Asian Pacific Journal of Allergy and Immunology (1986) 4:13-17

Regulation of Immunoglobulin Secretion by T Lymphocytes in Human Malaria

Sanit Makonkawkeyoon,* Watchara Kasinrerk * and Vicharn Vithayasai **

SUMMARY *In vitro* **studies were carried out on the nature of immunoglobulin synthesis and secretion by peripheral blood mononuclear leukocytes (PBMLs) and on the function of T and B cells from malaria patients. The mean values of secreted IgG and IgM concentrations of 22 malaria patient PMBLs were significantly higher than those of 20 normal PBMLs. When the suppressor T cell activity and the function of B cells in response to suppressor T cells were assayed by the cell co-culture technique, it was found that there was a decrease in suppressor T cell activity and the B cell function in response to normal suppressor T cells in malaria patients. The defects of these T and B cell functions may play some role in the immunological abnormalities seen in some malaria patients.**

The general features of immunity to malaria have been extensively reviewed elsewhere and it appears that malaria infection in man leads to a profound rise in the level of serum immunoglobulins and in their rates of synthesis.[1,2] Anti-malarial antibody has been demonstrated serologically in IgG, IgM and IgA.[3] However, since adsorption and catabolism experiments have shown that only a small part of the increased immunoglobulin is parasite specific,[2,4] presumably much of the remainder constitutes heterophile agglutinins, antiglobulins (rheumatoid factor),[5] and antibody to uninfected erythrocytes.[6] These immunological abnormalities may come from an alteration in immunoregulation.

In this study, the nature of immunoglobulin synthesis and secretion by PBMLs and the function of T and B cells from malaria patients were examined in order to determine whether there was any alteration of the immunoregulatory pathway.

MATERIALS AND METHODS

Subjects

Thirty-three blood samples from healthy blood donors from the Blood Bank Unit of Maharaj Nakorn Chiang Mai Hospital were used as the controls. Thirty-four blood samples from malaria patients from the Malaria Center Region II of Chiang Mai Province and the Mae Sod Hospital were studied. Seventeen malaria patients had positive blood films for *Plasmodium falciparum,* 16 positive blood films for *Plasmodium vivax,* and 1 positive blood film for both *P. falciparum* and *P.vivax* (mixed infection). All blood specimens were taken during acute attack and before treatment. The percentage of parasitaemia in these patients ranged from 0.03 to 0.43.

Human peripheral blood mononuclear leukocytes

Twenty millilitres of heparinised (10 U/ml of blood) venous blood was obtained from the subjects. The blood was diluted 1:2 with sterile 0.01 M phosphate buffered saline (PBS) pH 7.2 and centrifuged on Ficoll-Hypaque according to the method of Boyum (1968).[7] The peripheral blood mononuclear leukocytes (PBMLs) layer was collected, washed 6 times, and the cell concentrations adjusted with RPMI 1640 (Gibco, Grand Island Biological Co., Grand Island, N.Y.) containing 100 Units/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine and 10% heat-inactivated fetal calf serum (FCS, Gibco).

Induction of *in vitro* immunoglobulin secretion

A total of 1.0×10^6 PBMLs in 1 ml RPMI 1640 medium were incubated with or without pokeweed mitogen (PWM) at a final dilution of 1:200 at 37°C in

From the Department of Clinical Immunology, Faculty of Associated Medical Sciences* and the Department of Microbiology, Faculty of Medicine,** Chiang Mai University, Chiang Mai, Thailand.

5% CO_2 in air at 100% humidity for 0 (washing control) and 9 days. At the end of the culture periods, the culture supernatants were harvested, and the IgG and IgM concentrations were determined by sandwich ELISA. The culture supernatant of 0 day culture served as a control for the effectiveness of washing. Therefore, the concentrations (ng/ml) of IgG and IgM in culture supernatants directly secreted by PBMLs were corrected by subtracting the day 0 values from the corresponding day 9 values.

Sandwich ELISA for quantitation of IgG and IgM

The amounts of secreted IgG and IgM in culture supernatant and plasma were determined by sandwich ELISA.[8] Briefly, 200 μl of goat anti-human IgG (dilution 1:2,000) or anti-human IgM (1:4,000) were coated onto each well of a polystyrene microtitre plate at 37°C 3 hours, washed 4 times with PBS-Tween. Two hundred microlitres of diluted test culture supernatants or plasma were added, incubated 1 hour at 37°C, washed 4 times with PBS-Tween. Two hundred microlitres of goat anti-human IgG (1:8,000) or goat anti-human IgM (1:4,000) peroxidase conjugate were added, incubated 1 hour at 37°C, washed 4 times. Two hundred microlitres of substrate were added, and the reaction was stopped by 50 μl 8 N H_2SO_4 after 30 min. The reacting microtitre plate was read at 492 nm in a spectrophotometer (Titertek Multiskan, type 3 D-C, Flow Laboratories, Helsinki, Finland). The concentrations of IgG or IgM were calculated from a standard curve obtained using known concentrations of standard human IgG or IgM.

Preparation of T cells

Human T cells were isolated using AET (2-aminoethylisothiouronium hydrobromide)-treated sheep erythrocytes (E_{AET})[9]. Briefly, PBMLs (1.0×10^7/ml) were mixed with an equal volume of 1% E_{AET} suspension, incubated 10 minutes at 37°C, then centrifuged at $400 \times g$ for 2 minutes and incubated overnight at 4°C. The cell pellet was resuspended by gentle aspiration. The rosette and non-rosette forming cells were separated by Ficoll-Hypaque density gradient centrifugation. The E_{AET} were lysed with hypotonic ammonium chloride buffer solution. The remaining T cells were washed and counted.

Assay for suppressor T cell activity

The *in vitro* PWM-driven system was used to detect the activity of suppressor T cells. PBMLs from normal subjects (1.0×10^6/ml) were co-cultured with various concentrations of purified T cells from malaria patients or normal subjects in the PWM-driven system for 9 days. Normal PBMLs, normal PBMLs plus normal T-cells and normal PBMLs plus malaria patient T-cells were compared to determine the percentage suppression of IgG and IgM secretions.

Assay for B cell activity

In some experiments, PBMLs of malaria patients (1.0×10^6/ml) were co-cultured with various concentrations of purified T cells from malaria patients or from normal subjects in the PWM-driven system for 9 days. The percentage suppression of immunoglobulin secretion for each added T cell concentration was determined.

Statistical analysis

Differences in the IgG or IgM concentrations of various groups were analysed by the Student's *t*-test.

RESULTS

Plasma immunoglobulin concentrations in normal subjects and malaria patients

Plasma IgG and IgM concentrations from 20 normal healthy subjects and 22 malaria patients were determined by sandwich ELISA. The mean plasma IgG and IgM concentrations of malaria patients were higher than those of normal subjects ($p < 0.025$ and $p < 0.005$, respectively) (Fig.1). However, there was little difference ($p > 0.05$) in the mean concentrations of plasma IgG or IgM for malaria patients with *P. falciparum* or *P. vivax*.

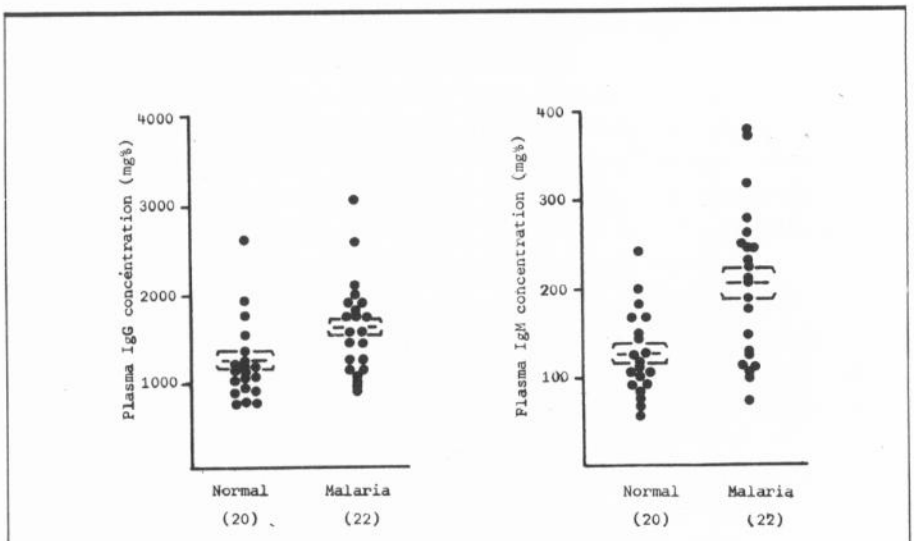

Fig. 1 Plasma IgG and IgM concentrations of normal subjects and malaria patients, mean ± SE.

Secreted immunoglobulins from PBMLs of normal subjects and malaria patients

PBMLs from twenty normal subjects and 22 malaria patients were assayed for their secreted immunoglobulins with or without PWM stimulation. After PWM stimulation, there were no differences in the mean values of secreted IgG or IgM ($p > 0.05$) amongst the PBMLs test groups, including those from patients infected with *P. falciparum* or *P. vivax*. However, without PWM stimulation, the mean values of secreted IgG and IgM from cells of malaria patients were significantly higher than those of normal subjects ($p < 0.005$ and $p < 0.025$, respectively) (Figs. 2,3).

Again, however, there was no significant difference between the PBMLs from patients infected with *P. falciparum* or *P. vivax*.

Assay for suppressor T cell activity

The activity of suppressor T cells was studied. With normal PBMLs, the mean percentages suppression of IgG and IgM secretion caused by T cells (4.0×10^5/ml) of normal subjects were higher than those caused by T cells (4.0×10^5/ml) of malaria patients ($p < 0.05$ and $p < 0.025$, respectively). At higher T cell concentrations (6.0×10^5/ml), the mean percentage suppression of IgM secretion caused by normal T cells was also higher than that caused by T cells from malaria patients ($p < 0.05$) (Figs. 4,5). Therefore, there was a decrease in the suppressor T cell activity in malaria patients.

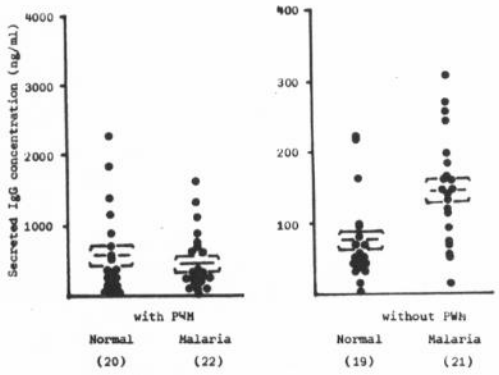

Fig. 2 Secreted IgG concentrations with and without PWM stimulation of normal and malaria patient PBMLs, mean ± SE.

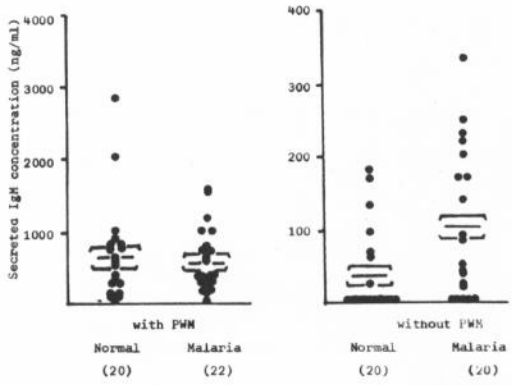

Fig. 3 Secreted IgM concentrations with and without PWM stimulation of normal and malaria patient PBMLs, mean ± SE.

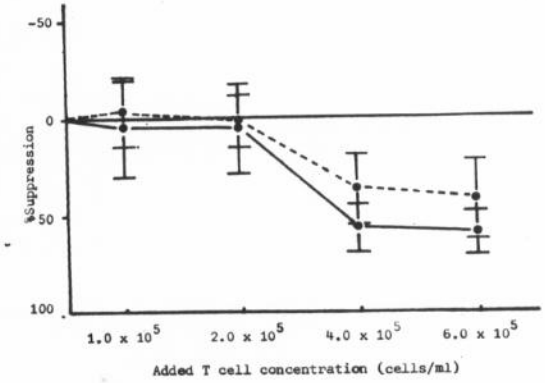

Fig. 4 Mean percentage suppression of IgG secretion of normal PBMLs by normal T cells (——) or malaria patient T cells (– – –), mean ± SD.

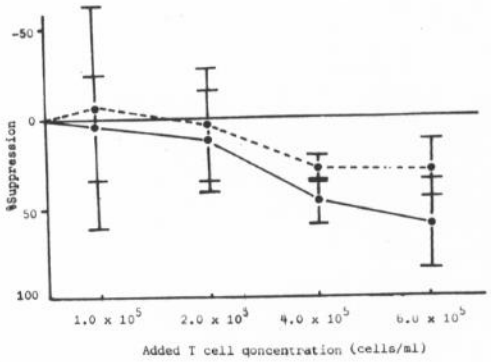

Fig. 5 Mean percentage suppression of IgM secretion of normal PBMLs by normal T cells (——) or malaria patient T cells (– – –), mean ± SD.

Assay for B cell activity

With PBMLs from malaria patients, the mean percentages suppression of IgG and IgM secretion caused by normal T cells were not significantly different from those caused by malaria patient T cells ($p>0.05$) (Figs. 6,7). However, with normal T cells, the mean suppressions of IgG and IgM secretions for normal PBMLs were higher than those for malaria patient PBMLs ($p<0.05$) (Figs. 8,9). These results indicated that the B cell function in response to normal suppressor T cells was diminished during malaria infection.

DISCUSSION

There is a reported increase of serum immunoglobulins in malaria patients.[2,10-13] This experiment confirms those reports which show that hypergammaglobulinaemia is a prominent feature in human malaria.

A comparison of IgG and IgM secretions from *in vitro* culture of PBMLs from normal controls and malaria patients revealed that, with PWM stimulation, there were no significant differences. However without PWM stimulation, there were significant increases of secreted IgG and IgM for malaria patient PBMLs compared to normal PBMLs, despite the fact that the percentages of B and T cells in the patient PBMLs were not different from the normal (data not shown). Since PWM is highly effective in the functional activation of human lymphocytes,[14] it is possible that PBMLs of both malaria

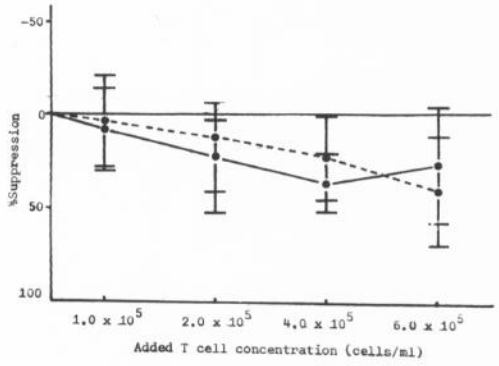

Fig. 6 Mean percentage suppression of IgG secretion of malaria patient PBMLs by normal T cells (——) or malaria patient T cells (– – –), mean ± SD.

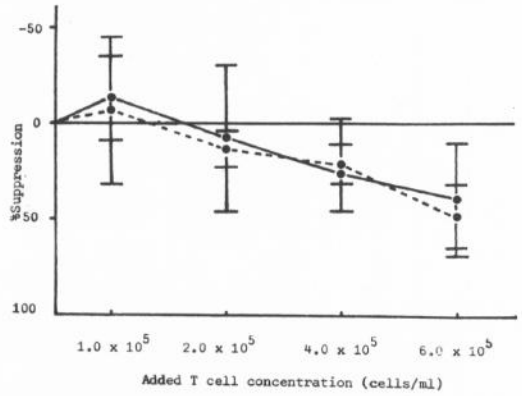

Fig. 7 Mean percentage suppression of IgM secretion of malaria patient PBMLs by T cell (——) or malaria patient T cells (– – –), mean ± SD.

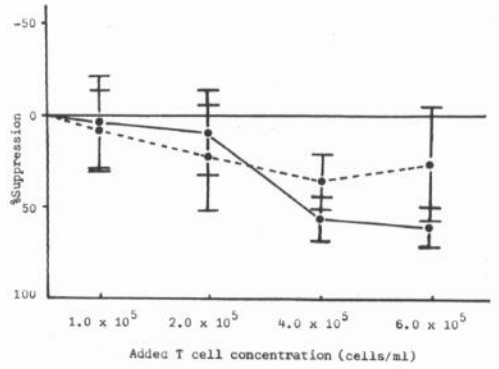

Fig. 8 Mean percentages suppression of IgG secretion of normal PBMLs (——) or malaria patient PBMLs (– – –) by normal T cells., mean ± SD.

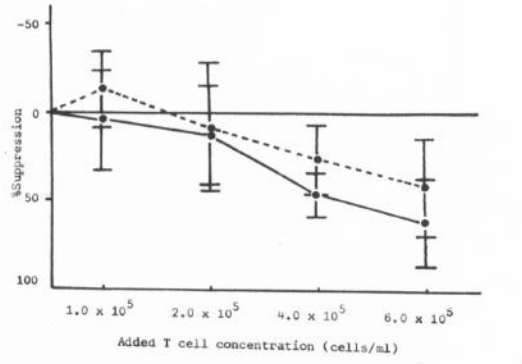

Fig. 9 Mean percentage suppression of IgM secretion of normal PBMLs (——) or malaria patient PBMLs (– – –) by normal T cells., mean ± SD.

patients and normal subjects were activated to secrete maximum immunoglobulins. This could explain the lack of significant difference between the two groups. The results from monitoring immunoglobulin secretion without PWM stimulation indicated that a polyclonal activation of B cells occurred during the course of malaria infection, with both *P. falciparum* and *P. vivax* infections.

It has been found that peripheral T cells, comprising a distinct subpopulation of prosuppressor cells, can be activated by PWM to function as suppressors of immunoglobulin synthesis.[14,15] When this suppression was used with normal PBMLs to assay suppressor T cell activity in our study, the mean percentages suppression of IgG and IgM secretion caused by normal T cells were higher than those for malaria patient T cells. This showed that suppressor T cell activity was diminished during malaria infection.

In order to assay for B cell function in response to suppressor T cells, PBMLs from malaria patients were examined for mean percentages suppression of IgG and IgM by normal T cells and by malaria patient T cells. There were no significant differences in the mean values. However, with normal T cells, the mean suppressions of IgG and IgM secretion for normal PBMLs were higher than those from malaria patient PBMLs. These results confirmed that the B cell function of malaria patients in response to normal suppressor T cells is decreased. These defects in suppressor T cell and B cell function in malaria patients may play some role in the hypergammaglobulinaemia phenomenon.

ACKNOWLEDGEMENTS

The authors are most grateful to the Foundation of Somdej Kromphaya Chainatnrenthorn which supported part of this work.

REFERENCES

1. Rowe DS. McGregor IA, Smith SJ, *et al.* Plasma immunoglobulin concentration in a West African (Gambian) community and in a group of healthy British adults. Clin Exp Immunol 1968; 3:63-79.
2. Cohen S, Butcher GA. Serum antibody in acquired malaria immunity. Trans R Soc Trop Med Hyg 1971; 65:125-35.
3. Targette GAT. Antibody response to *Plasmodium falciparum* malaria: Comparison of antibody titers and the antigenicity of different asexual forms of the parasite. Clin Exp Immunol 1970; 7:501-17.
4. Freeman T, Smithers SR, Targette GAT, Walker PJ. Specificity of immunoglobulin G in rhesus monkey infected with *Schistosoma mansoni, Plasmodium knowlesi* and *Trypanosoma brucei.* J Infect Dis 1970; 121: 401-6.
5. Greenwood BM, Bradley-Moore AM, Palit A, Bryceson ADM. Immunosuppression in children with malaria. Lancet 1972; 1:169-72.
6. Lustig, HJ, Nussenzweig V, Nussenzweig RS. Erythrocyte membrane associated immunoglobulins during malaria infection of mice. J Immunol 1977; 119:210-6.
7. Boyum A. Isolation of mononuclear cells and granulocytes from human blood. Scand J Clin Lab Invest 1968; 21 (Suppl. 97): 77-89.
8. Wakefield DF, Shelton MJ, Hosking CS. A quantitative ELISA for IgG in cell culture supernatants. Clin Chim Acta 1982; 123:303-10.
9. Pellegrino MA, Ferrone S, Dierich MP, Reisfeld RA. Enhancement of sheep red blood cell human lymphocyte rosette formation by the sulfhydryl compound 2-amino-ethylisothiouronium. Clin Immunol Immunopathol 1975; 3:324-33.
10. McGregor IA, Gilles HM, Walters JH, *et al.* Effects of heavy and repeated malaria infections of Gambian infants and children: Effects of erythrocytic parasitization. Br Med J 1956; 1:686-92.
11. Cohen S, McGregor IA, Carrington S. Gammaglobulin and acquired immunity to human malaria. Nature 1961; 129:733-7.
12. Tobie JE, Abele DC, Wolff SM, *et al.* Serum immunoglobulin levels in human malaria and their relationship to antibody production. J Immunol 1966; 97:498-505.
13. Collins WE, Contacos PG, Skinner JC, *et al.* Patterns of antibody and serum proteins in experimentally induced human malaria. Trans R Soc Trop Med Hyg 1971; 65: 43-58.
14. Waldmann TA, Broder S. Polyclonal B-cell activators in the study of the regulation of immunoglobulin synthesis in human system. Adv Immunol 1982; 32:1-64.
15. Broder S, Edelson RL, Lutzner DL, *et al.* The Sezary syndrome: a malignant proliferation of helper T cells. J Clin Invest 1976; 58:1297-1306.