In other studies, high-molecular-weight schizont proteins have been implicated as putative protective antigens of blood stage \textit{P. falciparum} (7), \textit{P. knowlesi} (8), and \textit{P. chabaudi} (16, 17) malaria parasites. The possibility of antigen processing was not investigated in those studies.

We have recently characterized a 230,000-mol wt schizont protein of the rodent malaria parasite, \textit{P. yoelii} (11), and demonstrated that it is processed in mature parasites to a series of discrete fragments. A monoclonal antibody against this \textit{P. yoelii} protein reacted with schizonts and merozoites to give an IIF staining pattern indistinguishable from that produced on \textit{P. falciparum} schizonts and merozoites using antibody 89.1. The 230,000-mol wt \textit{P. yoelii} protein and its fragments were purified by monoclonal antibody-Sepharose affinity chromatography, and was used effectively to immunize mice against challenge infection with \textit{P. yoelii}. The 230,000-mol wt antigen of \textit{P. yoelii} and the 195,000-mol wt antigen of \textit{P. falciparum} may be analogous proteins: both are of high molecular weight, both are schizont specific, both are strongly recognized by immune sera, and their subcellular localization is identical. Most importantly, both proteins are specifically processed to series of discrete fragments. In \textit{P. yoelii} the major antigenic product is of 90,000 mol wt, whereas in \textit{P. falciparum} it is of 83,000 mol wt in the absence of reinvasion. By analogy, the evidence indicates that the 195,000-mol wt protein of \textit{P. falciparum} may be a protective antigen, or a precursor of a protective antigen. It should be possible to purify this protein and test it in immunization studies on experimental hosts of \textit{P. falciparum}.

**Summary**

Stage-specific protein synthesis by the erythrocytic forms of the malaria parasite \textit{Plasmodium falciparum} was investigated by pulse labeling synchronous parasite cultures with $[^{35}\text{S}]$methionine at 6-h intervals during a complete 48-h developmental cycle. About 40 labeled parasite proteins could be immunoprecipitated with human immune serum, and most of these were associated with the schizont stage of development. In particular, one schizont protein was a 195,000-mol wt species against which a murine monoclonal antibody was produced. This monoclonal antibody, 89.1, reacted with the parasite membrane in schizonts and also with the surface of free merozoites in the indirect immunofluorescence test. In addition to the 195,000-mol wt protein, antibody 89.1 immunoprecipitated a series of lower-molecular-weight polypeptides from extracts of labeled asynchronous \textit{P. falciparum} parasite cultures. These were shown to be related to the 195,000-mol wt protein by peptide mapping. Pulse-chase labeling of synchronized cultures, and immunoprecipitation with antibody 89.1, showed that specific processing of the 195,000-mol wt polypeptide to the lower-molecular-weight products is concomitant with schizont maturation and merozoite release. It is sug-
gested that this P. falciparum protein may be analogous to a similarly processed 230,000-mol wt protective antigen of the rodent malaria parasite, P. yoelii.

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