Short Report: Analysis of Naturally Acquired Antibody Responses to the 19-kd C-Terminal Region of Merozoite Surface Protein-1 of Plasmodium vivax from Individuals in Sanliurfa, Turkey

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Abstract. Plasmodium vivax is the second most prevalent global Plasmodium species causing malaria after P. falciparum. These two Plasmodium spp. co-exist in most endemic areas, apart from west and central Africa, which has only P. falciparum. However, southeastern Turkey is one of the exceptional regions with the sole presence of P. vivax infection, where a thorough epidemiologic survey has not been performed. Here, we report for the first time the identification of naturally acquired antibodies against the 19-kd C-terminal region of the merozoite surface protein-1 of P. vivax (PvMSP119), using ELISA, from residents in the Sanliurfa region of southeastern Turkey. Among the 82 samples from patients with patent P. vivax malaria, 85% of the individuals were sero-reactive to PvMSP119. Particularly, 69.5% of the subjects were positive for IgM, 53.6% were positive for IgG (predominantly IgG1 and IgG3), and 7.3% were positive for IgA.

Although decades of malaria control strategies have almost eliminated malaria cases from Turkey, the disease has, however, been re-emerging since the early 1990s (http://www.saglik.gov.tr/EN). Almost all of the malaria cases that have been reported are from the southeastern part of Turkey, where there are favorable conditions for malaria transmission by the main vector, Anopheles sacharovi. According to recent WHO reports, endemic malaria was found in this region with a parasite index of > 10% (http://www.euro.who.int/malaria). Plasmodium vivax has continuously been identified as the only Plasmodium species in Turkey (F. Y. Zeyrek, unpublished data).1 Although southeastern Turkey is important in being one of the unique regions with infection with P. vivax alone, there has been no seroepidemiologic survey carried out. It is therefore important to study this area to understand the host immune responses against P. vivax that might help to develop vaccines against it.

The fact that people living in malaria-endemic regions develop immune responses to malaria parasites (i.e., antibody responses) because of their constant exposure to parasite antigens has led to the identification of several antigens from the Plasmodium spp. that could be considered potential vaccine candidates. Among them, the merozoite surface protein 1 (MSP1), an ~200-kd polypeptide, has been the most intensively studied blood-stage protein as a potential target for protective immunity.2 The antibodies to MSP1 protein have been found abundantly in naturally exposed people from endemic areas.3–7 Studies in animals and human phase I clinical trials have established MSP1 from different Plasmodium spp. as a strong vaccine candidate.8,9 Among the MSP1 protein fragments, the C-terminal MSP119 domain is highly conserved between parasitic strains.10 Moreover, as reported previously by different groups, the 19-kd C-terminal region of MSP 1 is a highly immunogenic conserved region of P. vivax.6,7,11–13

In this study, we investigated for the first time the residents of the Sanliurfa region in southeastern Turkey, where only the P. vivax malaria is endemic, for the presence of naturally acquired antibodies against the 19-kd C-terminal region of the MSP1 of P. vivax (PvMSP119) by using an ELISA. We aimed to evaluate these responses with respect to controlling parasitemia.

A total of 82 blood samples were collected from patients with patent P. vivax malaria at several National Malaria Control Centers within Siverek in Sanliurfa province during the peak season (July to December) of 2004. All sera were collected after informed consent from patients or from parents and kept at ~20°C. Patent infection was diagnosed by staining of the blood samples with 10% Giemsa solution and examination with standard light microscopy. The mean age was 20.7 ± 16.3 (SE) years (range, 0–65 years), and 53.7% of the subjects were male. The thick blood smear was used to calculate the level of parasitemia (asexual parasites/μL blood) as described before.14 Mean parasite density was 5,347.9 ± 6,421.5 parasites/μL (range, 160–25,560 parasites/μL). The majority of the patients (69.5%) had parasite densities of 150–5,000 parasites/μL, whereas only 9.8% of the patients had heavy parasitemia (>15,000 parasites/μL). Fourteen serum samples from individuals never exposed to P. vivax malaria were used as negative controls.

PvMSP119, a purified recombinant protein consisting of 90 amino acids (Leu1639–Ser1729) from the carboxy terminus of MSP1 expressed by Saccharomyces cerevisiae was used as an antigen for antibody assays.11,15 Ninety 6-well microtiter plates were coated with 0.4 μg/mL of antigen (PvMSP119) and incubated overnight at 4°C. The standard ELISA protocol was performed as described previously.16 The wells were loaded with 100 μL of serum (1:100 dilution in casein buffer) and incubated at room temperature for 1 hour. The plates were washed and incubated with peroxidase-conjugated goat anti-human IgG, IgM, IgA, and IgG subclasses (Zymed, South San Francisco, CA) at 37°C for 1 hour. After being washed, the plates were developed with substrate ABTS (KPL, Gaithersburg, MD), and the optical density (OD) was measured at 450 nm. Each sample was assayed in duplicate. The cut-off OD value for anti-PvMSP119 antibodies was set as...
the mean + 3 SD of log (unit + 1) values of the healthy control sera to obtain maximum sensitivity and specificity. The correlations between age, parasitemia, malaria exposure, and anti-PvMSP119 antibody level (ODs) were studied using Spearman rank correlation ($r$) test with the SPSS statistical package (SPSS version 10.0; SPSS, Chicago, IL). The correlations between parasite densities and anti-PvMSP119 antibody level (ODs) were also studied using the parametric $t$ test.

Our results showed that 85.4% of the people living in this region developed at least one of the antibodies (IgM, IgG, and IgA) against the PvMSP119 antigen. In particular, 69.5% of the individuals were positive for IgM, 53.6% of the individuals were positive for IgG, and 7.3% were positive for IgA type antibodies (Table 1). Among the IgG responders, IgG1 and IgG3 were the predominant subclasses (75% and 63.6%, respectively), followed by IgG2 and IgG4 (6.8% and 4.5%, respectively; Figure 1), suggesting high immune responsiveness to the PvMSP119 antigen in the Sanliurfa region.

We next analyzed the correlations between the antibody levels, age, and parasite levels. Although no significant correlation was found between the levels of anti-PvMSP119 antibody responses tested and age ($P > 0.05$), there were valuable correlations and implications between certain antibody responses and parasite densities. Accordingly, we found in our study that total plasma IgG and IgG1 levels showed a significant negative correlation with parasite density ($r = -0.245, P = 0.028$ and $r = -0.260, P = 0.018$, respectively), whereas others (IgM, IgG2, and IgG3) did not show any correlation with this parameter ($P > 0.05$; Figure 2). This finding suggests that increased levels of total IgG antibodies (mainly IgG1 isotype) against PvMSP119 may have a role in lowering parasite density. Supporting these data, our second analysis of the correlation between antibody responses and parasite levels among the antibody responders and non-responders showed a significant positive correlation between higher IgG1 levels and lower parasite densities ($t$ test, $t = 2.193$, $P = 0.031$). This effect might occur through prevention of the parasitic invasion of new erythrocytes. Nevertheless, further large scale studies that would include paired serum samples from the individuals during and after fever attacks will help to understand the protective efficacy of the antibodies against PvMSP119.

The predominant prevalence of anti-PvMSP119 antibody isotypes IgG1 and IgG3 were in accordance with previous observations (75% and 63.6%, respectively; Figure 1). The IgG1 isotype was the first to increase at an early age (0–4 years, 83.3%) and found to be at high levels at any given age groups. IgG3 prevalence was the second most predominant IgG isotype at an early age (0–4 years, 33.3%), but IgG3 levels showed a different pattern from IgG1 isotypes. The IgG3 isotype level was the highest at 15–29 years of age, but was found to be lower after 30 years of age. These results may suggest that the IgG3 isotype is closely related to the acute response to the PvMSP119 antigen. However, to determine any age-dependent change of the antibody levels, we need further studies using serum samples from asymptomatic individuals.

The IgM responses among the patients were > 64% in any age group tested, with a peak level of 85% in the 15–29 year olds (Table 1). Interestingly, although not statistically significant, the patients who had negative IgM levels had higher parasite levels compared with patients who had higher IgM levels (parasite levels: 6,460.86 ± 1,690.4 and 4,845.08 ± 730.5 parasites/μL, respectively; $t = 1.028, P = 0.307$). Higher IgM levels thus may suggest that rapid immature antibody responses will soon be seroconverted to the IgG antibodies and may predict the controlling role of anti-PvMSP119 IgM antibodies in lowering the parasite burden. This is in accordance with previous findings that individuals with patent P. vivax infection quickly develop IgG responses to PvMSP119 after a single exposure to malaria infection and as early as 3 days after the onset of symptoms. Children younger than 4 years of age had a higher frequency of serum positivity for antibodies (88.9%), with similar levels of IgM and IgG (66.7% and 66.7%, respectively). Of note, lower parasite levels were observed in comparison to the 5- to 14-year-old group, although this did not reach statistical significance (parasite levels of 0–4 and 5–14 year olds were 3,662.2 ± 1,442.3 and 6,202.6 ± 1,228.1 parasites/μL, respectively; $P > 0.05$). It was previously reported that transfer of specific anti-MSP119 antibodies from mother to child might occur during P. falciparum infection. We had only one child of 4 months of age in this group. Thus, further study is needed to determine whether PvMSP119-specific antibodies can be transferred from mother to child during $P$. vivax infection and could be associated with lower levels of parasite densities. We also observed that patients in the 15- to 29-year-old group with patent infection had a more acute immune response with higher levels of IgM responses and relatively lower levels of

### Table 1

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Number of subjects (%)</th>
<th>IgM</th>
<th>Total IgG</th>
<th>IgA</th>
<th>Total responders (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–4</td>
<td>9</td>
<td>6 (66.7)</td>
<td>6 (66.7)</td>
<td>1 (11.1)</td>
<td>8 (88.9)</td>
</tr>
<tr>
<td>5–14</td>
<td>31</td>
<td>20 (64.5)</td>
<td>13 (42)</td>
<td>2 (6.5)</td>
<td>24 (77.4)</td>
</tr>
<tr>
<td>15–29</td>
<td>21</td>
<td>17 (85)</td>
<td>10 (47.6)</td>
<td>1 (4.8)</td>
<td>19 (90.5)</td>
</tr>
<tr>
<td>30–65</td>
<td>21</td>
<td>14 (66.7)</td>
<td>15 (71.4)</td>
<td>2 (9.5)</td>
<td>19 (90.5)</td>
</tr>
<tr>
<td>Total</td>
<td>82</td>
<td>57 (69.5)</td>
<td>44 (53.6)</td>
<td>6 (7.3)</td>
<td>70 (85.4)</td>
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against it. --
P. vivax previously untouched region may help better understanding /H11505 (N
this region. To confirm this, we analyzed the presence of
spp. because
Plasmodium
mu
IGA responses without any cross-reactivity to other
Plasmodium
spp. infections from 82 patient samples
posing to vivax malaria in eastern Turkey develop antibodies
malaria is associated with serum antibod-
there was an analysis of the presence of other
Plasmodium
spp. infections from 82 patient samples
using polymerase chain reaction (F. Y. Zeyrek, unpublished data). Overall, our study showed that PvMSP1 is highly immu-
nullous antigen, PfMSP-1.
anti-PvMSP119 IgG antibody re-
dissimilarities to the potential candidate vaccine antigen PvMSP119. We are now undertaking studies to evaluate the immunogenicity of several other blood-stage antigens in this population. Further
P. vivax blood-stage antigens from this previously untouched region may help better understanding of host—P. vivax interactions and the development of vaccines against it.

REFERENCES
KD fragment of merozoite surface protein-1 (MSP-1 19) can play a protective role against blood-stage Plasmodium falciparum infection in individuals in a malaria endemic area of Africa. J Infect Dis 173: 666–672.


