IMMUNOLOGICAL EVALUATION OF CELL-MEDIATED AND HUMORAL IMMUNITY IN THAI PATIENTS WITH CEREBRAL AND NON CEREBRAL *PLASMODIUM FALCIPARUM* MALARIA: II. Evolution of Serum Levels of Immunoglobulins, Antimalarial Antibodies, Complement Fractions and Alpha Interferon

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INTRODUCTION

In human malaria, polyclonal B cell activation was proposed as the origin of the considerable elevation of serum immunoglobulins observed in these patients (Greenwood *et al.*, 1981). It was suggested that anti malarial antibodies only partially account for this phenomenon (Curtain *et al.*, 1964 ; Cohen *et al.*, 1961). In the group of patients with cerebral (CM) and non cerebral malaria (NCM) previously described (Brasseur *et al.*, 1983), we have investigated comparatively serum immunoglobulin and antimalarial antibody levels during the course of infection in order to determine if (a) the capability to raise an antibody response; and/or (b) antimalarial antibody levels could be associated to protection in these patients. Total C3 and C4 complement component levels were simultaneously determined. Another potentially protective mechanism may involve interferon mediated mechanisms during experimental malaria (Jahiel *et al.*, 1970). In these patients, we have confirmed that alpha interferon is detectable in the serum from the onset of infection.

MATERIALS AND METHODS

Patients with cerebral malaria (CM) or non-cerebral malaria (NCM) were described previously by Brasseur *et al.*, (1985). When not specified, data presented (initial values) apply to sera obtained in the first day of admission in the hospital. In addition, three patients with acute *P. vivax* malaria were included as controls.

Serum IgG, IgM and IgA levels were measured using heavy chain specific antibodies in an immunoephelometric method (Ritchie, 1967) by comparison with control sera from healthy adult French donors (mean values : IgG : 1200 ± 300 mg/100 ml; IgM : 110 ± 50 mg/100 ml; IgA : 275 ± 125 mg/100 ml.

Total antimalarial antibodies were detected by a counter immunoelectrophoresis method (CIEP) using soluble *P. falciparum* antigen (Druilhe *et al.*, 1978). Immunofluorescence detected antibodies (IFA) of the IgG or IgM class were measured using an indirect immunofluorescent assay with thin smears of *P. falciparum* blood stages from long term cultures of the FCR strain as antigen.

Malaria serum soluble antigens (MSSA) were detected using a CIEP method using a hyperimmune serum from an African donor.

C_3 and C_4 complement fractions were measured using the immunoephelometric method previously described (Ritchie, 1967) by comparison with control sera from healthy adult French donors (mean values : C_3 : 160 ± 70 mg/100 ml ; C_4 : 65 ± 70 mg/100 ml). Controls consisted in 17 healthy Thai individuals. In the latter group, mean values were : 276 ± 72 mg/100 ml for C_3 ; and 94 ± 17 mg/100 ml for C_4.

Interferon like activity in sera was measured as previously described (Rhodes-Feuillette *et al.*, 1979) on human embryonic AV3 cells by inhibition of vesicular stomatitis virus (VSV) cytopathic effect and read by two observers comparatively to a standard interferon. This test was completed by determination of the type of interferon. Dialysis at pH2 indicated that the acid-labile interferon activity was probably gamma interferon. In addition, seroneutralization assay was performed using anti-alpha and anti-gamma interferon antibodies.

RESULTS

IgG, IgM and IgA serum immunoglobulin levels

Fig. 1, 2 and 3 show the distribution of IgG, IgM and IgA serum levels in 97 patients. By comparison with healthy controls (in mg/100 ml : 1200 ± 300, 110 ± 50 and 275 ± 125 for IgG, IgM and IgA, respectively), IgG and IgM values were elevated in patients with acute malaria, whereas no significant difference was observed for IgA. No association of Ig values with parasitaemia levels was noted. No significant difference in immunoglobulins levels was observed between the two CM and NCM groups (Table 1).

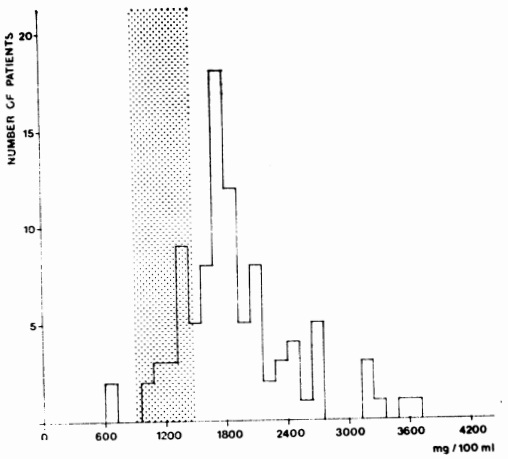

Fig. 1—Distribution of initial serum IgG levels in patients with cerebral and non cerebral *P. falciparum* malaria.

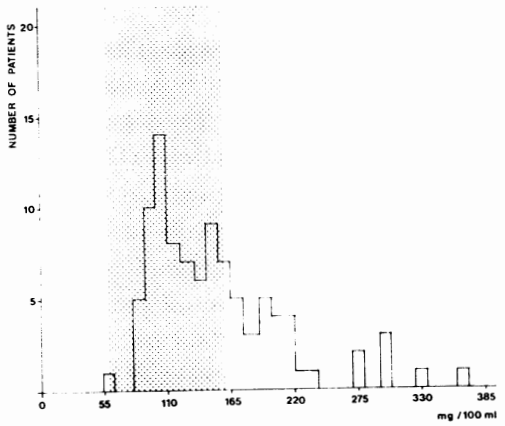

Fig. 2—Initial serum IgM levels in patients with cerebral and non cerebral *P. falciparum* malaria.

Antimalarial antibodies

Detection of antimalarial antibodies using CIEP was negative in 31/97 patients. Among them, 20 exhibited cerebral manifestations, 13 had an initial parasitaemia over 5 %, and 11 died within less than a week.

IFA-IgM antibodies were found in 62/97 patients (range 1/200 to 1/5400). IgM antibody levels were lower in the CM than in the NCM group (Table 1) ($p < 0.01$). IFA-IgG

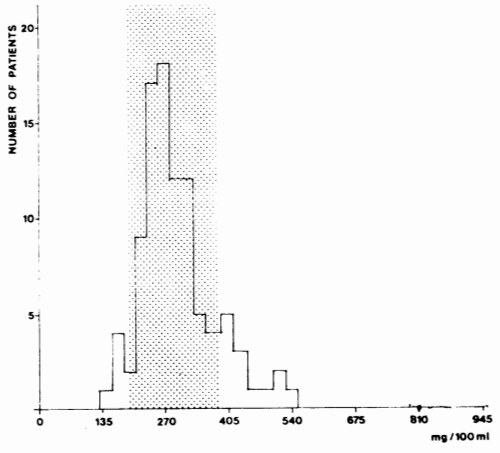

Fig. 3—Initial serum IgA levels in patients with cerebral and non cerebral *P. falciparum* malaria.

antibodies were present in all patients but two (1/200 to 1/48600). Mean IgG antibodies were non significantly lower in the CM than in the NCM group. It is of interest that for both IgG and IgM, mean antibody levels were much lower in the twelve CM or NCM patients with a lethal issue ($p<0.01$). Table 2 shows that a good correlation was found between data obtained with the CIEP and immunofluorescence techniques.

In 43 patients, a sequential study was performed. In 9/14 patients in which no antibodies were initially detected, the search remained negative 10-21 days later using CIEP.

Table 1

Mean values of immunoglobulins IgG, IgM, IgA, malarial fluorescent antibodies IgG, IgM, complement components C_3 and C_4 in 97 CM and NCM patients.

Patients	Immunoglobulins*			Malarial Immunofluorescent Antibodies**		Complement Components*	
	IgG	IgM	IgA	IgG	IgM	C_3	C_4
CM (40)	2036 (± 394)	147 (± 49)	331 (± 93)	1/6000 (± 6033)	1/230 (±331)	144 (± 70)	48 (± 26)
NCM	1885 (± 558)	158 (± 47)	284 (± 119)	1/7087 (± 8070)	1/551 (± 817)	153 (± 51)	48 (± 27)

* Expressed in mg/100 ml.
** Expressed as the last dilution with IFA positive.

C_3 and C_4 complement components

Table I shows that the mean C_3 and C_4 values were similar among patients from the CM and the NCM groups. In all patients, initial values were significantly lower than values obtained in control Thai individuals ($p < 0.01$, and $p < 0.1$, respectively). C_3 levels lower than 64 mg/100 ml were observed in 6 patients and found associated with high parasitaemia ranging from 6 to 35 %. In three patients with acute *P. vivax* infection, C_3 and C_4 mean values were 237 ± 89 mg/100 ml and 96 ± 27 mg/100 ml, respectively). An influence of the presence of MSSA was found on C_3 levels (C_3 values: in patients with MSSA: 127 ± 40 mg/100 ml ; in patients without MSSA: 175 ± 51 mg/100 ml, but non significantly. In patients in which sequential studies could be performed, a normalization of C_3 and C_4 values was observed by day 5 following treatment (Fig. 4).

Table 2

Correlation between the results of initial IFA and CIEP in malaria patients.

IFA	CIEP				Total
	0*	1	2	≥3	
0**	2***	0	0	0	2
1/200	6	1	0	0	7
1/600	4	6	0	0	10
1/800	10	12	1	0	23
1/5400	3	20	5	1	29
>1/16200	6	14	4	2	26
Total	31	53	10	3	97

*Number of precipitating bands in CIEP.
**Last dilution found positive in IFA.
***Number of patients.

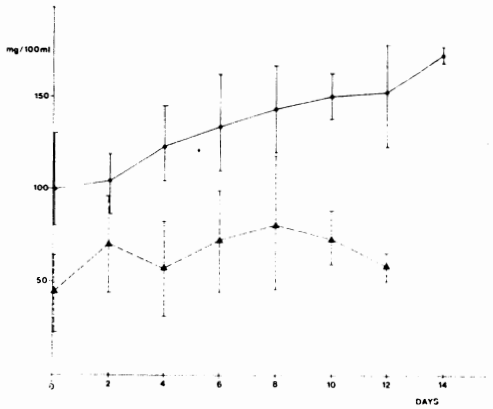

Fig. 4—Evolution of C_3 and C_4 serum levels in acute malaria patients. Expressed as mean values (C_3: —, C_4: ---) 1SD in 97 patients with cerebral or non-cerebral malaria.

Interferon like activity

Whereas no interferon like activity was detected in sera from healthy French controls, in all CM and NCM patients this type of activity was found. Levels of interferon varied between 40 and 640 units/ml, and no correlation was found with either antimalarial antibodies, complement or parasitaemia.

Table 3

Short term follow-up of serum interferon among 10 cerebral malaria patients.

Case No.	Day 0		Day 1		Day 2		Day 3	
	Titer*	Type**	Titer	Type	Titer	Type	Titer	Type
1	< 20	–	20	–	–	–	20	γ
2	40	α + γ	40	α + γ	40	α + γ	80	γ
3	40	α + γ	–	–	80	γ	–	–
4	–	–	20	–	40	α + γ	20	γ
5	–	–	40	γ	20	–	20	γ
6	–	–	40	γ	20	γ	20	γ
7	–	–	40	α + γ	–	–	–	–
8	–	–	160	α	–	–	20	γ
9	–	–	40	γ	40	γ	–	–
10	20	–	–	–	–	–	40	γ

* Evaluated in unit/ml.
** Determination by dialysis at pH 2 and specific seroneutralization.

In those patients followed over a several days period, a trend to the rise of circulating interferon was found. Table 3 shows that in 10 CM patients for which one or several sera were further available for testing, immune or gamma type interferon was found in all, and in five cases, alpha interferon was also found. Alpha interferon was found initially, and in two cases followed over 48 hrs, the molecular species of interferon varied from (alpha + gamma) types to gamma type interferon found alone.

DISCUSSION

Elevated Ig levels were reported for long in human malaria (Abele *et al.*, 1965; Cohen *et al.*, 1974). In the present series of Thai patients, IgG and IgM were found increased, whereas IgA levels were found within normal ranges. It is noteworthy that values in these patients were lower than those we found previously using the same methods in African patients from Burkina-Faso, or in Amerinds from French Guyana (Fribourg-Blanc *et al.*, unpublished data). This may be related to different epidemiological statuses, although normal range may differ in different populations (Grange *et al.*, 1983).

It was previously established that only a minor part of immunoglobulins produced in the course of malaria infection is specific for parasite antigens (Atkinson *et al.*, 1975). In our group of patients, similar IgG antibody levels were found among CM and NCM patients. In contrast, IFA-IgM antibodies were present at higher levels in NCM patients. It is also noteworthy that in 9 CM patients, no precipitating antibodies could be detected initially and 10 days later. This may be related to a defect in the specific humoral response in severe malaria cases, since it was observed that precipitating antibodies directed to La_1 antigen appeared simultaneously with IFA in NCM cases (Druilhe *et al.*, 1980).

Decreased complement component levels have been reported in experimental malaria in monkeys (Atkinson *et al.*, 1975; Glew *et al.*, 1975), in rodents (June *et al.*, 1979), and in man in the course of *P. vivax* (Neva *et al.*, 1974) and *P. falciparum* acute malaria (Williamson *et al.*, 1978; Greenwood *et al.*, 1974), in relation with a possible direct activation mediated by the classical pathway. Very low C_3 values were found in patients with high parasitaemia and detectable MSSA, and unrelated to the severity of clinical symptoms. The fall of complement levels appeared early and transient. The subsequent increase over normal levels observed at day five in treated patients after clearance of blood parasites may be due to increased synthesis.

Interferon like activity, which was not in healthy control donors, was detected in all CM and NCM patients, which confirms previous studies (Druilhe *et al.*, 1982).

No association was found between clinical severity, parasitaemia, antibody or complement levels, and interferon values. Interferon-like activities were of both alpha and gamma types.

SUMMARY

In Thai patients with *Plasmodium falciparum* malaria, IgG and IgM values were elevated, whereas IgA levels were within normal ranges. No association of Ig values with parasitaemia was noted. IFA-IgM antibody levels were lower in cerebral malaria (CM) than in the non cerebral malaria (NCM) group. IFA-IgG antibodies were present in all patients. The mean C_3 and C_4 values were similar among patients from the CM and NCM groups Interferon like activity was detected in all CM and NCM patients, and no correlation was found with either antimalarial antibodies, complement or parasitaemia.

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