SEROLOGIC RESPONSES TO RECOMBINANT PLASMODIUM VIVAX DUFFY BINDING PROTEIN IN A COLOMBIAN VILLAGE

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Abstract. The Plasmodium vivax Duffy binding protein (DBP) is essential during merozoite invasion into human erythrocytes. Because of its biological importance, the DBP is also seen to have potential use as a malaria blood-stage vaccine. We have used a soluble recombinant DBP (rDBP) containing the functional ligand domain to assess the natural immunogenicity of DBP in a low-endemic vivax malaria region. Human sera from adult residents from a Colombian village with unstable vivax malaria transmission reacted specifically with the rDBP as determined by ELISA. There was a significant positive correlation between increased antibody response (average, median, and percent positives) and age of patients, although the level of responses did vary considerably in their reactivity to the rDBP from negative to very high level within each age group. These data confirm previous findings on the serologic reactivity of the DBP in exposed populations and that immunologic boosting to the DBP occurs in malaria-endemic regions even with low-level transmission.

All malaria parasites undergo repeated cycles of asexual development to ensure their maintenance in the blood. During the obligatory process of invasion into a new erythrocyte, the merozoite of Plasmodium requires interaction with a specific set of erythrocyte surface receptors.1 These receptor-ligand interactions constitute a target of choice for chemotherapy or vaccination-based approaches to reduce or eliminate blood-stage malaria infections.2 The P. vivax Duffy binding protein (DBP) is essential in invasion. Its localization in the merozoite and gene structure have already been reviewed.1,3 Evidence that the DBP is the target of an effective immune response was suggested by hypervariability of amino acids in the critical ligand domain of the cysteine-rich DBL erythrocyte-binding domain.4,5 However, direct evidence for naturally occurring antibodies to the DBP from malaria-exposed people was only recently demonstrated.3 A recombinant protein corresponding to regions II-IV of the DBP was designed and was immunogenic in laboratory animals, generating antisera that reacted to P. knolwesi and P. vivax parasites. Additionally, human sera from a highly endemic region of Papua New Guinea reacted specifically to DBPII-IV, demonstrating an age-related increase in responsiveness. Here we present data showing that human sera from 92 people indigenous to regions endemic for low-level vivax malaria in Colombia reacted to this recombinant protein, and that a positive correlation was observed between antibody response and age.

MATERIALS AND METHODS

Recombinant protein expression and purification. The recombinant protein rDBP was expressed and purified as described earlier.1

Plasma samples. Protocols for this research were cleared by the University of Notre Dame Human Subjects Institutional Review Board and by the Ethical Committee of the School of Health at the Universidad del Valle (Cali, Colombia). After informed consent was obtained, a medical examination was performed on 92 outpatients from Zacarías, Colombia. Zacarías is a village situated in a malaria-endemic area located 8 km from Buenaventura, the main seaport on the Colombian Pacific coast. This region has an annual average rainfall of 6,895 mm with 87% relative humidity and a temperature of 26°C. The village was composed of 577 inhabitants at the time of the study (from three months to 96 years old), in which 93% of the residents are blacks of African origin, 5.6% are Spanish-Amerindians and 0.7% are Indians. Malaria transmission is present throughout the year as recorded by Malaria Eradication Service data. Epidemiologic studies carried out in the village reported a malaria prevalence of 48% for P. vivax, 41% for P. falciparum, and 3.7% for P. malariae, with 7.4% mixed infections.6

No individual had malaria symptoms when examined, but 4% were positive for P. vivax (range = 70–2,275 parasites/µl by thick blood film) and 3% for P. falciparum (range = 70–12,600 parasites/µl). Blood samples were collected in tubes containing heparin as an anticoagulant. Plasma were separated by centrifugation and kept at −70°C until used. Fifteen sera were collected from North American residents never exposed to malaria, pooled, and used as negative controls for an ELISA.

Enzyme-linked immunosorbent assay. Analysis by ELISA was performed as described earlier.1 Negative sera from 15 North American residents were used to calculate the cut-off value.

Statistical analysis. The antibody unit (AU) averages were transformed into log values for statistical comparisons of means, standard deviations (SDs), and medians. Statistical analysis was performed using Systat 5.2 for Macintosh software package (Systat, Inc., Evanston, IL). Multivariate analysis of variance and Tukey’s high significance degree post hoc test were used for analysis of the relatedness of antibody response with age groups. Two different age groupings were used to assess age-related immunoreactivity. Positive sera were determined after a cut-off value (3.12) that corresponds to mean + 2 SD of the AU from the 15 nonexposed North American residents used as negative controls.

RESULTS

The P. vivax DBP is a well-conserved molecule that plays an essential role during the blood-stage infection. The merozoite requires the presence of a Duffy blood group antigen as a receptor to invade human reticulocytes.7 Unlike P. fal-
Table 1
Sero-prevalence to the Plasmodium vivax Duffy binding protein in residents of Zacarias, Colombia, segregated by two different age groupings

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>n</th>
<th>% Positive</th>
<th>Mean ± SD</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>0—&lt;20</td>
<td>28</td>
<td>14.3</td>
<td>2.83 ± 0.42 (a)</td>
<td>2.68</td>
</tr>
<tr>
<td>20—&lt;40</td>
<td>37</td>
<td>40.5</td>
<td>3.22 ± 0.57 (b)</td>
<td>3.00</td>
</tr>
<tr>
<td>≥40</td>
<td>27</td>
<td>66.7</td>
<td>3.55 ± 0.56 (c)</td>
<td>3.60</td>
</tr>
<tr>
<td>&lt;25</td>
<td>39</td>
<td>18.0</td>
<td>2.88 ± 0.42 (a)</td>
<td>2.81</td>
</tr>
<tr>
<td>25—&lt;50</td>
<td>32</td>
<td>56.3</td>
<td>3.43 ± 0.64 (b)</td>
<td>3.23</td>
</tr>
<tr>
<td>≥50</td>
<td>21</td>
<td>57.1</td>
<td>3.45 ± 0.54 (b)</td>
<td>3.34</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>40.2</td>
<td>3.20 ± 0.59</td>
<td>2.99</td>
</tr>
</tbody>
</table>

* n = total number of patients analyzed; SD = standard deviation.

DISCUSSION

These results demonstrated that even low level and unstable transmission leads to a significant boosting of the antibody responses in residents of this region. Similar to the residents of a highly endemic region of Papua New Guinea, the residents of Zacarias demonstrated a transition to high-level antibody response only at mature adulthood, suggesting that chronic subclinical infections are responsible for boosting the antibody response. Together, these studies confirm the consistency of the antibody responses to the DBP from natural exposure. This inherent ability to boost the antibody response should be beneficial in the development of the DBP as a blood-stage vaccine against vivax malaria.

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REFERENCES