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Properties of epitopes of Pfs 48/45, a target of transmission blocking monoclonal antibodies, on gametes of different isolates of *Plasmodium falciparum*

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Summary We have studied the properties of epitopes on *Plasmodium falciparum* gamete surface protein Pfs 48/45, a target antigen of malaria transmission blocking antibodies. Using a two site immunoradiometric assay we have defined three spacially separate, non-repeated, epitope regions on the peptides representing this antigen. Epitope region I is a target of monoclonal antibodies (MoAbs) which strongly suppress infectivity of gametocytes of *P. falciparum* to mosquitoes; the effect is complement independent and is mediated as effectively by the monovalent Fab fragments as by intact MoAb. Epitope region II consists of two spacially close subregions, IIa and IIb; variant forms of epitopes IIa and IIb occurred in different isolates of *P. falciparum*. Epitope region III also showed slight structural modification between isolates. MoAbs against regions II or III were relatively ineffective in suppressing gametocyte infectivity compared to MoAbs against region I. However, certain combinations of MoAbs against regions II and III together acted synergistically to suppress infectivity to mosquitoes. All these epitopes failed to react with MoAb when the antigen was presented in reduced form. A fourth epitope, however, was identified which reacted strongly with MoAb when the antigen was presented in reduced form. The MoAb against this epitope had no effect on the infectivity of gametocytes of *P. falciparum* to mosquitoes.

Keywords; *Plasmodium falciparum* gamete, gametocyte, transmission blocking, monoclonal antibody, epitope

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Introduction

Transmission of malarial infections from a vertebrate host to mosquitoes can be prevented by antibodies against surface antigens of gametes of the malaria parasite (Carter *et al.* 1988). When present in a blood meal the antibodies interact with gametes of the parasite following their emergence from host RBC's in the mosquito midgut. Such antibodies may act to prevent fertilization of male and female gametes (Carter *et al.* 1979, Kaushal *et al.* 1983, Grotendorst *et al.* 1984); antibodies against surface antigens expressed in later development of the parasites in the mosquito midgut can act to interrupt the subsequent development of the zygote in a mosquito (Grotendorst *et al.* 1984, Vermeulen *et al.* 1985a). Several surface antigens on gametes of the human malaria parasite *Plasmodium falciparum* have been defined as targets of transmission blocking monoclonal antibodies (MoAbs) (Rener *et al.* 1983; Vermeulen *et al.* 1985a; Quakyi *et al.* 1987). These include a 230 kDa protein and a doublet of 48 and 45 kDa. Although the 230 kDa protein is immunologically distinct from the 48/45 kDa proteins, both sets of proteins generally occur as a complex on the gamete surface (Kumar, 1987; Rener *et al.* 1983). The proteins of the 48/45 kDa doublet are apparently closely related and are generally recognized immunochemically by the same MoAbs (Vermeulen *et al.* 1985a and present study). The properties of MoAbs reacting with the 48/45 kDa proteins have been studied both as regards their effects on the infectivity of gametocytes of *P. falciparum* to mosquitoes (Rener *et al.* 1983, Vermeulen *et al.* 1985a, Graves *et al.* 1985) and in immunoradiometric assays (IRMA) to define the epitopes recognized by these MoAbs (Carter *et al.* 1985; Vermeulen *et al.* 1985b).

The purpose of the present study has been to define the epitopes on the 48 and 45 kDa gamete surface protein recognized by currently available MoAbs against these proteins in relation to the effects of these MoAbs on infectivity of *P. falciparum* gametocytes to mosquitoes.

Following the system of nomenclature introduced by Kaslow *et al.* (1988) for an ookinete surface antigen of *P. falciparum*, we now designate the 48/45 kDa gamete surface protein of *P. falciparum*, Pfs 48/45 (*Plasmodium falciparum* sexual stage gamete surface antigen of 48 and 45 kDa).

Materials and Methods

ORIGIN OF LINES OF *P. FALCIPARUM* AND THEIR CULTIVATION FOR GAMETOCYTE PRODUCTION

Three cloned lines and one uncloned isolate of *P. falciparum* were used in this study. Clone 7G8 was derived from a Brazilian isolate (Burkot *et al.* 1984); clone LE5 from a Liberian isolate (Graves *et al.* 1985) and clone 3D7 (Walliker *et al.* 1987) from the Amsterdam Airport isolate NF54 (Ponnudurai *et al.* 1982). Uncloned isolate NF54 was also used in these studies.

Gametocytes were grown either manually in flasks as previously described (Ifediba & Vanderberg 1981) or in the automated mass culture system described by Ponnudurai *et al.* (1983) (line NF54 and clone 3D7 only).

ORIGIN OF MONOCLONAL ANTIBODIES (MOABS)

3E12 and 3G12 are mouse MoAbs both of isotype IgG1 from a hybridoma cell line derived by standard procedure (Köhler & Milstein 1975) by fusion of mouse myeloma cells NS1 with spleen cells of a Balb/c mouse immunized with gametes of *P. falciparum* clone 7G8.

MoAbs IIC5–B10 and IA3–B8 (both isotype IgG2a) and IID2–A10 (isotype IgG1) have been previously described (Rener *et al.* 1983; Carter *et al.* 1985); MoAbs 32F3 and 32F1 (both isotype IgG1) were described by Vermeulen *et al.* (1985a & 1985b) and were kindly given by T.Ponnudurai and J.H.E.Th.Meuwissen. Each of these MoAbs recognize one or both members of the Pfs 48/45 protein doublet by immunoprecipitation and/or Western blot.

PREPARATION OF PROTEIN A PURIFIED MOAB 3E12 AND FAB FRAGMENTS OF 3E12

MoAb 3E12 was purified from ascites fluid by precipitation with ammonium sulphate (Bethesda Research Labs. Gaithersburg MD) slowly dissolved in the ascites fluid to a final concentration of 300 mg/ml. The precipitated protein was spun and redissolved in distilled water and dialysed against borate buffered saline (BBS) (0·25M $Na_2B_4O_7 \cdot 10H_2O$; 0·1M H_3BO_3; 75 mMNaCl, pH 8·0). The BBS dialysed antibody was passed over a column of sepharose bound protein A (Pharmacia) in the presence of sheep haemoglobin as a visual marker. Following passage of the haemoglobin as a measure of the void volume of the column, the remaining protein on the column was eluted with 0·1 M acetic acid and the eluate neutralized immediately with 2M Tris. Most mouse IgG1 antibodies are retarded but not bound on protein A at pH 8·0; hence the above purification procedure. The main eluted protein peak, containing purified Mab 3E12, was dialysed and reconstituted to 7 mg protein per ml in 20 mm NaH_2PO_4, 20 mM L-cysteine HCl, 10 mM EDTA, pH 6·2. Three ml of this solution was incubated with 1 ml wet bead volume (in the same phosphate, cysteine, EDTA buffer) of Immobilized Papain beads (Pierce) for 5 h at 37°C. The beads were removed by centrifugation and the papain-digested solution of 3E12 antibody neutralized with 10 mM Tris HCl, pH 7·5, concentrated to 3 mls and dialysed against BBS, pH 8·0. The dialysed solution was then passed over a protein A sepharose column and 14 × 1 ml fractions collected by washing with BBS, followed by a further 13 × 1 ml fractions eluted with 0·1M acetic acid (and neutralized with 2M Tris). No protein was present in significant amounts in the first four fractions which were discarded. From each of the remaining fractions, 20–100 μl were separated on SDS-PAGE with a 5–15% acrylamide gradient (see below) without addition of reducing agent; the gel was stained with Coomassie Blue (Figure 1). Fractions 5 to 14 consisted of predominantly a single peptide of about 50 kDa on SDS-PAGE, as expected for an antibody Fab fragment. There were small amounts of intact antibody in the higher fractions at about 180 kDa on SDS-PAGE, amounting to less than 10% of the Fab protein by visual inspection, and also a peptide of about 28 kDa corresponding to the digested Fc fragment and representing less than half the amount of Fab protein in these fractions. Fractions above no. 17 consisted predominantly of the 28 kDa Fc and also relatively more of the intact undigested antibody, these being retarded by the protein A column, but were virtually free from Fab fragment protein. The apparent molecular weights of intact antibody and of Fab and Fc fragments are similar to those expected for mouse immunoglobulins (Harlow & Lane, 1988).

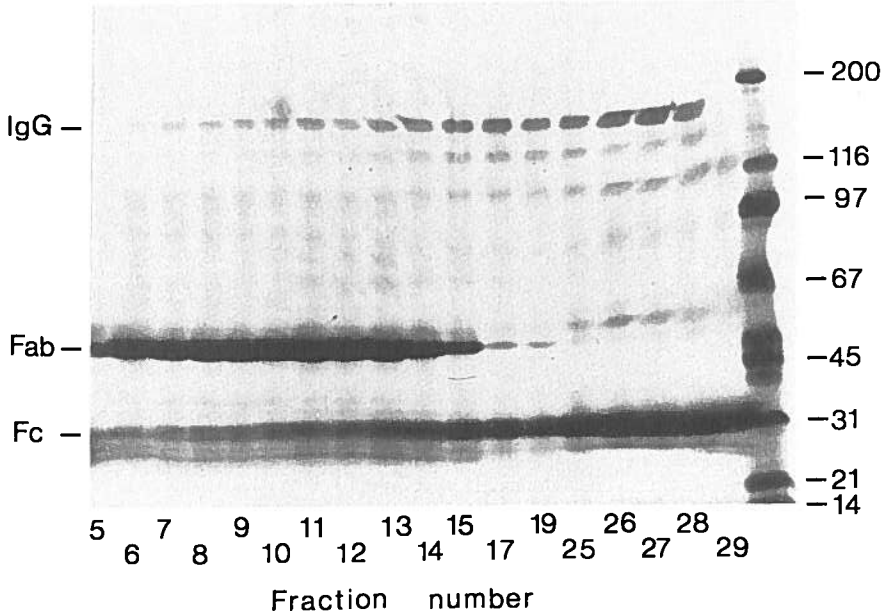

Figure 1. Coomassie stained SDS-PAGE, 5–15% polyacrylamide gradient, of fractions of papain-digested MoAb 3E12 after passing over a protein A sepharose column. Fractions 5–14 washed through with BBS; 15–27 eluted with 0·1 M acetic acid. Molecular weights ($\times 10^3$) are indicated on right hand side of gel. The positions of intact immunoglobulin G(IgG), Fab fragment and Fc fragments are indicated at the left hand side of the gel.

The fractions 5 to 14 containing the Fab fragments of 3E12, were combined and dialysed against medium 199. 3·8 ml of protein was recovered at a final protein concentration of 3·5 mg/ml. For use in membrane feeding experiments intact protein A purified MoAb 3E12 was also dialysed against medium 199 to a final concentration of 5 mg MoAb 3E12 per ml. Both intact MoAb 3E12 and 3E12 Fab fragments were used in membrane feeding experiments as described above.

SURFACE IMMUNOFLUORESCENCE REACTIONS OF MOABS WITH EXTRACELLULAR GAMETES OF *P. FALCIPARUM*

Surface immunofluorescence reactions of MoAbs 3E12 and 3G12 with female gametes of *P. falciparum* NF54 and 7G8 were carried out as previously described (Graves *et al.* 1985).

ASSAY FOR EFFECTS OF MONOCLONAL ANTIBODIES (MOABS) ON INFECTIVITY OF GAMETOCYTES OF *P. FALCIPARUM* TO MOSQUITOES

Gametocytes of *P. falciparum* lines NF54 or 7G8 were grown in manual culture and fed to *Anopheles freeborni* mosquitoes through a membrane feeding apparatus as previously described (Ifediba and Vanderberg 1981, Rener *et al.* 1983).

Each blood meal, presented to mosquitoes through a membrane feeding apparatus warmed to 37°C with a glass water jacket, contained the following components: 6 μl of 3E12 as protein-A purified MoAb or as Fab fragment at appropriate concentrations (see individual experiments Tables 1 and 2) in medium 199; 60 μl of packed human 0^+ RBC, 40 μl of packed gametocyte infected 0^+ RBC from culture, and 100 μl of human serum, either heat inactivated at 56°C for 30 min, or freshly drawn with active complement; control feedings consisted of the same combination, substituting 6 μl of medium 199 to replace antibody. MoAb 3G12 was tested using 60 μl of heat inactivated ascites fluid, 60 μl of packed human 0^+ RBC, 40 μl of packed gametocyte infected 0^+ RBC and 50 μl of freshly drawn human serum; control feedings consisted of the same combination substituting 60 μl of heat inactivated NS1 ascites fluid to replace the 3G12.

Following feeding, the mosquitoes were kept for 9 days at 27°C with 70–80% relative humidity; the mosquitoes were then dissected and their midguts examined for oocysts (the products of gamete fertilization during a blood meal containing gametocytes). Infectivity of the gametocytes to mosquitoes is expressed as the mean number of oocysts per midgut.

^{125}IODINE LABELLING OF MOABS USED IN IMMUNORADIOMETRIC ASSAYS (IRMA) AND ON IMMUNOBLOTS

Each MoAb used for IRMA, and as radiolabelled antibody in immunoblot, was labelled with ^{125}Iodine by the Iodogen method as previously described (Carter *et al.* 1985). Stock solutions of the radio-iodinated MoAbs were made up at 30 μg of MoAb protein (2 to 5 μC ^{125}Iodine bound per μg of MoAb protein) per ml of 10% calf serum in PBS pH 7·4 with 0·01% sodium azide.

TWO SITE IRMA

Two site IRMA were carried out with modification based on the method described by Carter *et al.* (1985). Gametocytes of *P. falciparum* lines 7G8 or NF54 (see above) were harvested on a Percoll gradient (Rener *et al.* 1983) and extracted in NETT (50 mM Tris, 150 mM NaCl, 5 mM EDTA, 0·5% Triton X-100, pH 7·0) containing protease inhibitors (5 μg each of pepstatin, leupeptin, chymostatin and antipain [Sigma Chemical Co.] per ml) in a suspension of 1×10^8 gametocytes per ml. After extraction with intermittent vortexing for 5 min at room temperature the suspension was centrifuged at 15 000 × g for 5 min and the supernatant retained as antigen extract. For use in the two site IRMA, described below, this extract was diluted in 10% calf serum in PBS, pH 7·4 to a final concentration equivalent to 2×10^6 gametocytes per ml.

The two site IRMA was set up as follows, modified from the method described by Carter *et al.* (1985). Round bottomed detachable polystyrene wells (Dynatech, UK, Immulon II, Removastrip U-wells) were incubated with 70 μl per well of protein A purified MoAbs (see below) at 10 μg MoAb per ml in PBS, pH 7·4, at 4°C overnight. The MoAb solution was removed and the wells were washed twice with 0·05% Tween-20 in PBS. The wells were then blocked against further adsorbtion of protein by incubation for 1 h at room temperature with 200 μl of 10% calf serum in PBS, pH 7·4. This solution was replaced by 50 μl of antigen extract at 2×10^6 gametocytes per ml, as described above, and incubated for 6 h at 4°C. After washing twice with 0·05% Tween-20 PBS, pH 7·4 50 μl of ^{125}I labelled MoAb (see below) at a 1:20 dilution of the stock in 10% calf serum in PBS,

pH 7·4, was added and incubated at 4°C overnight. The labelled MoAbs were removed and the wells were washed twice with 0·05% Tween-20 in PBS, pH 7·4 containing NaCl at a final concentration of 0·65% (high salt wash to reduce non-specific binding of ^{125}I-labelled MoAbs to proteins already bound to the plate) followed by 2 × washes with 0·05% Tween-20 in PBS, pH 7·4 (without added salt). The plates were then rinsed several times by emersion in distilled water, dried and individual wells detached and counted in a gamma counter. Each experimental combination was set up in triplicate wells and the mean value taken. Each combination of MoAbs was set up with or without antigen; the control values, without antigen, were subtracted from the values obtained in the presence of antigen. Values without antigen were between 50 and 500 cpm according to the preparation of ^{125}I labelled MoAb used; maximum counts in the presence of antigen were correspondingly between 3000 and 40 000 depending on the ^{125}I-labelled MoAb. Results are expressed for each ^{125}I-labelled MoAb as a percentage of the maximum binding achieved with that MoAb on the day of the experiment.

IMMUNOPRECIPITATION OF ^{125}IODINE LABELLED SURFACE ANTIGENS OF GAMETES OF *P. FALCIPARUM*

Gametes of *P. falciparum*, clone 3D7, were prepared and surface labelled with ^{125}Iodine as previously described (Rener *et al.*, 1983). Triton X-100 extracts of the radiolabelled gametes were immunoprecipitated with MoAb 3G12 covalently conjugated to CNBr activated sepharose (Pharmacia Fine Chemicals, 1983) or with MoAb IIC5–B10 followed by protein A sepharose as previously described (Rener *et al.*, 1983) and separated on 5% to 15% polyacrylamide on SDS-PAGE (Kaushal *et al.*, 1983).

SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE) AND IMMUNOBLOTTING OF GAMETOCYTE ANTIGEN WITH MOABS

Extracts of gametocytes of *P. falciparum* were separated on SDS-PAGE with a 5–15% acrylamide gradient (Kaushal *et al.* 1983) and electroblotted onto nitrocellulose (Towbin *et al.* 1979). Gametocytes of *P. falciparum* were grown and purified as described previously (Rener *et al.* 1983); 5×10^7 gametocytes were extracted in 1 ml of sample buffer containing 5% SDS, 10% glycerol and 50 mM Tris, pH 6·8, for separation on SDS-PAGE. Following electroblotting the nitrocellulose was cut into 2 cm strips each with the equivalent of 2×10^6 gametocytes. The strips were blocked by incubation with 5% Milk (Carnation, low fat) in PBS, pH 7·4, for 30 mins and washed × 3 with 0·05% Tween-20 in PBS, pH 7·4. The strips were then incubated overnight at 4°C, with rocking, with ^{125}I labelled MoAbs diluted 1 : 100 in 5% Milk in PBS, pH 7·4, from stocks (see above). The strips were washed × 5 with 0·05% Tween- 20/PBS pH 7·4, dried and exposed for autofluorography using an intensifying screen.

In immunoblot experiments with MoAb 3G12, which also included MoAbs 3E12 and IIC5-B10, reactivity of electroblotted antigen was demonstrated using horse radish peroxidase (HRP)-conjugated sheep antiserum to mouse IgG (generously given by Scottish Antibody Production Unit, Carluke, Lanarkshire, Scotland). After blocking with 5% Milk PBS, pH 7·4, as described above, the nitrocellulose strips with antigen were incubated at 4°C overnight with MoAb (protein A purified MoAb at 1 to 5 mg per ml diluted 1 : 500 in 5% Milk, PBS, pH 7·4). The strips were washed × 3 in 0·005% Tween-20

in PBS, pH 7·4, for 5 min each with rocking and incubated for 1 h at room temperature with a 1:100 dilution of HRP-conjugated sheep antiserum to mouse IgG in 5% Milk in PBS, pH 7·4. Following 3 × washes with rocking in 0·005% Tween-20 PBS, pH 7·4, for 10 min each, the strips were reacted with a solution of 50 mg of 3,3′-Diaminobenzidine tetrahydroxchloride dihydrate, and 20 mg Cobalt chloride and 20 μl of H_2O_2 (30% solution) (all from Sigma Chemical Co.) in 100 ml PBS pH 7·4. Bands usually developed in less than 1 min and the reaction was stopped by washing with water.

Results

MONOCLONAL ANTIBODIES 3E12 AND 3G12; ANTIBODIES AGAINST PFS 48/45, A GAMETE SURFACE PROTEIN OF *P. FALCIPARUM*

We have derived two mouse monoclonal antibodies (MoAbs) designated 3E12 and 3G12, both isotype IgG1, which react with the surface of live intact gametes of *P. falciparum* lines NF54 and 7G8 by immunofluorescence (data not shown). The MoAbs react with epitopes of Pfs 48/45 as shown by immunoblot (see below).

EFFECTS OF MOABS 3E12 AND 3G12 ON INFECTIVITY OF GAMETOCYTES OF *P. FALCIPARUM* TO MOSQUITOES

Gametocytes of *P. falciparum* isolate NF54 were fed to mosquitoes in the presence of protein A-purified MoAb 3E12 with or without the presence of active human

Table 1. Effect of monoclonal antibody 3E12 on infectivity of *P. falciparum* NF54 gametocytes to mosquitoes with and without active complement. Duplicate experiments were done; infectivity is expressed as mean number of oocysts per mosquito midgut. The number of mosquitoes infected/number dissected is given in parentheses

Concentration of 3E12 in blood meal (μg/ml)	Infectivity to mosquitoes			
	+ Complement		− Complement	
200	0	0	0	0
	(0/17)	(0/17)	(0/14)	(0/17)
40	0·33	0	0·4	0·42
	(4/18)	(0/10)	(4/13)	(2/12)
8	1·0	5·2	2·9	2·9
	(10/24)	(10/12)	(10/17)	(7/13)
1·6	6·3	11·5	10·8	6/2
	(18/22)	(7/15)	(15/15)	(12/13)
0·32	6·2	24·6	7·7	14·0
	(14/23)	(14/14)	(16/17)	(14/15)
M199 control	9·8	22·9	12·2	7·5
	(18/23)	(13/13)	(9/9)	(15/22)

complement. Certain mouse MoAbs activate human complement to suppress infectivity of malarial gametocytes to mosquitoes (Kaushal *et al.* 1983, Rener *et al.* 1983, Quakyi *et al.* 1987). As shown in Table 1, intact MoAb 3E12 suppressed infectivity of the gametocytes by 80 to 90% below control levels at a final concentration in the blood meal of 8 μg per ml; at concentrations above 40 μg per ml there was almost 100 percent suppression of infectivity. The level of suppression was not affected by the presence or absence of active human complement.

Fab fragments of 3E12, at molar concentrations equivalent to those of the intact MoAb, were as effective in suppressing infectivity of the gametocytes to mosquitoes as was the intact MoAb (Table 2).

When gametocytes of *P. falciparum* NF54 were fed in the presence of 3G12 ascites fluid diluted 1:2 in native human serum there was no loss of infectivity of the parasites to mosquitoes compared to controls (data not shown).

These results and those of previous studies are summarized for comparison in Table 3. It is clear that different MoAbs against Pfs 48/45 have different effects on the infectivity of *P. falciparum* gametocytes to mosquitoes and that these effects may depend on the line of parasite exposed to the antibody (see effects of MoAb IA3–B8). Two MoAbs, 3E12 and 32F3, were highly effective blockers of infectivity in complement-independent activities and 3E12 was equally effective as Fab fragments and this has also been found for 32F3 (T. Ponnudurai, personal communication); others, 32F1 and 3G12, had no transmission blocking effect at all. MoAbs IIC5–B10 and IA3–B8 had moderate or slight effects individually in the absence of active complement but were highly effective in suppressing gametocyte infectivity when present together. As with IA3–B8 alone, however, the activity of the mixture of IIC5–B10 and IA3–B8 was effective against the 7G8 line of

Table 2. Effect of Fab fragments of monoclonal antibody 3E12 on infectivity of *P. falciparum* NF54 gametocytes to mosquitoes; duplicate experiments were done; results expressed as in Table 1

Concentration of Fab fragments of 3E12 in blood meal (μg/ml)	Infectivity to mosquitoes	
120	0 (0/7)	0 (0/13)
24	0 (0/8)	0 (0/17)
5	0·2 (2/14)	6·1 (9/18)
1	13·0 (13/14)	9·9 (11/13)
0·2	17·9 (14/14)	9·8 (14/17)
M199 control	12·3 (11/12)	16·1 (14/15)

P. falciparum but not against the NF54 line. IA3–B8 was the only MoAb so tested which was significantly more effective in suppressing gametocyte infectivity in the presence of active human complement.

In an attempt to further understand the properties of the MoAbs against the Pfs 48/45 gamete surface protein, we have studied the epitopes recognized by these MoAbs on antigen from different lines of *P. falciparum* using immunoradiometric assays (IRMA) and immunoblots.

ANALYSIS OF EPITOPES RECOGNIZED BY MOABS ON PFS 48/45 BY IRMA

We have used MoAbs from among those described above in two site IRMA with antigen extracted in non-ionic detergent (Triton X-100) from lines 7G8 or NF54 of *P. falciparum* (Table 4).

The results presented define the presence of three apparently non-overlapping epitope

Table 3. Summary of effects of monoclonal antibodies against Pfs 48/45 in suppressing infectivity of *Plasmodium falciparum* gametocytes to mosquitoes

	Parasite clone tested			
	7G8		NF54	
Presence or absence of active complement	+C	−C	+C	−C
Monoclonal antibody				
3E12	ND	ND	+++[a]	+++[a]
3E12 (Fab)	ND	ND	+++[a]	ND
32F3	ND	ND	ND	+++[b]
IIC5–B10	+[c,d]	+[c,d]	+[d]	+[d]
IA3–B8	++[c,d]	+[c,d]	−[d]	−[d]
IIC5–B10+IA3–B8	+++[c,d]	+++[c,d]	+[d]	+[d]
32F1	ND	ND	ND	−[b]
3G12	ND	ND	−[a]	ND

In the studies referred to in this Table the final concentrations of purified antibody reported in the mosquito blood meals to give the effects indicated were: (a) (present study) 3E12 (intact MoAb) > 40 μg/ml; 3E12 (Fab fragment) > 24 μg/ml; 3G12 ascites fluid diluted 1:2 in human serum; (b) (Vermeulen *et al.* 1985a) 32F3 and 32F1,1 to 3 mg/ml; (c) (Rener *et al.* 1983) 0·5 to 4 mg/ml; (d), (Carter, unpublished results) 0·3 mg/ml.

+++ strong effect; suppressed > 98% below control values.

++ moderate effect; suppressed > 70% < 95% below control values.

+ slight effect; suppressed > 40% < 70% below control values.

− no apparent effect.

ND not done.

regions designated I, II and III. Of these, region II divides into two sub-regions, IIa and IIb. All regions and sub-regions appear to be represented once only on the antigen; thus, having bound the antigen in the first step of the two site IRMA a given MoAb was unable to react with it in the second step. MoAbs against different epitope regions, however, generally bound effectively in the second step. Two MoAbs were defined as reacting with the same epitope region if antigen bound by one MoAb was unable to react in the second

Table 4. Two site immunoradiometric assays (IRMA) with MoAbs against Pfs 48/45, using extracts of gametocytes from *P. falciparum* line 7G8 or line NF54. In each vertical column the same batch of ^{125}I labelled second MoAb was used in the same assay against 7G8 or NF54 antigen. The results are expressed as a percentage of the maximum counts achieved with that of ^{125}I labelled MoAb in the same assay on either 7G8 or NF54 antigen. Thus a figure of 100 represents the maximum amount of ^{125}I labelled MoAb which bound to antigen of either parasite line with any of the plate bound MoAbs indicated on the left hand side of the Table as plate-bound antigen-binding antibody. For most combinations two complete experiments were done; where a particular combination of MoAbs was omitted from one of the experiments it is indicated (ND) as not done. The epitope regions recognized by each MoAb, as deduced from interpretation of the results of the IRMA, are indicated as I, IIa, IIb, III

		^{125}Iodine-labelled, second antibody					
		I		IIa	IIb	III	
Epitope Region	Plate-bound first antibody	3E12	32F3	IA3–B8	32F1	IIC5–B10	Source of antigen
I	3E12	ND	ND	ND	ND	ND	7G8
		0·9	0·4	88·0	ND	23·0	
	32F3	ND	0·74	100	13·4	48·5	
		2·0	0·4	100	ND	32·0	
IIa	IA3–B8	ND	39·3	1·9	0·02	10·1	
		100	100	1·9	ND	42·0	
IIb	32F1	ND	5·0	0·6	0·05	3·5	
		4·3	4·0	1·2	ND	0·6	
III	IIC5–B10	ND	23·7	20·0	2·0	1·1	
		28·0	20·0	27·0	ND	−0·1	
I	3E12	ND	ND	ND	ND	ND	NF54
		0·8	0·7	0·5	ND	100	
	32F3	ND	2·4	−3·3	100	100	
		0·5	0·8	1·2	ND	96·0	
IIa	IA3–B8	ND	0·6	0·9	0·12	−0·17	
		0·2	0·8	0·4	ND	0·8	
IIb	32F1	ND	100	1·4	0·05	35·6	
		54·0	70·0	0·5	ND	12·6	
III	IIC5–B10	ND	76·3	2·7	16·4	8·3	
		63·0	44·0	0·2	ND	−0·5	

step with the other MoAb. According to these principles the results in Table 4 are interpreted in detail as follows.

MoAbs 3E12 and 32F3 each failed to bind in the second step to antigen bound by the other in the first step of the two-site IRMA; these MoAbs define epitope region I. Both 3E12 and 32F3, however, bound efficiently to antigen bound in the first step by MoAbs against other epitope regions e.g., IIC5–B10, against region III, or IA3–B8 against region IIa (with 7G8) or 32F1 against region IIb (with NF54). The converse of these situations was also true i.e., when antigen was bound by either 3E12 or 32F3, MoAbs against other epitopes generally reacted effectively against the bound antigen. Two MoAbs, IA3–B8 and 32F1, however, behaved differently depending upon the line of parasites used as a source of antigen; thus IA3–B8 did not bind to antigen of NF54 with any combination of MoAbs and 32F1 bound only weakly to antigen of 7G8 with MoAbs against other epitopes.

The results with IA3–B8 are explained by the complete absence of reactivity of this MoAb with NF54 antigen (as confirmed by its failure to react with gametes or gametocytes of NF54 in immunofluorescence reactions (data not shown) or with gametocyte antigen of NF54 on immunoblot (see below). Since both IA3–B8 and 32F1 reacted with antigen of 7G8 bound by the other MoAbs, i.e., 3E12, 32F3 and IIC5–B10 (confirmed by reactivity of both MoAbs with gametes of gametocytes of 7G8 by immunofluorescence (data not shown)) but each failed to bind to 7G8 antigen when the other was used in the first antigen-binding step, IA3–B8 and 32F1 both recognize the same epitope region, designated region II. Because the MoAbs behave differently, however, in reaction with antigen from 7G8 or from NF54, the epitopes recognized by IA3–B8 and

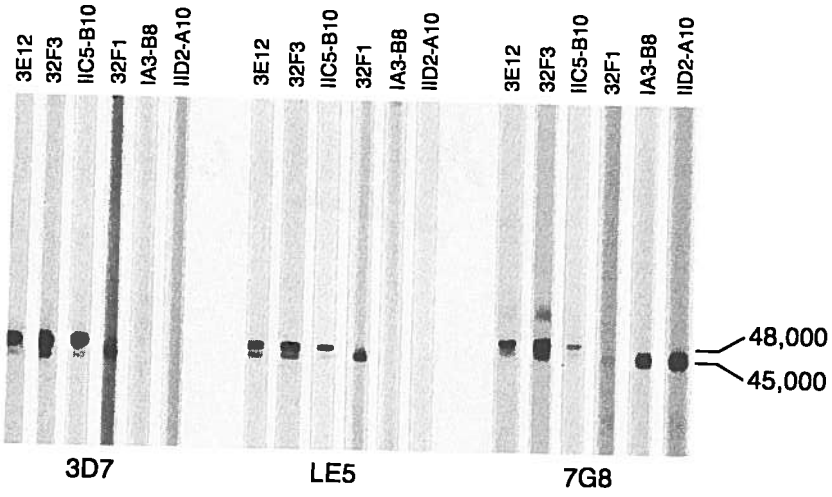

Figure 2. Immunoblot with MoAbs against *P. falciparum* gametocyte antigen separated without reducing agent on SDS-PAGE and transferred to nitrocellulose. The line of *P. falciparum* used as a source antigen is indicated at the bottom of each panel; the nitrocellulose strips were reacted with ^{125}Iodine labelled MoAbs as indicated at the top of each strip; the molecular weights of the peptides recognized are indicated on the right hand side; all antigen extracts were separated on the same SDS-PAGE.

32F1 must be in some way structurally distinct even though they appear to be spatially closely situated. Hence the designation of the two sub-region epitopes IIa, defined by IA3–B8, and IIb, defined by 32F1.

In addition to the marked differences between 7G8 and NF4 in region II, the properties of region III also differed, to a lesser degree, between the two lines of parasite. The relative efficiency of binding of IIC5–B10 to this epitope was consistently lower with antigen from 7G8 than from NF54. The properties of epitope region I, on the other hand, were similar in both lines of *P. falciparum*; MoAbs against region I bound strongly to antigen from either 7G8 or NF54.

ANALYSIS OF REACTIVITY OF MOABS WITH PFS 48/45 ON IMMUNOBLOT AND IMMUNOPRECIPITATION

The same MoAbs used in the IRMAs and also a sixth MoAb, IID2–A10 (isotype IgG1) (Carter *et al.* 1985) were reacted against antigen separated on SDS-PAGE and electroblotted onto nitrocellulose (Figure 2). The MoAbs were tested against antigen from 3 cloned parasite lines each from a different line of *P. falciparum*, 3D7 (a clone derived from NF54, Walliker *et al.* 1987), L.E5 (from Liberian isolate, Graves *et al.* 1985) and 7G8 (Burkot *et al.* 1984). The antigen extracts were all made and run under non-reducing conditions (as described in Materials and methods).

MoAbs 3E12 and 32F3 (epitope region I) reacted strongly with the 48 kDa peptide and less strongly with the 45 kDa peptide of Pfs 48/45 with antigen from all three clones. MoAb IIC5–B10 (epitope region III) reacted strongly with the 48kDa peptide but weakly

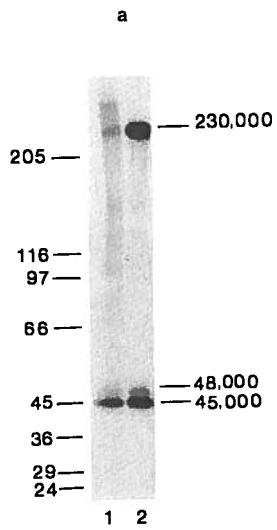

Figure 3a. Immunoprecipitation with MoAb 3G12, lane 1 and IIC5–B10, lane 2 of a Triton X-100 extract of gametes of *P. falciparum*, clone 3D7, surface labelled with ^{125}Iodine. The immunoprecipitates were separated on a 5 to 15% acrylamide gradient gel on SDS-PAGE, and their positions visualized by autofluorography. Standard molecular weight markers (× 1000) are indicated on the left and molecular weights of the radiolabelled gamete surface proteins immunoprecipitated on the right.

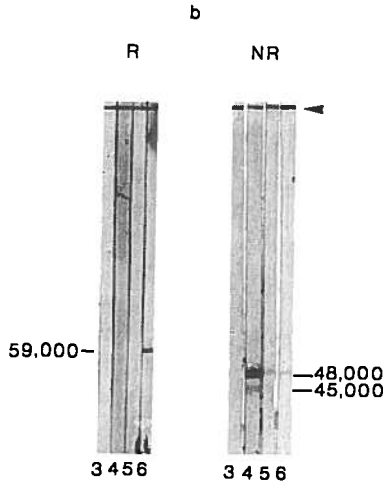

Figure 3b. Immunoblot with MoAbs against extracts of *P. falciparum*, clone 3D7, gametocytes separated with (panel R) or without (panel NR) reducing agent on SDS-PAGE and transferred to nitrocellulose. The strips were reacted with unlabelled MoAbs as follows: lanes 3, no antibody added; 4, 3E12; 5, IIC5–B10; 6, 3G12; the strips were then incubated with horse radish peroxidase-conjugated sheep antiserum to mouse IgG and the position of the antigen recognized by the MoAbs visualized by a colour reaction with horse radish peroxidase (see Materials and methods); the molecular weights of the reduced and non-reduced antigens are indicated on either side of the panels; the reduced and non-reduced antigens were run in separated sections of the same SDS-PAGE; the position of the top of the gel is marked by an arrow.

with the 45kDa peptide from all three clones; reactivity of IIC5–B10 with LE5, and especially 7G8, appeared to be weaker than with 3D7. MoAbs against epitope region II (32F1, IA3–B8 and IID2–A10) reacted predominantly with the 45kDa peptide and weakly with the 48kDa peptide of Pfs 48/45. MoAbs IA3–B8 and IID2–A10 (both of which can be concluded to react with epitope sub-region IIa in accordance with previous results (Carter *et al.* 1985)) were totally non-reactive with clones 3D7 and LE5 but reacted strongly with 7G8. MoAb 32F1 (epitope IIb) reacted strongly with 3D7 and LE5 and very weakly with 7G8. Reactivity of all epitopes with these MoAbs was destroyed when the antigens were extracted and separated on SDS-PAGE in the presence of a reducing agent (2-mercaptoethanol) (Figure 3 and data not shown).

Another MoAb, 3G12 (isotype IgG1) immunoprecipitated the 48 and 45 kDa proteins from extracts of surface radioiodinated gametes of *P. falciparum* (Figure 3a) and reacted with the 48 kDa peptide of Pfs 48/45 on immunoblot (Figure 3b). The epitope recognized by 3G12, however, is not destroyed under reducing conditions but gives an even stronger reaction than the non-reduced antigen (Fig. 3b). This epitope is, therefore, clearly distinct from those previously defined and is designated epitope region IV.

Discussion

Using MoAbs against the Pfs 48/45 gamete surface protein of *P. falciparum* we have defined several distinct epitope regions on the peptides comprising this target antigen of

malaria transmission-blocking antibodies. The same MoAbs have been tested in this or previous studies (Rener *et al.* 1983; Vermeulen *et al.* 1985a, Graves *et al.* 1985) for their effects on the infectivity of different lines of *P. falciparum* to mosquitoes. Combining present with previous observations we propose the following synthesis.

Pfs 48/45 consists of two peptides of apparent molecular size on SDS-PAGE of 48 kDa and 45 kDa when separated under non-reducing conditions and about 59 kDa and 53 kDa respectively under reducing conditions (Rener *et al.* 1983). The two peptides appear to be related being almost always recognized by the same MoAbs on immunoblot and are co-immunoprecipitated by these MoAbs from extracts in non-ionic detergents such as Triton X-100 (Vermeulen *et al.* 1985a and 1986 and present study).

In two site radioimmunoassay (IRMA) the MoAbs have been used to define three spacially separate epitope regions designated I, II and III. The reactivity of MoAbs with each of these regions is destroyed by reduction of the antigen (Vermeulin *et al.* 1985a and present study). None of the regions appears to be repeated on the target antigens according to the results of IRMA (present results and Carter *et al.* 1985, Vermeulen *et al.* 1985b). This suggests that the 48 kDa and 45 kDa peptides are dissociated from each other in the Triton X-100 extracts used in IRMA in these studies.

No difference was detected between lines of *P. falciparum*, NF54, LE5 and 7G8, in reactivity by IRMA or immunoblot with MoAbs against Pfs 48/45 epitope I. The results of IRMA suggest that MoAbs against Pfs 48/45 epitope region I bind more strongly to the antigen than do MoAbs against other epitopes.

Epitope region II divides into two subregions, IIa and IIb. Both subregion epitopes appear to be structurally distinct between different lines of *P. falciparum*. Subregion IIa is defined by MoAb IA3–B8 which reacted with gametocyte antigen of line 7G8 but not of NF54 (present study) L.E5 (present study and Graves *et al.* 1985). Subregion IIb is defined by MoAb 32F1 which reacted strongly with Pfs 48/45 from line NF54 by IRMA and on immunoblot but weakly in these tests with antigen from line 7G8. MoAb 32F1 reacted by IFA with gametocytes of both NF54 and 7G8 confirming the presence of subregion IIb in both lines of parasite.

Different forms of epitope region III also occurred between lines 7G8 and NF54. The difference between the lines in the form of this epitope was, however, relatively slight and was shown in IRMA and immunoblot by a reduced binding of MoAb IIC5–B10 to Pfs 48/45 from 7G8 compared to that from NF54.

A fourth epitope of Pfs 48/45 is defined by MoAb 3G12. In contrast to other epitopes of Pfs 48/45, discussed here, that recognized by 3G12 is not only stable in reduced form, but binds to antibody even more strongly than does the non-reduced antigen. The 3G12 epitope must, therefore, be structurally distinct from the others and is here designated Pfs 48/45 epitope region IV.

The binding of MoAbs to the 48 and 45 kDa proteins on immunoblot differed according to epitope region. Thus MoAbs against regions I, III and IV bound predominantly to the 48 kDa protein; those against region II bound mainly to the 45 kDa protein. This relative binding preference was not, however, apparent on immunoprecipitation of the proteins. The molecular basis of these observations is unclear but should become more so when the gene(s) coding these proteins have been cloned and sequenced.

The relationship between the properties of epitopes recognized by MoAbs against Pfs 48/45 and the effects of these MoAbs on infectivity of *P. falciparum* to mosquitoes is of interest. MoAbs against epitope region I appear to have the strongest transmission

blocking activity amongst those MoAbs so far studied (3E12, present study; 32F3 Vermeulen *et al.* 1985). These MoAbs can, in the absence of other antibodies or active complement, totally suppress infectivity of *P. falciparum* gametocytes to mosquitoes; 3E12 (present study) and 32F3 (T. Ponnudurai, personal communication) have both been shown to be as effective in monovalent form as Fab fragments, as is the intact MoAb. Almost total suppression of infectivity was achieved with a final concentration in the blood meal of 40 μg/ml of 3E12 MoAb or 24 μg/ml of 3E12 Fab fragments. It has been previously shown that antibodies against this epitope region prevent ookinete formation in the mosquito midgut presumably because they prevent fertilization of the gametes (Vermeulen *et al.* 1985a). The precise mechanism by which this may occur is unclear. The effect of the monovalent Fab fragments of 3E12 cannot be achieved by cross linking or agglutination reactions; nor indeed can that of the intact MoAb 3E12 as it reacts against an epitope which is not repeated on the target antigen; nor is complement-mediated killing involved in the suppression of gametocyte infectivity. The effect must be due to interference by the antibody with some essential biological function mediated on the gamete surface; the putative ligands involved in fertilization are a possible target.

MoAbs against epitope regions II and III were relatively inefficient in suppressing infectivity of *P. falciparum* gametocytes when presented individually in the absence of active complement. In combination, however, MoAbs IA3–B8 and IIC5–B10 (against regions II and III respectively) were highly effective in suppressing infectivity of *P. falciparum* to mosquitoes (Rener *et al.*, 1983). Similar synergistic effects of MoAbs were noted in studies on transmission blocking immunity in the chicken malaria parasite *P. gallinaceum* (Rener *et al.* 1980). In these studies the synergistic transmission blocking activity of two MoAbs was associated with a corresponding tight agglutination of male gametes of the parasite by the combination of MoAbs. We have not observed agglutination of male gametes of *P. falciparum* with IA3–B8 and IIC5–B10 or any combination of MoAbs. It is of interest to note, however, that the synergistic effect of the anti-Pfs 48/45 MoAbs is between antibodies against two different epitope regions. For epitopes which are not repeated on the target molecule this would be essential to achieve a network of cross-linked antibody and antigen. How this may function to suppress infectivity of the gametes to mosquitoes is not yet clear, but such cross linking would strongly impede the free movement of these molecules on the gamete surface.

Either alone or in combination with IIC5–B10, IA–B10 was without effect on the infectivity of lines of *P. falciparum* such as L.E5 (Graves *et al.* 1985) or NF54 (present study, T. Ponnudurai, personal communication) both of which are Pfs 48/45 epitope region IIa$^-$ and therefore do not react with IA3–B8. Alone amongst the MoAbs so tested, MoAb IA3-B8 was significantly more effective in suppressing the infectivity of parasites to mosquitoes in the presence, than in the absence, of active complement.

MoAb 3G12 against Pfs 48/45 epitope region IV had no detectible effect on the infectivity of *P. falciparum* gametocytes to mosquitoes. It may be relevant that this is the only epitope among those studied which is not dependent upon protein tertiary structure (i.e., the presence of intact disulphide bonds on the protein). Indeed following reduction of disulphide bonds, epitope IV was even more reactive with MoAb 3G12 than it was in the non-reduced protein. This could arise if the epitope represents a linear amino acid sequence or other structure (Pfs 48/45 contains both carbohydrates and fatty acid chains in its structure, Vermeulen *et al.* 1986; Carter, Fenton & Alano, unpublished data) which is partially obscured by the folding of the protein due to disulphide bonds.

The present analysis may help provide insight into which regions of Pfs 48/45 may be most important as target epitopes of transmission blocking antibodies for inclusion in a potential candidate antigen for a *P. falciparum* malaria transmission blocking vaccine. In a separate report (Foo *et al.* 1990) we present the results of a study of the diversity or conservation of the epitopes of Pfs 48/45 among natural isolates of *P. falciparum*.

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