Pfs2400 Can Mediate Antibody-dependent Malaria Transmission Inhibition and May Be the *Plasmodium falciparum* 11.1 Gene Product

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Summary

Monoclonal antibodies (mAb) have been raised against *Plasmodium falciparum* gametocyte stage protein extracts, in an effort to identify novel parasite antigens that might mediate malaria transmission-blocking immunity. mAb 1A1 identified Pfs2400, a sexual stage-specific antigen of greater than 2 megadaltons, that is associated with the outer leaflet of the parasitophorous vacuole membrane in mature circulating gametocyte-infected red blood cells. Upon induction of gametogenesis, Pfs2400 partitions between the gamete plasmalemma and the degenerating erythrocyte membrane. The antigen is no longer detectable in the fully emerged gamete. mAb 1A1 dramatically reduces the number of oocysts formed in *P. falciparum* gametocyte-fed mosquitoes. The cognate antigen is probably the product of the Pf11.1 gene (Scherf et al. 1988. *EMBO [Eur. Mol. Biol. Organ.] J.* 7:1129) on the basis that a peptide composed of two copies of the degenerate nine amino acid repeat sequence in the Pf11.1 protein, can inhibit binding of mAb1A1 to the native antigen. The mechanism of transmission inhibition mediated by the Pfs2400 is discussed.

Malaria transmission-blocking immunity is the concept that immunity can be induced in humans against the parasite stages that develop in the mosquito. The human immune factors could then function after a blood meal, against vulnerable gametes or later sporogonic stages, to inhibit development of the parasite in the mosquito (1). Several laboratories are presently trying to develop a transmission-blocking vaccine for the human malaria parasite, *Plasmodium falciparum* (2–6). A successful transmission-blocking component for a multisubunit vaccine, that would also target the malaria life cycle stages that develop in humans, is predicted to reduce the transmission of antigenic variants that arise naturally or as a result of drug or immune pressure (2, 7). Reducing the geographic spread of drug or asexual vaccine-resistant variants in human populations would be a major contribution to malaria control. Although sterile immunity is unlikely, especially in areas of high transmission, a transmission-blocking vaccine would probably have a significant effect on the number of malaria infections in areas of low transmission, such as Sri Lanka (8).

Within RBC, the malaria parasite multiplies asexually or differentiates sexually into male and female gametocytes. Gametocytes can continue developing only in a mosquito, where they emerge from the RBC and develop into extracellular gametes. Fertilization gives rise to zygotes which transform into motile ookinetes. These cross the gut wall and transform into oocysts. Sporozoites multiply within these structures, and after their release from mature, ruptured oocysts, can be inoculated into another individual to initiate a new infection. Any of these steps in sexual development represent potential targets for transmission intervention.

It has been shown for several species of *Plasmodia*, that antibodies induced by immunization or natural infection which are directed against gamete or zygote surface antigens, can indeed inhibit development of the malaria parasite when ingested with the blood meal (6, 9–13). These antibodies are effective at three distinct points after gametogenesis: (*a*) fertilization of the macrogamete by the microgamete (14); (*b*) transformation of the ookinete into an oocyst (14); and (*c*) production of sporozoites within oocysts (15). The *P. falciparum* gamete surface antigens, Pfs230 and Pfs48/45, function as targets of antigamete antibodies, but display limited immunogenicity in humans (16, 17). The early sexual stage antigen, Pfg27/25, is recognized by antibodies that can mediate transmission inhibition (5). However the antigen is not surface exposed during sexual development, and the mechanism

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of this inhibition may be mediated by cross-reacting antigens. The most promising target is the zygote surface antigen, Pfs25 (3, 4). Antibodies against this antigen inhibit both the development of the ookinete to an oocyst in the mosquito gut (14) and production of sporozoites (15). The Pfs25 is conserved between parasites isolated worldwide (18), and is highly immunogenic in experimental animals (3). However, a desirable feature of a third world vaccine is that a single inoculating dose should be effective and long-lasting, and the Pfs25 does not function as an immunogen in natural infections (19). Therefore, reinfection will not provide a natural boost, and alternative methods of administration must be devised to generate long-term protection from limited immunizations in the field.

We sought to identify novel sexual blood stage antigens that might render the parasite vulnerable to human antibodies and other immune factors in the mosquito blood meal, as these antigens might be expected to provide a boost upon parasite infection. Our goal was to produce mAbs that would recognize linear epitopes of gametocyte stage–specific antigens, that might be easily incorporated into a multisubunit peptide vaccine. A possible transmission-blocking target was selected for functional studies, on the basis of its localization within the parasite.

This paper reports the generation of a mAb that recognizes a linear sequence of the *P. falciparum* 11.1 gene product (20). The biochemical and immunological characterization of Pf11.1 has been obscured by its extensive cross-reactivity with other negatively charged *P. falciparum* antigens, including Pf155/RESA, the megadalton asexual stage antigen Ag332, and a 260-kD asexual stage antigen (21–23). Although the gene has been extensively studied, no specific protein product has been demonstrated. We show that the Pf11.1 gene product is a sexual blood stage antigen with an apparent monomer molecular mass of >2 megadaltons. The antigen, which we have named Pfs2400, appears in stage I gametocytes, accumulates during gametocyte maturation, and persists during emergence of the gametes. mAb1A1 significantly blocks development of the sexual parasites into oocysts in the infected mosquito, and the localization of this antigen during sexual development suggests a site of action for the mAb in blocking sexual development.

Materials and Methods

Parasites. The *P. falciparum* gametocyte-producing NF54 isolate and its clonal derivative, 3D7 (24), were cultured in human RBC and human serum from type A^+ donors, under conditions that promote gametocyte production (25), and modified by the addition of 10 mM glucose to the culture medium. To obtain pure gametocytes, any surviving asexual trophozoites and schizonts were selectively killed between days 12 and 14 of culture, by suspension in 5% (wt/vol) D-sorbitol in distilled water for 5 min at room temperature (26). Gametocytes and asexual rings were put back into culture for 2 d, after which, gametocytes were purified from asexual rings by Percoll gradient centrifugation (27). The final gametocyte preparation contained <1% contamination with asexual blood stages. In vitro emergence of gametes was induced by incubation of gametocytes in heat-inactivated FCS (14), and macrogametes were harvested by Percoll gradient centrifugation as described (28). Asexual parasites were obtained by maintaining the cultures at low parasitemias (<2%).

Immunizations. Purified gametocytes were pelleted at 37°C, washed once in gametocyte retarding buffer (GRB;[1] 170 mM NaCl, 10 mM glucose, 10 mM Tris, pH 7.4), and denatured by boiling in 3% SDS and 3 mM β-ME. The extract was diluted 30-fold with GRB, before intraperitoneal injection into BALB/c mice. Each immunizing dose consisted of $\sim 10^8$ gametocytes. The primary immunization was administered in CFA. Subsequent doses, administered at 5-wk intervals, were emulsified in incomplete Freund's adjuvant, except for the final boost, given intravenously, which consisted of the denatured parasite extract in PBS alone.

Hybridoma Screening. Immunized mouse spleen cells were fused with P3U1 myeloma cells (29). Undiluted hybridoma supernatants were screened for the presence of antigametocyte Ab by indirect immunofluorescence (IFA), on acetone-fixed blood smears of 3D7 gametocytes and asexual parasites (30).

MAb1A1. Log phase hybridoma cells, producing mAb1A1, were injected into pristane-primed BALB/c mice. MAb1A1, isotyped as an IgG1, was affinity-purified from ascites using the Affi-Gel protein A MAPS II kit (Bio-Rad Laboratories, Richmond, CA), according to the manufacturer's specifications.

Other Reagents. MAb7H11.E8, an IgG1 mAb against the *Plasmodium brasilianum* circumsporozoite protein was provided by Dr. Alan Cochrane (New York University Medical Center, New York). The 2.4/2.8 megadalton protein molecular weight marker, titin (31), was a kind gift of Dr. Kuan Wang (The University of Texas, Austin, TX). 18 amino acid peptides corresponding to each of the three consensus repeat sequences from the published *P. falciparum* 11.1 protein sequence (20) were synthesized. P3 is the three amino acid repeat peptide $(EKD)_6$. P6 is the six amino acid repeat peptide $(EEEKLT)_3$. P9 is the degenerate nine amino acid repeat peptide $[PEE(L/V)VEEV(I/V)]_2$.

Immunolocalization of the Pfs2400 during Sexual Development. 3D7 parasites were cultured under conditions that promote sexual differentiation (25). Under these conditions, the asexual parasitemia peaks at about day 5, and stage II gametocytes begin to appear in the culture at about day 7. Initial immunolocalization in developing sexual parasites, was performed by IFA on acetone-fixed blood smears (30) of these cultures and also of induced gametes, using mAb1A1 at 10 μg/ml. The gametocyte stages were classified by visual inspection of phase-contrast images, according to the description of Carter and Miller (32). Immunofluorescence of live gametes was performed after induction of gametogenesis, without further purification.

For immunoelectron microscopy, blood stage parasites and gametes were fixed and embedded in LR White resin (Polyscience, Warrington, PA) (33). Fixed *P. falciparum* sporozoites (Gary Long strain) were embedded in LR Gold resin (Polyscience), containing 0.5% benzoin methyl ether as an initiator. Sections were incubated for 20 min in PBS containing 5% nonfat dry milk and 0.01% Tween 20. Grids were then incubated with mAb1A1 in PBT (PBS containing 1% BSA and 0.01% Tween 20) for 2 h at room temperature. Control sections were incubated with normal mouse serum or PBT only. After washing in PBT, the grids were incubated for 1 h in 15 nm gold-labeled goat anti–mouse IgG (Janssen Pharmaceutical, Piscataway, NJ) diluted 1:20 in PBT, rinsed with PBT, fixed for 15 min in 2.5% glutaraldehyde, and then stained with 2% uranyl

[1] *Abbreviations used in this paper:* GRB, gametocyte retarding buffer; IFA, indirect immunofluorescence; PBT, PBS containing 1% BSA and 0.01% Tween 20.

acetate in 50% methanol and Reynold's lead citrate. Samples were examined under electron microscopes (model 100CX; JOEL Ltd., Tokyo, Japan; and model CEM 902; Zeiss, Oberkochen, Germany).

Western Blot Analysis. Gametocytes, macrogametes, asexually infected or control red blood cells were washed once in PBS at 37°C, and extracted with 1% NP-40, in 150 mM NaCl, 50 mM Tris, pH 8.0 for 1–2 h on ice. Protease inhibitors were included in the extraction buffer as described (34). NP-40 soluble extracts were boiled for 5 min in sample buffer (0.8% SDS, 1 M urea, 10 mM Tris, pH 8.0, 100 mM DTT), separated by SDS-PAGE, on a 3–10% polyacrylamide gradient gel prepared without a stacker, and transferred electrophoretically to nitrocellulose. Nitrocellulose strips were blocked with 3% BSA, 0.3% Tween 20 in 50 mM Tris, pH 7.4, 150 mM NaCl (BSA/Tris-buffered saline containing 0.3% Tween 20 [TTBS]) for 1 h at room temperature, and incubated with 25 μg/ml mAb in BSA/TTBS for 1.5 h. After extensive washing in BSA/TTBS, blots were probed with ^{125}I-rabbit anti–mouse IgG (New England Nuclear, Boston, MA) diluted to 0.1 μCi/ml in BSA/TTBS for 30 min, washed, dried, and autoradiographed.

Immunoprecipitation. NP-40 solubilized antigens were immunoprecipitated with mAb1A1, and then adsorbed onto protein G agarose (Pierce Chemical Co., Rockford, IL) according to the manufacturer's protocol. The immunoprecipitated product was visualized by Western blot analysis.

Radioimmunoassay. Polyvinylchloride microtiter plates were incubated overnight with 25 μg/ml of mAb1A1 in PBS. mAb1A1-coated plates were washed with PBS and blocked with 3% BSA/PBS. Plates were then incubated with NP-40 solubilized proteins at about 25 μg/ml in 0.5% NP-40, BSA/PBS, for 2 h at room temperature. Captured antigen was detected by counting, after binding of ^{125}I-labeled mAb1A1. To assay for peptide inhibition of mAb1A1 to the native antigen, labeled mAb1A1 at about 1.5 × 10^5 cpm/well (1.3 × 10^7 cpm/μg), was predetermined to produce a strong signal but not saturate the antigen binding sites. ^{125}I-labeled mAb1A1 was preincubated with peptides for 2 h at room temperature, before binding to parasite antigen trapped on plastic.

Transmission Inhibition Assays. Gametocytes of the *P. falciparum* strain NF54 were cultured in the Tipper system (35). 14 d after initiation of the culture, the parasitized red cell suspension (1.4 ml containing about 0.3 ml packed cells) of one tipper vessel was harvested and washed by centrifugation at 560 *g* for 2 min in 3 ml RPMI 1640 onto a cushion of 0.5 ml RBC, prewarmed to 37°C. 150 μl of the parasitized cell preparation were added to a prewarmed cocktail consisting of 70 μl of heat-inactivated human serum plus 40 μl of fresh frozen type A+ human serum as a source of complement, and 10 μl of mAb in PBS. Mixtures were distributed to prewarmed (37°C) membrane feeders. *Anopheles gambiae* mosquitoes were allowed to feed for 10 min, and fully fed mosquitoes were maintained at 26°C for 6 d. The midguts of 20 mosquitoes fed under each condition were examined for oocysts at this time, and the degree of transmission was calculated as the geometric mean of the number of oocysts. Transmission inhibition was assessed as the percent reduction in this number compared with the average oocyst numbers in control mosquitoes, fed in the absence of mAb1A1.

Results

mAb. Nine independent mAb were selected from a single fusion of mouse spleen cells immunized with denatured and reduced gametocyte protein extracts, that by IFA, reacted exclusively with gametocyte-infected RBC. One mAb (1A1), also demonstrated a fluorescence pattern consistent with a membrane or cytoskeletal association for the cognate antigen on acetone-fixed macrogametes. This antigen was chosen for further characterization, on the basis that it might be surface exposed and accessible to blocking antibodies in the mosquito blood meal.

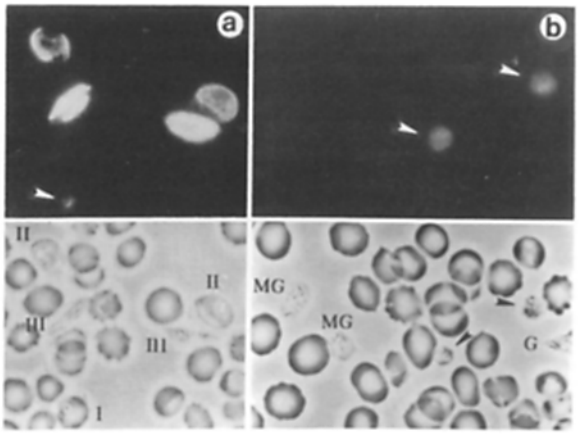

Figure 1. Immunolocalization of *P. falciparum* epitope recognized by mAb1A1, by indirect immunofluorescence. The second reagent was fluorescein-conjugated goat anti–mouse IgG. Fluorescence micrographs of (*a*) acetone-fixed gametocytes. Roman numerals refer to the distinct morphologic stages I–III of gametocyte development. (*White arrowhead*) Fluorescing stage I gametocyte. (*b*) Live, activated gametocytes. (*G*) Gametocyte. (*MG*) Macrogametes. (*Arrowheads*) Fluorescing activated gametocytes. Phase micrographs of the same fields are shown below.

Immunolocalization. IFA was used to establish the expression pattern of the epitope recognized by mAb1A1, during sexual development of *P. falciparum*. Immunofluorescence was positive for all sexual blood stage parasites (Fig. 1). mAb1A1 did not detect uninfected or asexually infected RBC. The faint fluorescence associated with the small intraerythrocytic parasite, that looks like a late ring/early trophozoite by phase-constrast (Fig. 1 *a*), is presumably a stage I gametocyte. The fluorescence intensity increased in the later gametocytes, from stage II onward, which are clearly distinguishable in the phase-contrast images. In these more mature gametocytes, the fluorescence pattern is consistent with an antigen localization surrounding the intraerythrocytic parasite. Thus, the antigen recognized by mAb1A1 is produced very early after commitment of the parasites to sexual development, and accumulates during gametocyte maturation.

The accessibility of the 1A1 epitope on the surface of live gametes was determined by IFA, as a preliminary evaluation of the possibility that this epitope might render the sexual parasite vulnerable to human antibodies in the mosquito blood meal. Gametocytes were induced to emerge from the infected RBC, and parasites were tested for reactivity with mAb1A1. As shown in Fig. 1 *b*, fluorescence was observed on the surface of some, but not all, of the live macrogametes. Intraerythrocytic gametocytes, visible in the same field by phase-contrast microscopy, were not detected by mAb1A1. These data indicate that mAb1A1 recognizes an epitope that is within the gametocyte-infected RBC, and is surface exposed during emergence of macrogametes. Its presence on only a subpopu-

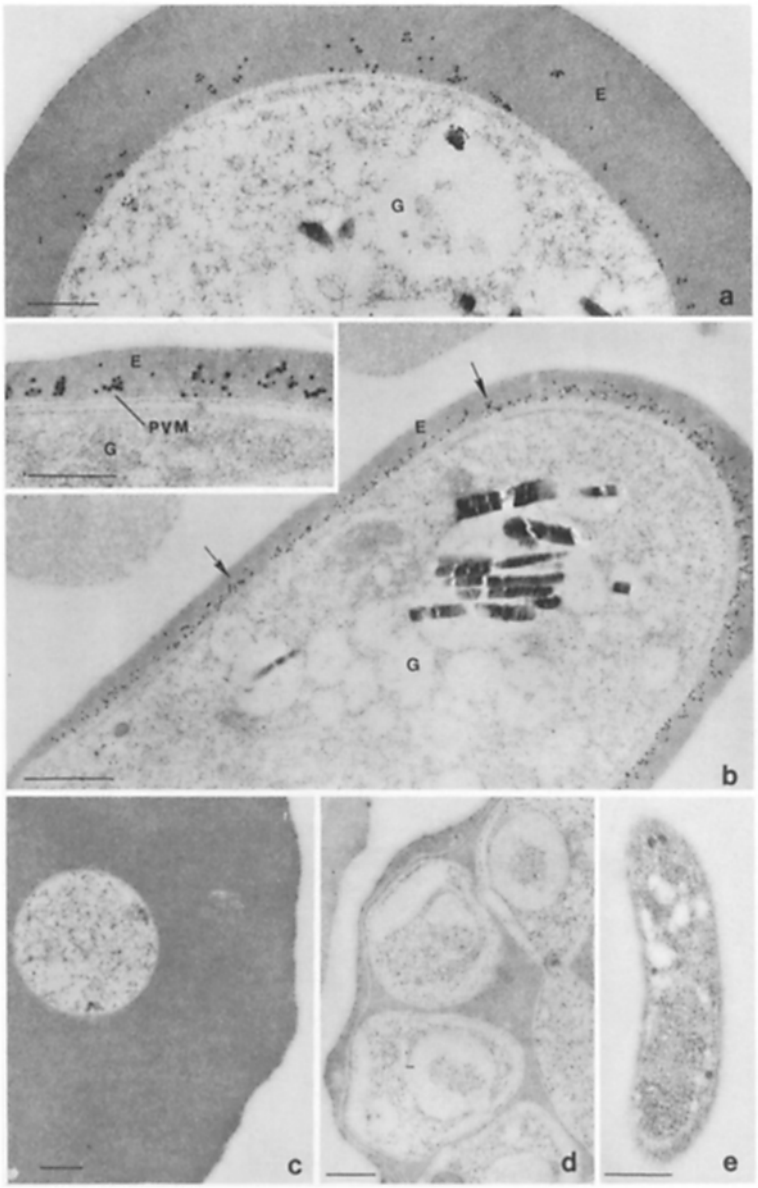

Figure 2. Electron micrographs of *P. falciparum* blood stages and sporozoite, incubated with mAb1A1, followed by gold-labeled goat anti-mouse IgG. (*a*) Erythrocyte infected with an immature gametocyte. (*b*) Mature gametocyte. (*c*) Asexual ring form. (*d*) Asexual schizont. (*e*) Sporozoite. (*E*) erythrocyte; (G) gametocyte; and (*PVM*) parasitophorous vacuole membrane. Gold particles (*arrows*) are seen in the erythrocyte cytoplasm adjacent to the PVM in the mature gametocyte (*b*). Bars: (*a*, *b inset*, *d*, and *e*) 0.5 μm; (*b* and *c*) 1.0 μm.

lation of macrogametes suggests that the antigen might be shed from the parasite surface, before fertilization.

Precise subcellular localization was determined by immunoelectron microscopy. As soon as sexual parasites could be distinguished morphologically from asexual trophozoites, gold label was observed. In immature gametocytes (Fig. 2 *a*), label was observed throughout the erythrocyte cytoplasm. Some label was found adjacent to the parasitophorous vacuole membrane. A few gold particles were also found in the parasite cytoplasm, apparently associated with cytoplasmic vacuoles.

Mature gametocytes were ultrastructurally characterized by a pellicular complex consisting of three sets of membranes (36). Immunoelectron microscopy showed that mAb1A1 binds to an abundant antigen in the erythrocyte cytoplasm that is associated exclusively with the outer leaflet of the parasitophorous vacuole membrane (Fig. 2 *b* and inset), consistent with the bright surface-like pattern observed by IFA. A few gold particles were seen in the gametocyte cytoplasm. No gold label was observed in asexual rings or schizonts (Fig. 2, *c* and *d*), and the epitope was also not detected in sporozoites (Fig. 2 *e*).

The mAb1A1 epitope was also localized in sexual parasites that had been induced to emerge from the infected erythrocyte. Fig. 3 *a* shows an activated gametocyte, with the gold label partitioning between the gamete plasmalemma and the RBC membrane. Some immunolabeling is scattered throughout the lysed erythrocyte cytoplasm, in association with remnants of erythrocyte cytoplasm and erythrocyte membrane. In this micrograph, the erythrocyte membrane has clearly lost its integrity, and appears to be pealing away from the emerging gamete. In extracellular gametes, the antigen is no longer detected (Fig. 3 *b*).

Pfs2400. The molecular weight of the cognate antigen was determined by immunoprecipitation and Western blot

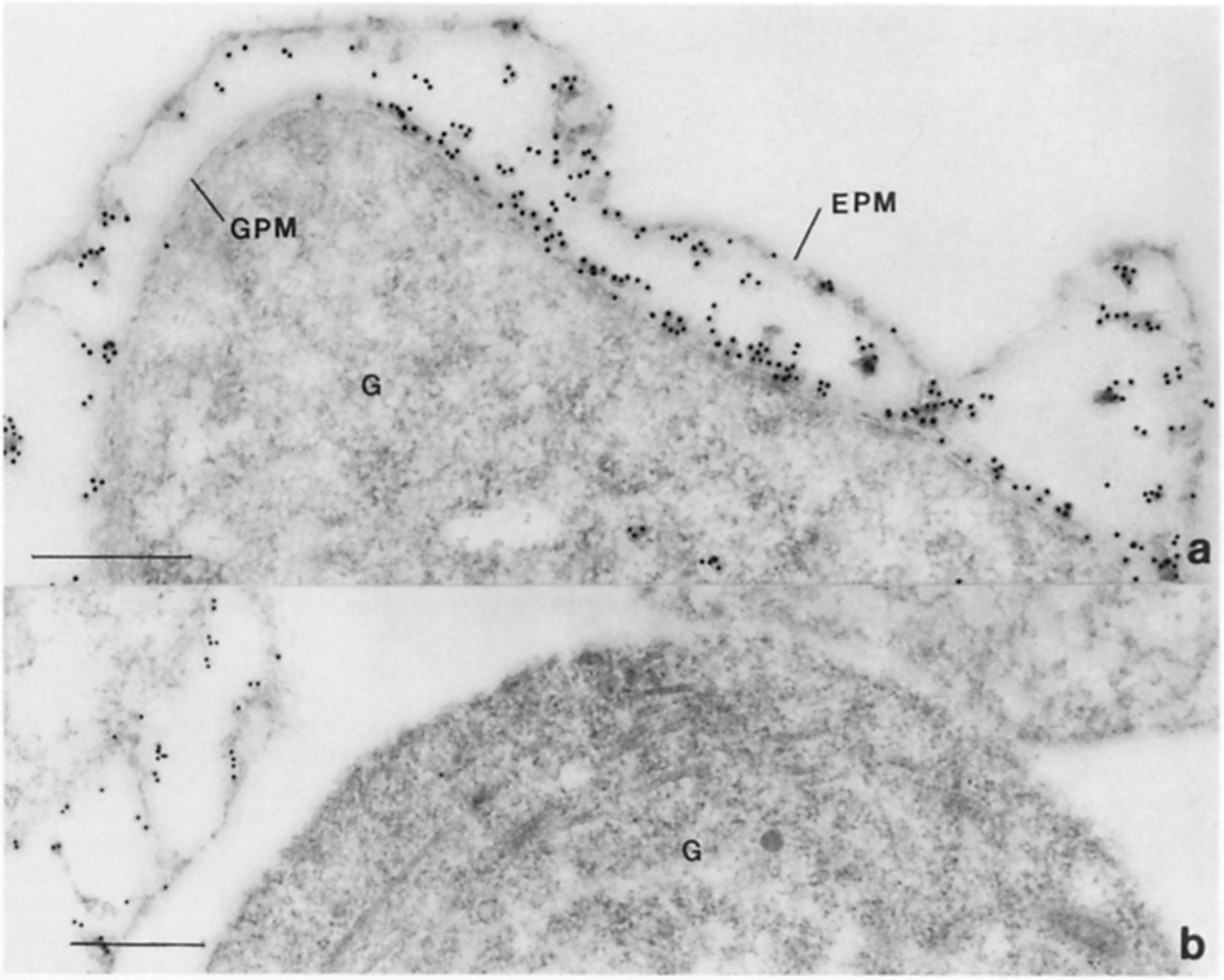

Figure 3. Electron microscopic localization of epitope recognized by mAb1A1 in an LR White section of a (*a*) *P. falciparum* gametocyte fixed after induction of gametogenesis. (*EPM*) Erythrocyte plasmalemma; (*GPM*) gamete plasmalemma. (*b*) Mature gamete. Bar, 1.76 μm.

analysis (Fig. 4). MAb1A1 detects a single parasite antigen that comigrates with the 2.4 megadalton subunit of the rabbit skeletal muscle protein, titin. The antigen is only present in protein extracts from gametocyte-infected RBCs and from emerging macrogametes. It can be detected by mAb1A1 under both reducing (Fig. 4) and nonreducing conditions (data not shown), indicating that the functional epitope is not dependent upon tertiary conformation or the presence of disulfide bonds. No reactivity was detected in protein extracted from RBCs infected with asexual stages or from uninfected RBCs. In keeping with the nomenclature for newly identified *P. falciparum* sexual stage antigens of unknown function, we refer to the antigen as Pfs2400 for sexual stage–specific antigen with an apparent M_r of 2400.

As many plasmodial antigens contain tandemly repeated amino acid sequences, which are frequently immunodominant, a RIA was performed to address the nature of the epitope recognized by mAb1A1 (Fig. 5). mAb1A1 was immobilized in plastic wells of microtiter plates, and used to capture native Pfs2400 from *P. falciparum* gametocyte protein extracts or control proteins. Probing with tracer amounts of ^{125}I-labeled mAb1A1 resulted in a strong signal when gametocyte proteins were the source of antigen, consistent with the possibility that the epitope might be present in multiple copies on the antigen. No Pfs2400 was detected in asexual stage proteins, BSA, or gametocyte culture supernatant.

To test directly for the release of Pfs2400 from extracellular gametes, gametocytes were induced to emerge, and both the supernatant and the macrogamete protein extract were assayed for the presence of the antigen by RIA. As seen in Fig. 5, Pfs2400 can be detected in both the supernatant and the cell extract, providing biochemical confirmation of our structural data that the antigen is shed from the parasite during emergence. No signal was detected in control FCS.

Pfs2400 Is the Product of the Pf11.1 Gene. To determine the identity of the antigen and the fine specificity of the epitope recognized by mAb1A1, we tested the possibility that the Pfs2400 is the product of the *P. falciparum* 11.1 gene, a recently characterized megadalton antigen distributed in small clusters surrounding parasitophorous vacuole membrane in mature gametocytes (37). mAb1A1 binding to native Pfs2400 was assayed in the presence and absence of synthetic peptides

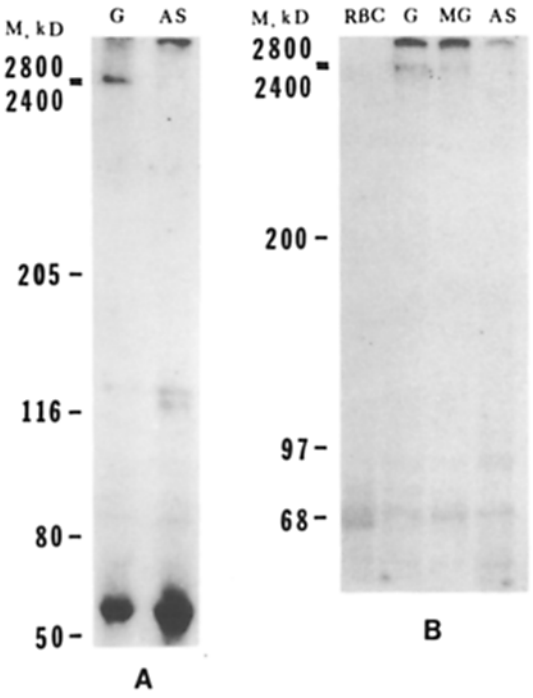

Figure 4. Identification of Pfs2400. (*A*) Immunoprecipitation of gametocyte proteins (*G*) and asexual trophozoite and schizont proteins (*AS*) with mAb1A1. Precipitated protein was visualized by Western blot. The 50-kD band is a mAb1A1 H chain from the immunoprecipitation reacting with the second reagent, ^{125}I goat anti-mouse IgG. (*B*) Western blot analysis of NP-40 protein extracts from uninfected red blood cells (*RBC*), gametocytes (*G*), emerging macrogametes (*MG*), and asexually infected red blood cells (*AS*). (*M*) Molecular weight standards. In control experiments, mAb 7H11.E8, against the circumsporozoite protein from *P. brasilianum*, showed no reactivity with any *P. falciparum* or RBC protein.

corresponding to each of the three blocks of tandem repeat sequences encoded by the Pf11.1 gene. As shown in Fig. 6, P9, consisting of two copies of the Pf11.1 degenerate nine amino acid repeat sequence, inhibited mAb1A1 binding to Pfs2400 in a dose-dependent manner. Complete inhibition was achieved at 50 μg/ml. The other two peptides, P3 and P6, corresponding to the three and six amino acid repeat sequences in the Pf11.1 antigen, respectively, had no inhibitory effect on mAb1A1 binding to Pfs2400 at concentrations as high as 200 μg/ml (Fig. 6, *inset*). Thus, Pfs2400 is most likely the product of the Pf11.1 gene. A peptide consisting of two linear repeats of the degenerate nine amino acid repeat sequence, PEE(L/V)VEEV(I/V), contains the minimum sequence necessary to inhibit mAb1A1 binding to the Pfs2400.

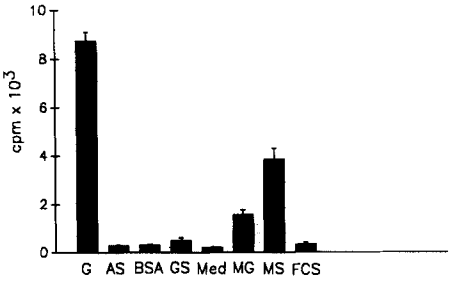

Figure 5. Pfs2400 occurs in gametocytes and emerging macrogametes, and soluble Pfs2400 is released from emerging gametes. Gametocyte proteins (G), asexual stage proteins (*AS*), control bovine serum albumin (BSA), gametocyte supernatant (*GS*), RPMI media including 10% human serum (*Med*), macrogamete proteins (*MG*), macrogamete supernatant (*MS*), or fetal calf serum (*FCS*) was added to mAb1A1-coated plastic. Protein sources containing Pfs2400 were detected by RIA, with ^{125}I-labeled mAb1A1. Error bars indicate the SD from the mean of triplicate assays.

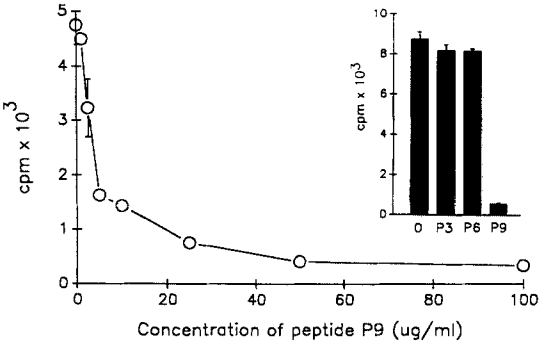

Figure 6. Competitive RIA reveals the fine specificity of mAb1A1. Increasing concentrations of synthetic peptide, P9, inhibit ^{125}I-labeled mAb1A1 binding to native pfs2400 in a dose-dependent fashion. Error bars represent the SD from the mean of triplicate assays. (*Inset*) Compares the effects of synthetic peptides P3, P6, and P9, on mAb1A1 binding to native antigen. (O) mAb1A1 binding to Pfs2400 in the absence of inhibitor. The sequences of the Pf11.1-derived repeat peptides are given in Materials and Methods.

Transmission Inhibition. As the surface of live macrogametes is accessible to binding by mAb1A1, at least during emergence from the RBC, we sought to determine the ability of mAb1A1 to block sexual development of the parasite in *P. falciparum* gametocyte-fed mosquitoes. Table 1 shows that, in three separate experiments, this reagent reduced the formation of oocysts within the mosquito midgut, compared with mosquitoes fed on control human serum. In two experiments, mAb1A1 reduced the mean number of oocysts formed by 70 and 80% at a final concentration of only 92.6 μg/ml. At lower concentrations (Expt. 3), mAb1A1 still reduced oocyst formation. Even at 1 μg/ml, oocyst numbers were reduced by 41%, compared with human control serum. In this experiment, an unrelated mouse mAb bearing the same IgG1 isotype as mAb1A1, had little effect on sexual development at 74 μg/ml.

Discussion

We have identified a linear epitope on the Pf11.1 gene product, that can mediate a substantial transmission blockade in an antibody-dependent fashion. The parasite load in these mosquito-feeding experiments is much higher than what is generally found in the field, where most mosquitoes are naturally infected with only one or a few oocysts (38). Mosquito feeding in the presence of a reduced number of gametocytes, will assess whether a 70–80% reduction in oocysts might represent real protection. MAb1A1, generated during this investigation, is specific for Pf11.1, a large sexual stage–specific antigen, containing at least three blocks of tandemly repeated amino acid sequences (20, 37). Using peptides corresponding

Table 1. *Transmission-inhibiting Activity of mAb1A1*

Sample	Final concentration	No. mosquitoes dissected	Percent infected	Mean no. oocysts/ mosquito	Mean percent reduction in oocysts
	IgG μg/ml				
Exp. 1					
NHS*		60	95	30.9	
MAb1A	92.6	20	90	9.3	70
Exp. 2					
NHS		60	98	45.2	
MAb1A1	92.6	20	85	9.2	80
Exp. 3					
NHS		60	100	102.5	
MAb1A1	44.4	20	100	42.7	58
	9.3	20	100	44.2	57
	1.0	20	100	60.0	41
MAb7H11·E8‡	74.1	20	100	96.1	6

* Normal human serum.
‡ Unrelated mAb.

to tandem copies of each of the known repeats, the fine specificity of mAb1A1 was shown to be directed against the nine amino acid repeat block.

The Pf11.1 locus is subject to DNA breakage and telomere healing, with concomitant loss of gene expression in laboratory isolates (37). Although the truncated Pf11.1 gene was observed in a laboratory clone that can initiate sexual development and generate gametocytes in culture, there was no evidence of the truncated gene in parasite DNA isolated directly from 15 patients (37), suggesting that Pf11.1 expression is necessary for some later step in sporogonic development, in vivo.

We suggest that mAb1A1 probably inhibits the formation of extracellular gametes, because the conserved but degenerate Pf11.1 nonapeptide repeats are briefly exposed on live sexual parasites during emergence of gametes from activated gametocytes. Perhaps mAb1A1 interferes with the complete shedding of the RBC and parasitophorous vacuole membranes.

Low concentrations (~10 μg/ml) of transmission-blocking mAb directed against *Plasmodium vivax* gamete surface antigens have been shown to increase the number of oocysts generated in mosquitoes fed on mature *P. vivax* gametocytes, perhaps by cross-linking male and female gametes to facilitate fertilization (11, 39). Transmission enhancement has not yet been observed in *P. falciparum*, and indeed, the presence of even lower concentrations (1 μg/ml) of mAb1A1 does not increase oocyst numbers in *P. falciparum*-infected mosquito. The data presented here indicate that mAb1A1 is probably functioning before gamete fertilization, unless the soluble Pf11.1 antigen has a role in sexual development in the mosquito midgut. Since low mAb1A1 concentrations in the blood meal still produce a dramatic reduction in the number of oocysts that develop in the infected mosquito, we will test the possibility that the antibody may be agglutinating activated gametocytes, and also assay the contribution of complement to the observed protection.

Western blot analysis of both reduced and unreduced blood-stage parasite extracts, with mAb1A1, demonstrated that the Pf11.1 gene product is about 2.4 megadaltons in both gametocytes and emerging gametes. The antigen does not appear to be processed while it is still associated with the activated sexual parasite. mAb1E10, the mAb reported to identify the Pf11.1 gene product, also recognizes a linear epitope contained within the nine amino acid repeat (37). However, mAb1E10 detects a smear from gametocyte protein extracts, that extends from the top to the 50-kD region of a gradient gel (37). It is possible that mAb1E10 cross-reacts with other negatively charged gametocyte stage antigens. This is supported by the finding that the detailed localization of the mAb1E10 epitope is slightly different from the even distribution of gold label around the parasitophorous vacuole membrane observed with mAb1A1. Alternatively, as the authors proposed, degraded antigen is responsible for the dramatic smear on Western blot analysis with mAb1E10 (37).

The Pf11.1 antigen has several features that make it an attractive candidate for a malaria transmission-blocking vaccine. The genomic sequence encoding the nine amino acid repeats is degenerate, but present in at least 20 isolates from eight geographically distinct locations (40, 20), and the encoded repeats can act as a target for mAb1A1-mediated inhibition of sporogonic development. In addition, antibodies against the nine amino acid repeats have been reported to be present in human immune sera (41, 42), indicating that Pf11.1 or one of the glutamate-rich, cross-reacting *P. falciparum*

antigens is immunogenic in a human infection. This suggests that it should be possible to identify a peptide containing a minimum epitope based on this sequence, which is immunogenic in humans. Further, since the antigen is expressed in blood stage parasites very early after sexual differentiation, reinfection might be expected to boost the antibody titers to transmission-blocking levels during the 12–14 d gametocyte maturation time, after a primary immunization with Pf11.1-derived epitopes. Whereas the Pf11.1 six amino acid repeat block is absent in some *P. falciparum* isolates, the three amino acid repeats are also conserved (20), and therefore represent a second potential transmission-blocking target. Experiments are in progress to test the capacity of peptides corresponding to these repeats to elicit polyclonal antibodies that, alone or in combination, can mediate a complete transmission blockade.

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References

1. Gwadz, R.W. 1976. Malaria: successful immunization against the sexual stages of *Plasmodium gallinaceum*. *Science (Wash. DC).* 193:1150.
2. Meuwissen, J.H.E.T. 1989. Current studies related to the development of transmission-blocking malaria vaccines: a review. *Trans. R. Soc. Trop. Med. Hyg.* 83(Suppl.):57.
3. Barr, P.J., K.M. Green, H.L. Gibson, I.C. Bathurst, I.A. Quakyi, and D.C. Kaslow. 1991. Recombinant Pfs25 protein of *Plasmodium falciparum* elicits malaria transmission-blocking immunity in experimental animals. *J. Exp. Med.* 174:1203.
4. Kaslow, D.C., S.N. Isaacs, I.A. Quakyi, R.W. Gwadz, B. Moss, and D.B. Keister. 1991. Induction of *Plasmodium falciparum* transmission-blocking antibodies by recombinant vaccinia virus. *Science (Wash. DC).* 252:1310.
5. Wizel, B., and N. Kumar. 1991. Identification of a continuous and cross-reacting epitope for *Plasmodium falciparum* transmission-blocking immunity. *Proc. Natl. Acad. Sci. USA.* 88: 9533.
6. Carter, R., N. Kumar, I. Quakyi, M. Good, K. Mendis, P. Graves, and L. Miller. 1988. Immunity to sexual stages of malaria parasites. *Prog. Allergy.* 41:193.
7. Miller, L.H., R.J. Howard, R. Carter, M.F. Good, V. Nussenzweig, and R.S. Nussenzweig. 1986. Research toward malaria vaccines. *Science (Wash. DC).* 234:1349.
8. Mendis, C., A.C. Gamage-Mendis, A.P.K. de Zoysa, T.A. Abhayawardena, R. Carter, P.R.J. Herath, and K.N. Mendis. 1990. Characteristics of malaria transmission in Kataragama, Sri Lanka: a focus for immuno-epidemiological studies. *Am. J. Trop. Med. Hyg.* 42:298.
9. Carter, R., L.H. Miller, J.Rener, D.C. Kaushal, N. Kumar, P.M. Graves, C.A. Grotendorst, R.W. Gwadz, C. French, and D. Wirth. 1984. Target antigens in malaria transmission blocking immunity. *Phil. Trans. R. Soc. Lond. Ser. B. Biol. Sci.* B307:201.
10. Mendis, K.N., Y.D. Munesinghe, Y.N.Y. de Silva, I. Keragalla, and R. Carter. 1987. Malaria transmission-blocking immunity induced by natural infections of *Plasmodium vivax* in humans. *Infect. Immun.* 55:369.
11. David, P.H., H.A. del Portillo, and K.N. Mendis. 1988. *Plasmodium vivax* malaria: parasite biology defines potential targets for vaccine development. *Biol. Cell.* 64:251.
12. Grotendorst, C.A., N. Kumar, R. Carter, and D.C. Kaushal. 1984. A surface protein expressed during the transformation of zygotes of *Plasmodium gallinaceum* is a target of transmission-blocking antibodies. *Infect. Immun.* 45:775.
13. Harte, P.G., N. Rogers, and G.A.T. Targett. 1985. Vaccination with purified microgamete antigens prevents transmission of rodent malaria. *Nature (Lond.).* 316:258.
14. Vermeulen, A.N., T. Ponnudurai, P.J.A. Beckers, J.-P. Verhave, M.A. Smits, and J.H.E. Th. Meuwissen. 1985. Sequential expression of antigens on sexual stages of *Plasmodium falciparum* accessible to transmission-blocking antibodies in the mosquito. *J. Exp. Med.* 162:1460.
15. Lensen, A.H.W., G.J.A. Van Gemert, M.G. Bolmer, J.F.G.M. Meis, D. Kaslow, J.H.E. Th. Meuwissen, and T. Ponnudurai. 1992. Transmission blocking antibody of the Plasmodium falciparum zygote/ookinete surface protein pfs25 also influences sporozoite development. *Parasite Immunol. (Oxf.).* 14:471.
16. Carter, R., P.M. Graves, I.A. Quakyi, and M.F. Good. 1989. Restricted or absent immune responses in human populations to *Plasmodium falciparum* gamete antigens that are targets of malaria transmission-blocking antibodies. *J. Exp. Med.* 169:135.
17. Graves, P.M., R. Carter, T.R. Burkot, I.A. Quakyi, and N. Kumar. 1988. Antibodies to *Plasmodium falciparum* gamete sur-

face antigens in Papua New Guinea sera. *Parasite Immunol. (Oxf.).* 10:209.
18. Kaslow, D.C., I. A. Quakyi, and D.B. Keister. 1989. Minimal variation in a vaccine candidate from the sexual stage of *Plasmodium falciparum. Mol. Biochem. Parasitol.* 32:101.
19. Quakyi, I.A., L.N. Otoo, D. Pombo, L.Y. Sugars, A. Menon, A.S. DeGroot, A. Johnson, D. Alling, L.H. Miller, and M.F. Good. 1989. Differential nonresponsiveness in humans of candidate *Plasmodium falciparum* vaccine antigens. *Am. J. Trop. Med. Hyg.* 41:125.
20. Scherf, A., C. Hilbich, K. Sieg, D. Mattei, O. Mercereau-Puijalon, and B. Muller-Hill. 1988. The 11.1 gene of *Plasmodium falciparum* codes for distinct fast evolving repeats. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:1129.
21. Petersen, C., R. Nelson, J. Leech, J. Jensen, W. Wollish, and A. Scherf. 1990. The gene product of the *Plasmodium falciparum* 11.1 locus is a protein larger than one megadalton. *Mol. Biochem. Parasitol.* 42:189.
22. Udomsangpetch, R., J. Carlsson, B. Wahlin, G. Holmquist, L.S. Ozaki, A. Scherf, D. Mattei, O. Mercereau-Puijalon, S. Uni, M. Aikawa et al. 1989. Reactivity of the human monoclonal antibody 33G2 with repeated sequences of three distinct *Plasmodium falciparum* antigens. *J. Immunol.* 142:3620.
23. Mattei, D., K. Berzins, M. Wahlgren, R. Udomsangpetch, P. Perlmann, H.W. Griesser, A. Scherf, B. Muller-Hill, S. Bonnefoy, M. Guillotte et al. 1989. Cross-reactive antigenic determinants present on different *Plasmodium falciparum* blood stage antigens. *Parasite Immunol. (Oxf.).* 11:15.
24. Walliker, D., I.A. Quakyi, T.E. Wellems, T.F. McCutchan, A. Szarfman, W.T. London, L.M. Corcoran, T.R. Burkot, and R. Carter. 1987. Genetic analysis of the human malaria parasite *Plasmodium falciparum. Science (Wash. DC).* 236:1661.
25. Ponnudurai, T., J.H.E. Th. Meuwissen, A.D.E.M. Leeuwenberg, J.P. Verhave, and A.H.W. Lensen. 1982. The production of mature gametocytes of *Plasmodium falciparum* in continuous cultures of different isolates infective to mosquitoes. *Trans. R. Soc. Trop. Med. Hyg.* 76:242.
26. Lambros, C., and J.P. Vanderberg. 1979. Synchronization of *Plasmodium falciparum* erythrocytic stages in culture. *J. Parasitol.* 65:418.
27. Carter, R., P.M. Graves, A. Creasey, K. Byrne, D. Read, P. Alano, and B. Fenton. 1989. *Plasmodium falciparum*: an abundant stage-specific protein expressed during early gametocyte development. *Exp. Parasitol.* 69:140.
28. Rener, J., P.M. Graves, R. Carter, J.L. Williams, and T.R. Burkot. 1983. Target antigens of transmission-blocking immunity on gametes of *Plasmodium falciparum. J. Exp. Med.* 158:976.
29. Potocnjak, P., N. Yoshida, R.S. Nussenzweig, and V. Nussenzweig. 1980. Monovalent fragments (Fab) of monoclonal antibodies to a sporozoite surface antigen (Pb44) protect mice against malarial infection. *J. Exp. Med.* 151:1504.
30. Pologe, L.G., A. Pvalovec, H. Shio, and J.V. Ravetch. 1987. Primary structure and subcellular localization of the knob-associated histidine-rich protein of *Plasmodium falciparum. Proc. Natl. Acad. Sci. USA.* 84:7139.
31. Kurzban, G.P., and K. Wang. 1988. Giant polypeptides of skeletal muscle titin: sedimentation equilibrium in guanidine hydrochloride. *Biochem. Biophys. Res. Commun.* 150:1155.
32. Carter, R., and L.H. Miller. 1979. Evidence for environmental modulation of gametogenesis in *Plasmodium falciparum* in continuous culture. *Bull. WHO* 57 (Suppl. 1):37.
33. Quakyi, I.A., Y. Matsumoto, R. Carter, R. Udomsangpetch, A. Sjolander, K. Berzins, P. Perlmann, M. Aikawa, and L.H. Miller. 1989. Movement of a falciparum malaria protein through the erythrocyte cytoplasm to the erythrocyte membrane is associated with lysis of the erythrocyte and release of gametes. *Infect. Immun.* 57:833.
34. Kumar, N., and R. Carter. 1984. Biosynthesis of the target antigens of antibodies blocking transmission of *Plasmodium falciparum. Mol. Biochem. Parasitol.* 13:333.
35. Ponnudurai, T., A.H.W. Lensen, A.D.E.M. Leeuwenberg, and J.H.E. Th. Meuwissen. 1982. Cultivation of fertile *Plasmodium falciparum* gametocytes in semiautomated systems. 1. Static cultures. *Trans. R. Soc. Trop. Med. Hyg.* 76:812.
36. Aikawa, M. 1988. Fine structure of malaria parasites. *In* Malaria. Vol. 1. W.H. Wernsdorf and J. McGregor, editors. Churchill-Livingstone, Edinburgh. 97–129.
37. Scherf, A., R. Carter, C. Petersen, P. Alano, R. Nelson, M. Aikawa, D. Mattei, L. Pereira da Silva, and J. Leech. 1992. Gene inactivation of Pf11-1 of *Plasmodium falciparum* by chromosome breakage and healing: identification of a gametocyte-specific protein with a potential role in gametogenesis. *EMBO (Eur. Mol. Biol. Organ.) J.* 11:2293.
38. Pringle, G. 1966. A quantitative study of naturally-acquired malaria infections in *Anopheles gambiae* and *Anopheles funestus* in a highly malarious area in East Africa. *Trans. R. Soc. Trop. Med. Hyg.* 60:626.
39. Peiris, J.S.M., S. Premawansa, M.B.R. Ranawaka, P.V. Udagama, Y.D. Munasinghe, M.V. Nanayakkara, C.P. Gamage, R. Carter, P.H. David, and K.N. Mendis. 1988. Monoclonal and polyclonal antibodies both block and enhance transmission of human *Plasmodium vivax* malaria. *Am. J. Trop. Med. Hyg.* 39:26.
40. Kahane, B., L. Sibilli, A. Scherf, G. Jaureguiberry, G. Langsley, L.S. Ozaki, M. Guillotte, B. Muller-Hill, L. Pereira da Silva, and O. Mercereau-Puijalon. 1987. The polymorphic 11.1 locus of *Plasmodium falciparum. Mol. Biochem. Parasitol.* 26:77.
41. Knobloch, J., M. Schreiber, S. Grokhovsky, and A. Scherf. 1987. Specific and nonspecific immunodiagnostic properties of recombinant and synthetic *Plasmodium falciparum* antigens. *Eur. J. Clin. Microbiol.* 6:547.
42. Koenen, M., A. Scherf, O. Mercereau, G. Langsley, L. Sibilli, P. Dubois, L. Pereira da Silva, and B. Muller-Hill. 1984. Human antisera detect a *Plasmodium falciparum* genomic clone encoding a nonapeptide repeat. *Nature (Lond.).* 311:382.