Plasmodium falciparum: exported protein-i, a blood stage antigen is expressed in liver stage parasites.
Plasmodium falciparum: Exported Protein-I, a Blood Stage Antigen, Is Expressed in Liver Stage Parasites

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After inoculation by Anopheles sp. mosquitoes, Plasmodium falciparum sporozoites rapidly make their way to the liver (Fairley 1947) where a single, uninucleate sporozoite develops during a minimum of 5-6 days to a mature liver stage schizont with 1-3 × 10⁶ uninucleate merozoites. There are no clinical or pathological manifestations associated with this stage of the parasite's life cycle, and thus the parasite developing within the liver is an attractive target for vaccine-induced protective immune responses. Infected hepatocytes are the target of protective immune responses induced by immunization with irradiated sporozoites, sporozoites that develop only partially in infected hepatocytes (Hoffman et al. 1989). However, so-called "erythrocytic stage" parasites such as the P. falciparum major merozoite protein-1 (PfMSP-1) are also expressed in infected hepatocytes (Szarfman et al. 1988a,b). As such, they also could be the targets of cellular or humoral immune responses that prevent the release of infectious merozoites from the liver. The current studies were undertaken to determine whether the erythrocytic stage P. falciparum exported protein I (Exp-1) is expressed in infected hepatocytes.

Exp-1 (Simmons et al. 1987), also called circumsporozoite related antigen (Coppel et al. 1985), QF116 antigen (Kara et al. 1988), or antigen 5.1 (Hope et al. 1984), is a 23-kDa P. falciparum blood stage protein that is secreted by the parasite into the host cell. It accumulates at the parasitophorous vacuole membrane and within vesicles in the infected red cell cytoplasm (Simmons et al. 1987). It contains a sequence of 15 amino acids with homology to the tandemly repeated tetramers of the P. falciparum circumsporozoite protein (PICSP) (Hope et al. 1985). The monoclonal antibody (mAb) 5.1, that recognizes this epitope in Exp-1 also reacts with sporozoites (Hope et al. 1985) and prevents the invasion of sporozoites into human hepatocytes (Mellouk et al. 1990), presumably by binding to CSP on the sporozoite surface. This cross-reaction has made it difficult to determine whether Exp-1 is itself expressed in infected hepatocytes. We raised antisera against a recombinant Exp-1 which does not contain the epitope cross-reactive with the PICSP and demonstrate expression of Exp-1 in infected hepatocytes.

The complete coding sequence of Exp-1 was amplified from total RNA isolated by the guanidinium method (Chirgwin et al. 1979) of the 3D7 clone of P. falciparum strain NF54 using the oligonucleotide primers 5'-GGAATTCATGAAAATCTTATCAGTA-3' and 5'-GGAAATTTCAATGGGCAGTTGACCACGACT-3', cloned into pUC 18 and sequenced (Sanger et al. 1977). The sequence is similar to previously published Exp-1 sequences and includes the change of Asp 136 to Gly which abolishes the 5.1 epitope (Simmons et al. 1987). The amplified Exp-1 sequence was cloned into the bacterial expression vector, pGEMEX (Promega), and expressed in Escherichia coli strain HMS174 (DE3) (Studier et al. 1990). In this system, recombinant protein is expressed as an insoluble fusion protein with a 260 amino acid hydrophobic leader from phase T7 gene 10. Recombinant Exp-1 (rExp-I) migrated at its expected size, 50 kDa, in 10% SDS-PAGE and, in immunoblotting, was recognized by the anti-Exp-1 mAb, mAbN1 (Gunther et al. 1991) (Fig. 1A).

The rExp-1 protein was electrophoresed from a polyacrylamide gel. In addition, the T7 hydrophobic leader protein without the Exp-1 insert was run on a polyacrylamide gel, and this protein and E. coli proteins that migrated at the same size as rExp-1 were electro-
A | B
---|---
180.0 | 190.0
116.0 | 125.0
84.0 | 88.0
54.0 | 65.0
48.5 | 56.0
36.5 | 38.0
26.5 | 33.5

**Fig. 1.** Western blot of electroeluted rExp-1 (A) and recombinant PfCSP (B) electrophoresed on 10% acrylamide SDS gels. The nitrocellulose strips were probed with (a) mAb NI, (c) NFS1 mAb against PfCSP, (b) mouse antisera against rExp-1, (c, g) sera of one of the immunized volunteers before immunization, and (d, h) sera of the same individual post immunization with irradiated sporozoites.

eluted. Anti-rExp-1 and control sera were obtained by immunizing BALB/c mice ip with 50-μg doses of either rExp-1 or a mixture of the T7 hydrophobic leader protein and the control E. coli proteins that ran at the same molecular weight as rExp-1. Proteins were emulsified in complete Freund’s adjuvant (first dose) and in incomplete Freund’s adjuvant (second and third doses).

Immunofluorescence assay (IFA) of *P. falciparum*-infected erythrocytes and fixed sporozoites was carried out as described (Szarfman et al. 1988a). For IFA of *in vitro*-infected hepatocytes, primary human hepatocytes were obtained in accordance with institutional review guidelines, processed, and infected with *P. falciparum* sporozoites as described (Rogers et al. 1992). Cryosections prepared from a *P. falciparum* (clone HB3)-infected chimpanzee liver biopsy that had been kept at −70°C since being obtained by Szarfman and colleagues (Szarfman et al. 1988a) were used in an IFA to evaluate the *in vivo* expression of Exp-1 antigen. The IFA was carried out as previously described (Mel-louk et al. 1990).

Control sera did not react with infected erythrocytes or hepatocytes by immunofluorescence or with extracts of infected erythrocytes by immunoblot (data not shown). The anti-rExp-1 serum did not react with recombinant PfCSP, falc 2.3 (Bathurst et al. 1992) by immunoblot (Fig. 1B, lane f), confirming that this serum does not cross-react with the PfCSP. In immunofluorescence, Exp-1 was not detected in sporozoites or in *in vitro*-infected hepatocytes at 2 days postinfection. However, Exp-1 was found in *in vitro*-infected hepatocytes at 4 and 6 days postinfection (results not shown).

**Fig. 2.** Immunofluorescence assay on (a) 3D7 *P. falciparum*-infected red blood cells and (b) chimpanzee liver biopsy 6 days postinfection with HB3 *P. falciparum* sporozoites. The slides were incubated with anti-rExp-1 serum and goat anti-mouse Ig fluorescein-conjugated second antibody.
SPECIFIC EXPRESSION OF Plasmodium falciparum EXP-1

TABLE I
Expression of Exp-I Antigen in Preerythrocytic and Erythrocytic Stages of P. falciparum

<table>
<thead>
<tr>
<th>Hepatic stage (days)*</th>
<th>Sporozoite</th>
<th>2b</th>
<th>4b</th>
<th>6b</th>
<th>Blood stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAbN1</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mouse anti-rPExp-1</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mouse anti-rPSSP2</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note. Mice were immunized with rPExp-1 or rPSSP2. Recognition of sporozoites, liver stage parasites, and infected erythrocytes in immunofluorescence assays are contrasted.

* Days after infection with P. falciparum sporozoites.

In vitro P. falciparum-infected human hepatocytes.

In vivo chimpanzee liver biopsy. 6 days postinfection with P. falciparum sporozoites.

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