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Immunology

Identification of a continuous and cross-reacting epitope for *Plasmodium falciparum* transmission-blocking immunity

(malaria/vaccine/monoclonal antibodies)

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ABSTRACT **Identification of continuous epitopes in the target antigens of *Plasmodium falciparum* transmission-blocking antibodies is likely to facilitate the production of a subunit peptide vaccine. Two such epitopes shared among several sexual-stage antigens were identified with murine monoclonal antibodies. An epitope recognized by four monoclonal antibodies capable of blocking infectivity of gametocytes in the mosquitoes is shared among three antigens (230, 48/45 doublet, and 27 kDa). These antigens are synthesized at different times during the development and maturation of gametocytes, and the blocking epitope appears conserved among parasites from diverse geographical locations. Immune response against such a unique epitope (continuous, cross-reacting, and conserved) is likely to be boosted by natural infection. The 27-kDa protein is reported here as a target of malaria transmission-blocking monoclonal antibodies, and the cross-reacting epitope represents an attractive candidate for a transmission-blocking vaccine.**

Development of malaria vaccines remains a highly desirable goal, as *Plasmodium* continues to become resistant to new-generation anti-malaria drugs. Various targets for current vaccine efforts include infective sporozoites, erythrocytic asexual stages, and sexual stages, which are responsible for malaria transmission. Some stumbling blocks in this unattained goal are antigenic variation and polymorphism of both T- and B-cell epitopes and the nature of the B-cell epitopes—predominantly conformational in most antigens identified thus far as the targets for immunity directed against the parasite stages found in the mosquito vector (1, 2). This form of immunity, known as transmission-blocking immunity (TBI), is largely antibody mediated and operates in the mosquito midgut to block either fertilization of female gametes by male gametes or subsequent zygote development (3). Target antigens of TBI have been defined by using monoclonal antibodies (mAbs). These antigens include a set of three gamete surface proteins of 230, 48, and 45 kDa identified by fertilization-blocking antibodies (4–6) and a single protein of 25 kDa present on the surface of zygotes/ookinetes, recognized by postfertilization blocking antibodies (7–9). The gamete surface antigens are produced by the blood-stage gametocytes and the 25-kDa protein predominantly after initiation of gametogenesis and fertilization in the mosquito midgut (10–12).

In almost every case, the epitopes recognized by transmission-blocking mAbs have been reduction sensitive and, hence, conformational in nature (3). The conformational nature of the epitopes recognized by transmission-blocking antibodies may largely account for the fact that such antibodies have not proven useful in cloning the genes from prokaryotic expression libraries. Nevertheless, even when genes are cloned by other approaches—i.e., oligonucleotides based on protein microsequence data, reproducing such conformational epitopes in the recombinant expression systems might pose the ultimate obstacle. Identification of reduction-insensitive continuous epitopes of blocking antibodies, on the other hand, would greatly facilitate development of a peptide-based subunit vaccine. The peptides, being chemically defined and generally stable, can be designed to contain the linear B-cell epitope and appropriate helper T-cell epitopes (which are also comprised of a linear stretch of 7–15 amino acids), to elicit an effective immune response (13). Results in this study describe *Plasmodium falciparum* transmission-blocking mAbs that recognize a continuous epitope shared among three gamete surface antigens (230, 48, and 45 kDa) and a 27-kDa protein produced during early gametocyte development (14).

MATERIALS AND METHODS

Parasites. *P. falciparum* gametocytes were obtained *in vitro*, as described (15). Parasites [isolate NF54 (Amsterdam Airport strain, probably from Liberia), LE5 (Liberian clone), 3D7 (a clone derived from NF54), 7G8 and ItD12 (clones of Brazilian isolates IMTM22 and Ituxi, respectively), and T4 (a clone of Thai isolate)] were obtained from the Malaria section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD, and CVD-1 (another clone of NF54) was obtained from the University of Maryland, Baltimore. Female gametes and zygotes were purified by Nycodenz discontinuous-gradient centrifugation after induction of gametogenesis and fertilization (5, 6).

Production of mAbs. BALB/cJ and CAF1/J (F_1 hybrid of BALB/cJ and A/J) mice were immunized i.p. with 3D7 gametes in complete Freund's adjuvant and boosted three times at 30-day intervals in incomplete Freund's adjuvant. The number of gametes varied between 5×10^6 and 1×10^7 for the primary immunization and 5×10^6 and 7×10^6 for the booster immunizations in all 10 fusions. Mice tail bleeds were tested by indirect immunofluorescence assay and immunoprecipitation for antibodies. Four days before fusion mice were given a final booster immunization (i.v.) in phosphate-buffered saline. Spleen cells were fused with myeloma cells P3-X63-Ag8.653 (American Type Culture Collection CRL 1580) and plated in two to six 24-well plates using hypoxanthine/aminopterin/thymidine-selective Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) NCTC-109 (GIBCO), 10% (vol/vol) EL-4 thymoma (American Type Culture Collection TIB 39) spent medium, 1% nonessential amino acids, and 10% fetal calf serum. Culture supernatants, 10–14 days after fusion, were screened for antibodies by

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Abbreviations: TBI, transmission-blocking immunity; mAb, monoclonal antibody.

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immunofluorescence assay using methanol-fixed gametes and gametocytes and by immunoprecipitation. Selected hybridomas were enriched for antigen-specific cells by stepwise cloning procedure (1×10^4 to 10 cells per well). Enriched cells were cloned by limiting dilution at 0.25 cell per well at least twice, expanded in culture, and injected i.p. into Pristane-primed CAF1 mice for ascitic fluid production. For isotype analysis, Immulon-2 plates were coated with purified class-specific heavy-chain reagents (rabbit anti-mouse IgM, IgG1, IgG2a, IgG2b, IgG3, and IgA) at 100 ng per well. After being blocked with phosphate-buffered saline/0.05% Tween 20/1% bovine serum albumin, culture supernatants were added and developed with peroxidase-conjugated goat anti-mouse immunoglobulin (A, G, M) using *o*-phenylenediamine as the substrate. Absorbance was read at 450 nm [Molecular Devices (Palo Alto, CA) V_{max} ELISA plate reader].

Radiolabeling and Processing of Parasite Antigens for Immunoprecipitation and SDS/PAGE. Live gametes were radioiodinated by the lactoperoxidase-catalyzed iodination procedure (16). Labeled parasites were extracted in Triton X-100 for immunoprecipitation (10) and SDS/PAGE analysis (17). To reduce proteins before immunoprecipitation, Triton X-100-solubilized parasite proteins were denatured in the presence of guanidine hydrochloride (6 M final concentration). Reduction occurred for 1 hr with 25 mM dithiothreitol followed by alkylation (50 mM iodoacetamide) to prevent reoxidation of −SH groups for 1 hr at room temperature in the dark. Denaturation of proteins was necessary for antigen reduction (unpublished work). Treated extracts were dialyzed for ≥24 hr at 4°C against two to three changes of buffer (10 mM Tris, pH 7.5/150 mM NaCl/1 mM EDTA/0.1% Triton X-100/2 mM phenylmethyl-sulfonyl fluoride/0.02% NaN_3). Gametocyte proteins were also labeled during biosynthesis, as described (10). Parasites were washed three times in leucine-free RPMI 1640 medium for labeling with [^{3}H]leucine and with methionine-free RPMI 1640 medium for labeling with Tran^{35}S-label. Labeled proteins were solubilized with Triton X-100 for immunoprecipitation as above.

Purification of Antigens. Radioiodinated gametes were extracted in Triton X-114 and subjected to five cycles of phase separation, as described (18). The 230-kDa protein is recovered in the aqueous phase, and the 48/45-kDa glycoproteins are recovered in the detergent phase after phase separation. Each phase was treated to reduce proteins, as described. The aqueous phase after treatment was used as a source of reduced 230-kDa protein, and the detergent phase was used for purifying reduced 48/45-kDa antigens by electroelution after SDS/PAGE. To purify the 27-kDa protein, Triton X-100 extract after protein reduction was subjected to SDS/PAGE, and the protein was purified by electroelution (Bio-Rad electro eluter).

Measurement of Infectivity of Gametocytes in Mosquitoes. Freshly drawn and washed human red blood cells, normal human serum, and mAbs were mixed in 1.5-ml Eppendorf tubes and warmed at 37°C. Cultures of mature gametocytes were centrifuged in prewarmed tubes at 1200 rpm for 2 min, resuspended to 50% hematocrit in heat-inactivated normal human serum (56°C, 30 min), and added to tubes containing mAb mixtures to yield a final hematocrit of 55% and 1:6–1:10 culture dilution in 1.0-ml total volume. Each suspension was fed to a cage containing ≈75 female *A. gambiae* G3 mosquitoes (starved for 8–10 hr) through a Baudruche membrane with the membrane feeder maintained at 39°C. Mosquitoes were allowed to engorge for 15 min. All unfed mosquitoes were removed, and the remainder was maintained at 26°C and 70% relative humidity. Eight to ten days after feeding, mosquitoes were dissected, and their midguts were examined for oocysts (product of sexual reproduction of parasites in midgut).

Epitope Mapping by One-Site and Two-Site ELISA. mAbs were purified from ascites by fast protein liquid chromatography by using a protein A-Superose 10/2 column (Pharmacia) and biotinylated at a ratio of 250 μg of sulfo-NHS-biotin (Pierce) per mg of mAb (19). Immulon-2 plates were coated overnight at 4°C with 1×10^5 gametes extract per well (sonicated in 50 mM sodium carbonate/sodium bicarbonate, pH 9.6, and centrifuged at $100,000 \times g$). After being blocked in phosphate-buffered saline/0.05% Tween 20/1% bovine serum albumin for 1–2 hr at 37°C the wells were incubated with antibodies for 2 hr at 37°C and developed by using peroxidase-conjugated ExtrAvidin (Sigma) and *o*-phenylenediamine as substrate. Absorbance was recorded at 450 nm. For two-site ELISA, wells were coated with mAb 6B6 at 2.5 and 5 μg/ml, blocked, and incubated with Triton X-100 extract of gametes (1×10^5 per well). After incubation with biotinylated 6B6 the wells were developed with peroxidase-conjugated ExtrAvidin and *o*-phenylenediamine.

RESULTS AND DISCUSSION

mAbs. Initially to search for reduction-insensitive epitopes in *P. falciparum* TBI target antigens, we attempted to bias the immune response in mice by immunization with gametes treated with 50 mM dithiothreitol for 5 min at 95°C to denature and reduce proteins. Only one of 371 hybrids (five fusions) obtained produced antibodies recognizing reduced forms of gamete surface antigens (the 230-, 48-, and 45-kDa proteins upon reduction migrate in SDS/PAGE with molecular sizes of 260, 59, and 53 kDa). A simple explanation for this low frequency of positive hybridomas could be poor immunogenicity of antigens rendered by the treatment. Another group of mice immunized at the same time with intact untreated purified gametes, however, yielded nine of 337 hybrids (five fusions) positive for the reduced forms of target antigens.

Recognition of Continuous and Shared Epitopes. Five clones designated 6B6, 16C3, and 19F1 (all IgG2b isotype), 5G3 (IgG3), and 11G12 (IgG1) produced antibodies that recognized reduced antigens and were selected for further study. All the mAbs recognized only the reduced form of 230- and 48/45-kDa gamete surface antigens in immunoprecipitation analysis (Fig. 1, lanes 2) and failed to recognize any of these antigens in nonreduced form (lanes 1). In addition, mAbs 6B6, 11G12, 16C3, and 19F1 also immunoprecipitated, from both nonreduced and reduced antigen preparations, a protein migrating in the 25- to 30-kDa (27-kDa) region of the gel. Parasite antigens in the extracts reduced after denaturation in the presence of 8 M urea or triethylamine, pH 11.5, showed reactivity patterns identical to those with 6 M guanidine hydrochloride (data not shown). To rule out the possibility that coimmunoprecipitation of different antigens is not from aggregation of antigens by the condition of denaturation and reduction, each protein was purified and then retested in immunoprecipitation analysis. Fig. 2 clearly demonstrates that purified antigens were independently immunoprecipitated by the mAbs and, hence, these mAbs recognize epitopes shared between various sexual-stage antigens. This cross-reacting epitope is unlikely to be carbohydrate in nature because the 230-kDa and the 27-kDa proteins are not glycoproteins.

In these studies, we have identified two shared continuous epitopes. An epitope (I) recognized by mAb 5G3 is shared between the 230- and the 48/45-kDa gamete surface antigens. The other epitope (II) recognized by mAbs 6B6, 11G12, 16C3, and 19F1 is shared between the gamete surface antigens (230, 48, and 45 kDa) as well as a 27-kDa antigen. Further experiments were undertaken to characterize the 27-kDa protein, as two totally unrelated proteins in this kDa range are present in the sexual stages of *P. falciparum*. One

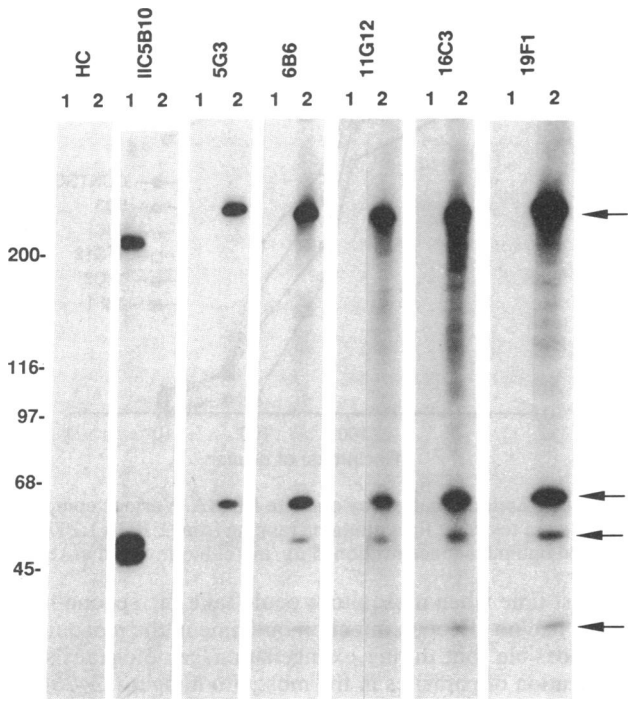

FIG. 1. Immunoprecipitation of *P. falciparum* gamete antigens by mAbs. Purified live gametes were radioiodinated with ^{125}I, and Triton X-100 extracts were treated to denature/reduce and alkylate proteins, as described. Untreated (lanes 1) and treated (lanes 2) extracts were immunoprecipitated with various mAbs (indicated on top of each pair of lanes) and analyzed by 5–15% SDS/PAGE under nonreducing conditions. HC is an unrelated mAb used as a negative control, and IIC5B10 is a mAb that recognizes a reduction-sensitive epitope in the 48/45-kDa antigens (4) and immunoprecipitates the 230-kDa and 48/45-kDa antigens because they exist as a stable membrane-bound complex (20). Positions of reduced antigens immunoprecipitated by mAbs are identified by arrows. Bio-Rad high-molecular-weight markers were used to calibrate gels (in kDa).

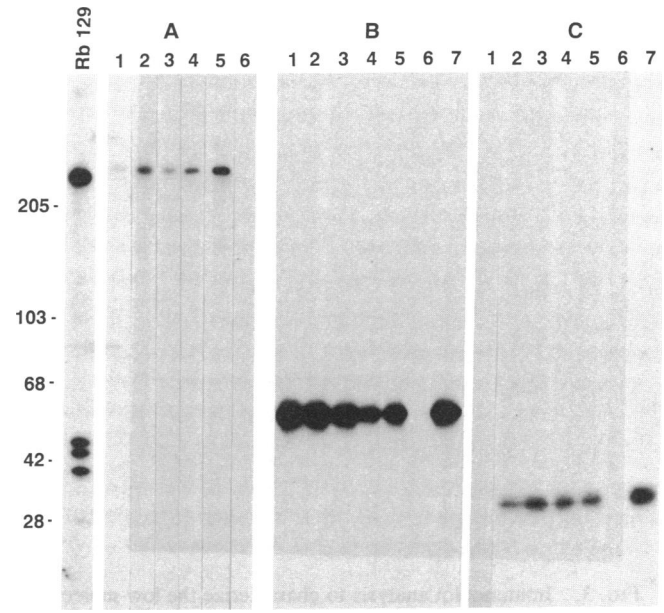

FIG. 2. Demonstration of cross-reacting continuous epitopes in antigens by immunoprecipitation. Separate antigen preparations containing reduced −230 kDa (*A*), −48/45 (*B*), and −27 kDa (*C*) proteins prepared as described, were analyzed by 5–15% SDS/PAGE under nonreducing conditions. Immunoprecipitation were done with mAbs 5G3 (lanes 1), 6B6 (lanes 2), 11G12 (lanes 3), 16C3 (lanes 4), 19F1 (lanes 5), a negative control mAb (lanes 6), and rabbit 129 (anti-*P. falciparum* gamete serum) (lanes 7 in *B* and *C*). Lane marked Rb 129 shows immunoprecipitation of nonreduced antigens from Triton X-100 extracts of radioiodinated gametes. Molecular-weight standards used were prestained high-molecular-weight markers from Bethesda Research Laboratories.

of them is the 25-kDa protein, another target of TBI, produced largely after initiation of gametogenesis and fertilization in the mosquito midgut (7–9, 11, 12), and the other one is an abundant (≥5%) internal 27-kDa protein produced soon after sexual commitment of parasites and continuing through gametocyte development and maturation in the vertebrate host (14, 21).

Proteins in Triton X-100 extracts of gametocytes were immunoprecipitated by using mAbs 1H12 [mAbs directed against 27-kDa protein (1H12 and 4B2) and against 25-kDa protein (1C7) were provided by R. Carter (University of Edinburgh, Scotland) and I. A. Quakyi (National Institutes of Health, Bethesda, MD), respectively] and 6B6. Immunoprecipitated proteins were fractionated by 12.5% SDS/PAGE under nonreducing conditions and transferred onto nitrocellulose membranes. Strips of nitrocellulose membranes were then reprobed for immunoreactivity with various mAbs. mAb 1C7 failed to react with the 27-kDa protein recognized by the epitope II mAb 6B6 (Fig. 3*B*, lane 2). Also, as shown in Fig. 5, the 27-kDa protein identified by mAb 6B6 is synthesized in the gametocytes in contrast to the 25-kDa protein, which is predominantly expressed by the parasites in mosquito midgut (11, 12). mAbs 1H12 and 4B2 directed against the gametocyte 27-kDa protein (14, 21) were likewise tested. Protein immunoprecipitated by 1H12 was recognized by biotinylated 6B6 (Fig. 3*A*, lane 4), and this binding was completely abolished by unlabeled excess mAb 6B6 (Fig. 3*A*, lane 3), providing further evidence for specificity of recognition of 27-kDa protein by mAb 6B6. Likewise, protein immunoprecipitated by mAb 6B6 was recognized by mAbs 4B2 and 1H12 (Fig. 3*B*, lanes 3 and 4, respectively). Proteins immunoprecipitated by mAbs 1H12 and 6B6 were also purified by electroelution from SDS/PAGE, labeled by radioiodination by the Iodo-Gen method (22), and retested in immunoprecipitation with various mAbs. The 27-kDa protein purified by 1H12 reacted with mAb 6B6 in immunoprecipitation and vice versa (data not shown). These studies clearly established that the 27-kDa protein recognized by the mAbs described here is immunologically similar to the 27-kDa internal protein in the gametocytes and gametes and not the zygote/ookinete 25-kDa antigen. Iodination of the 27-kDa protein, as shown in Figs. 1 and 2, is probably from labeling of a small fraction of this protein during surface radioiodination of gametes.

Blocking of Infectivity of Gametocytes to Mosquitoes. In membrane-feeding experiments, mAbs recognizing linear epitope II significantly blocked the infectivity of gametocytes in *A. gambiae* mosquitoes. The mAbs reduced the infection rate (percentage of mosquitoes infected) as well as numbers of oocysts per mosquito when compared with control mAb in parallel tests. Reduction in oocyst number with epitope II mAbs was independent of complement (Table 1). mAb 5G3, on the other hand, which recognizes epitope I did not affect the infectivity of *P. falciparum* gametocytes in the mosquitoes. Differences in level of blocking among the four mAbs recognizing epitope II could be from differences in the amount of immunoglobulins in the ascitic fluid or from differences in the affinities of mAbs of different isotypes. Protein A-purified mAb 6B6, when tested at various concentrations, produced nearly 100% blocking at 250 μg/ml, ≈85% blocking at 100 μg/ml, and ≈20% blocking at 10 μg/ml, results similar to those reported for mAbs directed against conformational epitope(s) in the 230-kDa protein (6). The epitope II mAbs (strongly positive in immunofluorescence

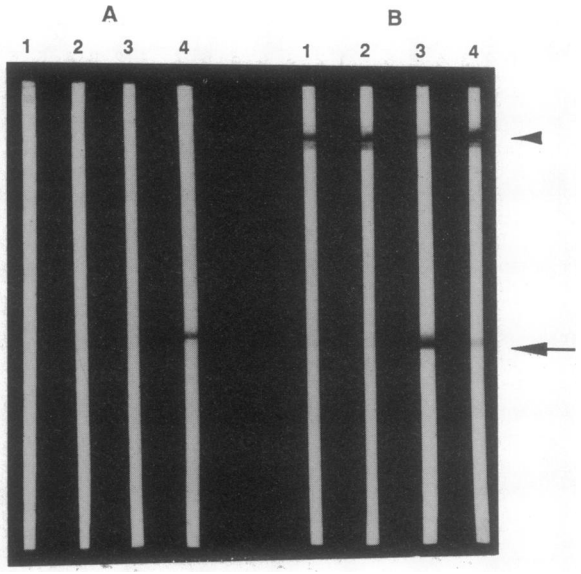

FIG. 3. Immunoblot analysis to characterize the low-molecular-size (≈27-kDa) protein. Triton X-100 extracts of purified *P. falciparum* gametocytes (3.5×10^7) were immunoprecipitated with mAbs 1H12 or 6B6, proteins separated by 12.5% SDS/PAGE under nonreducing conditions, and transferred onto nitrocellulose membranes. (*A*) Immunoblot of 27-kDa antigen immunopurified with mAb 1H12. Strips of membrane were probed with nonbiotinylated negative control mAb (lane 1), biotinylated mAb 14H10 (recognizes a conformational epitope in the 230-kDa antigen; unpublished work), and biotinylated mAb 6B6 in the presence (lane 3) or absence (lane 4) of 100-fold excess of nonbiotinylated 6B6. All strips were treated with 1:3000 dilution of ExtrAvidin–peroxidase and developed with diaminobenzidine as substrate. (*B*) Immunoblot of the 27-kDa antigen immunopurified with mAb 6B6. Strips were probed with no antibody (lane 1), mAb 1C7 (lane 2), mAb 4B2 (lane 3), and mAb 1H12 (lane 4). All strips were treated with 1:1000 dilution of peroxidase-conjugated goat anti-mouse immunoglobulins (A, G, M) and developed by using diaminobenzidine as substrate. The 27-kDa protein is indicated by an arrow. The band on top (arrowhead) in *B* is due to reactivity of conjugate to mouse immunoglobulins in the immunoprecipitates.

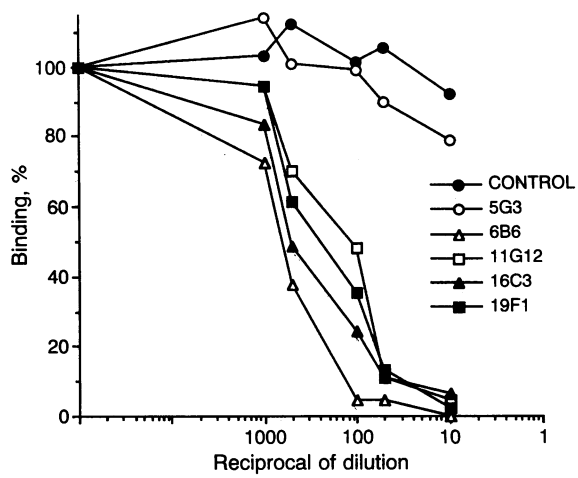

FIG. 4. Epitope analysis by one-site ELISA. Various competing ascites were tested at final dilutions ranging from 1:10 to 1:1000 and the fixed optimum concentration (5 μg/ml) of biotinylated mAb 6B6.

assay with methanol-fixed gametes and gametocytes) showed no evidence for surface reactivity when tested with intact live gametes. The mechanism of blocking thus remains mysterious. We have found ≈40% reduction in number of ookinetes (stages produced within 20–24 hr by the transforming zygotes) in the mAb 6B6-fed mosquitoes. Any ookinetes in the mAb-fed mosquitoes were retarded in their growth or morphologically deformed and failed to develop into oocysts. mAbs, when fed 4 days after the infectious blood meal (earliest time when mosquitoes could take the second blood meal), had no effect on infection outcome in the mosquitoes. It is possible that during exflagellation/gametogenesis and fertilization of parasites in the mosquito midgut (23–26), the linear epitope in the gamete surface antigens and in the internal 27-kDa protein might be transiently exposed and, thus, recognized by antibodies.

Epitope Mapping. A one-site ELISA was done to delineate the epitopes recognized by transmission blocking and nonblocking mAbs. Fig. 4 shows that all four transmission-blocking mAbs 6B6, 11G12, 16C3, and 19F1, which exhibited identical immunoreactivity patterns, appear to recognize the same or very closely linked epitope(s). Two-site ELISA also suggested that this epitope is not repeated in the antigens (data not shown). As expected, mAb 5G3, which shows a different immunoreactivity pattern and does not block transmission, also did not compete for binding with biotinylated 6B6. These mAbs thus recognize two linear epitopes (I and II), of which epitope II is a potent target of transmission-blocking antibodies.

Conservation of Blocking Epitope. The blocking epitope recognized by mAb 6B6 appears conserved in parasites from diverse geographical locations. A 27-kDa protein was immunoprecipitated by mAb 6B6 from biosynthetically labeled gametocytes from *P. falciparum* clones 3D7, T4, LE5, ItD12, and 7G8 (Fig. 5*A*). In other experiments, mAb 6B6-immunoprecipitated reduced forms of 230-kDa, 48/45-kDa, and the 27-kDa proteins from the extracts of surface radioiodinated gametes of 7G8, T4, and 3D7 parasites (Fig. 5 *B*).

Table 1. Effect of mAbs on infectivity of *P. falciparum* gametocytes in *A. gambiae*

	Control (C) and mAb infectivity									
	C	6B6	C	11G12	C	16C3	C	19F1	C	5G3
Native										
Oocysts,* no.	5.7	0.01	6.9	1.5	3.1	0.7	14.2	2.1	4.7	3.3
Infected/dissected†	80/98	2/104	89/111	50/95	107/173	44/161	83/96	42/80	87/135	69/120
Reduction,‡ %		99.8		78.3		77.4		85.2		29.8
Heat inactivated										
Oocysts,* no.	52.1	3.1	10.3	2.6	9.5	1.9	10.3	3.0	7.0	6.8
Infected/dissected†	36/45	23/42	55/75	38/75	76/98	50/95	55/75	47/75	85/103	65/90
Reduction,‡ %		94.0		74.8		80.0		70.9		2.8

Ascites containing control and test mAbs were tested at 1:4 dilution in membrane-feeding experiments. Ascites fluids were heat inactivated by incubation at 56°C for 45 min. Differences between controls and mAbs (6B6, 11G12, 16C3, and 19F1) are statistically significant, as revealed by χ^2 analysis (rate of infection, $P < 0.05$–$P < 0.0001$; number of oocysts per mosquito, $P < 0.05$–$P < 0.001$) (Statistix DOS version 2.0).
*Average (two to six independent infective feeds) of geometric means of total number of oocysts per mosquito.
†Total number of mosquitoes infected/total number of mosquitoes dissected.
‡Percent reduction in number of oocysts compared with controls.

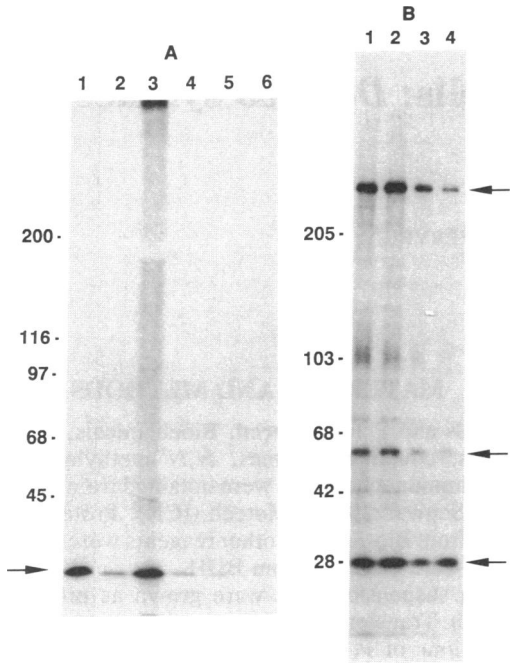

FIG. 5. Reactivity of mAb 6B6 with antigens in *P. falciparum* isolates of various geographic locations. (*A*) Gametocytes of *P. falciparum* clone 3D7 were labeled with Tran^{35}S-label (lane 1) and others; T4 (lane 2), LE5 (lane 3), ItD12 (lane 4), and 7G8 (lane 5) were labeled with [^{3}H]leucine. Labeled parasites were extracted in Triton X-100 and used for immunoprecipitation with mAb 6B6. Lane 6 shows immunoprecipitation of T4 parasite extract with an unrelated mAb. (*B*) Triton X-100 extracts of surface radioiodinated gametes of 7G8 (lane 2), T4 (lane 3), and 3D7 (lane 4) were denatured, reduced, and alkylated before immunoprecipitation with mAb 6B6. Lane 1 shows immunoprecipitation of 3D7 parasite extract with rabbit anti-gamete serum. The samples were analyzed by 5–15% SDS/PAGE under nonreducing conditions. Molecular weight standards used to calibrate the gel were high molecular weight (Bio-Rad, *A*) and prestained high molecular weight (Bethesda Research Laboratories, *B*).

Sexual reproduction of malaria parasites in the mosquito midgut is responsible for continued transmission of malaria from one infected host to another host. During fertilization of malarial gametes, genetic recombination occurs—possibly leading to selection of variants capable of survival in the immunocompetent human host (26, 27). Therefore, to hinder the processes responsible for procreation of such variants occurring naturally, as well as mutants selected due to immune and/or drug pressure, is highly desirable. Our results support the idea of developing a subunit vaccine against sexual stages. Identification of a specific continuous epitope shared among known major target antigens of TBI and a highly abundant 27-kDa protein in the sexual stages of parasite provides an ideal target for antibody-mediated immunity. Antigens exhibiting this cross-reactivity appear as early as the time of sexual differentiation of parasites during schizogony in the vertebrate host and are present during further maturation of gametocytes in the host (5, 10, 14, 21). Analysis of immune human sera has revealed that the three gamete surface proteins (230, 48, and 45 kDa) and the 27-kDa protein, which are all synthesized in the gametocyte, are targets of immune response during natural infection (28–30). In contrast, the 25-kDa zygote/ookinete protein, which is produced predominantly in the mosquito midgut stages, is not a target of immune response during natural infection (31). Thus an immune response against epitope II, which is shared among the 230-, 48/45- and 27-kDa antigens in vertebrate stages of parasite, will also have the advantage of boosting during natural infection. Further mapping of appropriate epitopes (B and helper T cells) will permit development of a malaria transmission-blocking subunit vaccine.

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