**Plasmodium falciparum** Rosette Formation Is Uncommon in Isolates from Pregnant Women

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Received 5 August 1999/Returned for modification 20 September 1999/Accepted 18 October 1999

We examined the formation of *Plasmodium falciparum* erythrocyte rosettes using parasite isolates from placental or peripheral blood of pregnant Malawian women and from peripheral blood of children. Five of 23 placental isolates, 23 of 38 maternal peripheral isolates, and 136 of 139 child peripheral isolates formed rosettes. Placental isolates formed fewer rosettes than maternal isolates (range, 0 to 7.5% versus 0 to 33.5%; \( P = 0.002 \)), and both formed fewer rosettes than isolates cultured from children (range, 0 to 56%; \( P < 0.0001 \)). Rosette formation is common in infections of children but uncommon in pregnancy and rarely detected in placental isolates.

Under in vitro conditions, *Plasmodium falciparum*-infected erythrocytes (*P. falciparum*-IE) may show rosette formation, the adherence of two or more uninfected erythrocytes to an erythrocyte containing a mature-stage parasite. Rosette formation has been associated with severity of malarial disease in young children in some studies (4, 10, 12, 15, 18, 20), but not all (1, 17), and has been proposed to contribute to microvascular obstruction in organs such as the brain (9). It was reported to be rare in isolates from pregnant Cameroonian women (11).

In regions of malaria endemicity, pregnant women are more susceptible to malaria infection than their nonpregnant counterparts (3). Placental malaria infection is clearly of major importance in the pathogenesis of malaria in pregnancy, with mature-stage IE frequently found in the placental intervillous spaces. Only a proportion of placental IE appear to be adherent to the syncytiotrophoblast (23, 24), and many of the erythrocytes in the intervillous spaces are uninfected. We postulated that placental accumulation of IE may result in part from rosette formation, which could lead to disturbances in blood flow (22). We therefore compared rosette formation by parasites cultured from peripheral blood of children and pregnant women with that of *P. falciparum* isolates obtained from placental blood at delivery.

Peripheral and placental blood samples were collected from pregnant women and peripheral blood was collected from children at Queen Elizabeth Central Hospital, Blantyre, Malawi, after obtaining informed consent. Peripheral blood was processed and cultured as described previously, in medium supplemented with 10% human blood group AB serum (2). Placental blood was collected from the cut maternal surface of the placenta, diluted to 5% hematocrit in complete medium supplemented with 10% AB serum, and examined for rosetting after incubation at 37°C for a minimum of 60 min. Rosetting assays were performed by staining IE with acridine orange and by examination with combined light and fluorescence micros-
TABLE 1. Rosetting by isolates from pregnant women and children

<table>
<thead>
<tr>
<th>Isolate group</th>
<th>No. of isolates</th>
<th>Median (IQR) of rosette formation</th>
<th>No. (%) of rosetting isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placental</td>
<td>23</td>
<td>0 (0–0)</td>
<td>5 (21.8)</td>
</tr>
<tr>
<td>Maternal peripheral</td>
<td>38</td>
<td>0.25 (0–4)</td>
<td>23 (60.5)</td>
</tr>
<tr>
<td>Antenatal</td>
<td>24</td>
<td>0.5 (0–4)</td>
<td>15 (62.5)</td>
</tr>
<tr>
<td>In labor</td>
<td>14</td>
<td>0.25 (0–3)</td>
<td>8 (57.7)</td>
</tr>
<tr>
<td>Children</td>
<td>139</td>
<td>5 (2.8–9.0)</td>
<td>136 (99.2)</td>
</tr>
</tbody>
</table>

*Values are the percentages of IE in rosettes. IQR, interquartile range.

Our findings suggest that rosetting is not important in the development of placental malaria, whereas it has been implicated in the pathogenesis of malaria disease in children (4, 16, 18). Our results are supported by the findings of Maubert et al., who examined parasites from the placentas of 23 Cameroonian women, none of whose parasites formed rosettes (11). In comparison, cryopreserved peripheral blood parasites (which may change phenotype after thawing [14]) from 1 of 12 women in labor and from 8 of 12 nonpregnant adults showed rosette formation. We could not test parasites from nonpregnant adults for rosetting, but children examined over the same period were almost always infected with rosetting parasites, and other studies suggest that *P. falciparum* rosetting is similar in adults and children (7, 8, 11, 15).

Placental infection may develop in part through adhesion to a ligand(s) such as CSA on the syncytiotrophoblast lining of the intervillous spaces, although not all isolates from pregnant women bind to CSA (2, 6). Our results suggest that parasites from the placenta have different adherence and antigenic characteristics from those infecting children (2). Both rosette formation and adhesion to CSA have been ascribed to *P. falciparum* erythrocyte membrane protein 1 (5, 13, 19). A possible explanation for the relative lack of rosetting in CSA binding parasites from pregnant women and the scarcity of CSA binding in isolates from children (17) would be that CSA binding and rosetting are mutually incompatible properties of parasitized erythrocytes. This possibility remains to be proven.

In conclusion, pregnant women are not commonly infected with parasites that form rosettes, and rosetting parasites are rarely found in the placenta, indicating that rosette formation is not a major factor in the pathogenesis of malarial infection of the placenta.

S.J.R. is the recipient of a Career Development Fellowship, and M.E.M. is the recipient of a Research Leave Fellowship in Clinical Tropical Medicine from the Wellcome Trust. J.G.B. was awarded an Australian National Health and Medical Research Council Medical Postgraduate Scholarship and received generous support from the District 9680 Rotary Against Malaria Programme, Sydney, Australia. We thank the staff of the Antenatal Clinic and Labour Ward of the Queen Elizabeth Central Hospital, Blantyre, Malawi, for friendly cooperation, and we thank all the women who participated. We are grateful for the enthusiastic support of V. Lema, Department of Obstetrics and Gynaecology, College of Medicine, University of Malawi; Terrie Taylor, Michigan State University; and Graham Brown, The Walter and Eliza Hall Institute of Medical Research.

REFERENCES


FIG. 1. Rosette formation (%) by IE cultured from children’s peripheral blood (1), cultured from maternal peripheral blood (2), or isolated from placent al blood (3).


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Editor: S. H. E. Kaufmann