Brief Definitive Report

THE THREE MAJOR ANTIGENS ON THE SURFACE OF *PLASMODIUM FALCIPARUM* MEROZOITES ARE DERIVED FROM A SINGLE HIGH MOLECULAR WEIGHT PRECURSOR

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The identification and characterization of antigens synthesized by the asexual blood stage of *Plasmodium falciparum* are crucial steps in the definition of those antigens important in the induction of protective immunity. Antigens synthesized in schizonts and expressed on the surface of merozoites are likely to be of major importance (1, 2). Recently, we described the use of a monoclonal antibody to identify a 195,000 mol wt protein that is synthesized in schizonts and then processed through a series of discrete fragments (3). On the surface of naturally released merozoites, the monoclonal antibody detected an 83,000 mol wt fragment (4).

We have now purified the 195,000 mol wt protein from detergent extracts of *P. falciparum*–infected cells and raised a polyvalent antiserum against this protein. This antiserum recognizes three polypeptides on the surface of the merozoite: the 83,000 mol wt polypeptide and two additional species of 42,000 and 19,000 mol wt. These three fragments of the 195,000 mol wt precursor are the major merozoite surface antigens recognized by human immune serum.

Materials and Methods

In Vitro Cultivation of P. falciparum and Collection of Merozoites. The West African (Wellcome) strain of *P. falciparum* was cultured and naturally released merozoites were harvested as described previously (3, 4).

Purification of the P. falciparum Protein Recognized by Monoclonal Antibody 89.1. Monoclonal antibody 89.1 was purified from the ascites fluids of mice carrying hybridoma WIC 89.1 by chromatography on protein A-Sepharose (5). The IgG was coupled to cyanogen bromide-activated Sepharose 4B according to the manufacturer's recommendations, using 10 mg protein/ml swollen gel.

Cell pellets from bulk cultures of *P. falciparum* containing ~3×10^{11} cells were extracted on ice in 100 ml 50 mM Tris-HCl, pH 8.2, 5 mM EDTA, 5 mM EGTA, 5 mM iodoacetamide, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM tosyl-L-lysine chloromethyl ketone (TLCK), and 0.5% (wt/vol) sodium deoxycholate. The extract was centrifuged at 100,000 *g* for 45 min and then the supernatant was passed through a 20-ml pre-column of Sepharose CL-6B and a column of antibody 89.1–Sepharose, equilibrated with a buffer containing 10 mM Tris-HCl, pH 8.2, 1 mM EDTA, 1 mM EGTA, and 0.5% (wt/vol) sodium deoxycholate. After uncoupling the pre-column, the antibody 89.1–Sepharose column was washed extensively with equilibration buffer. Material retained on the column was eluted with 50 mM diethylamine-HCl, pH 11.5, containing 0.5% (wt/vol) sodium deoxycholate. The eluate was concentrated by ultrafiltration using an Amicon XM50 filter (Amicon Corp., Danrers, MA). Protein concentration was estimated by the method of Peterson (6).

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Antisera. Protein eluted from the antibody 89.1–Sepharose column was used to prepare a polyvalent antiserum. An owl monkey (*Aotus trivirgatus*) was immunized intramuscularly with 100 μg of the protein together with 0.5 mg saponin as an adjuvant. On day 28 a boost with 100 μg protein and 0.5 mg saponin was given. On day 35 a serum sample was collected. A pool of sera was made from *P. falciparum*–immune donors (4).

Lactoperoxidase-catalyzed Iodination. The merozoites collected from a 24-ml culture were surface labeled with ^{125}I using the lactoperoxidase method and a detergent extract was prepared as described previously (4). To label proteins in an extract of infected erythrocytes, a pellet containing 10^8 cells, enriched for schizonts by centrifugation on Percoll (3), was lysed by the addition of 50 μl 10 mM Tris-HCl, pH 8.0, 0.2% (vol/vol) Nonidet P-40, and the proteins were labeled with ^{125}I using lactoperoxidase (7).

Immunoprecipitations, Electrophoresis, Peptide Mapping, and Western Transfer Analysis. Immunoprecipitations, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) using 7.5 and 12.5% slab gels, and the detection of labeled bands by autoradiography were performed as described previously (3, 4). Molecular weight markers were human spectrin heterodimer (240,000 and 220,000 mol wt), β-galactosidase (116,000 mol wt), phosphorylase *b* (93,000 mol wt), bovine serum albumin (68,000 mol wt), aldolase (39,000 mol wt), triose phosphate isomerase (27,000 mol wt), and lysozyme (15,000 mol wt). Peptide maps of labeled polypeptides were prepared after digestion with trypsin or chymotrypsin (Miles Laboratories, Slough, England), as described elsewhere (3). The Western transfer analysis was performed as described previously (8).

Results

Affinity Purification of the 195,000 Mol Wt Protein and its Fragments. An extract from 3.4×10^{11} erythrocytes infected with *P. falciparum* (3.7% parasitemia, of which 26% were schizonts) was passed through an antibody 89.1–Sepharose affinity column. Approximately 400 μg protein, representing 0.0034% of the total protein applied, was bound and specifically eluted from the column. In

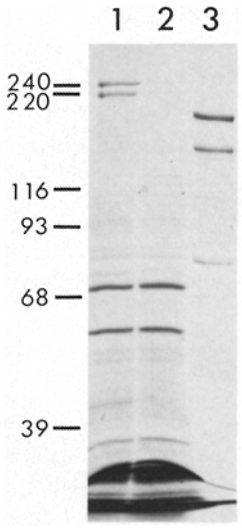

FIGURE 1. SDS-PAGE analysis of the monoclonal antibody affinity purification of the 195,000 mol wt protein and processing fragments from a detergent extract of *P. falciparum* schizonts. A detergent extract of infected erythrocytes (lane *1*) was subjected to centrifugation at 100,000 *g* and the supernatant (lane *2*) was applied to the column. After extensive washing the specifically bound material was eluted (lane *3*). Polypeptides were detected by Coomassie Blue staining. The positions of the molecular weight markers are indicated.

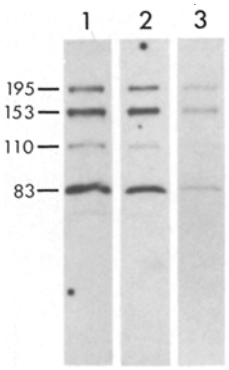

FIGURE 2. Autoradiograph of a Western blot of the specific eluate from the affinity column probed with monoclonal antibody 89.1 (lane *1*), polyvalent antiserum raised against the purified protein (lane *2*), and human immune serum (lane *3*), and developed with ^{125}I-labeled rabbit anti-mouse IgG (lane *1*) or ^{125}I-labeled protein A (lanes *2* and *3*). The molecular weights of the polypeptides are indicated.

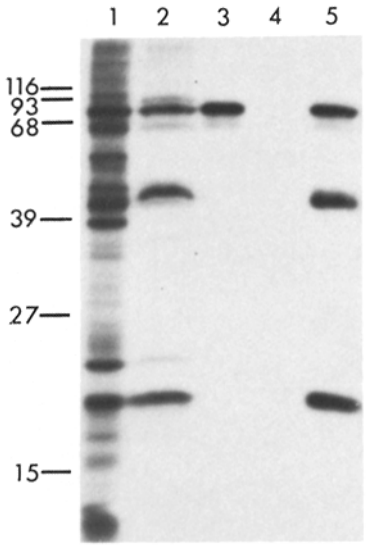

FIGURE 3. SDS-PAGE analysis of total ^{125}I surface-labeled merozoite proteins (lane *1*) and those immunoprecipitated by human immune serum (lane *2*), monoclonal antibody 89.1 (lane *3*), normal mouse serum (lane *4*), and polyvalent antiserum raised against the purified protein (lane *5*). Positions of the molecular weight markers are indicated.

addition to the 195,000 mol wt species, lower molecular weight polypeptides of 153,000, 110,000, and 83,000 mol wt were present in the eluate and were detected by Coomassie Blue staining after SDS-PAGE (Fig. 1). It has been shown previously by peptide mapping that these polypeptides are proteolytic fragments of the 195,000 mol wt protein (3).

Western Transfer Analysis of the Protein Eluted from the Antibody 89.1–Sepharose Column. No contaminants in the purified protein preparation were detected by Western transfer analysis. All of the polypeptides eluted from the affinity column at pH 11.5 reacted with the monoclonal antibody 89.1 (Fig. 2, lane *1*). An

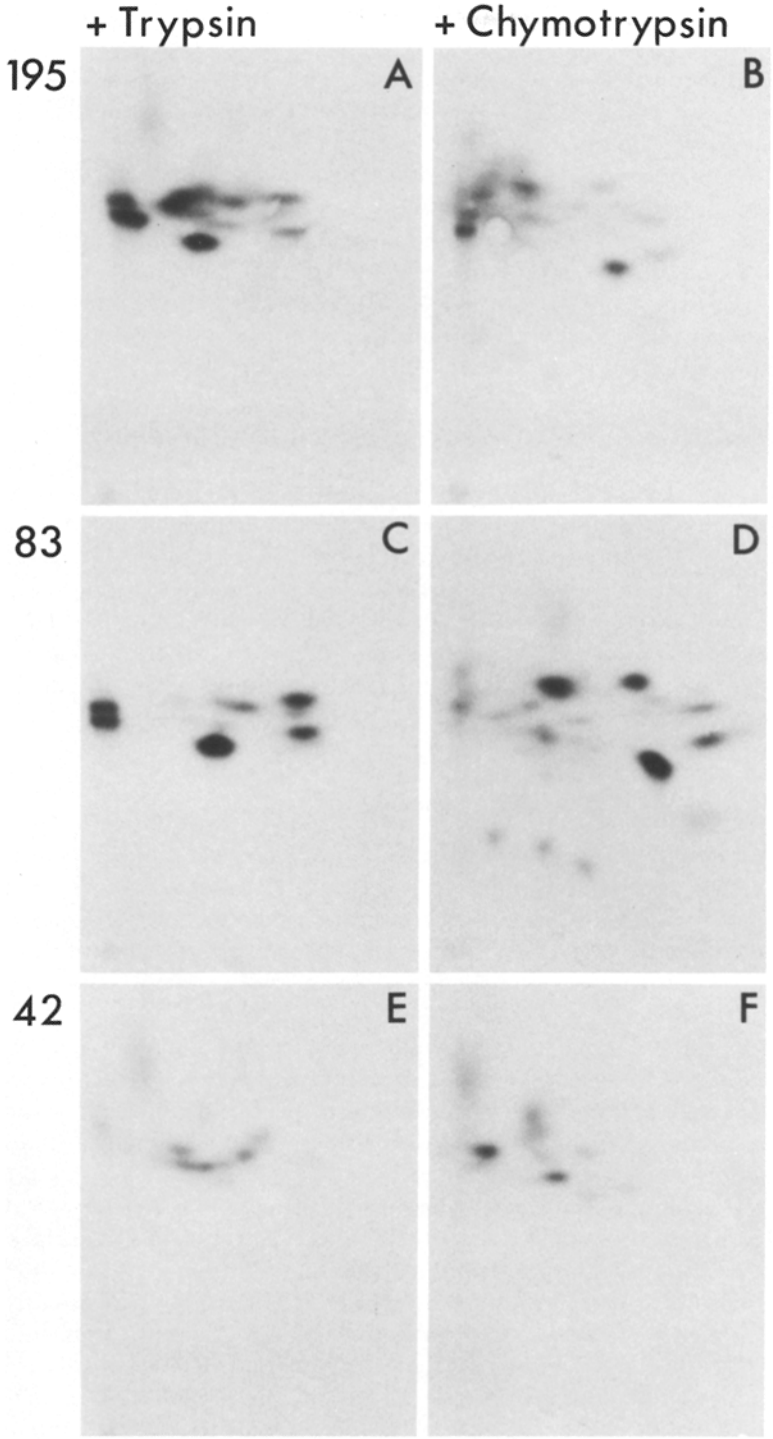

FIGURE 4. Peptide mapping of the polypeptides immunoprecipitated by polyvalent antiserum. The 195,000 mol wt protein (*A* and *B*) was immunoprecipitated from a detergent extract of schizont-enriched infected erythrocytes labeled with ^{125}I. The 83,000 mol wt polypeptide (*C* and *D*) and the 42,000 mol wt polypeptide (*E* and *F*) were precipitated from extracts of surface-labeled merozoites. The polypeptides were separated by SDS-PAGE and then digested with either trypsin (*A*, *C*, and *E*) or chymotrypsin (*B*, *D*, and *F*). Soluble peptides released by this treatment were separated in two dimensions on thin-layer plates and labeled peptides were detected by autoradiography.

identical pattern was observed when the transferred polypeptides were probed with either a polyvalent antiserum raised against the eluted material (Fig. 2, lane *2*) or human immune serum (lane *3*).

^{125}I-labeled Merozoite Surface Antigens. From extracts of surface-labeled merozoites (Fig. 3, lane *1*), three abundant proteins of 83,000, 42,000, and 19,000 mol wt were precipitated by human immune serum (Fig. 3, lane *2*). The monoclonal antibody 89.1 immunoprecipitated only the 83,000 mol wt species (Fig. 3, lane *3*), as found previously (4). The polyvalent antiserum raised against the purified protein recognized all the three major polypeptides precipitated by human immune serum (Fig. 3, lane *5*).

Peptide Mapping of the ^{125}I-labeled Polypeptides Recognized by the Polyvalent Antiserum Against the 195,000 Mol Wt Protein. The 195,000 mol wt protein, iodinated in extracts of schizonts and immunoprecipitated with the polyvalent antiserum, was compared by peptide mapping with the iodinated polypeptides precipitated by the same antiserum from extracts of surface-labeled merozoites. The soluble ^{125}I-peptides derived by tryptic or chymotryptic digestion of the 83,000 and the 42,000 mol wt fragments were represented among the peptides released from the 195,000 mol wt protein (Fig. 3). No soluble labeled peptides were released from the 19,000 mol wt fragment by extensive treatment with several proteases, although pronase and subtilisin digests each contained a single labeled peptide.

Discussion

A class of high molecular weight antigens has been implicated (8) in the induction of a protective immune response. In *P. falciparum*, the antigen is a 195,000 mol wt protein that is synthesized in schizonts and subsequently processed (3, 4). Here we investigate the terminal processing products of the 195,000 mol wt protein using a polyvalent antiserum raised against the monoclonal antibody affinity-purified protein.

The 195,000 mol wt protein, together with specific fragments of it, have been purified. Some of the breakdown was promoted during the extraction procedure but could not be prevented by the addition of protease inhibitors. No contaminant polypeptides could be detected after SDS-PAGE by Coomassie Blue staining or by reaction with specific antibodies.

The polyvalent antiserum raised against the purified protein reacted with the 195,000 mol wt protein in extracts of schizonts. From extracts of surface-labeled merozoites this antiserum precipitated 83,000, 42,000, and 19,000 mol wt species. This indicates that these polypeptides are terminal processing fragments derived from the 195,000 mol wt protein. The peptide maps indicate that the surface polypeptides are nonoverlapping fragments, which is consistent with the binding of the monoclonal antibody 89.1 to only the 83,000 mol wt species. The three major fragments derived from the high molecular weight precursor are the major species on the merozoite surface recognized by human immune serum. Thus the major response to merozoite surface antigens in immune individuals is directed against products from a single high molecular weight precursor.

Summary

A 195,000 mol wt *Plasmodium falciparum* protein and processing fragments derived from it have been purified by monoclonal antibody affinity chromatography. A polyvalent antiserum has been raised against the purified protein and used to identify the terminal processing products associated with the merozoite. Three unique fragments of 83,000, 42,000, and 19,000 mol wt are present and they represent the major surface antigens of *P. falciparum* merozoites.

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