Correlation of *Plasmodium falciparum* Gametocytemia with Complement Component Titers in Rural Nigerian School Children

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The relationship between *Plasmodium falciparum* gametocytemia and the complement components C3, C4, and C3b was investigated in 141 ambulant rural Nigerian school children. Three groups were studied. Group 1 included 42 children with circulating *P. falciparum* gametocytemia in which the mean titers of C3, C4, and C3b were 145.4 ± 22.6 mg/100 ml (72%), 60.5 ± 0.2 mg/100 ml (149%), and 64.0 ± 5.9 mg/liter (65%), respectively. These findings indicated marked C3b hypocomplementemia. Group 2 included 50 children who were used as controls and lacked malaria parasitemia. C3, C4, and C3b mean titers were 161.6 ± 28.9 mg/100 ml (80%), 56.8 ± 2.07 mg/100 ml (140%), and 84.8 ± 11.4 mg/liter (86%), respectively. Group 3 included 49 children with other blood stages of *P. falciparum* parasitemia but no gametocytes. C3, C4, and C3b mean titers in this group were 103.0 mg/100 ml (51%), 18.3 ± 1.2 mg/100 ml (45%), and 90.7 mg/liter (92%), respectively. Our findings suggest that C3b hypocomplementemia may be related to the advent of circulating *P. falciparum* gametocytes in children. We also confirmed C3 and C4 hypocomplementemia in acute human malaria. The implication of our findings in relation to neat in vitro culture of *P. falciparum* gametocytes or total inhibition of gametocytogenesis in malaria control is still speculative.

Hypocomplementemia has always been associated with experimental malaria. Monkeys infected with *Plasmodium knowlesi* have depressed total hemolytic complement levels. Hypocomplementemia was first associated with merozoite release, and in the terminal stage of the infection, a marked fall precedes death (6, 8; M. A. Ade-Serrano, Ph.D. thesis, University of Birmingham, Birmingham, England, 1976). Hypocomplementemia in human malaria was first reported by Cathorie (5) and Vincent (14). Their findings were confirmed by Dulaney et al. (7). Brueton and Greenwood (3) reported low C3, C4, and C1q levels and normal glycine-rich beta-glycoprotein levels in children with acute *Plasmodium falciparum* malaria. These findings were confirmed by Srichaikul et al. (13) and Ree (10). Williamson et al. (16) concluded that although the *P. falciparum* hypocomplementemia may be severe, it is also transient.

Many factors, both in vivo and in vitro, affect gametocytemia. They include the following: (i) low-temperature treatment (1); (ii) cloning of asexual parasites of rodents (15); (iii) host specificity, i.e., a strain of parasite may produce gametocytes when inoculated into one species of host but not into another; (iv) anti-malarial treatment (it has been suggested that several anti-malarial drugs may increase gametocyte production); (v) culture conditions (the Trager-Jenson culture system produced gametocytes consistently) (4, 12); and (vi) physiological condition and age of the host (2). Because any factor that inhibits gametocytemia may totally affect the further transmission of malaria, we were interested in the physical and biochemical factors that may elucidate the pathophysiology of gametocytogenesis, gametocytemia, and gametocyte maturation. The roles of age, parasite index, gametocyte density index, and *P. falciparum* malaria immunoglobulin G antibody titer in gametocytemia have not been significant (M. A. Ade-Serrano, G. C. Ejezie, and O. O. Kassim, Trop. Geog. Med., in press; M. A. Ade-Serrano and B. K. Adadevloh, manuscript in preparation). Complement amplifies immune responses; therefore, there is reason to search for any relationship between gametocytemia and complement component titers.

**MATERIALS AND METHODS**

A rural malarriometric survey was carried out on school children aged 5 to 14 in the Epe, Badagry, and Ikorodu areas of Lagos State (Ade-Serrano et al., in

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press). During this survey, anthropometric and basic hematological examinations were carried out. All anemic and clinically malnourished (kwashiorkor and marasmus) children were excluded. Three groups were studied. Group 1 included 42 children with gametocytokemia (P. falciparum only). Group 2 included 50 children without parasites in thick and thin blood smears. Group 3 included 49 children with other stages of P. falciparum parasitemia but no gametocytes.

**Measurements of C3 and C4.** Plasma collected in nonheparinized capillary tubes was separated by centrifugation and stored at -20°C until tested (within 48 h of collection). C3 and C4 were measured by radial immunodiffusion (Mancini) with specific antisera obtained from Hoechst Behringwerke. Pooling local Nigerian plasma served as the standard against which results were expressed as percentages and in absolute values.

C3b. C3b was measured with prepoured and prestandardized Hoechst Behringwerke Partigan plates.

**RESULTS**

The results of the survey are summarized in Table 1.

**C3.** In children with *P. falciparum* gametocytokemia (group 1), the mean titer of C3 was 72 ± 11.2%, which is expressed in absolute values as 145.4 ± 22.6 mg/100 ml (Fig. 1). The range was between 56 and 86% (113.1 to 173.7 mg/100 ml). The median was 70% (141 mg/100 ml). The C3 titer in this group was not related to parasitemia because the mean parasite rate was 0.5%. The findings were also unrelated to the sex and age of the children.

The control children in group 2 had a mean C3 titer of 80 ± 14.3% (161.6 ± 28.9 mg/100 ml) (Fig. 1). The median (79% [159.6 mg/100 ml]) almost corresponded to the mean, and the range was between 64 and 100% (129.3 to 202.0 mg/100 ml). None of the children in this group showed any parasites in three consecutive blood films.

The children in group 3 (those children with other stages of *P. falciparum* parasitemia but no gametocytes) had a mean C3 titer of 51% (103.0 mg/100 ml) (Fig. 1), with a range between 39 and 65% (78.8 to 131.3 mg/100 ml). The mean tallied with the median. The mean parasite count was 3.5%, and severe C3 hypocomplementemia (lower than 50% of the local standard) was associated with the higher parasite count. A total of 26 children in this group had C3 titers below 50% (101 mg/100 ml). The findings were unrelated to the sex and age of the children.

**C4.** The C4 titer (Fig. 2) showed a wide scatter in group 1 children. The mean titer was 149 ± 0.5% (60.5 ± 0.2 mg/100 ml), and the range was between 77 and 214% (31.3 to 86.9 mg/100 ml). In group 2 children, the mean C4 titer was 140 ± 5.1% (56.8 ± 2.07 mg/100 ml), and the range was between 82 and 197% (33.3 to 80.0 mg/100 ml). In Group 3 children, the mean C4 titer was 45 ± 2.9% (18.3 ± 1.2 mg/100 ml), and the range was between 31 and 80% (12.6 to 32.5 mg/100 ml). According to Student's t test, the mean titers for children in groups 1 and 2 were statistically significant (*P* < 0.002) as compared with those in children in group 3.

**C3b.** The children in group 1 (those with *P. falciparum* gametocytokemia) had a mean C3b titer of 65 ± 0.6% (64.0 ± 5.9 mg/liter) (Fig. 3). The mean C3b titers for children in groups 2 (control children without malaria parasitemia) and 3 (children with other blood stages of *P. falciparum* parasitemia but no gametocytes) were 86 ± 11.6% (84.8 ± 11.4 mg/liter) and 92% (90.7 mg/liter), respectively (Fig. 3). The mean C3b titer for children in group 1 as compared with those for children in groups 2 and 3 showed

**Table 1. Absolute values of C3, C4, and C3b in 141 rural Nigerian school children showing variation with *P. falciparum* gametocytomia, *P. falciparum* agametocytomia, and malaria apanasitemia**

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Complement component</th>
<th>Mean (mg/liter)</th>
<th>Median (mg/liter)</th>
<th>Standard deviation</th>
<th>Standard error of the mean</th>
<th>Range (mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (42)</td>
<td>C3</td>
<td>145.4 (72)</td>
<td>141.0 (70)</td>
<td>22.6 (11.2)</td>
<td>0.27</td>
<td>113.1-173.7 (56-86)</td>
</tr>
<tr>
<td></td>
<td>C4</td>
<td>60.5 (149)</td>
<td>63.3 (156)</td>
<td>0.2 (0.5)</td>
<td>0.01</td>
<td>31.3-86.9 (77-214)</td>
</tr>
<tr>
<td></td>
<td>C3b</td>
<td>64.0 (65)</td>
<td>61.1 (62)</td>
<td>5.9 (0.6)</td>
<td>0.02</td>
<td>50.3-78.9 (51-80)</td>
</tr>
<tr>
<td>2 (50)</td>
<td>C3</td>
<td>161.6 (80)</td>
<td>159.6 (79)</td>
<td>28.9 (14.3)</td>
<td>0.29</td>
<td>129.3-202.0 (64-100)</td>
</tr>
<tr>
<td></td>
<td>C4</td>
<td>56.8 (140)</td>
<td>58.1 (143)</td>
<td>2.07 (5.1)</td>
<td>0.1</td>
<td>33.3-80.0 (82-197)</td>
</tr>
<tr>
<td></td>
<td>C3b</td>
<td>84.8 (86)</td>
<td>85.8 (87)</td>
<td>11.4 (11.6)</td>
<td>0.23</td>
<td>70.0-108.4 (71-110)</td>
</tr>
<tr>
<td>3 (49)</td>
<td>C3</td>
<td>103.0 (51)</td>
<td>103.0 (51)</td>
<td>1.2 (2.9)</td>
<td>0.06</td>
<td>78.8-131.3 (39-65)</td>
</tr>
<tr>
<td></td>
<td>C4</td>
<td>18.3 (45)</td>
<td>17.1 (42)</td>
<td>1.2 (2.9)</td>
<td>0.06</td>
<td>12.6-32.5 (31-80)</td>
</tr>
<tr>
<td></td>
<td>C3b</td>
<td>90.7 (92)</td>
<td>88.7 (90)</td>
<td>77.9-108.5 (79-110)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Values are given in milligrams per 100 ml for C3 and C4 and in milligrams per liter for C3b. Values in parentheses denote the percentages of the absolute values as calculated by a local standard calibrated against Behringwerke-Hoechst standards.*
statistical significance ($P < 0.005$). Individual C3b titers in the children were not related to age, sex, size, or hematocrit, but children with circulating $P. falciparum$ gametocytes showed a greater fall in C3b hypocomplementemia. The mean parasite count was also lower in group 1 children than in group 3 children.

**DISCUSSION**

Our findings strongly suggest that a lowered C3b titer is associated with $P. falciparum$ gametocytemia. However, the design of our study could not provide any insight into which occurs first, the C3b hypocomplementemia or the $P. falciparum$ gametocytemia. In addition, there may be another age-related factor or factors which trigger both or each of our findings. It is well documented that $P. falciparum$ gametocytemia occurs more commonly in children than in adults (2). We used a microplate enzyme-linked immunosorbent assay to measure $P. falciparum$ immunoglobulin G antibodies in sera from these groups of children and found no correlation with gametocytemia, although age-related differences were shown (Adadevoh and Adadevoh, manuscript in preparation). We have further confirmed that in humans, $P. falciparum$ infection depresses complement factors. Srichaikul et al. (13) showed that total hemolytic complement activity was depressed in human malaria. Brueton and Greenwood (3) reported low C3, C4, and C1q levels and normal glycine-rich beta-glycoprotein levels in children with acute $P. falciparum$ malaria. These authors did not relate their findings to protein energy malnutrition, which depresses both C3 and C4 components (9), or to circulating gametocytes.

Williamson et al. (16) obtained titers of $84 \pm 15\%$ and $180 \pm 43\%$ for C3 and C4, respectively, on day 7. On day 14, titers were $81 \pm 19\%$ and $150 \pm 52\%$. They concluded that by 7 days after the onset of illness, serum C3 and C4 titers had returned to normal levels. Our titers for children with $P. falciparum$ gametocytemia (72\% [145.4 mg/100 ml] for C3 and 149\% [60.5 mg/100 ml] for C4) did not show any statistical differences as compared with those of Williamson et al. (16). None of the children in this study had been taking any anti-malarial drug when examined; hence, it may be conjectured that the fall in C3
and C4 levels occur with recovery, irrespective of treatment, because gametocytosis is induced and appears in the peripheral blood about 7 to 12 days after the asexual parasites are first seen or, more tenuously, that the induction of gametocytogenesis and the resulting gametocytemia induce greater resistance or abort an infection. This is in keeping with the concept of a successful parasite being more concerned with further transmission of itself than with killing its host. Gametocytemia, unlike schizotemia, is also associated with a lower parasite density (Ade-Serrano et al., in press) and a more negligible effect on host immunity (2).

Although our findings confirmed C3 and C4 hypocomplementemia, we found almost normal titers of C3b in our group 3 children who had acute malarial infection. Although our findings are single determinations in a complex, possibly constantly changing milieu, they may aid us in carrying out a further group of experiments, which will include the following: (i) serial determination of C3b titers in children with P. falciparum malaria until gametocytes appear in the spleen or the peripheral blood or both; (ii) relationship of these findings to types and titers of circulating specific immune complexes; (iii) in vitro culture of P. falciparum by a modification of the Trager-Jensen method (feeding parasites with human serum depleted of different serum factors, including C3b, and checking the rate and maturation of resulting gametocytes); (iv) study of the changes on maturing erythrocyte surfaces containing young gametocytes to check whether these surface changes are related to complement receptors or HLA surface antigens (11).

LITERATURE CITED