Transmission Blockade of *Plasmodium falciparum* Malaria by Anti-Pfs230-Specific Antibodies Is Isotype Dependent

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Malaria is transmitted from humans to mosquitoes through gametocytes that develop in the blood of infected patients. Gametogenesis of *Plasmodium falciparum* in the *Anopheles* mosquito midgut is accompanied by the emergence of the gametocytes that develop in the blood of infected patients. From that moment, the extracellular parasites become susceptible to immune factors, such as antibodies and complement, taken up during the blood meal from the vertebrate host.

Gametocytes of *P. falciparum* synthesize molecules with apparent molecular sizes of 230 and of 48 and 45 kDa (Pfs230 and Pfs48/45) after extraction with sodium dodecyl sulfate (SDS) and separation by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Both molecules are expressed on the surface of macrogametocytes or zygotes. Several monoclonal antibodies (MAbs) reactive to these sexual-stage molecules block transmission of the parasites from the vertebrate host to the mosquito vector. Some of these MAbs suppress the infectivity of malaria gametocytes for mosquitoes in the presence of active human complement.

By use of the parental hybridoma cell line 63F2A2 that produces specific antibodies of immunoglobulin isotype G1 (IgG1; 63F2A2.1) against Pfs230, we attempted to enrich for the synthesis of the downstream switch variant IgG2b and IgG2a monoclonal antibodies (MAbs) of the hybridoma cell line (63F2A2.2b and 63F2A2.2a, respectively). The parental IgG1 did not reduce the *Plasmodium falciparum* transmission in a bioassay irrespective of the presence of complement. MAbs 63F2A2.2b and 63F2A2.2a were effective in reducing the infectivity of *P. falciparum* parasites to *Anopheles gambiae* mosquitoes in membrane-feeding experiments. A transmission reduction of 91% was accomplished by the 63F2A2.2b switch variant, and a reduction of greater than 99% was accomplished by the 63F2A2.2a switch variant, but only in the presence of active human complement. Subsequently, the transmission-reducing effect of MAb 63F2A2.2b or 63F2A2.2a was confirmed in vitro by the rapid lysis of newly formed macrogametes or zygotes in the presence of active complement. MAB 63F2A2.1 did not lyse the newly formed macrogametes or zygotes irrespective of the presence of complement.

In this study, the importance of the immunoglobulin G (IgG) subclass for transmission-blocking activity is further analyzed by production and testing of a number of switch variants of the anti-Pfs230-specific MAB 63F2A2.

**MATERIALS AND METHODS**

**Parasites and Pfs230 extract.** Mature gametocytes of *P. falciparum* (isolate NF54) were produced in an automated large-scale culture system as described by Ponnudurai et al. (11). Gametocyte isolation and Triton X-114 extraction of Pfs230 was obtained as described previously (20). The freeze-dried aqueous-phase extract was diluted in phosphate-buffered saline (PBS) and used as the antigen in both the competition and the one-site Pfs230 ELISA.

**Antibodies.** (i) MAbs. The anti-Pfs230 MAbs 18F25, 63F2A2 (63F2A2.1), and 63F2A2.1, and 63F2B (all isotype IgG1) and anti-Pfs25 MAbs 32FB1 (isotype IgG1) have been described previously (20, 22). MAbs 18F25 was labelled with horseradish peroxidase by the periodate method described by Wilson and Na-kanke (24). The labelled MAb was dialyzed against PBS-thimerosal (0.01%), and fetal calf serum (1%) was added and samples were stored at 4°C. MAbs 32FB1 and 18F25 were labelled with fluorescein isothiocyanate (FITC) by the method described by Goding (5). Rat MAbs against different mouse immunoglobulin isotypes, i.e., IgG1, IgG2a, and IgG2b, and horseradish peroxidase-labelled monoclonal rat anti-mouse kappa light-chain antibodies were purchased from CLB (Amsterdam, The Netherlands).

(ii) Complement. Type AB blood from Dutch blood bank donors with no previous malaria exposure was allowed to clot at room temperature for 1 h, and aliquots of the pooled serum were stored at −20°C until required. Freshly thawed samples were used only once as a source of complement. The serum was heated at 56°C for 30 min to inactivate complement activity. The serum was also used as a malaria-negative control serum in the transmission and parasite lysis tests.

**Selection, enrichment, and purification of isotype switch variants.** Selection and enrichment of isotype switch variants were conducted by the technique of sequential sublining (2, 10). Briefly, the parental 63F2A2.1 hybridoma cells were grown in a 96-well microtiter plate (NUNC; Intermed), containing 1,000 cells per well, in Dulbecco’s modified Eagle’s medium supplemented with 20% fetal calf serum and 2.5 U of interleukin 6 per ml and cultured in a humidified 5% CO2-in-air incubator at 37°C. After 7 days, the culture supernatants were tested in the isotype-specific ELISA and cells from positive wells were redistributed into 50 cells per well. After a culture period of 4 days, the supernatants were tested, and cells of positive wells were subcultured at 2 cells per well and cloned by limiting dilution. Supercpantants were tested for the presence of the parental isotype and/or switch variants. Selected cloned lines were expanded in culture, and acetic fluids were produced from 106 hybridoma cells injected in pristane-primed BALB/c mice. IgG was purified from acetic fluid with a semiautomated fast-performance liquid chromatography system.

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RESULTS

Isolation and characterization of switch variants. When seeded at 1,000 cells per well, IgG2b switch variants were detectable from the parental 63F2A2.1 hybridoma after 7 days of culture in one to two wells per microtiter plate. Further cloning and subcloning resulted in the isolation of a 63F2A2 switch variant of the IgG2b subclass (63F2A2.2b). By use of a similar protocol, 63F2A2.2a MAb of the IgG2a isotype (63F2A2.2a) were obtained from the 63F2A2.2b-producing hybridoma. All switch variants were subjected to limiting dilution conditions to reach purity at the clonal level. The switch variants reacted only in the ELISA that was specific for the particular isotype (data not shown). Figure 1 shows similar binding profiles of the parental MAb 63F2A2.1 and its IgG2b and IgG2a switch variants in the Pfs230 one-site ELISA.

Transmission-blocking capacity of isotypes. The effect of IgG subclass specificity on transmission was analyzed in the biosay in the presence of active or inactivated human complement. In the presence of active complement, anti-Pfs230 MAbs 63F2A2.2b and 63F2A2.2a suppressed infectivity of *P. falciparum* for mosquitoes by 50% and 99%, respectively, at concentrations of 10 µg per feeder (Table 1). In the presence of inactivated complement, the different isotypes of MAb 63F2A2.2b was lower than that of MAb 63F2A2.2a.

![FIG. 1. Reactivity of IgG1, IgG2a, and IgG2b isotype variants of MAb 63F2A2 in the one-site Pfs230 ELISA. Protein A-purified MAbs were serially diluted and ranged in concentration from 10 µg/ml to 0.12 ng/ml in PTG. Results are expressed as the mean of duplicate incubations. OD, optical density.](image)

<table>
<thead>
<tr>
<th>Expt 1</th>
<th>Expt 2</th>
</tr>
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<tbody>
<tr>
<td>GMW</td>
<td>SD</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
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<tr>
<td>3</td>
<td>+</td>
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<td>4</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
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</table>

*Active (+) or inactivated (−) human complement.*

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TABLE 1. Effects of different isotypes of anti-Pfs230 MAb 63F2A2 on infectivity of *P. falciparum* gametocytes for *A. gambiae* mosquitoes in membrane-feeding experiments with active or inactivated complement
TABLE 2. Infectivity of *P. falciparum* gametocytes for mosquitoes in the presence of anti-Pfs230 MAb 63F2A2.2b or 63F2A2.2a at various concentrations with active or inactivated complement.

<table>
<thead>
<tr>
<th>MAb conc (µg/feeder)</th>
<th>Complement</th>
<th>Oocyst counta in presence of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAb 63F2A2.2b</td>
<td>MAb 63F2A2.2a</td>
</tr>
<tr>
<td></td>
<td>GMW SD R P/D</td>
<td>GMW SD R P/D</td>
</tr>
<tr>
<td>25</td>
<td>+</td>
<td>3.2 0.7 90.1 18/20 0 0 100 0/20</td>
</tr>
<tr>
<td>25</td>
<td>—</td>
<td>20.7 3.6 35.7 20/20 4.3 1.1 4.9 18/20</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>3.0 0.7 90.7 17/20 0.1 0.1 99.8 2/20</td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td>24.2 4.3 24.5 21/21 3.3 1.0 27.0 16/20</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>9.5 2.1 70.5 19/20 0.1 0.1 99.8 2/20</td>
</tr>
<tr>
<td>1</td>
<td>—</td>
<td>27.3 6.1 15.2 20/20 4.5 1.3 0.4 17/20</td>
</tr>
<tr>
<td>0.2</td>
<td>+</td>
<td>ND 0.9 0.3 80.1 12/20</td>
</tr>
<tr>
<td>0.2</td>
<td>—</td>
<td>ND 4.7 1.6 0 16/20</td>
</tr>
<tr>
<td>Controld</td>
<td></td>
<td>32.2 6.5 58/59 4.5 1.3 46/59</td>
</tr>
</tbody>
</table>

*a* Active (+) or inactivated (−) human complement.

*b* See Table 1, footnote b, for explanation of the different boxheads.

*c* ND, not done.

*d* Control is the mean of three feeders per experiment.

(ND), were then tested for the infectivity of *P. falciparum* gametocytes to mosquitoes. Ten anti-Pfs230 MAbs (isotype IgG2a) and one MAb of isotype IgG2b (MAb 12A1A5) were all effective in blocking transmission but only when active complement was added to the feeder, whereas 14 IgG1 isotype MAbs were not (Table 3).

**SIFA and complement-mediated lysis of macrogametes or zygotes.** The mechanism of complement-dependent transmission-blocking activity of MAbs 63F2A2.2b and 63F2A2.2a was further analyzed in vitro with activated gametocytes and a SIFA based on antibody reactivity to Pfs25, thereby monitoring development of macrogametes or zygotes 3 h after activation. The actual numbers of macrogametes or zygotes differ in several parasite batches, which makes direct comparison of data of independent experiments impossible. In a representative experiment (Fig. 2), no statistically significant differences were found in the presence of active complement (t = 4.43, df = 5, P = 0.007). Active complement in the presence of MAb 63F2A2.2b also had no effect, but in the presence of 63F2A2.2b or 63F2A2.2a, the number of macrogametes or zygotes was reduced substantially and almost completely in comparison with the effect of the control group (t = 6.62, df = 2, and P = 0.022 versus t = 6.47, df = 2, and P = 0.023, respectively).

The absence of lysis, fluorescence was clearly visible at the circumference of apparently intact parasites (Fig. 3A), whereas fluorescence after lysis revealed either no round forms or damaged effect of complement, a significant reduction was observed in the presence of active complement (t = 4.43, df = 5, P = 0.007). Active complement in the presence of MAb 63F2A2.2a had no effect, but in the presence of MAb 63F2A2.2b or 63F2A2.2a, the number of macrogametes or zygotes was reduced substantially and almost completely in comparison with the effect of the control group (t = 6.62, df = 2, and P = 0.022 versus t = 6.47, df = 2, and P = 0.023, respectively).

**Lysis of macrogametes or zygotes by the complement-fixing MAb 63F2A2.2a in the presence of active or inactivated complement is shown in fluorescent micrographs using SIFA and the FITC-labelled anti-Pfs230 MAb 18F25 (Fig. 3).** In the absence of lysis, fluorescence was clearly visible at the circumference of apparently intact parasites (Fig. 3A), whereas fluorescence after lysis revealed either no round forms or damaged

![Image A](A)

![Image B](B)

**FIG. 3.** Complement-mediated lysis of early *P. falciparum* macrogametes or zygotes in the presence of MAb 63F2A2.2a as observed after staining with FITC-labelled MAb 18F25. The macrogametes or zygotes remained visually intact when incubated with the IgG2a switch variant in the presence of inactivated complement (A) and were lysed in the presence of the IgG2a switch variant and active human complement (B).
round forms with fluorescent debris or fluorescence of incomplete round forms (Fig. 3B). With phase-contrast light microscopy, damaged parasites with extrusion of the pigment or localization of aggregated pigment at peripheral sites and loss of pigment motility were manifested.

DISCUSSION

In the present study, downstream IgG2b and IgG2a switch variant antibodies specific for Pf s230 were generated from the parent MAb, 63F2A2.1. In contrast to 63F2A2.1, both 63F2A2.2b and 63F2A2.2a were able to block the transmission of *Plasmodium falciparum* to *A. gambiae* mosquitoes in membrane-feeding experiments but only in the presence of active complement. Results from the one-site Pf s230 ELISA (Fig. 1) show that the dose-dependent bindings of MAbs 63F2A2.1, 63F2A2.2b, and 63F2A2.2a to Pf s230 are comparable. SIFA results suggest a complement-dependent disturbance of the sexual development of macrogametes or zygotes after binding of 63F2A2.2b or 63F2A2.2a switch variant MAbs. Even in the presence of active complement, parental MAb 63F2A2.1 did not prevent the development of parasites after activation of the mature gamocytes; however, irrespective of the presence of these MAbs, active complement shows a direct effect on macrogametes or zygote development (Fig. 2). This may be explained by an alternative activation of the complement cascade due to a direct interaction between C3 and the parasites similar to the stimulation observed with certain bacteria. Although some reduction in the number of viable parasites is accomplished by this activation of the alternative pathway of complement, this does not lead to transmission blockade. Whether this phenomenon applies to other epitopes of Pf s230 (20) remains to be determined.

All anti-Pf s230 MAbs of isotype IgG2b or IgG2a tested to date were effective in blocking transmission but only when complement was added to the feeder, whereas IgG1 isotype MAbs were not. To date, there is an absolute correlation between Pf s230-related transmission blockade by MAbs, the ability of the isotype to fix complement, and the need for active complement to show transmission blockade by Pf s230-binding antibodies. Quakyi et al. (15) already described two Pf s230-specific MAbs (both IgG2a) that suppressed infectivity of *P. falciparum* to *Anopheles freeborni* mosquitoes by a complement-mediated lysis of early macrogametes or zygotes. A comparable effect of complement-fixing MAbs of the IgG2a isotype against a *Plasmodium gallinaceum* protein that is analogous to Pf s230 was described by Kaushal et al. (7). Confirming our observations, Read et al. (17) also found in a recent study the complement-dependent suppression of transmission of gametocytes of *P. falciparum* 3D7 to mosquitoes with anti-Pf s230 MAbs of isotype IgG2a.

Generally, mouse IgG1 is known to be a poor binder of human complement factor Clq, whereas IgG2b and IgG2a bind Clq effectively. Binding of Clq is the first step in activation of the classical complement cascade, leading to the formation of the membrane attack complex. Mouse IgG2a is more effective than IgG2b in fixation of human complement factors such as Clq, C4b, and C3b (3, 8). Although complement fixation in binding tests is not directly proportional to complement activation, haemolysis experiments suggest that the fixation of C3b is directly proportional to activation of C3b and the terminal lytic sequence (3). The capacity of different murine isotypes to fix complement fits perfectly the ability of MAb 63F2A2 switch variants to interfere with complement.

These results may have important implications for the study of anti-Pf s230 antibodies that are present in 40 to 85% of the endemic human sera (6, 19). The presence of specific anti-Pf s230 antibodies in field sera has been associated with transmission-blocking activity by some investigators (6) but could not be confirmed by others (14) or in our study (20). Immunoprecipitation and ELISA (6, 20) are based on epitope recognition by antibody being independent of the isotype, while a Pf s230-based transmission blockade may be accomplished by an isotype-dependent effector mechanism. When Pf s230 components are used in the design of a transmission-blocking vaccine (15), these findings support the need for an immunization strategy (1, 21) which should induce antibodies of a complement-binding isotype.

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