SHORT REPORT: IgG1/IgG3 ANTIBODY RESPONSES TO VARIOUS ANALOGS OF RECOMBINANT yPfMSP119—A STUDY IN IMMUNE ADULTS LIVING IN AREAS OF PLASMODIUM FALCIPARUM TRANSMISSION

TAMSIR O. DIALLO, ANDRE SPIEGEL, ABABACAR DIOUF, RONALD PERRAULT, DAVID C. KASLOW, AND OLIVIER GARRAUD

Unité d’Immunologie, Institut Pasteur, Dakar, Sénégal; Unité d’Épidémiologie, Institut Pasteur, Dakar; Molecular Vaccine Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

Abstract. To further characterize protective-type (IgG1/IgG3) antibody responses to Plasmodium falciparum blood stage in putatively immune individuals’ plasma, we have tested for various analogs of the 19 kDa C-terminus of the MSP1 antigen obtained as secreted recombinant proteins from Saccharomyces cerevisiae. One of four proteins was then identified on the basis of consistent IgG3, along with less variable IgG1 recognition. This protein has thus been selected for further functional assays of IgG1/IgG3 antibodies.

The mechanisms for antibodies (Abs) in mediating protection to asexual blood stage Plasmodium falciparum parasite development presumably involve cytotoxic type Abs of the IgG1 and IgG3 subclasses, and IgG2 and IgG4 would act as antagonists.1–3 However, there are still many gaps in the detailed understanding of how natural protection is acquired and maintained in endemic areas. To identify mechanisms leading to the selection of specific responses of a given Ab class/subclass would be of interest to target the desired subclass production for Abs specific to “protective” epitopes in P. falciparum blood stage antigens (Abs) selected to serve as vaccine candidates.

To further characterize Ags which are consistently associated with cytotoxic, protective-type IgG1/IgG3 Abs, we have tested plasma obtained from a number of putatively immune Senegalese adults, originating from the villages of Dielmo where P. falciparum transmission is intense and perennial or Ndiop where transmission is low to moderate and seasonal.4,5 The individuals’ characteristics have been described previously, along with data showing that approximately two-thirds of them had specific Abs to MSP119.4–8 Anti-MSP119 Abs in the study population were mostly restricted to IgG1 and IgG3 subclasses, and IgG2 and IgG4 would be of interest to target the desired subclass production for Abs specific to “protective” epitopes in P. falciparum blood stage antigens (Abs) selected to serve as vaccine candidates.

For the present study, blood sampling and processing were done exactly as described.5 There were two different periods of sampling: in June, when P. falciparum transmission was moderate (Dielmo) or virtually absent (Ndiop) and in January, following the rainy season when P. falciparum transmission was maximum.4–6 MSP119 was obtained as secreted recombinant proteins from Saccharomyces cerevisiae and purified as described.12 Four analogs corresponding to amino-acid variations in the first (Q versus E) and second (KNG versus TSR) EGF-like motifs of MSP119 were obtained using similar procedures, namely Q-KNG, Q-TSR, E-KNG, and E-TSR.12 The concentration of the four recombinant proteins were adjusted to 1 mg/mL as measured by BCA (Pierce, Rockford, IL) and subsequently diluted for enzyme linked immunosorbent assays (ELISA). ELISA procedures were performed exactly as previously described with mouse anti-human IgG subclass Abs (Bionostics, Wyboston, Beds, UK) diluted 1:10,000 to 1:20,000 as appropriate.6 To minimize plate-to-plate and day-to-day variations, data were recorded as optical density values (OD). OD ratios > 1.5 were defined as positive responses which basically correspond to the mean ± 3 Standard Errors to the Mean (SEM).6 The Fisher’s exact test was used to compare groups (χ²).

Individual responses are shown in Figure 1. The difference in the frequency of IgG1 responses was not statistically significant among the four analogs although the response was slightly higher for Q-KNG (global test: χ² = 2; P = 0.57); in contrast, a significant difference was seen within IgG3 responses (global test: χ² = 8.28; P = 0.01). IgG3 responses to Q-KNG were significantly different from those to Q-TSR and E-TSR (P = 0.016 and P = 0.005, respectively) and more frequent than those to E-KNG, though not significantly different (P = 0.13). Because the cohort was being constituted over two consecutive years (1996 and 1997), we ascertained that the frequencies of responses from each year could be legitimately combined in the analyses. As the portion of “responders” versus “non-responders” in Dielmo and in Ndiop did not significantly differ from Year 1 to Year 2 (P values ranging from 0.35 to 0.91), data from Years 1 and 2 were subsequently considered together in each village and for each season. The comparison of IgG1 or IgG3 responses to all four yPfMSP119 analogs was not significantly affected by analyzing data site by site or season by season (P ranging from 0.16 to 1.0). Of note, neither anti-MSP119 specific IgG2/IgG4 was found in the present study.

An imbalance in IgG subclass distribution in Abs to P. falciparum blood stage Abs has been widely reported.13–14 In fact, protection against this stage seems to rely largely on specific IgG1 and IgG3 Abs, whose ratio (IgG1/IgG3) depends on the Ag used in the isotype-specific immunosays.14 Unlike other asexual blood stage Ags, MSP119 seems to elicit a restricted set of Ab responses in immune populations, with elevated IgG1/IgG3 and little if any IgG2 and IgG4; the yPfMSP119-Q-KNG proved to be optimally recognized by IgG1 and IgG3 in plasma from the selected study population. This suggests that subclass-specific responses can be influenced by the epitope they recognize and that certain measures of anti-MSP119 IgG3 might have been
IgG1/IgG3 RESPONSES TO P. FALCIPARUM MSP1<sub>19</sub> ANALOGS

<table>
<thead>
<tr>
<th>Classes tested</th>
<th>yPMSP1&lt;sub&gt;19&lt;/sub&gt; analogs</th>
<th>specific IgG1</th>
<th>specific IgG3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q-TSR</td>
<td>Q-KNG, E-TSR, E-KNG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q-KNG</td>
<td>Q-TSR, E-TSR, E-KNG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-TSR</td>
<td>Q-TSR, Q-KNG, E-KNG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-KNG</td>
<td>Q-TSR, Q-KNG, E-TSR</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Plasma samples from Dielmo (75) and Ndiop (70) are considered together in the present study, irrespective of the period of sampling. White boxes indicate Optical Density (OD) ratios $\geq 1.5$ but $< 2$. Grey and black boxes indicate OD ratios above the positivity threshold: $\geq 1.5$ and $>< 2$ (grey) and $= 2$ (black). The bottom lines indicate the % of positive responses in each column.

**Acknowledgments:** The authors would like to thank villagers from Dielmo and Ndiop; Drs. A. Tall (Institut Pasteur, Dakar) and J.-F. Trape (IRD/ORSSTOM, Dakar and Montpellier, France) and other Physicians and Health Care officers in Dielmo and Ndiop for providing access to blood samples in the villages and communication of clinical data; Drs. D. Fontenille and L. Lochouarn (ORSSTOM/IRD, Dakar) for entomological data; and Drs. P. David, O. Mercereau-Pujalson, S. Longacre, and H. Jouin (Institut Pasteur, Paris) for helpful comments.

**Financial support:** This work was supported in part by funding from the French Ministry of Cooperation and Development and from the Institut Pasteur, Paris.

**Authors’ addresses:** Tamsir O. Diallo, André Spiegel, Ababacar Diouf, and Ronald Perraut, Institut Pasteur de Dakar, PB 220, Dakar, Sénégal; David C. Kaslow, Virus and Cell Biology, Merck Res. Labs, WP16-101B, POB 4, 770 Summertown Pike, West Point, PA 19486. Olivier Garraud, GIMAP, Faculté de Médecine, 15 rue Ambroise Paré, 42023 Saint-Etienne, cedex, France.

Reprint requests: Olivier Garraud, GIMAP, Faculté de Médecine, 15 rue Ambroise Paré, 42023 Saint-Etienne, cedex, France.

**REFERENCES**


(classes tested are indicated as boxes. Plasma samples from Dielmo (75) and Ndiop (70) are considered together in the present study, irrespective of the period of sampling. White boxes indicate Optical Density (OD) ratios $< 1.5$ (below the positive threshold). Grey and black boxes indicate OD ratios above the positivity threshold: $\geq 1.5$ and $< 2$ (grey) and $\geq 2$ (black). The bottom lines indicate the % of positive responses in each column.)

**Figure 1.** Screening of individual IgG1 and IgG3 responses to four yPMSP1<sub>19</sub> analogs. Every individual plasma (diluted 1:200) included in the survey was tested for the presence of IgG1 and IgG3 specific to either Q-TSR, Q-KNG, E-TSR, and E-KNG analogs (recombinant proteins are described in the Materials and Methods). Individual responses for all four analogs and for the two IgG subclasses are tested as indicated by boxes. Plasma samples from Dielmo (75) and Ndiop (70) are considered together in the present study, irrespective of the period of sampling. White boxes indicate OD ratios $\geq 1.5$ but $< 2$. Grey and black boxes indicate OD ratios above the positivity threshold: $\geq 1.5$ and $< 2$ (grey) and $\geq 2$ (black). The bottom lines indicate the % of positive responses in each column.

<table>
<thead>
<tr>
<th>OD ratios</th>
<th>IgG1</th>
<th>IgG3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\geq 1.5$</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>$&lt; 2$</td>
<td>20.0</td>
<td>20.0</td>
</tr>
</tbody>
</table>


