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Effects of Transmission-Blocking Monoclonal Antibodies on Different Isolates of *Plasmodium falciparum*

PATRICIA M. GRAVES,[1]†* RICHARD CARTER,[1] THOMAS R. BURKOT,[2]† JOAN RENER,[1]‡ DEEP C. KAUSHAL,[1]§ AND JACKIE L. WILLIAMS[2]

Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205[1]; and the Departments of Entomology and Immunology, Walter Reed Army Institute of Research, Washington, DC 20307[2]

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The strain diversity in *Plasmodium falciparum* has been studied with respect to gamete surface antigens which are the targets of transmission-blocking antibodies. Of 12 isolates tested, 11 were positive by immunofluorescence with the three monoclonal antibodies studied. The exception was a Liberian isolate, two clones of which were found to react with only one of the three monoclonal antibodies. Antibodies IIC5-B10 and IA3-B8, which previously have been shown to act synergistically to block infectivity of 7G8, a Brazilian clone of *P. falciparum*, acted in an exactly similar way with another Brazilian isolate, It.D12, and an isolate from Thailand. In the presence of complement either IA3-B8 or a third antibody, IID2-A10, strongly suppressed infectivity of It.D12 as well as 7G8, but neither isolate was strongly suppressed by IIC5-B10. IA3-B8 and IID2-A10 did not react by immunofluorescence or immunoprecipitation with gametes of L.E5; IIC5-B10 reacted positively with L.E5 gametes in these tests. In the absence of complement, the combination of IA3-B8 and IIC5-B10 did not suppress infectivity of L.E5 to mosquitoes. In contrast to its effect on gametocytes of other isolates, IIC5-B10 in the presence of complement strongly suppressed infectivity of L.E5 to mosquitoes. These results imply that IA3-B8 and IIC5-B10 react with two structurally distinct epitopes on the surface of gametes of *P. falciparum* and that the properties of both epitopes on gametes of L.E5 differ from those on gametes of the other isolates tested.

It has been shown previously that two monoclonal antibodies (MAbs), IIC5-B10 and IA3-D8, reacted with gametocyte and gamete stage-specific antigens on a Brazilian clone of *Plasmodium falciparum* 7G8 and blocked transmission of this parasite to *Anopheles freeborni* mosquitoes when presented together with parasitized blood through a membrane feeding apparatus (6). The MAbs mediated a complement-independent suppression of infectivity to mosquitoes when mixed together but were without effect when tested individually in the absence of complement. One MAb, IA3-B8, also suppressed infectivity when administered by itself in the presence of active complement. Both MAbs precipitated the same set of three labeled proteins from surface radioiodinated gametes or zygotes of *P. falciparum* 7G8 with M_r values of 250,000, 59,000, and 53,000 under reducing conditions on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Three proteins of similar but not identical molecular weights have been identified on the surface of gametes and zygotes of *Plasmodium gallinaceum* by MAbs which block infectivity of this parasite to mosquitoes (4). These proteins, therefore, appear to represent a class of antigens on gametes of malaria parasites in general which are the targets of transmission-blocking antibodies.

The proteins, or more precisely the epitopes, identified by the transmission-blocking MAbs can only be considered as possible vaccination antigens if they are highly conserved worldwide. Therefore, additional isolates of *P. falciparum* from Africa, southeast Asia, South America, and Papua New Guinea were examined for the presence of strain diversity in the targets of transmission-blocking antibodies.

MATERIALS AND METHODS

Culture of *P. falciparum*. Parasites were grown by the method of Ifediba and Vanderberg (2) with modifications (1) as follows. Cultures of 15 ml were initiated in 75-cm^2 tissue culture flasks (Corning Glass Works, Corning, N.Y.) at 0.3% parasitemia in a 6% hematocrit of freshly drawn and washed erythrocytes. The RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) was supplemented with 32 mM sodium bicarbonate–25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; Ultrol grade; Calbiochem-Behring, La Jolla, Calif.)–50 μg of hypoxanthine (Sigma Chemical Co., St. Louis, Mo.) per ml–10 μg of gentamicin (GIBCO)–10% fresh frozen human serum. The gas mixture was 5% CO_2, 5% O_2, and 90% N_2. When the parasitemia reached approximately 1.5%, the hematocrit was reduced to 3% by doubling the volume of culture medium changed daily until the gametocytes were mature (usually between days 14 and 18).

All isolates were put directly from a natural human infection into culture and grown for various periods before testing. Clones were derived from two Brazilian and two African isolates (1, 5).

Indirect immunofluorescence. Immunofluorescence was performed on suspensions of live female gametes or zygotes prepared and separated as previously described (6). A total of 5×10^5 gametes (and 10^7 erythrocytes as carrier), suspended in 100 μl of RPMI 1640 medium, were incubated with 100 μl of a 60- to 100-μg/ml concentration of ammonium sulfate precipitated ascitic proteins in phosphate-buffered saline (PBS; pH 7.3) for 15 min. The samples were spun in an

* Corresponding author.
† Present address: Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea.
‡ Present address: Hazelton Laboratories America, Vienna, Va.
§ Present address: Central Drug Research Institute, Lucknow, India.

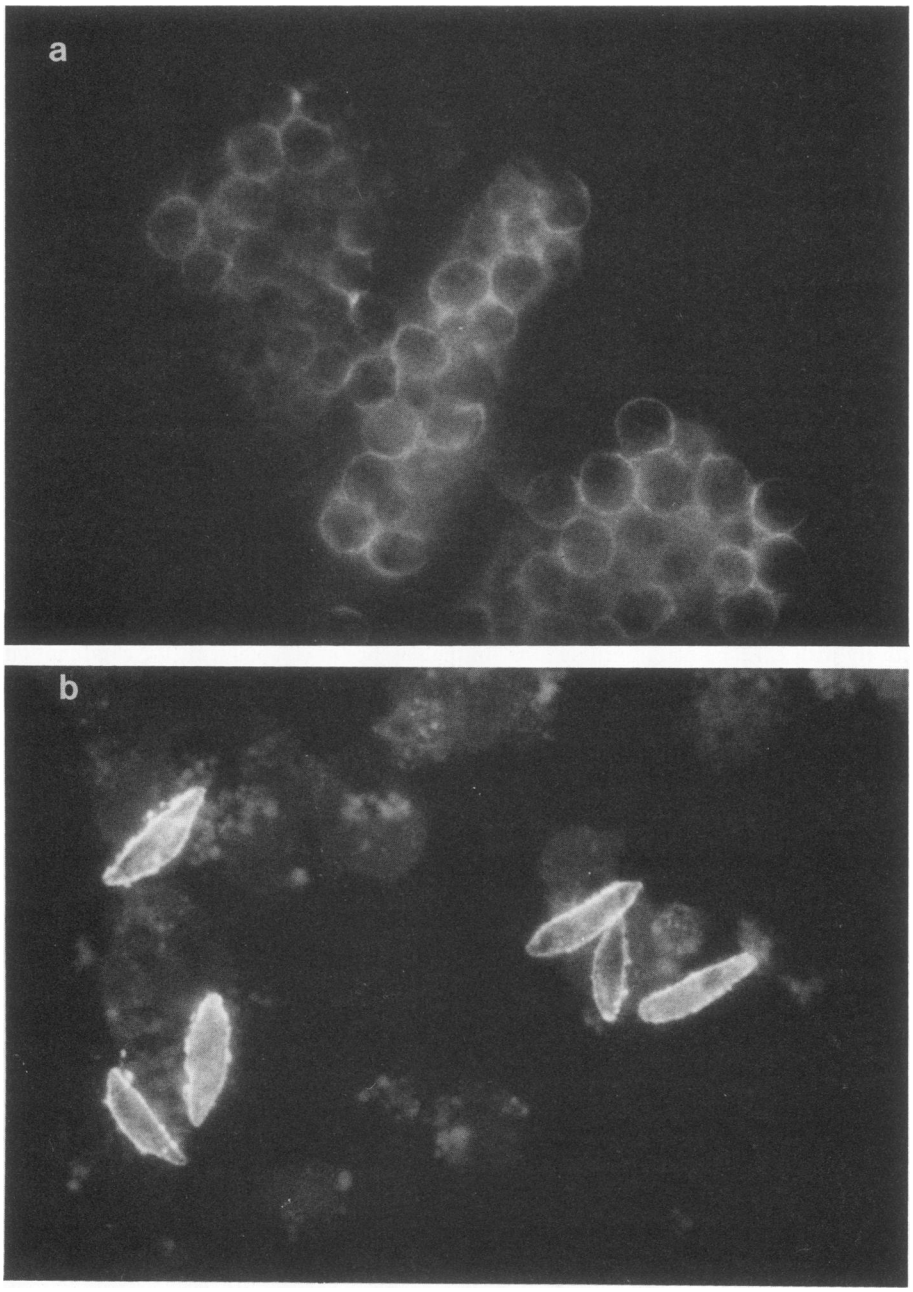

FIG. 1. (a) Immunofluorescence of extracellular female gametes and zygotes of *P. falciparum* clone L.E5 with MAb IIC5-B10. Magnification, 1000×. (b) Immunofluorescence of air-dried, acetone-fixed gametocytes of 7G8 with MAb IA3-B8. Magnification, 1000×.

Immufuge (Dade Co., Miami, Fla.) bench top centrifuge at 1000 × *g* for 1 min, washed three times in PBS, and then suspended in 50 μl of a 1:40 dilution in PBS of affinity-purified fluorescein isothiocyanate-conjugated goat anti-mouse K chain antibody for 15 min. Preparations were examined after three additional washes and suspension in 20 μl of PBS.

For immunofluorescence on fixed material, preparations of gametocytes were spotted onto multiwell slides, allowed to air dry, and then were fixed for 30 min in acetone at −20°C. MAbs at concentrations of 100 μg/ml or lower of ammonium sulfate-precipitated ascitic proteins in PBS were incubated for 30 min and washed, and then a 1:40 dilution of the fluorescent reagent (see above) was added for 30 min. Slides were counterstained with 0.01% Evans blue.

^{125}I surface labeling, immunoprecipitation, and SDS-PAGE of female gametes or zygotes of *P. falciparum*. Mature gametocytes were stimulated to undergo gametogenesis, and the gametes were purified on a discontinuous Percoll gradient (Pharmacia Fine Chemicals, Piscataway, N.J.) as previously described (6). Purified gametes were washed in RPMI 1640 medium, and 10^7 to 10^8 cells were labeled with ^{125}I by the lactoperoxidase method (3) and extracted with 1% Triton X-100 in PBS containing 10 mM HEPES and protease inhibitors (3). The antigen extract (100 μl) was preabsorbed with 100 μl of 50% protein-A Sepharose CL-4B (Pharmacia) in NETT buffer containing 1% bovine serum albumin. Approximately 5×10^4 trichloroacetic acid-precipitable counts of preabsorbed antigen were incubated for 1 h at 4°C with 60

TABLE 1. Indirect immunofluorescence of MAbs against live extracellular female gametes or zygotes of isolates and clones of *P. falciparum*

P. falciparum isolate (origination)	Indirect immunofluorescence of the following MAbs[a]:			
	IIC5-B10	IA3-B8	IID2-A10	NMS[b]
7G8 (Brazil)	+	+	+	−
It.D12 (Brazil)	+	+	+	−
It.F10 (Brazil)	+	+	+	−
T4 (Thailand)	+	+	+	−
BC1 (Cameroon)	+	+	+	−
L.E5 (Liberia)	+	−	−	−

[a] +, Positive reaction by indirect immunofluorescence; −, negative reaction by indirect immunofluorescence.
[b] Ammonium sulfate-precipitated normal mouse serum.

to 100 μg of MAb in the form of ammonium sulfate-precipitated ascitic proteins or with ammonium sulfate-precipitated normal mouse serum. The antigen-antibody mixture was added to 25 μl of packed beads of protein A-Sepharose and incubated for 2 h at 4°C with gentle rocking. The beads were washed, and the immune complexes were eluted as described by Kaushal et al. (3). Electrophoresis was carried out on 5 to 15% gradient slab gels.

Measurement of effect of MAbs on infectivity of *P. falciparum* gametocytes to mosquitoes. The production of monoclonal antibodies has been described previously (6). The MAbs used in this study were IA3-B8 and IIC5-B10, both isotypes $\gamma 2_a$, and IID2-A10, isotype γ1. To assess their effect on infectivity of *P. falciparum*, 30 to 40 μl of parasites and erythrocytes from culture (suspended to a 50% hematocrit in heat-inactivated human serum) were combined with 150 μl of freshly drawn defibrinated human blood (containing active complement) and 60 μl of MAb in a membrane feeder, on which *A. freeborni* mosquitoes were allowed to feed. In paired feeds designed to compare results obtained in the presence or absence of active complement, the fresh defibrinated blood was washed twice in Tris-buffered glucose-saline (pH 7.4; SA solution) (6), and the cells were suspended in either heat-inactivated or native human serum. The serum used in feed for the *P. falciparum* clone L.E5 was drawn from a single donor, with one sample being frozen immediately and the other heat-inactivated before freezing. Ammonium sulfate-precipitated ascites of the NS1 myeloma line was used as control in the L.E5 feeds. In feeds with *P. falciparum* clones 7G8 and L.E5, the final MAb concentration were as follows (in milligrams per milliliter): IIC5-B10 (0.6 to 2.8), IA3-B8 (0.7 to 2.9); and IID2-A10 (2.4 to 2.5). In feeds with *P. falciparum* lines It.D12 and T4 the final MAb concentration was 1.1 to 1.3 mg/ml. The controls were ammonium sulfate-precipitated normal mouse serum at a protein concentration of 1.1 to 1.3 mg/ml. In all experiments mosquitoes were dissected 8 to 9 days after feeding, and the

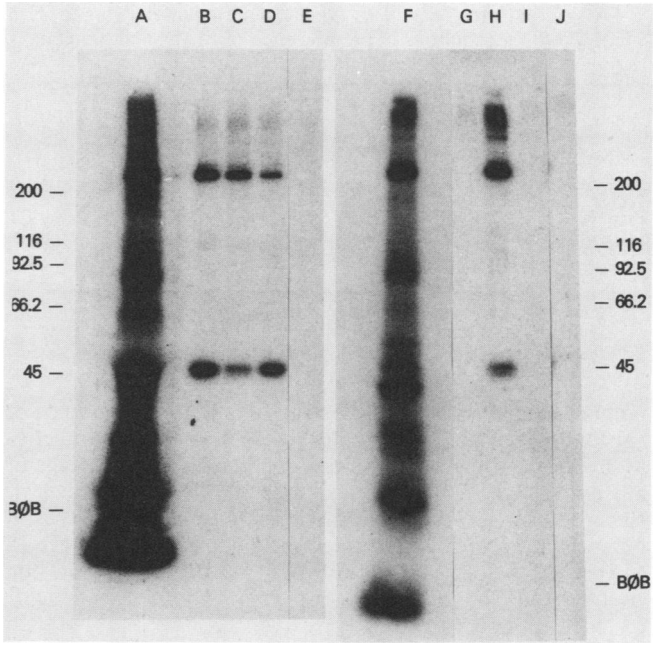

FIG. 2. Immunoprecipitation of ^{125}I-surface-labeled gametes of *P. falciparum*. SDS-PAGE under nonreducing conditions. Clone 7G8 antigen (lane A), immunoprecipitated with IA3-B8 (lane B), IIC5-B10 (lane C), IID2-A10 (lane D), and ammonium sulfate-precipitated normal mouse serum (lane E). Clone L.E5 antigen (lane F), immunoprecipitated with IA3-B8 (lane G), IIC5-B10 (lane H), IID2-A10 (lane I), and ammonium sulfate-precipitated normal mouse serum (lane J). The numbers at the side of the figure represent molecular weight × 10^{-3}.

TABLE 2. Indirect immunofluorescence of MAbs against air-dried, acetone-fixed gametocytes of isolates and clones of *P. falciparum*

P. falciparum isolate (origination)	Indirect immunofluorescence of the following MAbs[a]:			
	IIC5-B10	IA3-B8	IID2-A10	NMS[b]
7G8 (Brazil)[c]	+	+	+	−
It.D12 (Brazil)[c]	+	+	+	−
It.E1 (Brazil)[c]	+	+	+	−
It.F10 (Brazil)[c]	+	+	+	−
Honduras	+	+	+	−
Santa Lucia (El Salvador)	+	+	+	−
L.E5 (Liberia)[c]	+	−	−	−
L.G2 (Liberia)[c]	+	−	−	−
BC1 (Cameroon or Niger)	+	+	+	−
Nigeria 1	+	+	+	−
K.D7 (Kenya or Zaire)[c]	+	+	+	−
Tanzania 1	+	+	+	−
T4 (Thailand)	+	+	+	−
V1 (Vietnam or Cambodia)	+	+	+	−
FCQ.30 (Papua New Guinea)	+	+	+	−

[a] +, Positive reaction by indirect immunofluorescence; −, negative reaction by indirect immunofluorescence.
[b] Ammonium sulfate-precipitated normal mouse serum.
[c] Cloned line.

TABLE 3. Infectivity of *P. falciparum* It.D12 gametocytes to *A. freeborni* mosquitoes in the presence of MAbs and human complement

Antibody	No. of feeds	No. dissected	Mean no. of oocysts per infected mosquito	% Infected
NMS[a] (control)	5	77	14.3	63.6
IID2-A10	2	33	1.0	3.0[b]
IA3-B8	5	70	6.1	31.4[b]
IIC5-B10	5	75	4.3	50.7
IA3-B8 + IIC5-B10	5	60		0.0[b]

[a] Ammonium sulfate-precipitated proteins from normal mouse serum.
[b] Significantly different from control ($P < 0.001$ by the chi-square test).

TABLE 4. Infectivity of *P. falciparum* T4 gametocytes to *A. freeborni* mosquitoes in the presence of MAbs and human complement

Antibody	No. of feeds	No. dissected	Mean no. of oocysts per infected mosquito	% Infected
NMS[a] (control)	3	54	12.0	51.9
IA3-B8	3	27	3.6	29.6
IIC5-B10	3	42	10.8	28.6[b]
IA3-B8 + IIC5-B10	3	45	1.0	2.2[c]

[a] Ammonium sulfate-precipitated proteins from normal mouse serum.
[b] Significantly different from control ($P < 0.05$ by the chi-square test).
[c] Significantly different from control ($P < 0.001$ by the chi-square test).

number of oocysts per midgut was examined in samples of approximately 10 to 15 mosquitoes. Suppression of infectivity by the MAbs, expressed as a decline in the percentage of mosquitoes infected, was evaluated by the chi-square test. Because of the variation in the infectivity of the cultures in different experiments, the infection rates in the presence of MAbs were compared only with those obtained in the controls run simultaneously.

RESULTS

Immunofluorescent reactions of MAbs. In indirect immunofluorescence at concentrations of 60 to 100 μg/ml, MAbs IIC5-B10, IA3-B8, and IID2-A10 gave uniform strong surface fluorescence reactions (Fig. 1) with extracellular female gametes or zygotes of all isolates tested except for one (Table 1). Gametes of the cloned African line L.E5, although positive with IIC5-B10, were completely negative in reaction with IA3-B8 or IID2-A10. All controls were negative.

A wider range of isolates was tested by immunofluorescence on air-dried, acetone-fixed gametocytes (Table 2). In this case all isolates were positive when tested with MAbs at 60 to 100 μg/ml, with the exception of L.E5 and L.G2, both clones of the Liberia isolate (5), which were totally negative. Immunofluorescence tests were performed at least twice for each isolate or clone.

Immunoprecipitation of labeled antigen by MAbs. MAb IIC5-B10 precipitated three proteins of apparent M_r under nonreducing conditions of 235,000, 49,000, and 46,000 from the surface of ^{125}I surface-labeled gametes of clone L.E5 (Fig. 2). These proteins are equivalent to those precipitated from 7G8 gametes as shown previously (6). Their apparent M_r values under reducing conditions were 260,000, 59,000, and 53,000, respectively. Neither IA3-B8 nor IID2-A10 precipitated labeled material from gametes of L.E5.

Effects of MAbs on infectivity to mosquitoes of different isolates of *P. falciparum*. In the presence of complement, the infectivity of strain It.D12 (Table 3) was partially suppressed by IID2-A10 or IA3-B8 alone, but not by IIC5-B10; in combination, IA3-B8 and IIC5-B10 completely abolished infectivity. Similar results were obtained with these MAbs in the presence of complement against strains T4 and 7G8 (Tables 4 and 5, respectively), although with complement, infectivity of 7G8 was even more effectively suppressed by either IID2-A10 or IA3-B8 than was It.D12. In the absence of complement, neither MAb alone was effective against 7G8, although the combination of IA3-B8 with IIC5-B10 was as effective with as without complement.

The minimum effective concentration in the absence of complement of ammonium sulfate-precipitated ascites of MAbs IIC5-B10 and IA3-B8 was tested (Table 6). In equal concentrations, this combination of MAbs completely suppressed the infectivity of 7G8 at 60 μg/ml (total combined concentration), and suppression was still very effective at 15 μg/ml.

The effect of these MAbs on infectivity of strain L.E5 differed from that of the other isolates (Table 7). IID2-A10 alone was ineffective with or without complement; although IA3-B8 appeared to have some suppressive effect in the absence of complement (as measured by the percentage of mosquitoes infected), this was not borne out by the oocyst densities achieved. IIC5-B10, on the other hand, gave partial suppression of infectivity of L.E5 without complement and almost complete suppression with complement. In the absence of complement, the combination of IIC5-B10

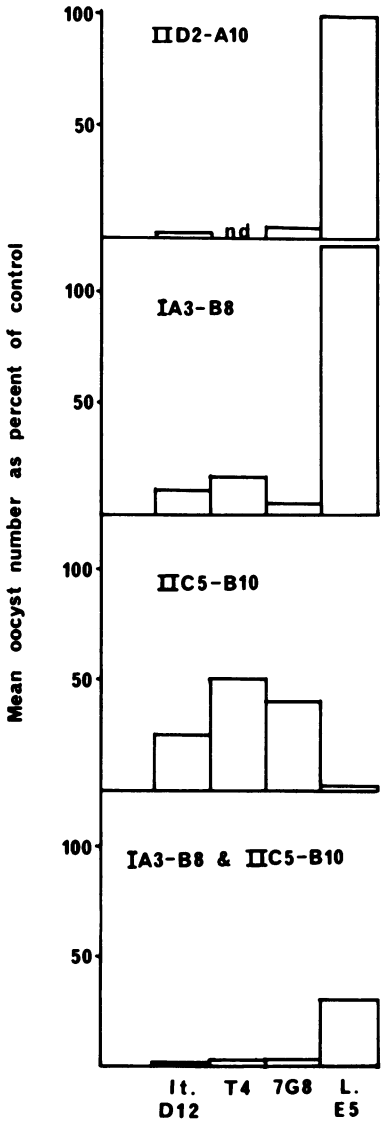

FIG. 3. Infectivity of strains It.D12; T4, 7G8, and L.E5 to mosquitoes in the presence of MAbs and active complement. The results are given as the overall weighted mean oocyst number per mosquito expressed as a percentage of the mean number of oocysts observed in simultaneous control feeds without antibody. nd, Not done.

TABLE 5. Infectivity of *P. falciparum* 7G8 gametocytes to *A. freeborni* mosquitoes in the presence of MAbs with or without human complement[a]

Antibody	Complement	No. of feeds	No. dissected	Mean no. of oocysts per infected mosquito	% Infected
NMS/SA[b]	−C	8[c]	133	11.6	51.9
(Control)	+C	8	150	16.2	62.0
IID2-A10	−C	4	57	10.3	40.4
	+C	4	50	1.5	8.0[d]
IA3-B8	−C	8	77	6.3	42.9
	+C	8	93	2.0	18.3[d]
IIC5-B10	−C	8	84	10.0	48.8
	+C	8	92	11.8	32.6[e]
IA3-B8	−C	8	88	1.1	8.0[d]
+ IIC5-B10	+C	8	101	1.2	5.0[d]

[a] Results obtained in feeds with MAbs were compared with the appropriate control feed, i.e., with or without complement. The effect of complement was assessed by comparing the results obtained for the same MAb with or without complement.
[a] Either (i) ammonium sulfate-precipitated proteins from normal mouse serum or (ii) Tris-glucose-saline buffer (pH 7.4 SA solution [1])
[b] Four feeds with the antibodies IA3-B8, IIC5-B10, and the two combined have been reported previously (1).
[d] Significantly different from control ($P < 0.001$ by the chi-square test).
[e] Significantly different from control ($P < 0.05$ by the chi-square test).

and IA3-B8 did not significantly affect infectivity of L.E5, although with complement moderate suppression occurred.

DISCUSSION

Results of this study indicate that MAbs IA3-B8 and IIC5-B10, previously shown to act in synergism to block infectivity of *P. falciparum* to mosquitoes, each recognize a distinct epitope on the target antigen. This may be concluded from the fact that IA3-B8 failed to react by either immunofluorescence or immunoprecipitation with gamete surface antigens from one isolate of *P. falciparum* from Liberia, but did react with all other isolates tested. MAb IIC5-B10, on the other hand, reacted by these tests with all isolates examined, including the Liberian isolate. A third MAb (IID2-A10) showed the same pattern of reactivity as IA3-B8 and probably recognizes the same epitope.

These data demonstrate the conservation of the IIC5-B10 epitope among15 different clones or isolates of *P. falciparum* from Central and South America, Africa, South east Asia, and Papua New Guinea. The epitope that reacts with IA3-B8 and IID2-A10 also appears to be generally conserved, being recognized by these MAbs in all but two clones (L.E5 and L.G2) from the Liberian isolate.

TABLE 7. Infectivity of *P. falciparum* L.E5 gametocytes to *A. freeborni* mosquitoes in the presence of MAbs with or without human complement

Antibody	Complement	No. of feeds	No. dissected	Mean no. of oocysts per infected mosquito	% Infected
NS1 (control)[a]	−C	7	82	2.0	51.2
	+C	7	85	3.4	52.9
IID2-A10	−C	5	39	2.0	35.9
	+C	5	35	3.4	60.0
IA3-B8	−C	7	61	4.1	26.2[b]
	+C	7	84	3.8	58.3
IIC5-B10	−C	7	79	2.4	29.1[b]
	+C	7	86	1.0	1.2[c]
IA3-B8	−C	7	82	4.7	37.8
+ IIC5-B10	+C	7	73	1.7	31.5[b]

[a] Ammonium sulfate-precipitated ascites from the NS1 myeloma line.
[b] Significantly different ($P < 0.05$ by chi-square test).
[c] Significantly different ($P < 0.001$ by chi-square test).

Several isolates were tested for their infectivity to mosquitoes in the presence of these MAbs (Fig. 3). The effect of the MAbs on infectivity reflected their reactivity with the isolates by IFAT and immunoprecipitation. *P. falciparum* lines It.D12 from Brazil and T4 from Thailand responded to the MAbs in a way generally similar to that previously shown for line 7G8 from Brazil (6). Thus, in the presence of complement, IIC5-B10 was relatively ineffective in suppressing infectivity, IA3-B8 and IID2-A10 were more infective, and the combination was highly suppressive against all three isolates. Clone L.E5 of the Liberian isolate, on the other hand, was of greatly reduced infectivity in the presence of IIC5-B10 with complement but was affected little by IA3-B8 or IID2-A10. In the absence of complement, the combination of IIC5-B10 and IA3-B8, which completely suppressed infectivity of line 7G8, was totally ineffective in suppressing the infectivity of L.E5.

The results with L.E5 involving IA3-B8 and IID2-A10 are readily accounted for by the failure of these MAbs to react with their target epitope on gametes of L.E5, presumably because of its variation, inaccessibility, or absence on ga-

TABLE 6. Infectivity of *P. falciparum* 7G8 gametocytes to mosquitoes in the presence of different concentrations of MAbs without complement

Antibody[a]	Final MAb conc. (mg/ml)	No. dissected	% Infected[b]	Mean no. of oocysts per infected mosquito	Mean no. of oocysts per mosquito as percentage of control
Control (IID4)[c]	2.5	25	36.0	11.7	
IA3-B8	1	25	36.0	8.9	76.2
IIC5-B10	1	14	42.9	6.3	64.3
IA3-B8 + IIC5-B10	1	18	0.0[d]		0.0
IA3-B8 + IIC5-B10	0.25	24	4.2[d]	1.0	1.0
IA3-B8 + IIC5-B10	0.06	23	0.0[d]		0.0
IA3-B8 + IIC5-B10	0.015	20	30.0	1.7	10.7
	0	31	45.2	6.4	69.1

[a] Antibody in the form of ammonium sulfate-precipitated ascitic proteins.
[b] Mean of two separate experiments.
[c] IID4 is a MAb reacting with zygotes of *P. gallinaceum* which does not cross-react with *P. falciparum*.
[d] Significantly different from control ($p < 0.01$ by χ^2 test)

metes of this line of parasites. The response of L.E5 to IIC5-B10, namely suppression of its infectivity by this MAb in the presence of complement, in contrast to the relatively minor effect of IIC5-B10 on infectivity of other isolates was unexpected; no difference were detected between isolates in their reactivity to IIC5-B10 by IFAT or, where tested, by immunoprecipitation. We propose that the cell surfaces of gametes of L.E5 are different from those of gametes of other isolates tested in such a way that IIC5-B10 is able to fix complement efficiently. It is unknown whether such a difference is related to the variation of L.E5 in reaction with IA3-B8 and IID2-A10 or whether two distinct mutations are involved.

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