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Regulation of the immune response in *Plasmodium falciparum* malaria: IV. T cell dependent production of immunoglobulin and anti-*P. falciparum* antibodies *in vitro*

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SUMMARY

T cells from patients with acute *Plasmodium falciparum* malaria were investigated for induction of immunoglobulin- or anti-malaria antibody secretion *in vitro*. Stimulation of autologous T/B cell mixtures (2T:1B) with low concentrations of *P. falciparum* antigen and cultured for 12 days gave rise to a T-dependent IgG secretion which was significantly elevated over that in medium controls. This was achieved with both a crude *P. falciparum* antigen and a partially purified preparation enriched in Pf 155, a merozoite-derived antigen deposited in the red cell membrane at invasion (Perlmann *et al.*, 1984). Control antigen (RBC ghosts) induced IgG secretion only when added at high concentrations (> 10 μg/ml). Neither of the antigens induced IgG secretion at concentrations of ≤ 10 μg/ml in control cultures of lymphocytes from patients with *P. vivax* malaria. Supernatants from cultures of *P. falciparum* patients frequently contained anti-*P. falciparum* antibodies when nanogram quantities (10–100 ng/ml) of either one of the two malaria antigen preparations was used for stimulation. No anti-*P. falciparum* antibodies were induced by the control antigen at any concentration. The induced anti-*P. falciparum* antibodies were directed to intracellular parasites and, at lower frequencies, to Pf 155 as detected on the surface of infected erythrocytes. The induction *in vitro* of anti-*P. falciparum* antibodies appeared to be correlated with the presence of such antibodies in the sera of the lymphocyte donors. The lymphocytes of only one out of eight *P. vivax* patients responded to antigen stimulation by secreting anti-*P. falciparum* antibodies. However, this donor (but not any of the others), was also *P. falciparum* seropositive. Taken together, these results indicate that the induction of anti-*P. falciparum* antibody secretion reflects a secondary response *in vitro* of cells primed *in vivo*. The present experimental system should be well suited to map parasite antigen for their capacity to induce T cell dependent responses in *P. falciparum* malaria.

Keywords *Plasmodium falciparum* malaria immunoglobulin synthesis parasite antigen

INTRODUCTION

Although protective immunity to malaria parasites is regulated by the T cell system and may in part

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be cell-mediated (Jayawardena, 1981; Weidanz & Grum, 1983; Allison & Eugui, 1983), there is good evidence that antibodies play an important role in providing protection (Cohen, 1979). However, efficient and long lasting antibody responses in malaria infections are T dependent (Jayawardena, 1981; Weidanz & Grum, 1983; Taylor & Siddiqui, 1979; Allison & Engui, 1983). It has been shown that proliferative lymphocyte responses *in vitro* to crude malarial antigens of patients with acute *P. falciparum* malaria are shortlived and generally low, suggesting the occurrence of suppressor mechanisms (Taylor & Siddiqui, 1979; Brasseur *et al.*, 1983; Troy-Blomberg *et al.* 1983; 1984; Theander *et al.*, 1986). Suppression may involve both accessory cells and T cells. However, little is known how T cells regulate antibody production in human plasmodial infections. In this study we have developed a T/B-cooperation system allowing assay of the T cell dependent production of anti-malaria antibodies *in vitro*. For lymphocyte stimulation and assessment of antibody specificity we used both a crude *P. falciparum* antigen and a more defined preparation, enriched in a merozoite-derived heat-stable polypeptide (Pf 155, M_r 155, 000 D) deposited in the membrane of invaded erythrocytes (Perlmann *et al.*, 1984; Berzins *et al.*, 1985).

MATERIALS AND METHODS

Patients. Blood samples from a total of 19 patients with acute *P. falciparum* were included in this study. Four out of these were Africans hospitalized in Sweden, whereas the others were Colombians (South America).

Controls. Eight donors acutely infected with *P. vivax* malaria were used as a control group. They were all Colombians living in the same area as the Colombians with acute *P. falciparum* malaria.

Preparation and fractionation of peripheral blood lymphocytes. Human peripheral blood lymphocytes (PBL) were isolated from defibrinated blood by gelatin sedimentation, carbonyl iron treatment and Ficoll-Isopaque (Pharmacia Fine Chemicals, Uppsala, Sweden) centrifugation (Perlmann *et al.*, 1976). T cells were separated by rosette formation with neuraminidase treated (NANAase) sheep red blood cells (SRBC). E-rosette forming cells (T cells) were separated from non-E-rosetting cells by Ficoll isopaque density centrifugation. SRBC attached to T lymphocytes were lysed by osmotic lysis (Jonsdottir *et al.*, 1979). B cell enriched fractions were collected from the interphase, and contained 45% cells with surface immunoglobulin (SIg^+), 5–6% T cells, and 10–12% monocytic cells while the rest were large granular lymphocytes (LGL) and null cells.

Adherent cells. These were obtained from peripheral blood mononuclear leukocytes (MNL) by incubation for 1–2 h at 37°C in tissue culture flasks (Falcon Plastics, Oxnard, California, USA), in 50% heat-inactivated fetal bovine serum (FBS). Non-adherent cells were washed off and adherent cells were recovered after overnight incubation at 4°C (Troye-Blomberg *et al.*, 1983).

Culture conditions for antigen induced T dependent B cell activation. T cells supplemented with 5% autologous adherent cells were mixed with autologous B cells at a ratio of 2T/1B cell and a final concentration of 5×10^5 lymphocytes/ml. Whenever enough E^- cells were available these were cultured in parallel without T cells. Culture medium was Hepes buffered RPMI 1640 (Biocult Laboratories, Paisley, Scotland) supplemented with 2 mM L-glutamine, 25 μg/ml gentamycin and 10% FBS (TCM). The cell mixtures were put into round-bottomed tissue culture tubes (A/S Nunc, Roskilde, Denmark) in the presence or absence of antigen (10 μg/ml, 100 ng/ml or 10 ng/ml). After incubation for 4 days at 37°C in humidified air plus 5% CO_2, the medium was removed and fresh TCM without antigen was added. After further incubation for 12 days the supernatants were harvested and stored at −20°C until analysed for total immunoglobulin and anti-malaria antibodies.

Antigen preparations. Antigen was obtained from cultures *in vitro* in human bloodgroup 0^+ erythrocytes (Trager & Jensen, 1976) of a Tanzanian strain of *P. falciparum*, maintained in culture since 1978 (established by Dr J. H. Th. Meuwissen, Nijmegen, The Netherlands). The parasitaemias of the cultures were 5–10% at the time of preparation. Sonicates of parasite antigen were prepared from schizont-enriched fractions as previously described (Troye-Blomberg *et al.*, 1983; Wahlgren *et al.*, 1983). As control antigens sonicates of normal RBC ghosts were used (Wahlgren *et al.*, 1983).

A partially purified antigen preparation enriched in Pf 155 was obtained by adsorbing spent *P.*

falciparum culture medium to polyacrylamide beads conjugated with human glycophorine and subsequent elution with 3 M KSCN as described (Perkins, 1984). As shown in immunoblotting the major components in this antigen material are Pf 155 and two polypeptides that migrate faster (135 kD, 120 kD) (Berzins *et al.*, 1985; Udomsangpetch *et al.*, 1986).

Indirect immunofluorescence (IIF). Indirect IIF was performed on air-dried monolayers of infected erythrocytes by sequential incubation with supernatants from antigen stimulated- or non-stimulated cells, biotinylated goat antibodies to human Ig and fluorescein isothiocyanate (FITC) conjugated avidin. Isotyping of bound antibodies was done with FITC-conjugated affinity purified rabbit antibodies specific for human γ- or μ-chain (Wahlgren *et al.*, 1986). Antibodies to intracellular parasites were determined on unfixed erythrocytes, while antibodies to Pf 155 in the membrane of infected erythrocytes (E_i) were determined on glutaraldehyde (GA) fixed erythrocytes counterstained for intracellular parasites with ethidium bromide (Perlmann *et al.*, 1984). For inhibition of IIF, active supernatants were preincubated with different concentrations of *P. falciparum* or RBC ghost antigens for 30 min at room temperature before testing.

Determination of immunoglobulin concentrations. The concentrations of IgG or IgM in supernatants were determined in an enzyme-linked immunosorbent assay (ELISA) (Engvall & Perlmann, 1972), using rabbit anti-human $F(ab')_2$ antibodies on the solid phase and ALP-conjugated rabbit antibodies specific for human γ- or μ-chain as the indicator reagents. As standards for each test different dilutions of purified IgG or IgM myeloma proteins were used. The amount of immunoglobulin found in supernatants was calculated from the standard curves.

Statistical analysis. This was by paired or unpaired *t*-tests as indicated.

RESULTS

P. falciparum *antigen-induced immunoglobulin secretion* in vitro. Optimal culture conditions and kinetics were first determined by mixing varying numbers of T cells, reconstituted with 5% autologous macrophages (Troye-Blomberg *et al.*, 1983), with a constant number of autologous B cells and incubating these mixtures for 7–12 days with or without antigens. As the highest amounts of immunoglobulin or anti-*P. falciparum* antibodies were usually induced at a T/B ratio of 2:1, this ratio was used in all subsequent experiments. After removing excess antigen by changing the medium on day 4, measurable amounts of IgG or antibodies were found in stimulated lymphocyte cultures on day 12 and this day was chosen for harvesting the supernatants.

Figure 1 shows a summary of the IgG concentrations detected in culture supernatants of antigen exposed (10 ng/ml) lymphocytes from patients acutely infected with either *P. falciparum* or *P. vivax*. The crude *P. falciparum* antigen induced a significant IgG secretion over what was found in the medium controls (659 ± 113 ng/ml; $P < 0{\cdot}01$). For both the crude antigen and the Pf 155 enriched preparation, this secretion was statistically significant when 100 ng/ml were used for stimulation ($P < 0{\cdot}01$ and $P < 0{\cdot}001$, respectively; data not shown). In contrast, 10 μg/ml or more of the control (RBC ghost) antigen was required to induce significant IgG secretion ($P < 0{\cdot}05$, paired *t*-tests; data not shown). In lymphocyte cultures from *P. vivax* patients neither of the antigens induced IgG secretion over that found in the medium controls when added at concentrations of < 10 μg/ml. However, this was the case when they were added at concentrations above 100 μg/ml. For this group of donors, the spontaneous IgG secretion was significantly higher (1775 ± 381 ng/ml, $P < 0{\cdot}001$) than that of the acute *P. falciparum* patients (Fig. 1). The reasons for this are not known. Under the present experimental conditions very little IgM was found in either stimulated or control culture supernatants on day 12 (200–400 ng/ml) (data not shown).

*Anti-*P. falciparum *antibody reactivity in lymphocyte supernatants after antigen exposure.* Table 1 summarizes the results of the experiments in which different culture supernatants were tested for anti-*P. falciparum* antibodies. After exposure of the lymphocyte to low doses (10–100 ng/ml) of either crude or Pf 155 enriched *P. falciparum* antigen, large proportions of the supernatants contained antibodies to total (=intracellular) parasites (approximately 70% and 40%, respectively.) When the same supernatants were tested for antibodies to parasite antigens in the membrane of infected erythrocytes (mainly Pf 155, see Perlman *et al.*, 1984; Berzins *et al.*, 1985;

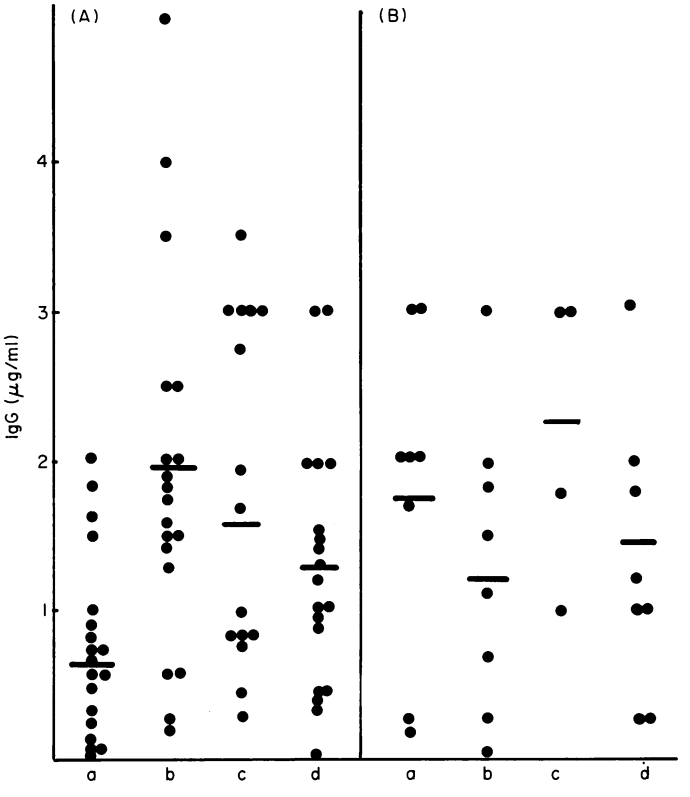

Fig. 1. Scatter diagram of IgG concentrations in culture supernatants of lymphocytes exposed to different antigen preparations. (A) patients with acute *P. falciparum* malaria; (B) patients with acute *P. vivax* malaria. Dots represent the concentration of total IgG (μg/ml) in each supernatant, and horizontal lines the arithmetic means after 12 days of culture with a change of medium on day 4. a, medium control; b, crude *P. falciparum* antigen; c, Pf 155 enriched; and d, RBC-ghost antigen. All antigens were added at a concentration of 10 ng/ml.

Table 1. Anti-*P. falciparum* antibodies in supernatants* of antigen treated lymphocytes obtained from patients with acute *P. falciparum* or *P. vivax* infections

	Immunofluorescence			
	Parasites†		E_i-Membrane‡	
Antigen§	*P. falciparum*	*P. vivax*	*P. falciparum*	*P. vivax*
Crude *P. falciparum*	13/19‖	1/8	6/19	0/8
Pf 155 enriched	6/16	0/8	2/16	0/8
RBC ghost	0/19	0/8	0/19	0/8

* Collected after 12 days with change of medium on day 4.

† IIF of unfixed and air dried erythrocytes.

‡ IIF of GDA-fixed and air dried erythrocytes, (E_i = infected erythrocytes).

§ Antigen dose for stimulation 10 ng protein/ml.

‖ Number positive/total number tested.

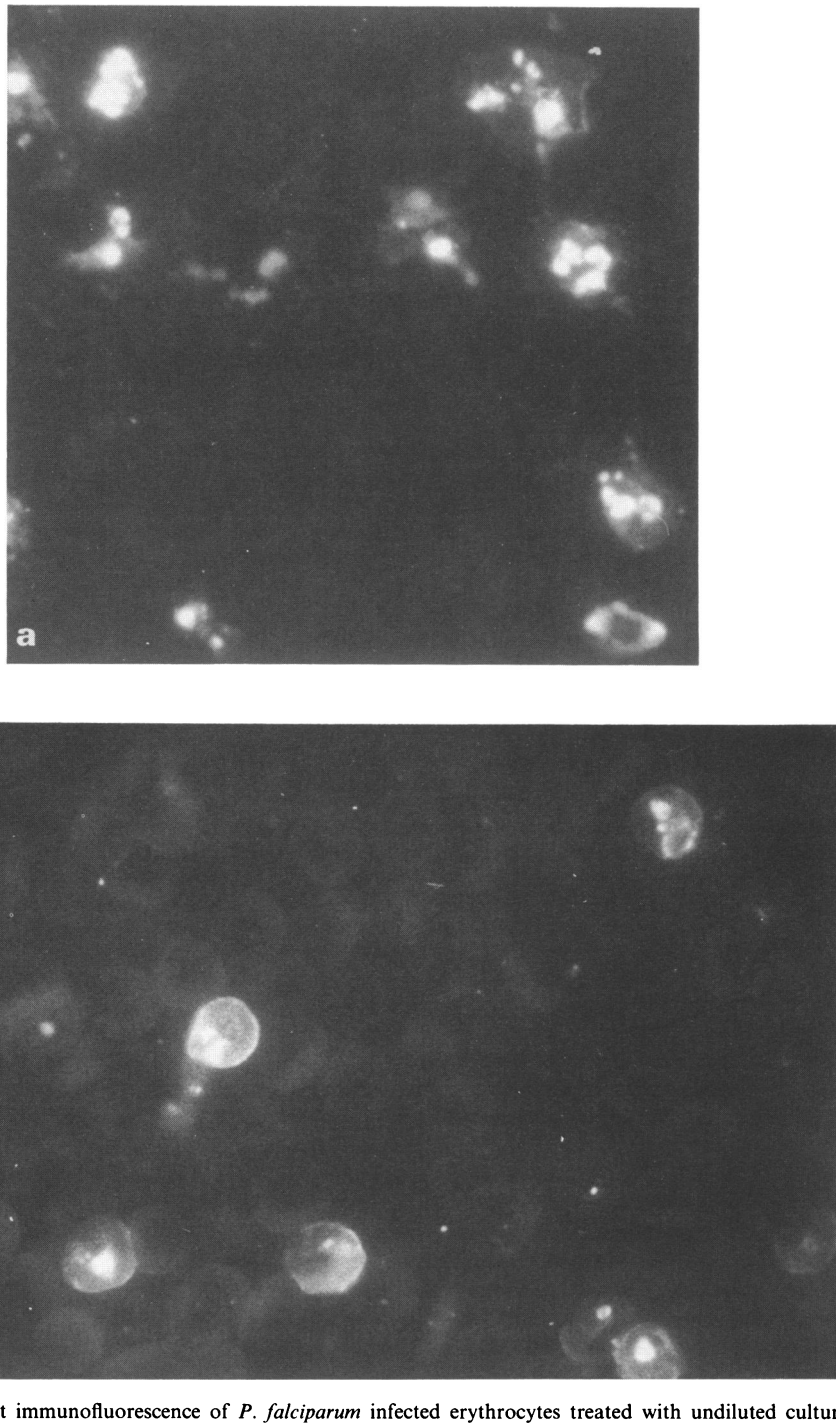

Fig. 2. Indirect immunofluorescence of *P. falciparum* infected erythrocytes treated with undiluted culture supernatants of lymphocytes obtained from a patient with acute *P. falciparum* malaria. The lymphocytes were stimulated with crude *P. falciparum* antigen. (a) IFF of intracellular parasites in unfixed and air dried erythrocytes. (b) IFF of parasite antigen (Pf 155) in the membrane of infected erythrocytes after glutaraldehyde fixing and air drying. In (b), the parasites were counterstained with ethidium bromide.

Udomsangpetch *et al.*, 1986) the corresponding numbers were 30% and 10%, respectively. Culture supernatants of lymphocytes exposed to RBC-ghost antigen did not contain any anti-*P. falciparum* antibodies. For the *P. vivax* group, only one supernatant out of eight contained anti-*P. falciparum* antibodies which stained intracellular parasites when the lymphocytes had been exposed to the crude *P. falciparum* antigen.

The results recorded in Table 1 were obtained by assaying undiluted culture supernatants from lymphocytes stimulated with low doses (ng) of antigen. Titration experiments indicated that the antibody reactivities frequently persisted at dilutions up to 1:5 but were in most instances lost at dilutions $\geq$ 1:25. Supernatants of lymphocytes stimulated with higher antigen doses ($\geq$10 μg) contained the same amounts of anti-*P. falciparum* antibodies, or none at all, as detected by the methods used here, in spite of increased concentrations of secreted IgG.

Figure 2a shows the typical IIF pattern seen with supernatants reacting with intra-cellular parasites. The antibodies seemed to react with the surface of mainly late stage parasites. On GA-fixed and air dried preparations, the staining was restricted to the membrane of infected erythrocytes, reflecting the presence of antibodies to Pf 155 (Fig. 2b.). Isotype determination of the staining antibodies revealed that both IgG and IgM antibodies bound to intracellular parasites whereas only IgG antibodies bound to parasite antigen in the membrane of infected erythrocytes.

To establish the specificity of the staining reactions the aliquots of *P. falciparum* antigens used for induction of antibody secretion *in vitro* were used to inhibit IIF. Table 2 shows the results of a typical experiment in which the supernatants from four donors were tested for antibodies. Both the crude *P. falciparum* antigen and the partially purified preparations inhibited IIF staining at 100 μg/ml and 25 μg/ml, while only weak inhibition or none was found at 5 μg/ml. Equivalent concentrations of RBC-ghost antigen did not inhibit. Similar results were obtained with other supernatants and tests for antibodies to intracellular parasites (data not shown).

Correlation between antibody secretion in vivo *and antibody activity in serum.* All sera from the

Table 2. Pf 155-immunofluorescence of lymphocyte culture supernatants preincubated with antigen

Antigen	Dose (μg/ml)	Donor 1	Donor 2	Donor 3	Donor 4
—	—	+	+	+	+
Crude P.	100	−	−	−	−
	25	−	−	−	−
	5	+	+	+	+
Pf 155-	100	−	−	−	−
enriched	25	−	−	−	−
	5	+	+	+	+
RBC ghost	100	+	+	+	+
	25	+	+	+	+
	5	+	+	+	+

Supernatants were from lymphocytes stimulated with crude *P. falciparum* antigen (10 ng/ml) and harvested after 12 days with change of medium on day 4. All lymphocyte donors were from patients with acute *P. falciparum* infection. The total concentrations of IgG at harvest were 0·6, 1·7, 1·1 and 1·1 μg/ml for donors 1–4, respectively. All supernatants were tested at a final dilution of 1:1 in indirect IIF on GDA-fixed and air dried infected erythrocytes. For inhibition, supernatants were preincubated for 30 min with an equal volume of antigen at the concentration shown.

Table 3. Antibody secretion in lymphocyte cultures and serum titres

Donor	Stimulating antigen	Antibodies in culture supernatants		Serum titres	
		Parasites*	E_i-Membrane†	Parasites	E_i-Membrane
P. falciparum	Crude Pf	+	−	> 1/15000	0
P. falciparum	Crude Pf	+	+	> 1/15000	1/3000
P. falciparum	Pf 155 enriched	+	+	> 1/15000	1/15000
P. vivax	Crude Pf	−	−	0	0

Supernatants from lymphocytes stimulated with *P. falciparum* antigen (10 ng/ml) as indicated and harvested after 12 days, with change of medium on day 4. Negative medium controls were run in parallel (not shown).

* Indirect IIF, unfixed and air dried erythrocytes.

† GDA-fixed and air dried erythrocytes (E_i = infected erythrocytes).

P. falciparum patients used in this study contained antibodies to intracellular parasites and approximately 50% also contained antibodies to parasite antigens in the membrane of infected erythrocytes (Pf 155). Only one of the sera from the acute *P. vivax* patients contained anti-*P. falciparum* antibodies reactive with intracellular parasites. There was a correlation between antibody reactivity in the sera and antibody secretion by lymphocytes after antigen exposure *in vitro*. As seen from the typical results in Table 3, both the crude *P. falciparum* antigen and the Pf 155-enriched preparation induced secretion of anti-*P. falciparum* antibodies to intracellular parasites after stimulation of the T cells from all three *P. falciparum* patients. Secretion of antibodies to parasite antigens in the membrane of infected erythrocytes was only induced in lymphocytes from donors whose sera had elevated anti-Pf 155 titres. This was seen for six out of 10 Pf 155 seropositive donors tested. Conversely, none of the supernatants from seronegative donors contained anti-Pf 155 antibodies. *Plasmodium vivax* donors were negative in both IIF assays, with exception of the one who also was *P. falciparum* seropositive and whose lymphocytes secreted antibodies to total *P. falciparum* antigen.

*T cell dependency of anti-*P. falciparum *antibody secretion* in vitro. The T-dependency of the induction of the anti-*P. falciparum* antibody secretion was tested by setting up parallel cultures of B cells without T cells. The typical results obtained with the lymphocytes from three *P. falciparum* patients are shown in Table 4. Anti-*P. falciparum* antibodies were only found in the cultures containing T and B cell mixtures exposed to *P. falciparum* antigen. No *secretion* of antibody was induced in the B cell cultures, although these contained 5–6% T cells, nor in the cultures containing T cells and macrophages only. The same results were seen with an additional five responding donors so tested (data not shown).

DISCUSSION

We have earlier reported that T cells from the peripheral blood of patients with acute *P. falciparum* infection respond with a disease related stimulation of DNA-synthesis when exposed to *P. falciparum* antigen *in vitro*. No such responses were obtained in controls with T cells from acutely infected *P. vivax* patients (Troye-Blomberg *et al.*, 1983; 1984). The malarial antigen used in these studies was a preparation containing both plasmodial and erythrocyte derived material (Wahlgren *et al.*, 1983). In the present study we demonstrate that the same antigen preparation induces immunoglobulin (IgG) and anti-*P. falciparum* antibody secretion in cultures of autologous T/B cell mixtures derived from the peripheral blood of *P. falciparum* patients. Since supernatants only were positive in IIF when tested undiluted, this suggested that only a minor part of the induced IgG was

Table 4. T cell dependency of secretion of immunoglobulin or anti-*P. falciparum* antibodies *in vitro*

Donor	Antigen*	Cell fraction	Immunoglobulin in culture supernatants (ng/ml)		Antibody in culture supernatants	
			IgG	IgM	Parasites†	E_i-membrane‡
1	Crude Pf	B	400	< 100	−	−
		T	< 100	< 100	−	−
		2T/1B	2200	400	+	+
	Pf155 enriched	B	200	100	−	−
		T	< 100	< 50	−	−
		2T/1B	2000	500	+	+
	RBC ghost	B	400	150	−	−
		T	< 100	< 50	−	−
		2T/1B	600	300	−	−
2	Crude Pf	B	250	200	−	−
		T	< 100	< 50	−	−
		2T/1B	1800	500	+	+
	Pf155 enriched	B	< 150	< 100	−	−
		T	< 50	0	−	−
		2T/1B	2000	350	+	+
	RBC ghost	B	300	100	−	−
		T	< 100	0	−	−
		2T/1B	700	300	−	−
3	Crude Pf	B	600	350	−	−
		T	200	< 100	−	−
		2T/1B	2500	500	+	+
	Pf155 enriched	B	650	180	−	−
		T	200	< 100	−	−
		2T/1B	2800	400	+	+
	RBC ghost	B	500	300	−	−
		T	300	100	−	−
		2T/1B	1000	500	−	−

* Antigen dose for stimulation was 10 ng protein/ml.
† Indirect IIF of unfixed and air dried erythrocytes.
‡ Indirect IIF of GDA-fixed and air dried erythrocytes.
E_i = infected erythrocytes.

anti-*P. falciparum* specific antibodies. Similar results were obtained by exposing the lymphocytes to a partially purified antigen preparation, highly enriched in Pf 155, a merozoite derived antigen (Perlmann *et al.*, 1984). In contrast, RBC-ghost antigen preparations induced significant immunoglobulin secretion in T/B cell mixtures, but no anti-*P. falciparum* antibodies, indicating that *P. falciparum* exposed donors also are sensitized to normal red blood cell components (Rosenberg *et al.*, 1972).

A prerequisite for the induction of anti-*P. falciparum* antibodies *in vitro* appeared to be that the donors had detectable amounts of anti-*P. falciparum* antibodies in their sera. The induced antibodies were primarily of IgG isotype. Under the assay conditions used here hardly any IgM was produced. The results suggest that antibody secretion *in vitro* reflects a secondary response of primed cells *in vivo*. This conclusion was also supported by the results of assaying the cultures for secretion of antibodies to Pf 155 in a modified IIF which makes use of GA-fixed and air dried E_i as the substrate (Perlmann *et al.*, 1984). Secretion of antibodies with specificity for this particular

antigen was found only among donors with elevated serum titres to Pf 155. The importance of priming *in vivo* for the outcome of the experiments *in vitro* has also been reported for other antigen systems such as tetanus toxoid, poliovirus and hepatitis B viruses (Volkman, Allyn & Fauci, 1982; Emini, Jameson & Wimmer, 1983; Hellström, Sylvan & Lundbergh, 1985; Lum & Culbertson, 1985).

Anti-*P. falciparum* antibody secretion *in vitro* was induced by low antigen concentrations (10–100 ng protein/ml). These concentrations were much lower than those required for optimal DNA-synthesis in T cells (approximately 5 μg antigen protein/ml) (Troye-Blomberg *et al.*, 1983; 1984). The differences in dose-response for the two assays suggest that the T cell derived lymphokines needed for B cell differentiation are secreted without proliferation. Lymphokines from antigen or mitogen stimulated T cells have been shown to induce antibody secretion in other antigen specific systems in the absence of intact T cells (Tan *et al.*, 1985). Whether or not this also applies to our malaria system is under investigation. High antigen doses induced polyclonal IgG secretion, also seen with the RBC ghost antigen, and in lymphocyte cultures of both *P. falciparum* and *P. vivax* patients. Whether or not specific anti-*P. falciparum* antibody secretion is actually suppressed when elevated antigen doses were used for stimulation (Volkman *et al.*, 1982; Lum & Culbertson, 1985) is under investigation. Polyclonal lymphocyte activation by *P. falciparum* antigen as reflected by increased ^{3}H-thymidine incorporation and increased immunoglobulin secretion has been reported (Greenwood, Aduloju & Platts-Mills, 1979; Ballet *et al.*, 1981; Kataaha *et al.*, 1984). It has also been proposed that crude *P. falciparum* antigen preparations contain mitogenic components (Greenwood *et al.*, 1979). The importance of using low antigen doses for the induction of specific antibodies rather than polyclonal IgG has also been reported for other *in vitro* systems (Friedman *et al.*, 1983; Hellström *et al.*, 1985; Lum & Culbertsson, 1985).

The T cell dependency of antibody secretion in the present investigation was studied by setting up B cell cultures in the absence of added T cells. Under the present experimental conditions little immunoglobulin and no anti-*P. falciparum* antibodies were found in the supernatants collected from these cultures. Furthermore, neither immunoglobulin nor antibodies were detected in the supernatants from T cells cultured in the absence of B cells, arguing against passive transfer of cytophilic antibodies in the positive cultures. However, further experiments are needed to establish optimal experimental conditions for the induction of B cells to secrete anti-*P. falciparum* antibodies without T cell help.

In recent years, rapid progress has been made in the isolation and characterization of *P. falciparum* antigens which are believed to give rise to protective immunity and therefore may be suitable candidates for malaria vaccines (Miller, David & Hadley, 1984; Anders, 1985; Nussenzweig & Nussenzweig, 1985; Targett & Sinden, 1985). While the reactivity of these components with anti-malarial antibodies has been studied extensively, their capacity to induce T cell help and memory, assuring long lasting vaccination effects and good anamnestic responses, is largely unknown. The present system *in vitro*, applied in conjunction with T cell cloning, should be well suited for further investigations of the antigenic fine structures inducing the appropriate T cell responses.

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