Clin. exp. Immunol. (1985) **59,** 571–576.

Complement changes in falciparum malaria infection

P. PHANUPHAK,* MATTANA HANVANICH,* REUTAI SAKULRAMRUNG,† P. MOOLLAOR,* V. SITPRIJA* & D. PHANTHUMKOSOL‡ *Departments of* **Medicine and* † *Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok and* ‡ *Phra-Pok-Klao Hospital, Chandhaburi Province, Thailand*

(Accepted for publication 18 October 1984)

SUMMARY

Complement profiles were sequentially studied in 183 Thai adults infected with *Plasmodium falciparum*. On the first day of admission, CH_{50}, C1q, C4 and C3 were low in 65%, 8%, 19% and 62% of cases, respectively. All patients with low C1q or C4 also had low C3 and CH_{50}. Simultaneous reduction of C1q, C4, C3 and CH_{50} were found in 10 instances. Factor B was not reduced in any of the patients indicating that only the classical pathway is activated during acute falciparum malaria infection. The incidence and the degree of hypocomplementaemia were higher in patients with cerebral, renal and hepatic complications although significant difference was seen only for C3. After 3–4 days of effective anti-malarial treatment, normalization of C1q and C4 was found in almost all instances whereas C3 and CH50 remained low in 27% and 54% of the cases, respectively. Normalization of C3 was achieved at 4 weeks after discharge while low CH_{50} still persisted. The reasons for the persistently low CH_{50} remain unknown.

Keywords falciparum malaria hypocomplementaemia classical pathway activation

INTRODUCTION

Changes in the complement system following malarial infection have long been recognized. Total haemolytic complement activity (CH_{50}) along with several components of the classical complement pathway have been found to decrease during acute malarial infection (Dulaney *et al.*, 1948; Rosenberg *et al.*, 1973; Greenwood & Brueton, 1974; Adam *et al.*, 1981). Components of the alternate complement pathway however, were largely undiminished (Greenwood & Brueton, 1974; Petchclai *et al.*, 1977). The degree of hypocomplementaemia was found to correlate with various complications of malaria such as disseminated intravascular coagulation, jaundice and cerebral malaria (Dulaney *et al.*, 1948; Srichaikul *et al.*, 1975; Petchclai *et al.*, 1977; Adam *et al.*, 1981) although controversy exists (Greenwood & Brueton, 1974). In contrast to the small and selected series previously reported, we report here the sequential changes of the classical and alternate complement pathways in 183 unselected Thai adults infected with *Plasmodium falciparum* from the day of admission to 4 weeks after recovery.

MATERIALS AND METHODS

Patients. A total of 183 Thai adults hospitalized for the treatment of falciparum malaria were included in this study. The biographic data of these patients have been previously described

Correspondence: Dr Praphan Phanuphak, Division of Allergy and Clinical Immunology, Department of Medicine, Chulalongkorn Hospital Medical School, Bangkok 10500, Thailand.

(Phanuphak *et al.*, 1983). In brief, 148 were uncomplicated malaria with 35 complicated cases. The complications consisted of 20 with cerebral malaria, nine with jaundice, four with renal failure and two with massive intravascular haemolysis, although overlap of complications occurred. The mean duration of symptoms prior to admission was 5·0 days (s.d. = 3·5 days). The degree of parasitaemia ranged from 470/mm^3 to 437,580/mm^3 with a mean of 45,846/mm^3 (s.d. = 80,391).

Serum collection. Venous blood was obtained from the patient on the day of admission (S1) and again on the 3rd or 4th day after admission (S2) when the parasitaemia had been almost cleared, i.e., prior to discharge from the hospital. All patients were treated with a 14 day course of quinine as well as other supportive measures started on the first day of admission. All patients were asked to return for follow-up 2 and 4 weeks after discharge. However, only 84 and 40 patients returned for 2(S3) and 4 week (S4) follow-up, respectively. Sera were separated and stored in a −70°C freezer prior to being shipped under dry ice to the Immunology Laboratory in Bangkok.

Complement determinations. CH_{50} was assayed according to the method of Mayer (1971) using locally prepared haemolysin. Antigenic concentrations of C1q, C3 and C4 were determined by radial immunodiffusion using standards and antisera purchased from Behringwerke AG, Marburg, FRG. A standard for, and antiserum to, factor B was purchased from Pel-Freez Biologicals, Rogers, Arkansas, USA.

Normal controls. Sera from 100 healthy volunteer blood donors at the National Blood Bank Center served as normal controls for the various complement levels. The age and the sex ratio of the normal controls were comparable to those of the patients. The only differences were that the controls were residents of Metropolitan Bangkok and were free of malarial infection at the time of blood donation. Any complement levels lower than mean − 1·5 s.d. were considered abnormal in this study (only 2–4% of the normal controls had complement levels lower than those cut-off levels).

RESULTS

Complement profiles on admission

On admission, 119 out of the 183 patients (65%) had low CH_{50}. Forty-eight patients (26%) had CH_{50} below the measurable or threshold level, i.e., lower than 10 units/ml. Eleven of these patients were from the group with complicated malaria (11 of 35 or 31%) whereas 37 were from the uncomplicated group (37 of 148 or 25%). The distribution of CH_{50} values in 35 complicated and 148 uncomplicated malaria patients on the first day of admission is shown in Fig. 1.

Clq, C4 and C3 on admission were abnormally low in 14, 36 and 114 patients corresponding to 7·7%, 19·7% and 62·3% of the total patients. All of the patients with low C1q or C4 also had low C3. Simultaneous reduction of Clq, C4 and C3 were found in 12 patients. Six of these 12 patients had complicated malaria, i.e., a 17% (six of 35) incidence as compared to the 4% (six of 148) incidence in the uncomplicated group. It is interesting to note that two out of the three deaths in our study had such a profound complement change whereas the third death had only low C3 and CH_{50}. Factor B, however, was not reduced in any of the patients.

Inspite of the abnormally low C1q andC4 in certain malaria patients on admission, the mean values of C1q, C4 and factor B in the entire group of patients were higher than those of the normal controls (Table 1). This was due to several high values reflecting an acute phase response to infection. However, the mean value of C3 in the entire group of patients was significantly lower than normal controls ($P < 0{\cdot}001$). In addition, mean C3 levels in the complicated malaria cases was lower than that of the uncomplicated cases, although the difference was not statistically significant (Table 1). Nevertheless the complement levels did not correlate with the degree of parasitemia or with the levels of circulating immune complexes present in the patients as determined by a liquid phase C1q binding test (data not shown).

Sequential changes in complement levels after anti-malarial treatment

Most of the low C1q, C4 and C3 levels on admission returned to normal 3–4 days after initiation of anti-malarial chemotherapy (S2) (Table 2). Almost none had low C1q or C4 when they returned for the 2 and 4 week follow-up (Table 2). Only 20% (17 of 84) and 10% (four of 40) of the patients

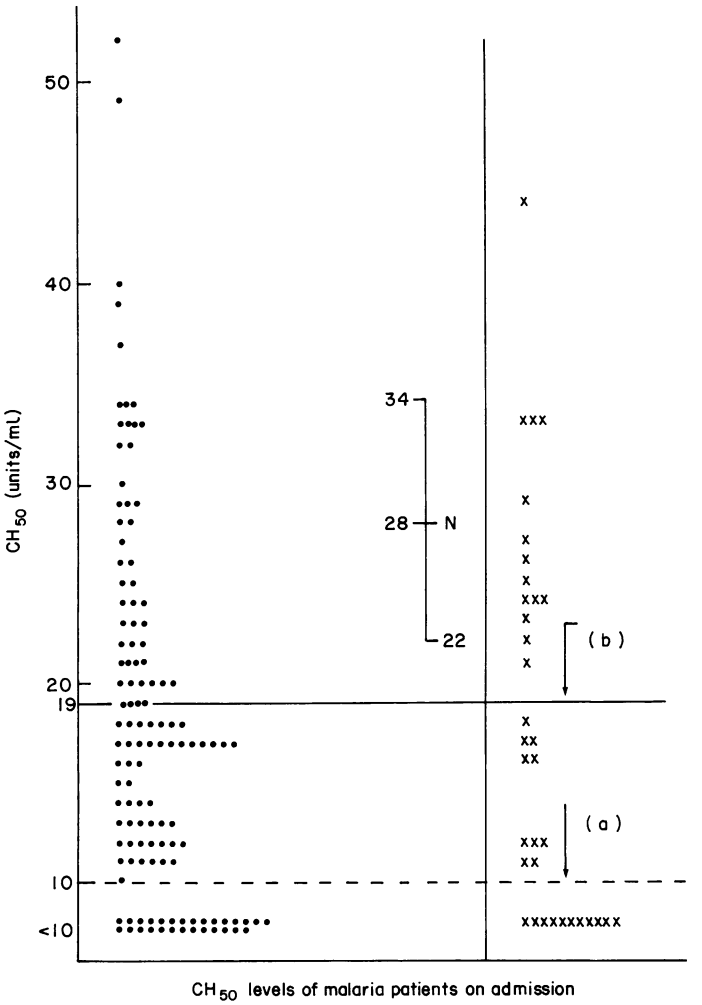

Fig. 1. CH_{50} levels of 148 uncomplicated (●) and 35 complicated (x) falciparum malaria patients on admission. Ten units per millilitre was the lowest measurable level in the assay system. CH_{50} levels of 100 normal controls (N) are illustrated by longitudinal line representing mean ± s.d. (28 ± 6 units/ml). The lowest limit of normal CH_{50} was set at 19 units/ml (mean − 1·5 s.d.). (a) = measurable threshold level; (b) = lowest limit of normal CH_{50}.

Table 1. Levels of various complement components in 183 falciparum malaria patients on the first day of admission

Complement	Normal values ($n=100$) (mean ± s.d.)	Malaria patients		
		Total ($n=183$)	Uncomplicated ($n=148$)	Complicated ($n=35$)
C1q	17·0 ± 2·7 mg%	21·0 ± 7·4	21·1 ± 6·9	20·7 ± 9·2
C4	18·0 ± 6·7 mg%	24·1 ± 20·8	24·0 ± 20·3	24·6 ± 22·6
C3	128·0 ± 29·0 mg%	81·5 ± 31·1*	83·2 ± 30·2*	74·2 ± 33·9*
Factor B	13·0 ± 5·2 mg%	20·1 ± 6·7	20·0 ± 6·2	20·0 ± 8·6

* Significantly lower than normal controls ($P < 0·001$).

continued to have low C3 at 2 (S3) and 4 weeks (S4) after discharge, respectively. Unlike the complement components, the incidence of low CH_{50} did not decline with time up to 4 weeks of follow-up (Table 2).

Most of the low complement components in S2, S3 or S4 were continuations from S1, although some instances of hypocomplementaemia started in S2. Two of the three deaths were among those who continued to have low C1q, C4, C3 andCH_{50} in S2, and although their erythrocytic forms of malarial parasites had already been eradicated these patients died of complications.

Sequential changes of C3 levels after *P. falciparum* infection are illustrated in Fig. 2. It is evident that levels of C3 gradually increased with time after acute infection, reaching normal levels 4 weeks after infection. C3 levels in S2 and S3 were still significantly lower than normals although the level in S2 had already increased considerably from the initial level (S1). When C3 levels in complicated and uncomplicated cases were compared, there was a suggestion of lower C3 in the complicated group during S1 and S2 although the difference was not statistically significant (Fig. 2).

DISCUSSION

We have demonstrated that acute falciparum infection is often associated with profound hypocomplementaemia. C1q, C4 and C3, but not factor B, were the complement components mostly affected, suggesting that only the classical pathway of complement was involved. Our observations are in agreement with other earlier reports (Greenwood & Brueton, 1974; Petchclai *et al.*, 1977; Adam *et al.*, 1981) except that our patient population was larger and unselected for any particular complication or severity.

It is difficult to accurately determine the onset of hypocomplementaemia in human malaria due to uncertainty as to the precise time of infection as well as the variable time intervals after infection before diagnosis can be established. The first blood samples (S1) in our study were obtained 5 days (s.d. = 3·5) after the onset of clinical symptoms and hypocomplementaemia was already apparent in the majority of these samples. This roughly correlates with Adam *et al.* (1981) who found that hypocomplementaemia could be detected as early as 3 days after the onset of clinical symptoms, reaching a nadir on day 9.

In experimental murine malaria, June *et al.* (1979) found that C3 levels began to fall 9 days after *P. berghei* infection in OF_1 mice and returned to normal within 3 days after anti-malarial treatment. Our study, the first large scale prospective study of complement changes in falciparum malaria showed results similar to experimental malaria in mice; most of the complement components normalized within 3–4 days after effective anti-malarial therapy. Persistently low C1q, C3 and C4 after treatment may be a bad prognostic sign since two out of the four patients whose C1q, C3 and C4 remained simultaneously low 3–4 days after quinine treatment died of complications, even

Table 2. Prevalence of low C1q, C4, C3, factor B and CH_{50} at different time periods after malarial infection

Complement components	Number with low complement levels (percentage)			
	S1 (n = 183)	S2 (n = 170)	S3 (n = 84)	S4 (n = 40)
C1q	14 (7·7%)	4 (2·4%)	0 (0%)	0 (0%)
C4	36 (19·7%)	6 (3·5%)	1 (1·2%)	0 (0%)
C3	114 (62·3%)	45 (26·5%)	17 (20·2%)	4 (10·0%)
Factor B	0 (0%)	0 (0%)	0 (0%)	0 (0%)
CH_{50}	119 (65·0%)	91 (53·5%)	55 (65·5%)	27 (67·5%)

S1 = On the day of admission; S2 = 3–4 days after treatment, just before discharge; S3 = 2 weeks after discharge and S4 = 4 weeks after discharge.

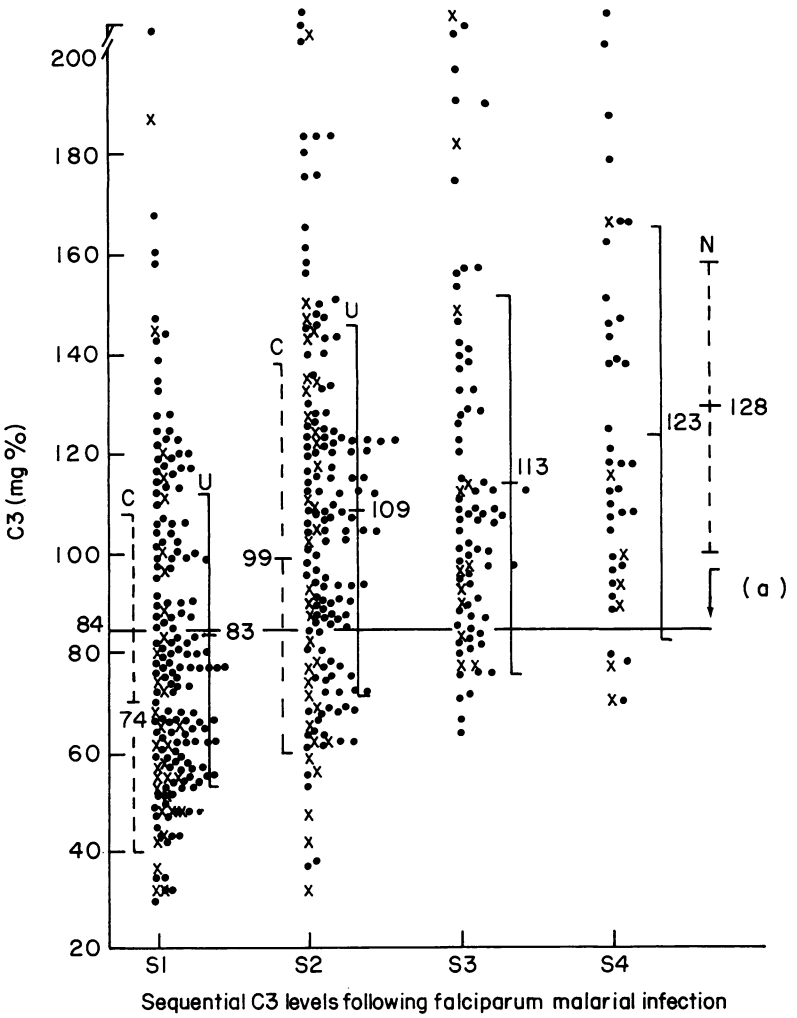

Fig. 2. Sequential C3 levels following falciparum malarial infection. S1 = on the day of admission, S2 = 3–4 days after treatment, just before discharge, S3 = 2 weeks after discharge, S4 = 4 weeks after discharge. Mean and standard deviation of the complicated cases (C, represented by x) and the uncomplicated cases (U, represented by ●) are separately illustrated on S1 and S2 but are collectively presented on S3 and S4. Mean C3 level of normal controls (N) was 128 mg% (s.d. = 29). The lowest limit of normal C3 was set at 84 mg% (mean − 1·5 s.d.). (a) = lowest limit of normal C3.

though they were free from the asexual forms of the malarial parasites. However, in general it took longer (i.e., 4 weeks) for C3 to return to normal (Fig. 2).

We were surprised to see that functional CH_{50} in the majority of patients remained low for up to 4 weeks after hospital discharge. The low CH_{50} was not accompanied by low C1q or C4 but was occasionally accompanied by low C3. We have made several spot checks and were certain that low CH_{50} was not due to mishandling of the serum specimens. It is possible, although unlikely, that the low CH_{50} observed was caused by the concomitant reduction of other complement components not determined in this study, such as C2, C5 or C8. Another possibility is the presence of some undefined anti-complementary activity in the serum similar to that suggested by Gewurz & Ertel (1973). However, in our studies, patient's serum did not interfere with the CH_{50} of the normal serum when mixed in equal proportion, suggesting that there was no anti-complementary activity in

patient's serum. As a result, the possibility of *in vivo* correction of hypocomplementaemia with exchange transfusion was not investigated.

Although the clinical significance of hypocomplementaemia in malarial infection remains obscure, it is clear that many of the complications of malarial infection are mediated through the activation of complement by immune complexes (June *et al.*, 1979; Adam *et al.*, 1981). However, like others (Greenwood & Brueton, 1974; Ganguly *et al.*, 1980), we could not find any significant correlation between hypocomplementaemia and malarial complications, possibly due to some high values within the group reflecting an acute phase response to infection.

An interesting area for research is why complement is decreased during malarial infection. Possibilities are (a) decreased complement synthesis from malaria infected hepatocytes, (b) increased complement catabolism as a result of malarial infection, (c) immune consumption of the complement due to complement fixing anti-merozoite antibodies and the released merozoites (Cooper & Fogel, 1966; Neva *et al.*, 1974), or soluble malarial antigen–antibody complexes (June *et al.*, 1979) or (d) some undefined anti-complement activity. However, we found no correlation between hypocomplementaemia and the degree of parasitaemia or the level of circulating immune complexes in our studies.

We acknowledge the support of Rachadapiseksompoj–China Medical Board Grant No. 76-357. We also thank all hospital staff of the Phra-Pok-Klao Hospital for assistance in the study; Miss Aroon Prakobkarndee for excellent technical assistance; Mrs Vallapa Kaewmora for preparing the manuscript; and Dr Bruce Reynolds for critical review of the manuscript.

REFERENCES

ADAM, C., GENITEAU, M., GOUGEROT-POCIDALO, M., VERROUST, P., LEBRAS, J., GILBERT, C. & MOREL-MAROGER, L. (1981) Cryoglobulins, circulating immune complexes, and complement activation in cerebral malaria. *Infect. Immun.* **31,** 530.

COOPER, M.R. & FOGEL, B.J. (1966) Complement in acute experimental malaria. II. Alterations in the components of complement. *Milit. Med.* **131 (suppl.),** 1180.

DULANEY, A.D., PRIEST, J.B., ALMEDA, M.L. & PARKER, B. (1948) The complement content of human sera with especial reference to malaria. *J. clin. Invest.* **27,** 320.

GANGULY, N.K., CHANDANANI, R.E., MAHAJAN, R.C., SHARMA, S. & VASUDEVA, V. (1980) Immunoglobulins and C3 levels in *Plasmodium vivax* infection and their relationship to haemagglutination antibody titres. *Indian J. Med. Res.* **71,** 505.

GEWURZ, H. & ERTEL, N. (1973) Non-immune activation of complement: two new phenomena. In *'Non-specific' Factors Influencing Host Resistance* (ed. by W. Braun & J. Ungar) p. 340. S. Karger, Basel.

GREENWOOD, B.M. & BRUETON, M.J. (1974) Complement activation in children with acute malaria. *Clin. exp. Immunol.* **18,** 267.

JUNE, C.H., CONTRERAS, C.E., PERRIN, L.H., LAMBERT, P.H. & MIESCHER, P.A. (1979) Circulating and tissue-bound immune complex formation in murine malaria. *J. Immunol.* **122,** 2154.

MAYER, M.M. (1971) Complement and complement fixation. In *Experimental Immunochemistry* (ed. by E.A. Kabat) p. 133. Charles C. Thomas, Publisher, Springfield.

NEVA, F.A., HOWARD, W.A., GLEW, R.H., KROTOSKIW, A., GAM, A.A., COLLINS, W.E., ATKINSON, J.P. & FRANCK, M.M. (1974) Relationship of serum complement levels to events of malaria paroxysm. *J. clin. Invest.* **54,** 451.

PETCHCLAI, B., CHUTANONDH, R., HIRANRAS, S. & BENJAPONGS, W. (1977) Activation of classical and alternate complement pathways in acute falciparum malaria. *J. Med. Assoc. Thai.* **60,** 174.

PHANUPHAK, P., TIRAWATNPONG, S., HANVANICH, M., PANMUONG, W., MOOLLAOR, P., VEJJAJIVA, S., SITPRIJA, V., INTARAPRASERT, R. & PHANTHUMKOSOL, D. (1983) Autoantibodies in falciparum malaria: a sequential study in 183 Thai patients. *Clin. exp. Immunol.* **53,** 627.

ROSENBERG, E.B., STRICKLAND, G.T., YANG, S.L. & WHALEN, G.E. (1973) IgM antibodies to red cells and autoimmune anemia in patients with malaria. *Am. J. Trop. Med. Hyg.* **22,** 146.

SRICHAIKUL, T., PUWASATTEN, P., PUWASATTEN, P., KARNJANAJETANEE, J. & BOKISH, V.A. (1975) Complement changes and disseminated intravascular coagulation in *Plasmodium falciparum* malaria. *Lancet*, **i,** 770.