Brief Definitive Report

TARGET ANTIGENS OF TRANSMISSION-BLOCKING IMMUNITY ON GAMETES OF PLASMODIUM FALCIPARUM*

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Malaria, once successfully controlled and even eradicated in many tropical regions by the use of residual insecticides, is again highly prevalent due to the development of insecticide-resistant mosquitoes and to logistical problems of maintaining long-term anti-malaria compaigns. Today over one billion people are at significant risk from this debilitating and often fatal disease. In areas where residual spraying was previously successful the induction of tranmission-blocking immunity in the human population could be expected to have equivalent effect.

Transmission of malaria from man to mosquito is mediated by the sexual stages of the malaria parasite (gametocytes), which circulate as intraerythrocytic parasites in the blood of the host. Gametocytes undergo gametogenesis and the extracellular gametes fertilize in the midgut of the mosquito during a blood meal. It has been shown in several species of animal malaria that immunization of the host with extracellular gametes of the parasites can suppress infectivity of subsequent blood infections to the mosquitoes (1–3). Anti-gamete antibodies inhibit fertilization of gametes released in the blood meal ingested by the mosquito leading to sterilization of the parasites in the vector.

Using in vitro grown gametocytes of *Plasmodium falciparum*, the most dangerous of the human malarias, we report here the identification of target antigens of monoclonal antibodies (MAbs) that suppress the infectivity of these parasites to mosquitoes.

Material and Methods

Malaria Culture. Cultures of *P. falciparum* were grown in human erythrocytes based on the method described by Ifediba and Vanderberg (4).

Purification of Female Gametes of P. falciparum. Female gametes of *P. falciparum* were prepared from cultures in which the majority of gametocytes were morphologically mature. The cultures were centrifuged at 1,000 *g* for 5 min and the cells resuspended in a solution that stimulates gametogenesis (7 mM Tris, 117 mM NaCl, 8 mM glucose, 30 mM $NaHCO_3$ with 10% human serum added) (5); the final pH of the suspension was pH 8.1. After incubating for 30 min at room temperature the cells were resuspended to a 20% hematocrit and separated on a discontinuous Percoll (Pharmacia Fine Chemicals. Piscataway, NJ) gradient (15%, 30%, 42%, 54%, 60% Percoll in RPMI 1640 medium) by centrifuging at 16,000 g (Sorvall HB-4 swing-out head; DuPont Instruments-Sorvall

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Biomedical Div., Dupont Co., Wilmington, DE) for 10 min. The extracellular gametes separated in a layer at the 30%/42% interface.

Production of MAbs. Mature gametocytes of *P. falciparum*, Brazilian isolate Ituxi 084, were harvested and gametogenesis stimulated (5), and gametes and gametocytes were partially purified by density gradient centrifugation as described above. A suspension of 5×10^6 cells of this preparation in 0.5 ml of a 1:100 dilution in phosphate-buffered saline (PBS), pH 7.4, of reconstituted lyophylized *Bordetella pertussis* (0.167 opacity units of *B. pertussis* equivalent to $\sim 10^8$ organisms) was inoculated into a BALB/c mouse. The *B. pertussis* was inactivated with 0.01% thimerosal and freeze-dried for storage as lot 7b. 3 mo later a similar number of parasites combined from seven different isolates of *P. falciparum* (IMTM2, IMTM25, IMTM27, IMTM29, and Ituxi 084, all isolated from Brazil; L, isolated from Liberia, West Africa; and V1, isolated from South East Asia) were inoculated intravenously into the mouse. 3 d later spleen cells from the immunized mouse were fused with myeloma cells of the line P3-NS1/1-Ag4-1(NS1) (6) and hybrid cell lines (hybridomas) grown as previously described (7). Supernatants from the hybridoma cultures were screened by indirect immunofluorescence (IF) against air-dried female gametes of *P. falciparum* prepared from a mixture of cultures of the isolates used in immunization. Hybridoma cultures that gave positive reactions were cloned by limiting dilution and screened by IF with live female gametes of a West African isolate of *P. falciparum* (BC1) to identify those hybridomas that secreted antibodies reacting with surface antigens on the malarial gametes. Cloned hybridomas that produced positive reactions were grown as ascites tumors in pristane primed BALB/c mice as a source of MAbs. Proteins from the ascitic fluids were precipitated with 30% ammonium sulfate and dialyzed against PBS, pH 7.4.

Assay for Effect of MAbs on Infectivity of Gametocytes of P. falciparum to Mosquitoes. Fresh defibrinated human blood was spun at 1,000 *g* for 5 min at room temperature and the cells washed in a solution containing 8.5 mM Tris, 140 mM NaCl, 9 mM glucose, pH 7.4 (SA solution) and resuspended to a 50% hematocrit in heat-inactivated normal human serum or in serum from freshly drawn defibrinated human blood as source of active complement. To 150-μl samples of this suspension of uninfected erythrocytes in normal serum was added 60 μl of the appropriate MAb in the form of ascites proteins, ammonium sulfate precipitated and dialyzed against PBS, pH 7.4. Mixtures of MAbs consisted of 30 μl of each. The concentrations of proteins in the original solutions of MAbs in PBS were between 3 and 8 mg/ml for IA3-B8, between 1 and 3 mg/ml for IIC5-B10 and 3–8 mg/ml for other MAbs. In samples without MAbs 60 μl of SA solution was substituted. Gametocytes from cultured *P. falciparum* (line 7G8, a clone of Brazilian isolate IMTM 22) were spun at 1,000 *g* for 5 min at room temperature, resuspended to a 50% hematocrit in normal human serum (inactivated at 56°C for 30 min), and 30 μl added to each mixture of MAbs and uninfected erythrocytes in human serum prepared as described above. These suspensions were immediately presented to cages of *Anopheles freeborni* mosquitoes through water-jacketed membrane feeders at 40°C and equipped with nylon monofilament mesh (pore size 74 μm) (Sargent-Welch Co., Skokie, IL). Mosquitoes were allowed to feed for 10 min and the fed mosquitoes kept at 27°C and 60–70% relative humidity. 8–9 d after feeding the mosquitoes were dissected and their midguts examined for oocysts (products of parasite fertilization).

Surface Radioiodination, Immunoprecipitation, and Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) of Female Gametes of P. falciparum. Female gametes were labeled with ^{125}I by the lactoperoxidase method and immunoprecipitation with MAbs with SDS-PAGE carried out as previously described for zygotes of *P. gallinaceum* (8).

Results

The PBS-dialyzed ammonium sulfate-precipitated MAbs from ascitic fluid were tested for their effect on the infectivity of gametocytes of *P. falciparum* line 7G8 to *An. freeborni* mosquitoes. Following preliminary tests two MAbs (IA3-B8 and IIC5-B10, both isotype γ_{2a}) were identified that appeared to act synergistically

TABLE I

The Effect of Monoclonal Antibodies on the Infectivity to Anopheles freeborni Mosquitoes of Gametocytes of Plasmodium falciparum Grown in Culture Complement Inactivated

Experiment number	Monoclonal antibodies				SA solution
	IIC5-B10	IA3-B8	IIC5-B10 + IA3-B8	Other*	
1	2.8 (20)	5.3 (9)	0.3 (12)	24.8 (9)	13.3 (19)
2	0.1 (7)	1.0 (12)	0.0 (12)	1.5 (12)	0.7 (11)
3	17.3 (12)	5.4 (8)	0.1 (10)	46.6 (10)	24.0 (16)
4	3.0 (6)	8.2 (6)	0.0 (15)	1.1 (14)	6.0 (12)
Mean number of oocysts per gut	6.3 (45)	4.3 (35)	0.1 (49)	16.0 (45)	12.4 (58)

Infectivity is expressed as the mean number of oocysts (products of parasite fertilization) per mosquito. Four replicate experiments are represented; the number of mosquitoes examined is indicated in parenthesis after the value for infectivity.

* "Other" represents infectivity in the presence of MAbs found to be without suppressive effect on infectivity in preliminary tests.

The human serum in the experiments represented here was heat inactivated before use.

TABLE II

The Effect of Monoclonal Antibodies on the Infectivity to Anopheles freeborni Mosquitoes of Gametocytes of Plasmodium falciparum Grown in Culture Active Complement Present

Experiment number	Monoclonal antibodies				SA solution
	IIC5-B10	IA3-B8	IIC5-B10 + IA3-B8	Other	
1	0.1 (10)	0.2 (10)	0.0 (10)	7.2 (10)	9.0 (20)
2	0.1 (12)	0.1 (12)	0.0 (12)	2.5 (12)	4.0 (14)
3	25.3 (11)	2.0 (8)	0.1 (18)	48.9 (14)	32.5 (28)
4	2.1 (8)	0.9 (17)	0.3 (14)	8.1 (7)	6.4 (12)
Mean number of oocysts per gut	7.2 (41)	0.7 (47)	0.1 (54)	19.6 (43)	16.5 (74)

The four replicate experiments represented were each run simultaneously with the corresponding experimental replicate in Table I. In these experiments, however, the human serum used in the membrane feeding was freshly drawn and in its native state (ie. complement systems intact).

The results are expressed as in Table I.

to suppress infectivity of the parasites to mosquitoes. These two MAbs were subsequently tested in four replicate experiments conducted as two parallel series. In one series human serum, heat inactivated at 56°C for 30 min, was used (Table I); in the other fresh serum from human blood drawn and defibrinated on the day of the experiment was used as a source of active complement (C) (Table II). In the absence of active C (Table I) suppression of infectivity by individual MAbs was relatively weak. When IIC5-B10 and IA3-B8 were combined, however, infectivity was consistently suppressed by ~99%. The suppression achieved by the mixed MAbs was thus clearly synergistic. Similar synergistic suppression of infectivity by IA3-B8 and IIC5-B10 was achieved when active C was present (Table II). Under these conditions, however, IA3-B8 consistently

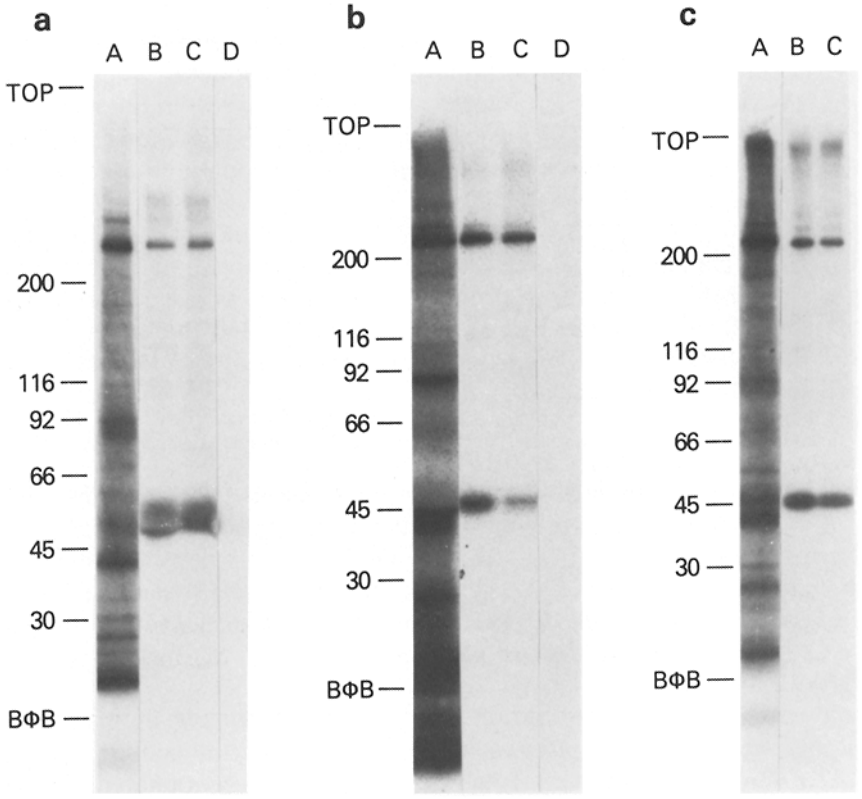

FIGURE 1. Antigens precipitated from extracts of surface radioiodinated female gametes of *Plasmodium falciparum* by transmission-blocking monoclonal antibodies (MAbs) and separated by SDS-PAGE. (a) *P. falciparum* isolate BC1; SDS-PAGE under reducing conditions. Antigen extract (lane *A*); precipitated with IA3-B8 (lane *B*); with IIC5-B10 (lane *C*) and with ammonium-sulfate precipitated normal mouse serum (lane *D*). (*b*) *P. falciparum* line 7G8; SDS-PAGE under nonreducing conditions. Antigen extract (lane *A*); precipitated with IA3-B8 (lane *B*); with IIC5-B10 (lane *C*); with ammonium-sulfate precipitated normal mouse serum (lane *D*). (*c*) *P. falciparum* isolate BC1; SDS-PAGE under nonreducing conditions. Antigen extract (lane *A*); precipitated with IA3-B8 (lane *B*); with IIC5-B10 (lane *C*). Molecular weights are indicated in kilodaltons.

suppressed infectivity by at least 90%, demonstrating a C-mediated effect by this MAb. PBS-dialyzed, ammonium sulfate-precipitated MAbs that failed to suppress infectivity in preliminary tests were included as simultaneous controls in each experiment.

Extracts of ^{125}I surface-labeled female gametes of *P. falciparum* were immunoprecipitated with IIC5-B10 or IA3-B8 and separated on SDS-PAGE. The same three proteins were precipitated from gametes of an African isolate, BC1, by both MAbs and had apparent molecular weights under reducing conditions of 255, 59, and 53 kilodaltons (Fig. 1 *a*, lanes *B* and *C*). None of the labeled proteins were immunoprecipitated by ammonium sulfate-precipitated normal mouse serum (lane *D*). Equivalent proteins were precipitated from the Brazilian isolate 7G8 by IA3-B8 and IIC5-Bio (Fig. 1 *b*, lanes *B* and *C*). Resolution of the

proteins of 59 and 53 kilodaltons in the immune precipitates was obscured by the presence of the heavy chain of mouse immunoglobulin in the same region of the gel. Under nonreducing conditions the immunoglobulins migrated higher up the gel and the gamete proteins were clearly resolved in the immune precipitates (Fig. 1 *b* and *c*, lanes *B* and *C*). Under these conditions the apparent molecular weights of the three precipitated proteins changed to 235, 49, and 46 kilodaltons respectively.

Both IIC5-B10 and IA3-B8 reacted by indirect immunofluorescence (IF) with air-dried male gametes of *P. falciparum*, demonstrating the presence of the target antigens on male as well as female gametes of the parasite. Neither MAb precipitated labeled material from surface-radioiodinated human erythrocytes; nor did they precipitate labeled material from gametes of *P. gallinaceum* or react with them by IF on live gametes.

Discussion

Our results have demonstrated that a combination of two MAbs, both of isotype γ_{2a}, against surface antigens of gametes of *P. falciparum* mediated almost total suppression of infectivity of a Brazilian isolate of this parasite to mosquitoes. In the absence of C either MAb alone had a generally slight effect. The suppressive effect of the mixture was thus clearly synergistic. In the presence of active C, however, one MAb, IA3-B8, was consistently 10 times more effective than in the absence of C.

Each MAb precipitated the same three proteins from female gametes of *P. falciparum* with apparent molecular weights under reducing conditions on SDS-PAGE of 255, 59, and 53 kilodaltons. The MAbs precipitated apparently equivalent proteins from gametes of Brazilian and African isolates. Although male gametes could not be obtained in quantities sufficient for immunochemical analysis the MAbs reacted with these stages by IF, demonstrating the presence of the target antigens on male as well as female gametes.

We previously reported two MAbs that synergized to suppress infectivity to mosquitoes of the chicken malaria *P. gallinaceum* (7) and have subsequently identified the target antigens of MAbs that suppressed infectivity of *P. gallinaceum* to mosquitoes (9). These MAbs reacted by agglutination and IF with male and female gametes of the parasite and each precipitated three proteins of apparent molecular weights on reducing SDS-PAGE of 240, 56, and 54 kilodaltons from both male and female gametes that were designated PgZ-1, PgZ-13a, and PgZ-13b respectively. In both *P. falciparum* and *P. gallinaceum* all three proteins precipitated by the transmission-blocking MAbs had higher apparent molecular weights under reducing compared with nonreducing conditions on SDS-PAGE.

It appears, therefore, that the three proteins in these molecular weight regions represent a set of antigenically related molecules present on both male and female gametes of malaria parasites, and which are targets of transmission-blocking MAbs. By analogy with the system used for *P. gallinaceum* (8) we designate the 255-, 59-, and 53-kilodalton proteins on gametes of *P. falciparum* PfZ-1, PfZ-13a, and PfZ-13b, respectively. The identification of these targets of transmission-blocking immunity in *P. falciparum* could provide the basis for developing a transmission-blocking vaccine against this disease.

Summary

Three proteins of apparent molecular weights on reducing SDS-PAGE of 255, 59, and 53 kilodaltons have been identified as the targets on gametes of *P. falciparum* malaria of two monoclonal antibodies (MAbs) that act synergistically to block transmission of the parasites to mosquitoes.

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