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Antibodies to *Plasmodium falciparum* gamete surface antigens in Papua New Guinea sera

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Summary Sera from individuals living in malaria endemic areas of Papua New Guinea were tested for their effect on infectivity of *Plasmodium falciparum* gametocytes grown in culture to *Anopheles freeborni* mosquitoes. Consistent reduction of infectivity to less than 5% of control was observed with nine out of the 41 sera from the endemic area tested and also with three out of seven sera tested from individuals rarely exposed to malaria infection. Gamete surface antigens recognized by the sera were investigated by immunoprecipitation from ^{125}I surface-labelled gametes extracted in SDS and Triton X-100. The main antigens recognized were of the same mol. wt (230, 48 and 45 kD) as those known to be targets of transmission-blocking monoclonal antibodies. A significant negative correlation was observed between the total ct/min immunoprecipitated from surface-labelled gametes by the sera and the average number of oocysts per gut observed in membrane feeding experiments with these sera. Spearmann's rank correlation coefficient indicated that suppression of infectivity correlated strongly with the presence of antibodies against the 230 kD protein; there was no significant correlation between suppression and antibodies to the 48/45 kD proteins. The antibody response to the different gamete surface antigens varied greatly in sera from the endemic areas suggesting that individuals respond differently to each gamete antigen.

Keywords: *Plasmodium falciparum*, transmission blocking, gamete, Papua New Guinea

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Introduction

Transmission of malaria in human populations takes place when anopheline mosquitoes become infected with the parasites following a bloodmeal on individuals with parasites circulating in their blood. Within the mosquito bloodmeal the intracellular male and female gametocytes emerge from their host erythrocytes and fertilization of gametes takes place usually within 10–20 min after infection. The zygotes then transform into motile forms called ookinetes which invade the mosquito midgut wall to continue development on the serosal surface of the midgut adjacent to the haemocoel.

Antigens on the surface of gametes and zygotes of the human malaria *Plasmodium falciparum* have been shown to be the targets of monoclonal antibodies (MoAb) which when ingested together with the parasites in a bloodmeal can block their infectivity to mosquitoes (Rener *et al.* 1983, Vermeulen *et al.* 1985). These antigens include a protein of 230 kD and two proteins closely related to each other of 48 and 45 kD, all of which are present on the surface of the gametes as they emerge into a mosquito bloodmeal. Another protein of 25 kD which appears on the zygote surface some hours after gametogenesis in the mosquito midgut is also a target of transmission-blocking antibodies (Vermeulen *et al.* 1985).

The gamete surface proteins of 230, 48 and 45 kD are already present in the gametocytes of *P. falciparum* prior to gametogenesis (Kumar & Carter 1984, Vermeulen *et al.* 1986). The 25 kD zygote surface protein, on the other hand, is synthesized almost entirely after gametogenesis although low levels of synthesis appear to take place in the gametocytes (Vermeulen *et al.* 1986). The presence of the 230, 48 and 45 kD proteins in the gametocytes means that these proteins are potentially capable of inducing an immune response during natural infections of *P. falciparum*. In the present study we have looked for the presence of antibodies against those antigens in human populations in Papua New Guinea (PNG) where intense transmission of malaria takes place.

Materials and methods

Venous blood samples of 5–10 ml were collected in 1984 from consenting adults attending the outpatients department at Yagaum Health Centre, Madang, Papua New Guinea. Blood samples were also drawn in August 1985 from a cross-sectional sample of individuals over the age of 4 years attending malariometric surveys in the villages of Agan (Madang Province) and Tau (East Sepik Province). In both villages malaria is highly endemic, although transmission is more intense in Tau. The crude parasite rate at the time of the survey in Agan was 30% (21·4% *P. falciparum*, 1·4% *P. vivax* and 8·6% *P. malariae*, $n=70$). In Tau the crude parasite rate was 50% (47·9% *P. falciparum*, 6·3% *P. vivax*, 12·5% *P. malariae*, $n=48$). At Goroka hospital, situated in the Eastern Highlands province at an altitude too high for malaria transmission, sera were collected from adults who had recently visited a coastal area and who presented to the outpatients department with suspected malaria. In all cases blood was allowed to clot and the serum collected and frozen at −20°C until shipped to the National Institutes of Health (NIH), Bethesda, Maryland where it was heat inactivated and stored at −20°C.

Isolates of *P. falciparum* were grown in culture for production of gametocytes either in flasks as previously described (Rener *et al.* 1983) or in the tipper system (Ponnudurai *et al.*

1982). The NF54 isolate was kindly provided by Dr T. Ponnudurai; the 3D7 clone was derived from NF54 by Dr D. Walliker by the limit dilution technique.

Gametocytes from 14–16-day-old cultures grown in O+ erythrocytes were suspended for 30 min at 20% haematocrit in Tris saline glucose (TSG; 7 mM Tris, 120 mM NaCl, 8 mM glucose) with 30 mM $NaHCO_3$ and 10% non-immune heat-inactivated human serum, adjusted to pH 8·3. An extract of homogenized *Anopheles freeborni* pupae (200 pupae per 10 ml TSG, pH 7·4, centrifuged at 15 000 *g* and filtered through 45 μ filter) which contains a potent exflagellation-stimulating factor (Nijhout 1979) was added at a 1:5 dilution. After separation on a three-step Nycodenz (Nycomed UK Ltd, Sheldon, Birmingham, UK) gradient (Vermeulen *et al.* 1985) the purified extracellular gametes and zygotes (containing not more than 20% of erythrocytes and other material) were surface radioiodinated with ^{125}I by the lactoperoxidase method (Rener *et al.* 1983). In initial experiments the labelled gametes were extracted in 1% Triton X-100 with protease inhibitors as previously described (Kumar & Carter 1984). In experiments with the sera from Agan, Tau and Goroka, the labelled cells were extracted first in 1 ml of 2% sodium dodecyl sulphate (SDS) in phosphate-buffered saline (PBS) for 5 min with occasional vortexing at room temperature. Then a volume of 10% Triton X-100 in PBS was added equal to 0·2 of the volume of 2% SDS and the mixture vortexed for 1 min at room temperature. The 2% SDS is added to break the complex that exists between the 230 kD and 48/45 kD gamete proteins (Kumar 1987) and thus allows these proteins to be immunoprecipitated independently by antibodies. Subsequent addition of Triton X-100 neutralizes the SDS in mixed micelles preventing it from disrupting the antibodies themselves during immunoprecipitation.

Amounts of antigen extract containing 10^5 trichloracetic acid precipitable ct/min were used for immunoprecipitation with 20 μl of human serum. The mixture was incubated for 1 h at 4°C and 50 μl of a 25% suspension of protein-A-Sepharose CL 4B (Pharmacia) in NETT (0·15 M NaCl, 5 mM EDTA, 50 mM Tris, 0·5% Triton, pH 7·4) added and incubated overnight at 4°C with rocking. Following incubation the protein-A-Sepharose beads absorbed with immune complexes were washed twice with 5% low-fat milk powder in NETT, once with NETT + 0·5 M NaCl and twice with NETT in a fresh tube. The beads were extracted in sample buffer (without reducing agent) and the extracts separated on SDS-PAGE on 5–15% acrylamide gradient gels as previously described (Rener *et al.* 1983). Gels were dried on Whatman CF11 filter paper and autoradiographed.

In some experiments, individual radiolabelled protein bands on the dried down gels were identified following autoradiography and the bands cut out and counted in a gamma counter. The amount of radioactivity immunoprecipitated of a specific protein band was expressed as a percentage of the amount of radioactivity in the corresponding protein band in the antigen sample used for immunoprecipitation.

The sera were also tested for their effects on the infectivity of gametocytes of *P. falciparum* to *Anopheles freeborni* mosquitoes. Suspensions of 14–16-day-old cultured cells, fresh human erythrocytes and sera were fed to the mosquitoes through water-jacketed membrane feeders as previously described (Rener *et al.* 1983, Graves *et al.* 1985). Each bloodmeal contained 40 μl of non-immune serum (any blood type), 60 μl of packed O+ type human erythrocytes, freshly drawn and washed in heat-inactivated non-immune serum (any blood type), 70 μl of Papua New Guinea serum (heat-inactivated) and 70 μl of a suspension of *P. falciparum* gametocyte infected O+ cells at 60% haematocrit in non-immune serum. The cultured cells in this suspension were mixed with up to 4 volumes of

uninfected freshly drawn O+ erythrocytes. Controls consisted of at least two different non-immune (USA) sera included in duplicate in each experiment instead of the PNG serum.

Mosquitoes were kept at 26°C and 80% relative humidity for 8–10 days after which they were dissected and oocysts (products of parasite fertilization) on their stomachs were counted.

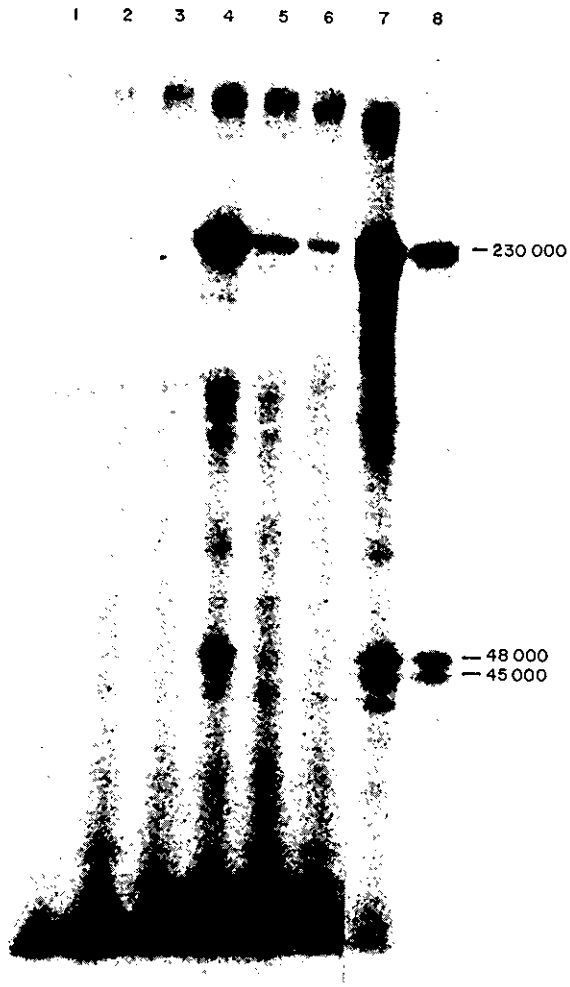


Figure 1. *Plasmodium falciparum* (NF54-3D7) gamete surface proteins from gametes surface-labelled with ^{125}I, extracted with Triton X-100 and immunoprecipitated with the following sera from Yagaum Health Centre, Madang. Track 1: control USA serum; tracks 2–6: sera JMS/2 (50, 42, 49, 40, 39); track 7: rabbit anti-gamete serum; track 8: anti-gamete MoAb (IIC5-B10). (This MoAb immunoprecipitates the 230 kD and 48 and 45 kD gamete surface proteins as a complex from Triton X-100 extracts; the target epitopes, however, are all on the 48/45 kD proteins and not on the 230 kD protein.)

Results

Initial studies with the panel of sera from adults attending Yagaum Health Centre, Madang, showed that several of them precipitated antigens from ^{125}I surface labelled gametes which had been extracted in 1% Triton X-100 (Figure 1). The main surface antigens recognized were of the same mol. wt (230 and 48/45 kD under non-reducing conditions) as the target antigens of anti-gamete MoAb and of sera from rabbits which had been immunized with gametes of *P. falciparum* (Figure 1). Experiments in which the

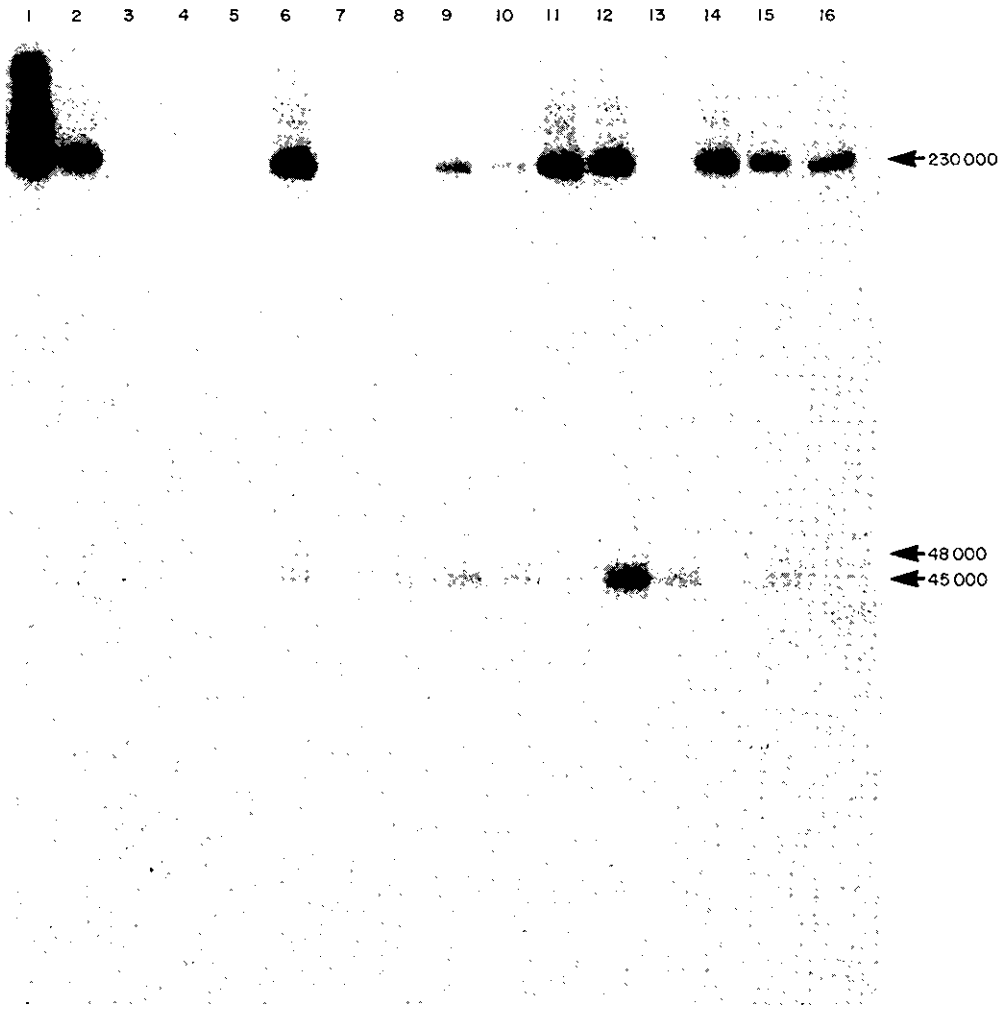


Figure 2. *Plasmodium falciparum* gamete surface antigens labelled with ^{125}I, extracted with SDS and Triton X-100 and immunoprecipitated with the following sera from Agan (A) and Tau (T) villages and Goroka hospital (G). Tracks 1–15: T547, T510, three non-immune USA sera, G15, G13, G11, G8, G7, G6, A67, A41, A34, A29; track 16: ^{125}I labelled gamete antigen extract. The presence of SDS disrupts the complex between the 230 kD and 48/45 kD gamete surface proteins.

Table 1. Gamete surface antigens immunoprecipitated by Papua New Guinea sera and the effects of the sera on the infectivity of *P. falciparum* to *Anopheles freeborni* mosquitoes

Serum*	Age (years)	Parasite status‡	Infectivity (per cent of control)	Antigen immunoprecipitated (per cent of antigen added)§	
				230 kD	48/45 kD
T447	50	Neg	0·6	22·0	<4
T448	46	Pf	1·0	0·4	<4
A 67	15	Neg	1·0	19·0	28·0
T438	54	Neg	1·2	1·0	<4
T450	21	Pf+Pv	1·4	3·5	<4
T489	55	Neg	1·4	0·4	<4
G 15		Pf+Pv	3·5	23·0	7·0
T449	12	Pf	3·8	0·4	<4
G 8		Pv	3·9	6·7	14·4
G 13		Pf+Pfg	4·3	0·0	<4
T427	55	Neg	4·7	0·4	<4
T547	20	Neg	4·9	43·3	5·6
T510	32	Neg	5·9	14·8	6·7
T426	7	Pf	11·0	11·1	<4
G 6		Neg	11·7	12·4	3·7
G 11		Pf+Pfg	11·9	3·3	9·1
G 7		Neg	12·5	4·1	13·0
T374†	41	Neg	18·0	0·6	2·2
T353	11	Pf	20·0	14·6	3·0
T378	4	Pf+Pm	27·0	5·9	4·6
A 29	45	Neg	29·0	17·3	9·2
A 21	25	Neg	30·5	14·7	10·0
T333†	50	Neg	33·0	1·1	3·4
T400	10	Pf	35·0	1·1	<4
A 34	18	Pf	38·0	9·6	4·3
T314†	23	Neg	46·0	0·6	1·5
T370†	74	Neg	52·0	0·7	3·5
A 5†	12	Pf	56·0	13·8	20·0
T362†	58	Neg	59·0	0·8	1·5
T380†	51	Neg	60·0	1·4	2·1
T451	14	Neg	65·0	3·0	<4
T452	4	Pf	66·0	4·1	<4
T315†	20	Neg	67·0	0·4	1·9
T376†	14	Pf+Pm	68·0	0·4	2·1
G 5		Neg	68·0	1·2	0·8
T373	45	Neg	70·0	2·3	3·2
T350†	37	Neg	73·0	0·9	3·4
A 3	50	Neg	89·0	14·3	4·4
T377†	11	Pf+Pm	124·0	0·3	1·8
T336†	10	Pf	126·0	0·4	2·3
A 41	33	Neg	138·0	1·0	24·8
T384†	9	Neg	152·0	<2	<2

Table 1 (*cont.*)

T375†	20	Pf+Pm	155·0	0·3	1·5
A 40	19	Neg	170·0	0·4	1·4
T381	50	Neg	195·0	0·7	2·6
A 68	14	Neg	197·0	0·5	5·0
A 65	19	Neg	204·0	0·6	2·5
A 20†	24	Neg	224·0	0·4	2·7

* T, Tau; A, Agan; G, Goroka.

† Serum tested once only.

‡Blood slide results at time of bleeding. Neg, Negative; Pf, *P. falciparum*; Pfg, *P. falciparum* gametocytes; Pm, *P. malariae*; Pv, *P. vivax*.

§ Values are given as <4 or <2 when the absolute value for ct/min of immunoprecipitated antigen was too low for more accurate relative values to be determined.

Papua New Guinea sera were fed to mosquitoes together with *P. falciparum* gametocytes indicated that those sera which precipitated gamete surface antigens also blocked infectivity of parasites to mosquitoes. Thus of the sera in Figure 1, sera JMS/2·39 and 49 blocked infectivity of the parasites to mosquitoes by at least 95% compared to controls, serum JMS/2·40 blocked by 80% but sera JMS/2·50 and 42 each blocked less than 15% compared to controls.

In view of these preliminary results, further studies were made on a panel of 41 sera taken from a cross-section of ages (>4 years) in two village populations (Agan and Tau) in endemic areas. In addition seven sera from non-immunes attending Goroka hospital were included. Results of immunoprecipitations from gametes extracted in SDS followed by Triton X-100 with some of these sera are shown in Figure 2. It should be noted that protein A binds only to IgG subclasses 1, 2 and 4, and that the protein A will not be in excess if the concentration of these IgG subclasses in the sera exceeds 1·25 g/100 ml. Thus the immunoprecipitations may be regarded as a comparative, rather than a fully quantitative, measure of the amount of anti-gamete antibody in the sera. Many sera precipitated the 230 kD protein and/or amounts of the 48/45 kD doublet (Table 1).

One panel of 18 sera (11 from Agan, 3 from Tau and 4 from Goroka) was tested simultaneously in two feeding experiments with *P. falciparum* (NF54-3D7); the same sera were tested together in a single immunoprecipitation experiment. Results from sera used in this panel are therefore directly comparable with each other (Figure 3). A strong negative correlation ($r=0·807$) was found between the total ct/min immunoprecipitated by the sera and the average number of oocysts per gut observed in membrane feeds with these sera (mean of two experiments) (Figure 3). In this experiment, the ct/min associated with each antigen separately were not measured; nevertheless the regression coefficient was highly significant ($t=5·47$, $P<0·001$).

In order to compare the results obtained during different feeding experiments, in which control infectivity varied, results with individual sera (oocysts per gut) were normalized by expressing them as a percentage of the mean control infectivity in each experiment. These normalized infectivities, ranked in order of magnitude of suppression, were correlated with the amount of antigen counts immunoprecipitated for the 230 kD and 48/45 kD gamete surface proteins standardized as described in the Materials and

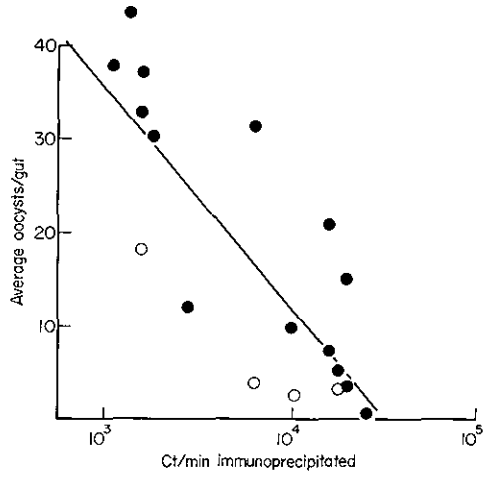


Figure 3. Relationship between average oocysts per gut (mean of two experiments) observed on stomachs of *Anopheles freeborni* mosquitoes after feeding on cultured *P. falciparum* gametocytes in the presence of Papua New Guinea sera, and the number of ct/min immunoprecipitated from ^{125}I surface-labelled gametes by the sera. All sera were tested simultaneously. (●) Sera from Agan and Tau; (○) sera from Goroka. The regression line is described by the equation $y = 106{\cdot}8 - 23{\cdot}76x$. $t = 5{\cdot}47$, $P < 0{\cdot}001$. The correlation coefficient $r = -0{\cdot}807$.

methods section (Table 1). Except where indicated, the normalized infectivity values are the mean of at least two experiments. Nine out of 41 sera from endemic areas consistently reduced infectivity to less than 5% of control, as did three out of the seven sera from individuals from a non-endemic area. Spearmann's rank correlation coefficient showed there to be a significant negative correlation between infectivity and counts associated with the 230 kD protein ($\rho = -0{\cdot}384$, $P < 0{\cdot}01$). Correlation with the 48/45 kD doublet was not significant ($\rho = -0{\cdot}142$, $P = 0{\cdot}17$). For serum donors living in the endemic areas of Agan and Tau, there was no significant correlation between age and the amount of 230 kD antigen immunoprecipitated ($\rho = 0{\cdot}019$).

Discussion

The results of this study have shown that antibodies are generated to surface antigens of the gametes of *P. falciparum* in many individuals exposed to malaria transmission in Papua New Guinea. The main gamete surface antigens recognized by their sera were the 230, 48 and 45 kD proteins, each of which has been previously shown to be a target of MoAb which block infectivity of *P. falciparum* to mosquitoes (Rener *et al.* 1983, Vermeulen *et al.* 1985, Quakyi *et al.*, in press). In the present study a strong correlation was found between the presence of antibodies against gamete surface antigens and suppression of infectivity of *P. falciparum* to mosquitoes by Papua New Guinea sera. In particular, suppression of infectivity correlated strongly with the presence of antibodies against the 230 kD protein. There was no significant correlation between suppression and antibodies to the 48 and 45 kD proteins, although such a correlation would not be

expected in this case because of the small number of individuals responding to this antigen.

It is not clear what effect such anti-gamete antibodies may have on the natural transmission of malaria in Papua New Guinea. However, results from the Goroka sera, collected from individuals rarely exposed to malarial infection, suggest that anti-gamete malaria transmission-blocking antibodies may be produced even during a primary attack of malaria. Rapidly induced, effective transmission-blocking antibodies have been reported during primary attacks in a high proportion of *P. vivax* infections in Sri Lanka (Mendis *et al.* 1987). It appears, therefore, that anti-gamete transmission-blocking antibodies may be readily induced during human malaria infections. However, enhancement of infectivity was also observed with several of the sera in this study, and has been previously reported in sera from *P. vivax* infected people in Sri Lanka (K. N. Mendis, personal communication). These effects of transmission blocking or enhancement during infection must be further elucidated since they are of great importance to the effectiveness of malaria transmission-blocking vaccines based on gamete surface antigens.

Our results also indicate that the antibody response to the different gamete surface antigens varied considerably between different sera. Discrepancy in response to these antigens varied from those sera in which only antibodies to the 230 kD protein appeared to be present to others, more rarely, in which only the 48/45 kD proteins were recognized. There was no relationship between age and recognition of particular antigens. Such results are hard to explain as a consequence of variable degrees of exposure to different gamete antigens and suggest that individuals have responded differently to each gamete antigen.

Genetically restricted responses to malarial antigens have been shown in congenic strains of mice differing in the major histocompatibility (MHC) locus (Good *et al.* 1986, Del Giudice *et al.* 1986). This locus controls the recognition of helper T-cell epitopes on target antigens and thus largely determines whether a particular MHC haplotype can mount an antibody response to a particular antigen. Similar genetically determined differences in humans could account for the different responses to the gamete surface antigens. Such a situation could complicate the use of a malarial vaccine based on these antigens, if certain individuals were genetically incapable of mounting an immune response to specific malarial antigens. We are now investigating whether an association exists in humans between HLA type and antibody response to gamete surface antigens in *P. falciparum*.

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