CONCISE REPORT

Invasion of Erythrocytes by Plasmodium falciparum Malaria Parasites: Evidence for Receptor Heterogeneity and Two Receptors

By Graham H. Mitchell, Terence J. Hadley, Mary H. McGinniss, Francis W. Klotz, and Louis H. Miller

Plasmodium falciparum malaria parasites with different capabilities of invading sialic acid-deficient erythrocytes were identified. Thai-2 parasites cultured in Tn erythrocytes invaded neuraminidase-treated and Tn erythrocytes twice as efficiently as Thai-2 parasites cultured in normal erythrocytes and seven to ten times more efficiently than a cloned line of Camp parasites cultured in normal erythrocytes. All three parasite lines required sialic acid for optimal invasion, but Thai-2 parasites cultured in Tn erythrocytes were of the Tn phenotype. To remove normal erythrocytes, C.L. blood was incubated with the anti-M monoclonal antibody (a gift of J.J. Moulds, Gamma Biologicals, Houston, Tex) and passed over an immunoaffinity column containing goat antimouse immunoglobulin covalently coupled to Sepharose 4B (Cappel, Cochranville, Penn). Fewer than 1% of the effluent cells possessed M antigens as determined by immunofluorescence.

Tn erythrocytes from donor C.L. were agglutinated by monoclonal antibody, indicating that approximately 90% of CL. erythrocytes were of the Tn phenotype. To remove normal erythrocytes, C.L. blood was incubated with the anti-M monoclonal antibody (a gift of J.J. Moulds, Gamma Biologicals, Houston, Tex) and passed over an immunoaffinity column containing goat antimouse immunoglobulin covalently coupled to Sepharose 4B (Cappel, Cochranville, Penn). Fewer than 1% of the effluent cells possessed M antigens as determined by immunofluorescence.

Invasion Assay. Malaria parasites (merozoites) invade erythrocytes and develop sequentially into ring-forms, trophozoites, and schizonts. Each schizont in culture produces approximately ten to twenty new merozoites, which are released when infected erythrocytes rupture and invade other erythrocytes. Schizont-infected erythrocytes were obtained and purified (99% purity) on Percoll-sorbitol.

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Results

In preliminary experiments four different lines of *P. falciparum*, including the cloned Camp line, were cultured in erythrocytes from donor C.L. (90% Tn). Only the Thai-2 line survived and multiplied. Thai-2 parasites maintained in culture with Tn erythrocytes (Thai-Tn) were compared with Thai-2 parasites maintained in normal erythrocytes and with Camp parasites maintained in normal erythrocytes. Thai-Tn parasites invaded neuraminidase-treated erythrocytes with an average efficiency of 45%, in contrast to Camp parasites, which invaded neuraminidase-treated erythrocytes with an average efficiency of about 5% (Table 1). Thai-2 parasites invaded neuraminidase-treated erythrocytes with an average efficiency of 28% (Table 1). All three lines tested required sialic acid for optimal invasion. Similar differences were obtained with Tn erythrocytes (Table 2). Thai-Tn parasites invaded Tn erythrocytes seven to eight times more efficiently than did Camp parasites and two to three times more efficiently than did Thai-2 parasites. Furthermore, treatment of Tn erythrocytes with neuraminidase decreased their susceptibility to invasion by Thai-Tn parasites by approximately 40% but had no effect on the susceptibility of these erythrocytes to invasion by Camp parasites. Treatment of normal erythrocytes with trypsin decreased susceptibility to invasion by all three lines tested to about 10% of normal (Table 1).

Discussion

Thai-Tn parasites invaded neuraminidase-treated erythrocytes with 45% efficiency and Thai-2 parasites invaded neuraminidase-treated erythrocytes with 28% efficiency. Although both parasites required sialic acid on the erythrocyte membrane for optimal invasion, their ability to invade neuraminidase-treated erythrocytes suggests that they can also utilize an erythrocyte ligand which is independent of sialic acid. The low efficiency of invasion of neuraminidase-treated erythrocytes obtained with Camp parasites may be due to residual sialic acid on the erythrocyte membrane after neuraminidase treatment, or to a low affinity interaction with another ligand. Treatment of Tn erythrocytes with neuraminidase enhanced their susceptibility to invasion by Thai-Tn parasites but not by Camp parasites, further indicating that Thai-Tn and Camp parasites have different receptors and that Thai-Tn parasites can utilize a sialic acid-independent ligand. The increased invasion of Tn erythrocytes by Thai-Tn parasites following neuraminidase treatment may be due to the decrease in negatively charged residues (sialic acid), making a second erythrocyte ligand more accessible.

The data presented here is the first reported indication that there is receptor heterogeneity among *P. falciparum* parasites. Previous reports suggested that two receptors may be involved in invasion.3,13,14 Friedman et al reported that the effect of neuraminidase on invasion could be partially reversed by incubating neuraminidase-treated erythrocytes with α1 acid glycoprotein.7 This effect, which required sialic acid on α1 acid glycoprotein, did not occur when α1 acid glycoprotein was incubated with trypsin-treated erythrocytes. Other investigators suggested that, in addition to sialic acid, the hydrophobic Tα peptide of glycoporphin A may be a ligand necessary for invasion because the Tα peptide inhibits invasion in vitro.13,14 However, the specificity of inhibition by the hydrophobic Tα peptide has been questioned by Breuer et al, who found that hydrophobic haptenes coupled to bovine serum albumin also inhibit invasion.17 The Tα peptide of glycoporphin A remains associated with the membrane after trypsin treatment of intact erythrocytes and therefore is probably not the trypsin-sensitive ligand utilized by Thai-Tn, Thai 2, and possibly Camp parasites.

The data presented here suggest that *P. falciparum* ma-

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**Table 1.** Invasion of Enzyme-Treated Normal Erythrocytes by Various Lines of *Plasmodium falciparum*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment No.</th>
<th>Invasion Expressed* as % of Control</th>
<th>Ratio of Thai-Tn/Camp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuraminidase</td>
<td>1</td>
<td>1</td>
<td>Camp: 13</td>
</tr>
<tr>
<td>Thai-2</td>
<td>7</td>
<td>33</td>
<td>54</td>
</tr>
<tr>
<td>Thai-Tn</td>
<td>6</td>
<td>28</td>
<td>53</td>
</tr>
</tbody>
</table>

*Invasion rates obtained with test RBCs are expressed as a percentage of invasion rates obtained with normal, untreated RBCs and are calculated as follows:

\[
\frac{\text{% of test RBCs infected}}{\text{% of normal RBCs infected}} \times 100
\]

Invasion rates obtained with normal RBCs were greater than 10% in most experiments. The data are presented for three lines of parasites: Camp, Thai-2, and Thai-Tn (Thai-2 parasites cultured in Tn RBCs).

The ratios of the invasion rates of Thai-Tn parasites to Camp parasites in the same population of RBCs.

**Table 2.** Invasion of Purified Tn Erythrocytes and Purified Tn Erythrocytes Treated with Neuraminidase by Various Lines of *Plasmodium falciparum*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment No.</th>
<th>Thai-Tn</th>
<th>Camp</th>
<th>Thai-Tn/Camp</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Neuraminidase</td>
<td>4</td>
<td>2</td>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>20</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>Thai-Tn</td>
<td>5</td>
<td>1</td>
<td>29</td>
<td>29</td>
</tr>
</tbody>
</table>

*†Same as Table 1.
REFERENCES


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RECEPTOR HETEROGENEITY OF P. FALCIPARUM

Malaria parasites express at least two different receptors for two different ligands on the erythrocyte membrane. One receptor binds to a ligand that is dependent on sialic acid, and a second receptor binds to a ligand that is independent of sialic acid and is sensitive to cleavage by trypsin. Our observations suggest that Thai-Tn parasites have either a greater number of receptors for the sialic acid-independent ligand than Camp parasites or receptors of higher affinity. The location of the sialic acid-independent ligand is unknown. The biologic evidence for two receptors and for receptor heterogeneity may be important to future biochemical characterization of receptors and to vaccine development if receptors are to be used as immunogens.
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