A Single Fragment of a Malaria Merozoite Surface Protein Remains on the Parasite During Red Cell Invasion and Is the Target of Invasion-inhibiting Antibodies

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Summary
A complex of polypeptides derived from a precursor is present on the surface of the malaria merozoite. During erythrocyte invasion only a small fragment from this complex is retained on the parasite surface and carried into the newly infected red cell. Antibodies to this fragment will interrupt invasion.

Proteins on the surface of malaria merozoites are targets of an immune response and are candidate vaccine components. The precursor to major merozoite surface antigens, referred to here as MSP1 (merozoite surface protein 1) is synthesized during schizogony and is present on the merozoite as a complex of fragments derived by proteolytic processing (1, 2). An 83-kD fragment, MSP183 can be isolated from culture supernatants, and mAbs specific for MSP183 do not react with newly invaded (ring-stage) parasitized erythrocytes in an immunofluorescence (IF) assay (2-3), suggesting that this part of the complex is shed during the invasion process. A second group of MSP1-mAb do react in IF with ring forms, and all appear to react with reduction-sensitive epitopes on a COOH-terminal 42-45-kD fragment (MSP142) and a 16-19-kD polypeptide subfragment (MSP119) from the cysteine-rich COOH terminus of the precursor (2, 4-6). There is evidence that a MSP1-specific immune response can protect against blood-stage challenge (1, 7), and certain mAbs to either the Plasmodium falciparum MSP1 or homologues in other species have been shown to inhibit the growth of the parasite in vitro (8-9) or protect on passive transfer in vivo (10-12). The function of MSP1 is unknown, although it may be involved in red cell invasion, possibly in a receptor role (13).

Here we demonstrate that MSP142 is specifically carried into the red cell and that antibodies to this fragment can inhibit invasion. These observations suggest that processing must take place for merozoite invasion to proceed.

Materials and Methods

Antibodies. MSP1-specific mAbs were used: 12.1 and 13.2 (anti-MSP183); 13.1 and X509 (anti-MSP142 N-terminus); 7.5, 12.8, and 12.10 (anti-MSP142 COOH terminus and MSP119) (2). mAb 13.4 (anti-MSP2, merozoite surface protein 2, 46-kD antigen) and 13.3 (anti-lactate dehydrogenase) were used as controls (14). X509 is a product of a human EBV-transformed cell line derived from a Gambian donor (Blackman, M.J., et al., manuscript in preparation).

Parasites. P. falciparum, strain FCB-1, and clones T9/94 and T9/96 were maintained essentially as described previously (3), and synchronized by a combination of sorbitol and Percoll treatments when appropriate.

Immunoelectron Microscopy. P. falciparum (FCB-1 strain) cultures ~1 h after invasion and containing 50-60% ring forms were fixed, cryosectioned and incubated with ascitic fluid containing the mAbs. Antibody binding was revealed by incubation with rabbit anti-mouse Ig and protein A-gold. After washing in PBS, sections were stained with 2% uranyl acetate and viewed in a JEOL 100B electron microscope (15).

Production of Merozoites and Saponin-lysed Rings. Merozoites were harvested at 1-1.5 h intervals from cultures enriched for schizonts (85-90% parasitaemia at a hematocrit of ~0.2%) and washed in PBS containing the following protease inhibitors: 1 mM PMSE, 2 mM tosyl-L-lysine chloromethyl ketone (TLCK), 0.1 mM tosyl-L-phenylalanine chloromethyl ketone (TPCK), and chymostatin, leupeptin, antipain, and aprotinin (all at 10 μg/ml).

To prepare ring-stages, schizonts were cultured in the presence of fresh, washed erythrocytes at 2% hematocrit (15-20% parasitaemia). After 7 h the cells were centrifuged twice over 67.5% isotonic Percoll to remove any remaining schizonts. The resulting pellets containing only uninfected erythrocytes and ring-stage parasites (with <0.01% schizont contamination) were washed twice in serum-free medium and resuspended in ice-cold PBS containing protease inhibitors (PBS/PI). Saponin (10% wt/vol in PBS, BDH Ltd., Poole, UK) was added slowly with gentle mixing to a final concentration of 0.005%, and the suspension was incubated in ice until lysis began. An equal volume of PBS/PI was added, and
then the parasites and red cell membranes were pelleted by centrifugation at 15,000 g for 15 min at 4°C. After two further washes pellets were solubilized directly into SDS sample buffer for analysis by SDS-PAGE and immunoblotting.

**Metabolic Labeling and Immunoprecipitation.** Synchronous cultures containing 30–35-h-old trophozoites (6% hematocrit, 10% parasitemia) were incubated in cysteine-free complete medium containing 1.85 MBq/ml [35S]cysteine (>37 TBq/mmol, Amersham International plc, Amersham, UK). The cultures were diluted with complete medium 2 and 12 h later and mature schizonts were purified over 63% Percoll, washed three times in warm serum-free medium, then divided into two cultures in normal complete medium. One culture was used to produce merozoites, the other was supplemented with fresh erythrocytes to produce ring-stages. The parasites were harvested, frozen at −70°C, and then thawed directly into lysis buffer (50 mM Tris-HCl, pH 8.2, containing 5 mM EDTA, 5 mM EGTA, 0.5% wt/vol sodium deoxycholate, and supplemented with the protease inhibitors leupeptin, aprotinin, PMSF, and TLCK). Labeled proteins were immunoprecipitated and analyzed by SDS-PAGE.

**Merozoite Invasion Inhibition Test.** Synchronous parasites (T9-94 and T9-96 clones) at ~1% schizont parasitemia were cultured in triplicate in medium supplemented with purified Ig (14). 24 h later the number of ring-stage parasites was counted in Giemsa-stained thin film smears.

**Results**

The presence of antigens in newly invaded parasites was studied by immunoelectron microscopy (immunoEM) (Fig. 1). The three mAbs specific for MSP119 reacted with the parasite membrane of newly formed ring stages (Fig. 1, a–d). mAbs specific for MSP143 (Fig. 1 e) or the NH2-terminal part of MSP142 (Fig. 1 f) did not react, nor did a mAb reacting with MSP2 (Fig. 1 g). mAb 7.5 reacted better with ring-stages 1 h after invasion than those 2 h after invasion and did not react with older parasites, suggesting that the antigen subsequently disappeared.

To investigate the fate of different MSP1 fragments during invasion, lysates of merozoites and ring-stages were probed by immunoblotting. mAb 12.8 reacted with both MSP142 (39 kD under nonreducing conditions) and MSP119 in

![Figure 1. Immunoelectron micrographs of newly formed *P. falciparum* ring-stages in freshly invaded erythrocytes labeled with the mAbs (a) 7.5 (1 h after invasion), (b) 7.5 (2 h after invasion), (c) 12.10, (d) 12.6, (e) 12.1 (f) 13.1, and (g) 13.4, or (h) with ascites fluid. The antibodies are directed against MSP119 (a–d), MSP142 (e), the NH2-terminal end of MSP142 (f), and to MSP2 (g). Bars, 0.5 μm.](image1)

![Figure 2. Reactivity of mAbs with extracts of either merozoites (lanes 1, 3, and 5) or early ring-stages (lanes 2, 4, and 6) of *P. falciparum* clone T9-96. The lysates were electrophoresed on a 7.5–15% polyacrylamide gel, transferred to nitrocellulose, and then probed with either mouse mAb 12.8 (lanes 1 and 2), mouse mAbs 12.8 ± 12.1 (lanes 3 and 4), or human mAb X509 (lanes 5 and 6). The position of standard molecular weight markers is indicated.](image2)

![Figure 3. mAb 12.8 immunoprecipitation of proteins labeled with [35S]cysteine in schizonts and present in subsequent merozoite (lanes 1 and 3) and ring (lanes 2 and 4) stages. Immunoprecipitates were dissolved in SDS sample buffer in the absence (lanes 1 and 2) or presence (lanes 3 and 4) of mercaptoethanol and electrophoresed on a 10–15% polyacrylamide gel. Labeled polypeptides were detected by fluorography. The arrow indicates the position of MSP143.](image3)

380  Malaria Merozoite Protein Processing
Merozoite invasion was inhibited by mAbs 12.8 and 12.10, specific for MSP1_{19}. Fig. 4 shows the inhibition relative to a control Ig using mAb 12.10 and two different parasite strains. At 500 μg/ml the antibody inhibited invasion by 72% (T9-94 strain) and 50% (T9-96 strain), respectively.

Discussion

We have used mAbs to trace some of the MSP1 fragments during erythrocyte invasion. Only MSP1_{19} is detectable in the newly invaded red cell; mAbs specific for the NH\textsubscript{2} terminus of MSP1_{42} do not react by IF and immunoEM, and MSP1_{42} could not be detected by immunoblotting or immunoprecipitation. The ring-stage MSP1_{19} is indistinguishable from that on the merozoite surface and has been shown by metabolic labeling to be derived from the invading merozoite. Since MSP1 may be membrane-bound by a glycosyl phosphatidylinositol anchor (16) this is presumably intact on ring-stage MSP1_{19}. MSP1_{42} to MSP1_{19} processing appears to occur, at least in part, at erythrocyte invasion and is intrinsic to this process. Some but not all merozoites isolated have MSP1-derived fragments that have already undergone the MSP1_{42} to MSP1_{19} processing, and therefore, this step can occur before invasion; the cleavage may be triggered by interaction of the free merozoite with erythrocyte surface components; alternatively, the presence of MSP1_{19} in merozoite extracts may be partly or wholly artefactual, resulting from proteolysis during the isolation procedure. Certainly, different methods of merozoite isolation result in differing relative amounts of MSP1_{42} and MSP1_{19} (see e.g., reference 2). The apparent specificity and reproducibility of the proteolytic cleavage suggest that this process is an integral part of the asexual blood-stage cycle.

We have shown that antibodies to conserved epitopes on \textit{P. falciparum} MSP1_{19} (albeit at high concentration) can inhibit red cell invasion. Recently it has been shown that a mAb protective on passive transfer against \textit{P. yoelii} (10) is directed against the homologous MSP1 cysteine-rich domain (17). These findings suggest that an antibody response to MSP1_{19} may interrupt the malaria asexual blood stage cycle.

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