Clin. exp. Immunol. (1988) **71**, 377–382

Cell-mediated immune responses to *Plasmodium falciparum* antigens in adult Gambians

E. M. RILEY, S. JEPSEN,* G. ANDERSSON,† L. N. OTOO & B. M. GREENWOOD *Medical Research Council Laboratories, PO Box 273, Fajara, Near Banjul, The Gambia, * WHO National Malaria Diagnostic Centre, Statens Seruminstitut, Amager Boulevard 80, 2300 Kobenhavn S, Denmark and † Department of Immunology, University of Stockholm, S 106-91 Stockholm, Sweden*

(Accepted for publication 30 September 1987)

SUMMARY

Peripheral blood mononuclear cells from clinically immune Gambian adults were assayed for *in vitro* proliferation in response to crude and partially purified *Plasmodium falciparum* antigens. Lymphoproliferative responses to malaria antigens, lectin mitogens and *Candida albicans* were compared with those of control donors with no previous exposure to malaria. Cells of malaria-immune individuals were significantly more responsive to conconavalin A, and less responsive to phytohaemagglutinin, than cells from the control donors in both non-immune human serum and autologous serum. Cells from a proportion of immune donors proliferated in response to soluble malaria antigens but a substantial minority did not. Young adults and women were over-represented in the non-responding population. Responses to soluble malaria antigens were depressed in autologous serum compared with normal human serum. Both immune and control cells produced low levels of γ-IFN when stimulated with crude *P. falciparum* schizont antigens. Approximately half the immune donors, and none of the controls, produced significant levels of γ-IFN in response to purified soluble malaria antigen or malaria parasite culture supernatant. There was no direct correlation between lymphoproliferation and γ-IFN production.

Keywords Malaria *Plasmodium falciparum* lymphocyte proliferation γ-interferon

INTRODUCTION

In view of the rapid progress that is being made in purifying individual malaria antigens and cloning the relevant genes, and the probability that clinical trials of malaria vaccines will be initiated in the near future, it is essential that meaningful test systems are developed for assessing the immunogenicity of potential vaccine antigens and for determining their ability to induce clinically effective protective immunity. Immunity to malaria has, until recently, been measured almost exclusively by assays of humoral immunity. Although, on a population basis, *Plasmodium* specific antibody levels show show some correlation with the acquisition of clinical immunity, these same measurements are of little use in predicting resistance to infection in individual cases (McGregor, 1986). There is, however, good evidence from studies in experimental animal models that T cells are intricately involved in regulation of immune responses to malaria (Jayawardena *et al.*, 1982) and that an intact T cell system is required for maintenance of antimalarial immunity (Jayawardena, 1981).

In vitro investigation of human cell-mediated responses to malaria antigens has indicated that specific activation of T cells is transient and limited in acutely infected parasitaemic individuals (Troye-Blomberg *et al.*, 1984) but that specific lymphoproliferation can be induced by crude parasite extracts and soluble purified antigen in peripheral blood lymphocytes (PBL) from recently recovered individuals (Wyler & Oppenheim, 1974; Bygberg *et al.*, 1985; Troye-Blomberg *et al.*, 1983). These studies have been performed largely with cells from non-immune donors (from non-endemic areas) with limited exposure to *P. falciparum*, although one recent study did include a number of fully immune individuals (Theander *et al.*, 1986b). However, very little is known about the nature of cell mediated immunity in clinically immune adults living in endemic areas. We report here a study of cellular responsiveness to crude and purified *P. falciparum* antigens in healthy, clinically immune adults living in rural Gambia.

MATERIALS AND METHODS

Subjects

Between February and May 1986, blood was taken from 32 adult Gambians (17 female and 15 male, age range 16–60 years) living in a rural village close to the Gambia river. In this region

malaria is holoendemic but most of the transmission occurs during the rainy season (June to November). Thick blood films were examined at the time of sampling. None of the donors gave a history of an attack of clinical malaria during the last year and all can be considered to be clinically immune.

Fifteen healthy adult Europeans (5 female, 10 male, age range 24–45 years) who had lived in malaria endemic areas for less than 5 years, who had been taking regular chemoprophylaxis (proguanil or pyrimethamine and dapsone) and who had not experienced symptomatic infection with malaria served as the control group. The possibility that antimalarial drugs could interfere with the proliferative responses of control lymphocytes was considered, but recent studies by Bygbjerg, Odum & Theander (1986) suggest that prophylactic dosages of antimalarial drugs have no significant effect on *in vitro* responses to either mitogens or malaria antigens.

Lymphocyte cultures

Fifteen ml of venous blood was diluted 1 in 2 with RPMI 1640 (Flow, Irvine, UK) containing 10 u/ml sodium heparin. Mononuclear cells were isolated by sodium metrizoate density gradient centrifugation (Lymphoprep, Nygaard, Norway), washed three times in RPMI 1640 with 5% normal human A+ serum and resuspended in complete tissue culture medium (RPMI 1640 plus 2 mM L-glutamine, 100 u/ml Penicillin and Streptomycin, 30 mM Hepes, 0·22% sodium bicarbonate) containing either 10% normal A+ human serum or 10% autologous plasma, at a concentration of 7×10^5 cells/ml. One hundred and fifty μl of cell suspension was aliquoted into each well of a 96-well, round-bottomed microtitre plate (Linbro Chemical Co, New Haven, USA) together with 20 μl of mitogen or antigen. Twenty μl of culture medium was added to unstimulated control wells. Each agent was tested in triplicate. Plates were incubated at 37°C in 5% CO_2, 95% air for 4 or 7 days. One μCi of ^{3}H-thymidine (specific activity 2 Ci/mmole; Amersham International Plc, UK) was added to each well for the last 18 h of incubation. Cells were harvested onto glass fibre filters (Titertek, Flow, UK) using a minifold harvester (Sleicher and Schull, W. Germany). Cellular incorporation of ^{3}H-thymidine was measured by liquid scintillation counting.

Antigens and mitogens

The following mitogens and antigens were used:

(a) Concanavalin A (Con A) (Flow) final concentration in well = 12 μg/ml.

(b) Pokeweed mitogen (PWM) (Gibco Ltd, Uxbridge, UK) final dilution in well = 1:850 w/v of stock solution.

(c) Phytohaemagglutinin (PHA) (Difco, Detroit, USA) final concentration in well = 12 μg/ml.

(d) *C. albicans* extract (10% w/v; Hollister-Stier, Elkhart, Indiana, USA) was used at a final dilution of 1:80.

(e) Crude schizont antigen was obtained from a continuous culture of *P. falciparum*. Schizont enrichment was performed by plasmagel sedimentation. The schizont-enriched fraction was washed, sonicated and stored at −20°C. The protein concentration of the final preparation was approximately 4·0 g/l. The optimal, non-mitogenic, dilution was 1:400. A similar preparation of uninfected red cells was used as a control.

(f) Culture supernatant from the same isolate of *P. falciparum* was collected whenever the parasitaemia of the continuous culture exceeded 12%. Pooled supernatant was centrifuged, filtered and stored at −20°C. The supernatant was used at a final dilution of 1:80.

(g) Purified soluble antigen was prepared as described elsewhere (Jepsen & Andersen, 1981, Jepsen, Hindersson & Axelsen, 1986). Briefly, pooled supernatants from continuous cultures of a Tanzanian isolate of *P. falciparum* were affinity purified against malaria immune serum from Liberian adults coupled to a CNBr·Sepharose 4B column. The eluted antigens were concentrated, dialysed and analysed by crossed immunoelectrophoresis (Jepsen & Axelsen, 1980). The final product contains seven separate soluble antigens. The protein concentration of the stock solution was 3·0 g/l and it was diluted 1:80 in the culture wells.

Optimal dilutions for antigens were determined as the dilution which failed to induce proliferation in naive/control human lymphocytes cultured for 4 days and caused maximum proliferation of immune lymphocytes at 7 days.

Antibody assay

Total antimalarial serum antibody levels were measured by indirect immunofluorescence. Doubling dilutions of serum were applied to acetone fixed, air-dried slides of cultured late stage (trophozoites/schizonts) parasites and incubated for 30 min in a moist chamber. After washing in PBS, fluorescein-conjugated sheep anti-human Ig antiserum was applied for 30 min. Slides were extensively washed, counterstained with Evans blue and examined under UV light. The titre is given as the greatest dilution at which specific surface immunofluorescence of parasites was detectable.

Estimation of γ-interferon in culture supernatants

γ-interferon (γ-IFN) was assayed by a two-site enzyme-linked immunosorbent assay (ELISA) using two mouse monoclonal antibodies to human γ-IFN to one of which alkaline phosphatase was conjugated. Bound enzyme was detected with *p*-nitrophenylphosphate and absorbance read at 405 nm. IFN units were determined by reference to standard curves prepared with the international human γ-IFN standard GG 23-901-530 kindly supplied by the National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA.

Statistical analysis

Results are expressed as Δ counts per min (Δ ct/min) which represents ct/min of stimulated wells minus ct/min of unstimulated wells. Differences between immune and control groups were analysed using a non-parametric Wilcoxon rank sum test. Differences within the immune group were analysed by the Student's *t*-test.

RESULTS

Parasitaemia and antibody titres

Only one adult Gambian had parasitaemia (*P. falciparum*, 20 per μl) at the time that blood samples were collected. However, all had antibody titres in excess of 1:160 with a small number of individuals having exceedingly high antibody levels (up to 1:20480). Thick films from all the European controls were negative and all but one of the European controls had antibody titres of 1:10 or less, confirming their minimal exposure to plasmodial antigen. One expatriate control had a titre of 1:80.

Table 1. Lymphocyte proliferation responses to mitogen stimulation

	A^+ serum		Autologous plasma	
	Immune	Control	Immune	Control
Con A	30,459 ±4,394	15,057 ±3,730	37,116 ±4,106	22,057 ±3,367
PHA	34,843 ±4,582	56,098 ±6,536	39,812 ±2,351	68,369 ±8,733
PWM	10,464 ±1,278	9,478 ±1,633	10,191 ±1,274	9,092 ±1,573

Change in ct/min (ct/min mitogen stimulated cultrues − ct/min unstimulated cultures), mean ± s.e. Immunes $n = 32$, controls $n = 15$, after 4 days in culture. Background values of unstimulated cultures in A+ (autologous) serum: Immunes 731 ± 154 (665 ± 122); Controls 371 ± 42 (714 ± 177).

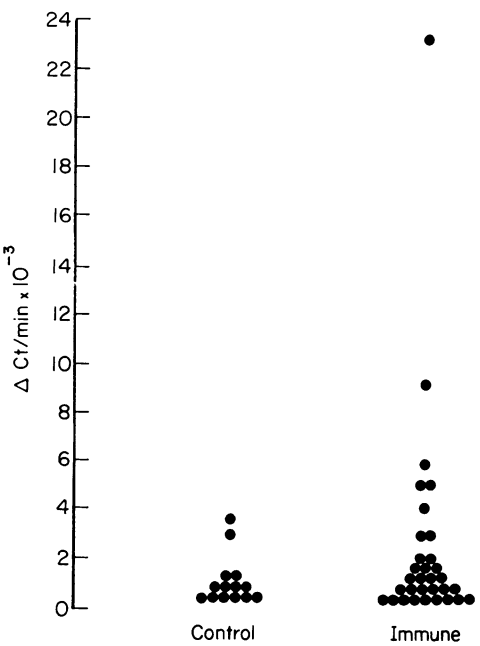

Fig. 1. Proliferative response of peripheral blood mononuclear cells to a crude preparation of *P. falciparum* schizont antigen. ^{3}H-thymidine incorporation by control and immune cells after 7 days culture (ct/min antigen-stimulated wells − ct/min unstimulated wells). Each point represents the mean response in triplicate wells for one individual donor.

Cellular response to mitogens

Responses to Con A, PHA and PWM were measured after 4 days (Table 1). Con A responses were significantly higher in the immune Gambian group than in the control group in both A^+ serum ($P < 0.05$) and autologous plasma ($P < 0.025$). In contrast PHA responses were significantly lower in the immune group than in the control group (in A^+ serum $P < 0.025$; in autologous plasma $P < 0.01$). Responses to PWM in the two groups were not significantly different ($P > 0.1$).

Response to specific antigens

Cells were cultured *in vitro* for 4 or 7 days. No responses to specific antigens were seen after 4 days incubation (data not shown). Results from 7-day cultures are given in Table 2.

Responses to *Candida* antigen preparations were not significantly different in the two populations. However, in both the Gambian and the control groups, responses were significantly higher in A^+ serum than in autologous plasma ($P < 0.05$ in each case). No age or sex differences were noted in the response to *Candida* antigens (data not shown).

At schizont antigen concentrations that were not mitogenic to naive cells, no significant differences in cellular proliferation were observed overall between the immune group and the control group in either A^+ serum or autologous plasma ($P > 0.1$). However, cells from certain individual immune donors did proliferate when cultured with schizont antigen (Fig. 1). Within the two groups there was no significant difference between responses in A^+ serum and autologous plasma.

Proliferative responses to the *P. falciparum* culture supernatant were minimal in both the Gambian and control groups. The difference between the means of the two groups in A^+ serum is just significant ($0.05 > P > 0.025$). There is no significant difference between responses in A^+ serum and in autologous plasma in either group ($P > 0.1$).

Responses to the partially purified soluble malaria antigen preparation were significantly higher in the immune group than the control group in both A^+ serum ($P < 0.005$) and autologous plasma ($P < 0.05$). Within the immune group the range of responses to soluble antigen was extremely wide (Fig. 2a). While the cells of most immune donors gave responses which were higher than the normal range (mean ± 2 s.d. of control group) a substantial minority (34%) gave responses of less than 5,000 ct/min. When divided into three age groups (Fig. 2b) (less than 20 years, 20 to 40 years and above 40 years) the mean response of the oldest age group is significantly higher ($P < 0.05$) than that of the youngest group. When responses of male and female donors are compared, responses of male donors are significantly greater than those of female donors ($P < 0.025$) (Fig 2c). Of the 16

Table 2. Lymphocyte proliferation in response to antigen stimulation

	Immune		Control	
	A+	Autologous	A+	Autologous
Culture s/n	803 ±148	613 ±233	365 ±94	197 ±88
Schizont Ag	3,045 ±913	993 ±262	1,666 ±596	997 ±486
Soluble Ag	6,936 ±1,029	2,971 ±677	1,228 ±464	875 ±326
Candida Ag	29,188 ±3,357	19,594 ±2,909	28,145 ±7,832	13,898 ±4,763

Change in ct/min (ct/min stimulated cultures − ct/min unstimulated cultures) after 7 days, mean ± s.e. Immunes $n = 32$, controls $n = 15$. Background values of unstimulated cultures in A+ (autologous) serum: Immunes 1716 ± 321 (1326 ± 244); Controls 472 ± 46 (961 ± 152).

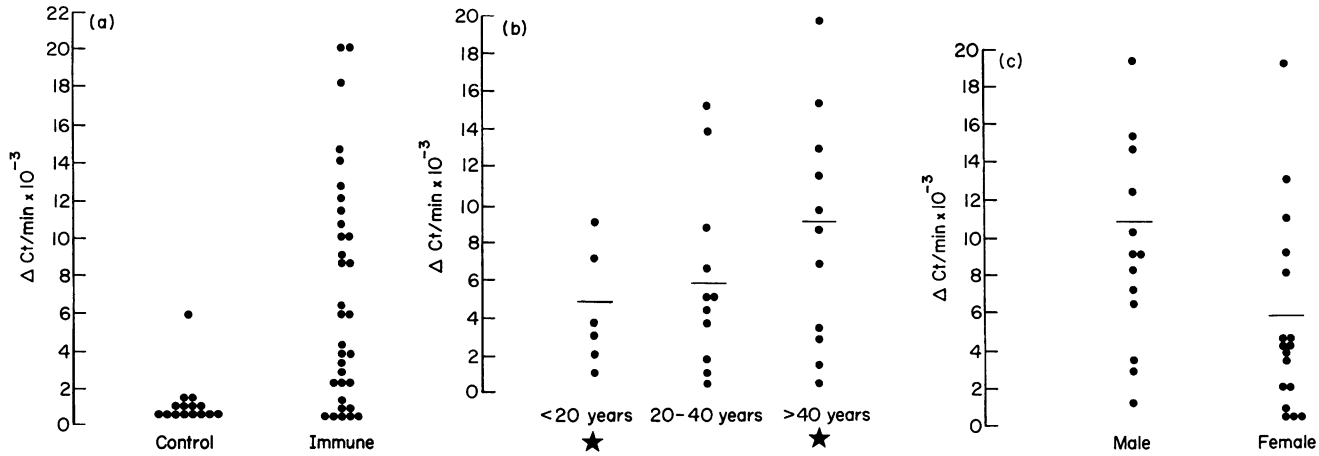

Fig. 2. Proliferative response of peripheral blood mononuclear cells to affinity purified *P. falciparum* soluble antigen after 7 days in culture. Each point represents the mean response in triplicate wells for one individual. (a) Comparison of ^{3}H-thymidine incorporation by cells from control and immune donors. (b) Comparison of ^{3}H-thymidine incorporation by cells from immune donors of different ages—less than 20 years, 20 to 40 years and greater than 40 years. Difference between starred (*) groups is statistically significant ($P<0{\cdot}05$). (c) Comparison of ^{3}H-thymidine incorporation by cells from immune male and immune female donors. The difference between the two groups is significant ($P<0{\cdot}025$).

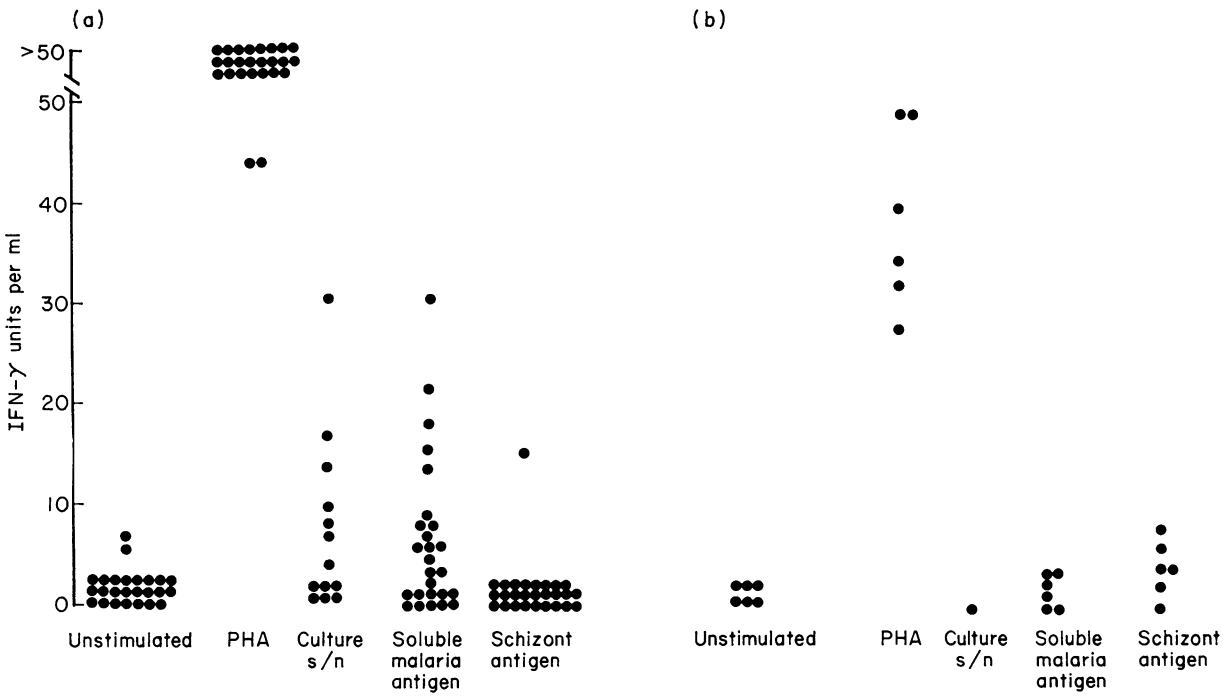

Fig. 3. Production of γ-IFN by peripheral blood mononuclear cells cultured for 7 days with mitogen or *P. falciparum* antigens. Each point represents iu/ml γ-IFN in pooled culture supernatants from triplicate wells from one individual. (a) Immune. (b) Control.

donors whose responses to soluble antigen fall within the control range, 12 are female. The response of immune cells was consistently higher in normal A$^+$ serum than in the donors own plasma and when compared in a paired *t*-test the difference is significant ($P<0{\cdot}005$, $r=0{\cdot}54$). In the control group the mean response was slightly higher in A$^+$ than in autologous plasma but the correlation coefficient of 0·14 suggests that this is due to random variation rather than to a consistent trend.

γ-interferon production

Culture supernatants were collected immediately prior to cell harvesting (i.e. at 7 days) from a random sample of the immune and control plates. The results are shown in Fig. 3. Cells from both Gambian and control donors produced large amounts of γ-IFN when stimulated with PHA. Control donors failed to produce γ-IFN when stimulated with soluble malaria antigen or malaria culture supernatant but produced low levels of γ-IFN in

response to schizont antigen stimulation. This suggests that schizont antigen concentrations which are not mitogenic may still non-specifically induce interferon production. Approximately half the Gambian donors tested produced significant amounts of γ-IFN in response to malaria culture supernatant or soluble malaria antigen, or both. There was no overall correlation between lymphoproliferation and interferon production (for soluble malaria antigen the correlation coefficient $(r) = -0{\cdot}13$, $n = 25$), although individuals producing large amounts of γ-IFN in response to soluble malaria antigen tended to show moderate levels of proliferation. Many of those whose cells proliferated well (> 10,000 ct/min) produced little or no γ-IFN and non-responders in the proliferation assay also failed to produce γ-IFN. Conversely all individuals producing γ-IFN in response to malaria culture supernatant failed to proliferate (ct/min < 2,000).

DISCUSSION

The soluble antigen preparation used in this study has previously been demonstrated to be free of non-specific, polyclonal stimulating activity (Bygbjerg *et al.*, 1985; Theander *et al.*, 1986b) and this was confirmed in the experiments reported here. Results obtained with the crude schizont antigen preparation demonstrate the difficulty in detecting specific antigen-induced responses to a stimulating agent which contains both antigens and mitogens. High concentrations (> 25 μg/ml) of crude schizont antigen caused both sensitized and unsensitized lymphocytes to proliferate (data not shown) whilst at nonmitogenic concentrations (< 10 μg/ml) cells of a few individual immune donors proliferated but failed to produce interferon. These results are comparable with those of Troye-Blomberg *et al.* (1983) who were unable to differentiate specific responses to crude schizont antigen from non-specific mitogenic responses. Crude antigen preparations contain components which induce non-specific polyclonal lymphocyte activation and which may favour the expression of specific suppressor mechanisms which inhibit *in vitro* cell proliferation (Jayawardena, Waksman & Eardley, 1978). Preliminary data suggests that immune lymphocytes respond less well to purified malaria antigens when cultured together with the crude schizont preparation than when cultured with purified antigen alone (E.M. Riley—unpublished observations) and similar effects have been shown in mice with extracts of *P. berghei* (Khansari, Segre & Segre, 1981).

The purified malaria antigen suspension used in these experiments contains several separate proteins with molecular weights ranging from 56 to 300 kd. The two major components are an 85 kd amphiphilic glycoprotein and a 116 kd glycoprotein with partial immunological cross-reactivity with the Pf155 merozoite antigen described by Perlmann *et al.*, (1984). The remaining antigens have not yet been fully characterized but are believed to include both heat stable exoantigens ('S antigens'—Wilson *et al.*, 1969) and subunits of other asexual stage antigens (S. Jepsen & P. Jakobsen—unpublished data).

A sizeable minority (34%) of immune donors did not show any significant proliferative response to soluble malaria antigens. However, when the immune population were stratified by age and sex it became clear that young donors showed significantly lower proliferative responses than did older donors and female donors gave lower responses than male donors. Of the 16 low responders (< 6,000 ct/min) 12 were females aged between 18 and 45 years. We are currently investigating the possibility that this hyporesponsiveness is related to pregnancy.

The difference in proliferative responses between young adults (under 20 years of age) and those over 40 years is interesting. Several parameters of humoral immunity to falciparum malaria—e.g. natural acquisition of antibodies to sporozoite surface antigens—have been shown to be age-related, with age differences persisting to late adult life, particularly in areas with moderate to low malaria transmission (Druilhe *et al.*, 1986). This may reflect the cumulative effects of repeated exposure to infection and periodic immunosuppression due to clinical malaria. If this is so, then development of cellular immunity—including expansion of the malaria-specific T helper cell clones believed to be involved in antibody synthesis—may also be a gradual, exposure-related phenomenon.

Inhibition of cellular proliferative responses by regulating mechanisms may also explain the low responses to specific malaria antigens in a clinically immune population. Specific suppression of cellular antimalarial responses during acute infection has been reported (Bygbjerg *et al.* 1983; Troye-Blomberg *et al.*, 1983; Troye-Blomberg *et al.*, 1984), although Troye-Blomberg *et al.* (1984) report that immune adults from Liberia and Columbia showed strong and long-lasting responses to relatively crude antigen preparations. All our samples were taken in the second half of the dry (low transmission) season when donors had not been exposed to infection for several months. Ballet *et al.* (1985) have demonstrated that lymphoproliferative responses to *P. falciparum* merozoites wane rapidly following emigration from endemic areas, which may imply that the specific cellular response to malaria antigens is transient—again possibly due to continuing immunoregulation.

The lack of a direct correlation between lymphoproliferation and γ-IFN production in response to antigen stimulation was not unexpected since Troye-Blomberg *et al.* (1985) describe similar findings in malaria-immune individuals and acute malaria patients. However the ability to produce γ-IFN after exposure to malaria antigens may be an important indicator of cellular immunity to malaria since γ-IFN is a potent activator of monocytes and macrophages, which have been shown to be involved in parasite killing (Ockenhouse, Schulman & Shear, 1984).

Existing data on responses to mitogens during acute malaria infection are confusing, with some workers reporting decreased responses and others being unable to demonstrate any change (Troye-Blomberg *et al.*, 1983; Theander *et al.*, 1986a; Greenwood *et al.*, 1972). Mitogen responses have not been specifically examined in healthy, malaria-immune cell donors and the differences described here may reflect basic differences in the status of the immune system in the two populations. Marked differences in 'normal' immunological parameters have been described between European and African populations particularly with respect to the distribution of lymphocyte subsets and it is possible that prolonged exposure to immunomodulatory infections such as malaria may be partly responsible for these differences (Greenwood & Whittle, 1981).

The effects of autologous plasma on cell proliferation are interesting. Mitogen responses were unaffected by serum source, however, the response to *Candida* antigens is greater in normal

human serum than in autologous plasma in both groups and in the malaria-immune group responses to malaria antigens were higher in normal serum than in 'immune' serum. This suggests that the inhibitory effect of autologous serum is antigen specific. Blocking of antigenic epitopes by specific antibody is one obvious mechanism by which immune serum may interfere with antigen presentation and impair cellular responses. If confirmed, this may help to explain why some individuals with high antimalarial antibody titres may still be susceptible to infection.

ACKNOWLEDGMENTS

We would like to thank Idrissa Sambou and Herbert Robinson for their technical assistance and Momodou Sanyang and the people of Brefet for their patience and co-operation.

REFERENCES

BALLET, J.J., DRUILHE, P., VASCONCELOS, I., SCHMITT, C., AGRAPART, M. & FROMMER, D. (1985) Human lymphocyte response to *Plasmodium falciparum* merozoite antigens. A functional assay of protective immunity? *Trans. R. Soc. Trop. Med. Hyg.* **79,** 497.

BYGBJERG, I.C., JEPSEN, S., THEANDER, T.G., ODUM, N. & KAPPELGAARD, E. (1983) Lymphocyte blast transformation with soluble antigens of *Plasmodium falciparum* during and after acute malaria. Second International Conference on Malaria and Babesiosis, **4,** 9.

BYGBJERG, I.C., JEPSEN, S., THEANDER, T.G. & ODUM, N. (1985) Specific proliferative response of human lymphocytes to purified soluble antigens from *Plasmodium falciparum in vitro* cultures and to antigens from malaria patients sera. *Clin. exp. Immunol.* **59,** 421.

BYGBJERG, I.C., ODUM, N. & THEANDER, T.G. (1986) Effect of pyrimethamine and sulphadoxine on human lymphocyte proliferation. *Trans. R. Soc. Trop. Med. Hyg.* **80,** 295.

DRUILHE, P., PRADIER, O., MARC, J.P., MILTGEN, F., MAZIER, D. & PARENT, G. (1986) Levels of antibodies to *Plasmodium falciparum* sporozoite surface antigens reflect malaria transmission rates and are persistent in the absence of infection. *Infect. Immun.* **53,** 393.

GREENWOOD, B.M., BRADLEY-MOORE, A.M., PALIT, A. & BRYCESON, A.D.M. (1972) Immunosuppression in children with malaria. *Lancet,* **i,** 169.

GREENWOOD, B.M. & WHITTLE, H.C. (1980) Immunology of Medicine in the Tropics. *Current Topics in Immunology,* **14,** 1.

JAYAWARDENA, A.N. (1981) Immune responses in malaria. In *Parasite Diseases.* (ed. by J.M. Mansfield), p. 85. New York: Marcel Decker Inc.

JAYAWARDENA, A.N., MURPHY, D.B., JANEWAY, C.A. & GERSHON R.K. (1982) T cell-mediated immunity in malaria. I. The Ly phenotype of T cells mediating resistance to *Plasmodium yoelii. J. Immunol.* **129,** 377.

JAYAWARDENA, A.N., WAKSMAN, B.H. & EARDLEY, D.D. (1978) Activation of distinct helper and suppressor T cells in experimental trypanosomiasis. *J. Immunol.* **121,** 622.

JEPSEN, S. & ANDERSEN, B.J. (1981) Immunoadsorbent isolation of antigens from the culture medium of *in vitro* cultivated *Plasmodium falciparum. Acta Pathol. Microbiol Immunol. Scand. [C],* **89,** 99.

JEPSEN, S. & AXELSEN, N.H. (1980) Antigens and antibodies in *Plasmodium falciparum* malaria studied by immunoelectrophoretic methods. *Acta. Pathol. Microbiol. Immunol. Scand. [C),* **88,** 263.

JEPSEN, S., HINDERSSON, P. & AXELSEN, N.H. (1986) Computer controlled affinity chromatographical purification of soluble *Plasmodium falciparum* antigens from supernatants of *in vitro* cultures. *Trans. R. Soc. Trop. Med. Hyg.* **80,** 534.

KHANSARI, N., SEGRE, M. & SEGRE, D. (1981) Immunosuppression in murine malaria: A soluble immunosuppressive factor derived from *Plasmodium berghei* infected blood. *J. Immunol.* **127,** 1889.

MCGREGOR, I.A. (1986) The development and maintenance of immunity to malaria in highly endemic areas. *Clin. in Trop. Med. and Comm. Dis.* **1,** 29.

OCKENHOUSE, C.F., SCHULMAN, S. & SHEAR, H.L. (1984) Induction of crisis forms in the human malaria parasite *Plasmodium falciparum* by γ-interferon activated, monocyte-derived macrophages. *J. Immunol.* **133,** 1601.

PERLMANN, H., BERZINS, K., WAHLGREN, M., CARLSSON, J., BJORKMANN, A., PATARROYO, M.E. & PERLMANN, P. (1984) Antibodies in malarial sera to parasite antigens in the membrane of erythrocytes infected with early asexual stages of *Plasmodium falciparum. J. exp. Med.* **159,** 1686.

THEANDER, T.G., BYGBJERG, I.C., ANDERSEN, B.J., JEPSEN, S., KHARAZMI, A. & ODUM, N. (1986a) Suppression of parasite specific response in *Plasmodium falciparum* malaria. A longitudinal study of blood mononuclear cell proliferation and subset composition. *Scand. J. Immunol.* **24,** 73.

THEANDER T.G., BYGBJERG, I.C., JEPSEN, S., SVENSON, M., KHARAZMI, A., LARSEN, P.B. & BENDTZEN, K. (1986b) Proliferation induced by *Plasmodium falciparum* antigen and interleukin production by lymphocytes isolated from malaria-immune individuals. *Infect. Immun.* **53,** 221.

TROYE-BLOMBERG, M., ANDERSSON, G., STOCZKOWSKA, M., SHABO, R., ROMERO, P., PATARROYO, E., WIGZELL, H. & PERLMANN, P. (1985) Production of IL-2 and IFN-γ by T cells from malaria patients in response to *Plasmodium falciparum* or erythrocyte antigens *in vitro. J. Immunol.* **135,** 3498.

TROYE-BLOMBERG, M., PERLMANN, H., PATARROYO, E. & PERLMANN, P. (1983) Regulation of the immune response in *Plasmodium falciparum* malaria. II. Antigen specific proliferative responses *in vitro. Clin exp Immunol.* **53,** 345.

TROYE-BLOMBERG, M., ROMERO, P., PATARROYO, M.E., BJORKMAN, A. & PERLMANN, P. (1984) Regulation of the immune response in *Plasmodium falciparum* malaria. III. Proliferative response to antigen *in vitro* and subset composition of T cells from patients with acute infection or from immune donors. *Clin. exp. Immunol.* **58,** 380.

WILSON, R.J.M., MCGREGOR, I.A., HALL, P., WILLIAMS, K. & BARTHOLOMEW R. (1969) Antigens associated with *Plasmodium falciparum* infections in man. *Lancet,* **iii,** 201.

WYLER, D.J. & OPPENHEIM, J.J. (1974) Lymphocyte transformation in human *Plasmodium falciparum* malaria. *J. Immunol.* **113,** 449.