Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 43 (1986)
Heft: 3

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DOI: https://doi.org/10.5169/seals-313634

Acta Tropica *43*, 255–262 (1986)

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Influence of circulating malarial antigens on cell mediated immunity in acute *Plasmodium falciparum* malaria

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Summary

In a group of Thai patients with *P. falciparum* acute malaria, circulating malarial antigens (CMA) were detected in 27/33 cerebral malaria (CM) cases and 31/43 noncerebral cases. Delayed cutaneous responses to phytohemagglutinin and candidin were found frequently negative in patients with CMA, especially in the CM group. Mean in vitro lymphocyte proliferative responses to lectins were lower in the group of patients with CMA. An inhibitory activity on proliferative responses to phytohemagglutinin of lymphocytes from healthy individuals was exerted by sera containing CMA. Data suggest that CMA from *P. falciparum* may suppress in vivo and in vitro cell mediated immune reactions.

Key words: circulating malarial antigens; *P. falciparum;* cell mediated immunity.

Introduction

An influence of acute malarial infection on nonspecific T cells dependent immune responses has been observed in rodents experimentally infected with *Plasmodium berghei* and *yoelii* (Jayawardena et al., 1975; Lelchuk and Playfair,

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1980; Weinbaum et al., 1978). Generally, T cell mediated immune functions were found impaired in these models. In the primate *Aotus trivirgatus, P. falciparum* infection led to an impairment of lymphocyte responses to lectins, and this phenomenon was found to be related to parasitemia levels (Taylor and Siddiqui, 1979). In the naturally occurring *P. falciparum* infection in man, limited alterations in the number of circulating T cell and, inconsistently, a decrease of in vitro T lymphocyte reactivity to stimulants were reported (Greenwood et al., 1977; Wells et al., 1979; Wyler, 1976). We have recently identified the impairment of cell mediated responses in a large group of patients with cerebral malaria and/or high parasitemia (Brasseur et al., 1983): alterations were predominantly observed in the expression of delayed cutaneous reactions in the presence of phytohemagglutinin (PHA) and antigens. In vitro responses to mitogens and antigens were less frequently impaired. No consistent modification of blood lymphocyte counts, including T cells, was observed. In this group of patients a correlation was found between high parasitemia and in vivo and in vitro depression of immune responses. In addition, a direct role of the parasite was further suggested by the restoration of responses which was observed within a few days following the clearance of the parasite from the blood. However, in the CM subgroup, the consistent initial suppression of cutaneous delayed reactions was not correlated with parasitemia. For this reason, we have considered the hypothesis that parasitemia did not reflect the actual "load" of parasite derived products. We have therefore looked for the presence of circulating malarial antigens (CMA) in the serum of infected patients. Our data are consistent with an effect of parasite derived substances on non malaria specific cell mediated functions in *P. falciparum* infected patients.

Material and Methods

Patients

Serum from 86 patients admitted in the Pra Pokklao Hospital, Chantaburi, East-Thailand, with acute *P. falciparum* malaria were examined for the presence of *P. falciparum* circulating antigens. Asexual forms of *P. falciparum* were demonstrated and counted on blood smears. Among these, 33 patients had cerebral malaria (CM) (21 male and 12 female adults) defined as being unrousably unconscious in the absence of any other cause of neurological disorder (Brasseur et al., 1983). 53 patients were noncerebral (NCM) cases (25 male and 27 female adults; one 12-year-old child). In the adults, the mean age was 28 (range: 16–68 years).

Unless stated, all data presented in this report were obtained during the initial phase (less than 24 h) following the admission in the hospital, i.e. prior to or within 24 h from the start of therapy which included quinine and symptomatic intensive care in cerebral cases. Due to practical difficulties, every study could not be performed in each patient. The number of individuals subjected to a given test is therefore variable.

Control sera were obtained from volunteers living in Chantaburi. For all individuals studied, informed consent of the donor or its legal representative was obtained.

Cutaneous delayed reactions to phytohemagglutinin and soluble antigens

Among the 86 patients examined for CMA, skin tests were performed in 53 individuals. Among them, 16 had cerebral malaria, and 37 had acute malaria without cerebral involvement. Skin tests

were performed on the forearm by intracutaneous injection of 0.1 ml of PHA or antigen solution: PHA (HA 16, Wellcome, Beckenham, England); Tuberculin purified protein derivative (PPD) (Institut Pasteur, Paris), Candidin (CCD) (Institut Pasteur), streptokinase-streptodornase (SKSD) (Varidase, Lederle, Madrid, Spain), were injected at a final concentration of 10 μg/0.1 ml, and 10 IU/0.1 ml, respectively. Local skin induration and erythema were measured 48 h later. In 53 patients, positivity (i.e. mean size of skin induration over 5 mm) or negativity (less than 5 mm) was recorded.

Determination of the number and reactivity of blood lymphocytes

Total and differential blood leucocytes counts were performed in hemacytometers and blood smears. Mononuclear cells were isolated from heparinized blood on Ficoll-metrizoate gradients. T cells were counted following rosette formation using aminoethylisothioronium-treated sheep erythrocytes (AET-E).

Lymphocyte cultures were performed in microplates (0.2×10^6cells in 0.2 ml per well) in RPMI-1640 medium supplemented with glutamin (Gibco, glutamine 100 mM, Glasgow, Scotland; 1:100), antibiotics and 10% heat inactivated human serum from AB Rh^+ donors. Three-day cultures were performed in the presence of PHA (PHA-P, Difco, Detroit, USA, 1/600), and Concanavalin A (Con A, Pharmacia, Uppsala, Sweden, 10 μg/ml). Seven-day cultures were stimulated with CDD (Institut Pasteur, Paris, France, 100 IU/ml) and SKSD (Varidase, 250 μg/ml). All cultures were performed in triplicate. Twelve hours before harvesting, 2 μCi of 3-H thymidine (3 H-T) was added in each well. The cultures were harvested with an automatic cell-harvester, and the incorporated 3-HT was counted in a scintillation spectrophotometer. Results are expressed in Δ cpm i.e. (incorporation in stimulated cultures) minus (incorporation in control unstimulated cultures).

Detection of circulating malarial antigens in serum

P. falciparum CMA were determined in duplicate using a counter immunoelectrophoresis method performed on cellulose acetate strips, and previously used for the detection of malarial antibodies (Druilhe and Monjour, 1975; Druilhe et al., 1978). CMA were revealed using human immunoglobulin (Ig) preparations with high antimalarial antibody titers: pooled ammonium sulphate precipitated Igs were obtained from hyperimmune African donors selected because of their elevated serum antibody level to CMA. It was determined by immunofluorescent antibody test and compared with a reference serum of known content. Igs were absorbed with normal Thaï human sera.

Statistics

Statistical analyses were performed by the chi-square test of Fisher and Yates, and the Student's "t" test.

Results

Relationship between the presence or absence of P. falciparum CMA and the parasitemia level

CMA were found in 58/86 patients and in all but five with a parasitemia over 3%. CMA were also present in 23 patients with a parasitemia lower than 1%. Irrespective of the level of parasitemia, the presence of CMA was more frequent among CM than NCM patients but the difference was not statistically significant (27/33 versus 31/43 chi-square test, $\chi^2 = 0.98$). No relationship was found between the presence of CMA and the level of serum antibodies to *P. falciparum* blood stages. In patients with CMA IgM antibody titers (geometric mean: 79) ranged from 0 to 400 and IgG antibody titers (geometric mean:

2680) from 0 to 16,200. In the group without CMA, geometric means and ranges were 32 (0 to 1800) and 721 (0 to 48,600) for IgM and IgG antibody titers, respectively.

Cutaneous delayed hypersensitivity tests in P. falciparum infected patients without CMA

Most patients without CMA (12/15) exhibited positive responses to PHA compared to 25/38 in the group with CMA. However, the difference between the two groups was not significant ($\chi^2 = 1.03$) (Table 1). The only CM case in which CMA were not detected exhibited negative skin tests with all stimulants. The significance of skin tests was emphasized by the fact that all normal individuals (15/15) responded to PHA, while only half (CM, 53%) and two thirds (NCM, 65%) of the individuals with CMA responded to PHA (Table 1, χ^2-test, 2 P<0.01).

Data on cutaneous responses to antigens suggest also an influence of CMA. However, less rigorous conclusions can be reached than in the case of PHA since only 11/15, 12/15 and 4/15 normal adults responded to CDD, SKSD and PPD, respectively (p>0.1).

Table 1. Cutaneous delayed hypersensitivity reaction in patients with and without CMA. Results are expressed as the ratio of the number of individuals to the total number tested for each group and stimulant.

		CMA		Positive delayed cutaneous response to:			
				PHA	CDD	SKSD	PPD
Patients	NCM	+	a	25/38 (65%) d	11/33 (33%)	6/38 (16%)	2/33 (6%)
	CM	+	a	8/15 (53%) c	1/14 (7%)	0/15 (0%)	1/13 (7%)
	NCM	0	a	12/15 (80%) e	6/14 (43%)	6/15 (40%)	0/12 (0%)
	CM	0	b	0/1	0/1	0/1	0/1
Control	–	0		15/15	11/15	12/15	4/15

Chi-square:
(a) χ^2: 1.03, 2P>0.1, not significant
(b) χ^2: 6.80, 2P<0.01, significant
(c) χ^2: 2.40, 2P>0.1, not significant
(d) χ^2: 0.71, 2P>0.1, not significant
(e) χ^2: 9.13, 2P<0.005, significant

Table 2. Effect of sera containing CMA on proliferative response of normal lymphocytes to PHA

Patients	Donor's type	Parasitemia (a)	CMA	Inhibition of in vitro proliferative response of normal lymphocytes to PHA (b) by patient serum	In vitro response of patients lymphocytes to PHA (c)
1	NCM	7	+	13	4.218
2	NCM	7	+	34.8	3.574
3	NCM	8	+	48.9	2.968
4	CM	46	+	0	3.035
5	CM	5.8	+	50.7	6.772
6	CM	3.5	+	36.1	0.417
7	CM	60	+	50.4	3.370
8	NCM	1	0	3.7	4.220
9	NCM	4.3	0	19.2	4.798

(a) expressed in percentage of red blood cells
(b) expressed in percentage
(c) expressed as the value: Ln (3H-thymidine) count per minute incorporated in stimulated cultures minus Ln (3H-thymidine) count per minute incorporated in control unstimulated cultures

Blood lymphocyte counts and in vitro lymphocyte reactivity to lectins and soluble antigens in P. falciparum infected patients with and without CMA

The mean in vitro response to PHA and Con A in the group with CMA was slightly lower than in the group without CMA. However, four patients with proliferative responses to PHA lower than two standard deviations from the mean value (3.099±1.01) i.e. lower than 1.079, were found in the group of patients with CMA. In the case of the soluble antigen CDD, the proliferative mean response was lower in the group of patients with CMA (0.356±0.574) than in the group without CMA (1.068±0.747) (t-test, $P < 0.001$). The lack of in vitro response in many patients and normal control rendered studies with SKSD and PPD inconclusive.

Effects of the serum of patients with CMA on in vitro responses of lymphocytes from healthy donors to PHA and tetanus toxoid

Table 2 shows that sera from malaria patients with CMA added at a final concentration of 5% (in addition to 5% normal serum from healthy individuals) were able to inhibit the response of normal lymphocytes to PHA, and that the serum inhibitory activity was linked to the patients' lymphocyte proliferative response to PHA thus suggesting a role of CMA in vivo.

Results of sequential studies performed in two CM patients are presented in Fig. 1 and confirm in time this relationship by showing a parallel evolution between the serum inhibitor activity and the level of response of patient's lymphocytes.

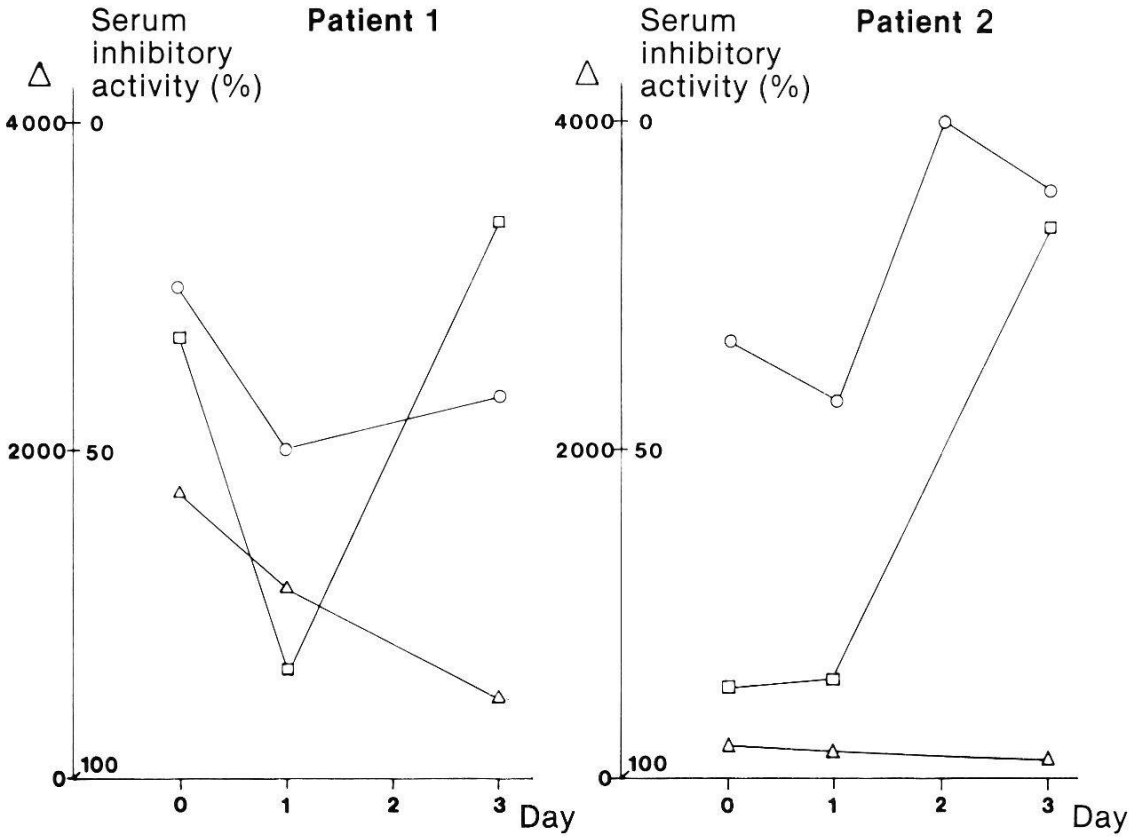

Fig. 1. Lymphocyte responsiveness and serum inhibitory activity in two patients in the days following cerebral malaria attack.

○ = Serum inhibitory activity on the proliferative response of normal lymphocytes expressed as the percentage of inhibition at a concentration of 5% in culture.

□ = Proliferative lymphocyte response to PHA of patient's lymphocytes.

△ = Proliferative lymphocyte response to CDD of patient's lymphocytes.

△:△ cpm (see Material and Methods section).

Discussion

In the present report, the influence of *P. falciparum* CMA on non malaria specific cell mediated responses of patients with acute malaria was investigated. A large group of patients was studied during the initial phase of the attack. They included high and very high parasitemia and CM cases. We have previously reported that the impairment of cell mediated responses was a direct function of parasitemia only in patients with noncerebral malaria (Brasseur et al., 1983). In an attempt to obtain further insight in the mechanism of this phenomenon, the influence of MCA in the serum of patients was studied.

In this study, no quantification of the level of antigens was attempted and we have only defined the presence or absence of CMA. Our findings are in agreement with several reports in human (McGregor et al., 1968; Perrin et al., 1982) as well as in experimental malarial infection in animals (Seitz, 1972, 1976). The detection of CMA in active malaria was found to be related to high parasitemia. The method used provides no information on the free or com-

plexed form of antigens (Allison et al., 1969; Houba et al., 1976). We may only assume that the detected material was present in sera in a circulating form, therefore it was not deposited in organs. Such deposition has been shown to play an important role in some manifestations of *P. falciparum* malaria, including renal and cerebral forms (Adam et al., 1981; Houba et al., 1976; June et al., 1979).

In our group of patients the major immunological defect observed was a decreased or abolished delayed cutaneous response to PHA and this was statistically significant only in MCM and CM patients with CMA when compared to healthy control individuals (Table 1). The positivity of skin tests during recovery, previously described in several patients under successful therapy, suggested that the parasite or its products may play a direct role (Brasseur et al., 1983). The present study provides some evidence for the association between the presence of CMA and altered delayed cutaneous reactions. An effect of the parasite on the number of circulating T cells could not account for the influence on skin reactions since blood T cells counts were within normal ranges in the two groups with and without CMA.

Little information is available on the effects of the presence of CMA in humans. Investigations reported here provide additional evidence for their role in the pathogenicity of acute *P. falciparum* malaria. It is of interest that CMA were found in most (27/33) CM patients in our group, whatever was the initial parasitemia. This may reflect a heavy parasitic load which may be sequestered in the deep capillaries, and not be seen in the peripheral blood, thus, the quantitiy of circulating CMA may be directly related to the total parasitic load in the patients.

In vitro, the inhibitory activity of sera on responses of normal lymphocytes suggests a direct effect of malarial products on lymphocyte activation mechanisms. The fact that this influence was observed for proliferative responses to both nonspecific and antigen (CDD) specific responses suggests a common mechanism of inhibition. Current studies aimed at isolating and characterizing *P. falciparum* derived material acting either on antigen specific or nonspecific immune responses may provide purified material for future studies.

Acknowledgments

The authors thank Dr. D. A. Warrell, Dr. N. J. White and the medical staff of Pra Pokklao Hospital, Chantaburi, East Thailand, for allowing us to study their patients, and for their help in collecting the samples.

This study was supported by grants from Ministère des Relations Extérieures and INSERM, Paris, France, and in cooperation with Wellcome Mahidol University, Oxford Tropical Medicine Research Programme.

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