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Impairment of *Plasmodium falciparum*-Specific Antibody Response in Severe Malaria

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Serum antibody response to plasmodial antigens was investigated in 97 Thai patients with *Plasmodium falciparum* malaria. No difference in immunoglobulin G (IgG) antibody levels was detected between groups without or with cerebral manifestations of malaria (n = 40). In patients with the most severe form of the disease, i.e., those who died despite adequate therapy (n = 12), antibody detected in the immunofluorescent-antibody test was found at lower levels than in those who recovered (geometric means: IgG = 1/420 versus 1/3,800; IgM = 1/15 versus 1/70); similarly, precipitating malarial antibodies were present in only 1 of these 12 patients, while they were detectable in 65 of the remaining 85 patients (76.5%). In contrast, anticytomegalovirus antibody levels were similar in the different groups of patients. Results show that depression of antibody response may extend to antiplasmodial responses during severe malaria. The link between fatality and a low level of antibody production suggests that an appropriate immune response to malarial antigens may be required to achieve recovery with drug treatment and provides a new direction for malaria therapy research.

A malaria-related suppression of the immune system has been reported in numerous studies with experimental models (11). In *Plasmodium falciparum*-infected individuals, more limited studies have revealed the occurrence in some instances of a suppression of antibody responses to vaccines (6, 7) and altered cell-mediated responses to antigens and to lectins. In one such study, we observed that T-cell unresponsiveness was infrequent. It occurred mainly, if not only, in patients with very high parasite loads (3).

Since all studies with human subjects were performed with nonmalarial antigens, it is important to determine whether this state of immune unresponsiveness may also extend to specific responses to malarial antigens. Such a depression, if it exists, may interfere with attempts to immunize patients living in endemic areas against malaria and might also be a factor favoring the survival of the parasite in otherwise immunocompetent hosts.

We report here results showing that antibody responses against blood-stage malarial antigens are altered in patients with very severe *P. falciparum* malaria.

MATERIALS AND METHODS

Patients. We studied 97 Thai patients admitted to Phra Pokklao hospital, Chantaburi, East Thailand, with acute *P. falciparum* malaria. All had asexual *P. falciparum* parasites in Giemsa-stained blood smears. Forty patients had cerebral malaria (CM), defined as unarousable unconsciousness in the absence of any other detectable cause of neurological disorder (10). Thirty-nine of these patients were adults (mean age, 27 years; range, 15 to 60 years), and one was a child of 10 years. Twenty-six of these CM patients had been hospitalized in the past year with a previous malaria attack, diagnosed on the basis of a positive blood smear. Ten died despite antimalarial treatment and intensive care. Of the 57 patients without cerebral manifestations (NCM patients), 52 were adults (mean age, 28 years; range, 15 to 68 years) and 5 were children aged 9 to 12 years. None of these patients showed clinical signs of malnutrition at the time of the study. Two of the NCM patients died.

Normal control individuals were healthy Thai volunteers living in Chantaburi (mean age, 25 years; range 17 to 30 years). Informed consent of the donor or of the legal guardian was obtained from all individuals studied.

Determination of serum immunoglobulin levels. Serum immunoglobulin G (IgG), IgM, and IgA levels were measured by using heavy-chain-specific antibodies and an immunonephelometric method (9).

Determination of *P. falciparum* antibodies. *P. falciparum*-specific IgG and IgM antibodies were determined in an immunofluorescent-antibody test (IFAT) using as antigen acetone-fixed thin smears of blood-stage *P. falciparum* from cultures of the FCR3 strain and fluorescein isothiocyanate-labeled goat anti-human IgG or IgM diluted 1/200 in phosphate-buffered saline (Institut Pasteur, Paris). This procedure has been used daily for more than 10 years. Titration of over 1,000 serum samples from French adults without previous malaria has shown that specific anti-malarial antibodies correspond to titers of ≥1/200, and titers in positive cases range between 1/200 and 1/50,000 (unpublished data). For a given group of patients, results are expressed as the geometric mean of reciprocal titers. Precipitating malarial antibodies were detected by using a countercurrent immunoelectrophoresis (CIEP) assay as previously described (5).

Delayed cutaneous reactions to PHA and CDD. Skin tests were performed on forearm skin by intracutaneous injections of 0.1 ml of phytohemagglutinin (PHA, 10 IU/ml) (PHA-HA 16; Wellcome, Beckenham, England) and 0.1 ml of candidin (CDD, 1/1,000; Institut Pasteur, Paris). Local skin induration and erythema were measured 48 h later by the same investigator. Two perpendicular diameters were recorded; the longest diameter of the induration was measured, and a second measurement was made perpendicular (at right angles) to it at the midline of the first measurement. Values of the two measurements were added, and then the sum was divided by two to give the value used for comparison of induration among the various subjects. Positivity and

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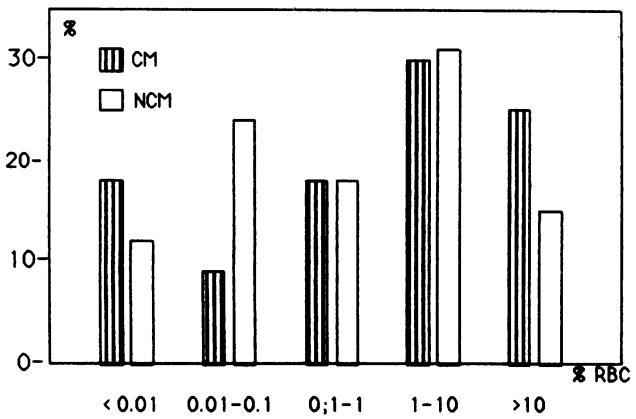

FIG. 1. Distribution of parasitemia in the CM and NCM cases. Results are expressed as the percentage of CM (33 patients) and NCM cases (57 patients) in the different classes of parasitemia.

negativity were defined as a mean diameter of skin induration greater and less than 5 mm, respectively.

In vitro lymphocyte proliferative responses to PHA and CDD. Seven-day-duration cultures of mononuclear cells isolated on Ficoll-metrizoate were performed in RPMI 1640–10% AB serum medium as previously described (3). Responses to CDD (100 IU/ml) or PHA-P (1/600) were assessed on the basis of [^{3}H]thymidine incorporation, which was counted in a liquid scintillation spectrophotometer and expressed as counts per minute per 2×10^5 mononuclear cells.

SMA. *P. falciparum* antigens circulating in the serum (SMA) were detected by using a CIEP method and a pool of human immunoglobulins with high titer of antibodies to SMA (1).

Antibodies to CMV. Levels of antibody to cytomegalovirus (CMV) were determined by enzyme-linked immunosorbent assay using commercially available reagents (Virion S.A.) with serum samples diluted 1/100. Results are expressed in arbitrary units as compared with values of known negative and positive (titer = 100 units) controls (CRTS, Lille, France).

Statistical analysis of results. Chi-square analysis was used to test parameter associations. Comparisons between groups were performed by using the two-tailed Student *t* test. Dependence of variables was estimated by using the correlation coefficient *r*, therefore assuming normal distribution for both variables. The probability (*P*) of *r* being equal to 0 was given when *P* was <0.1.

RESULTS

With the patients we studied, no direct relationship was found between levels of peripheral blood parasitemia and the presence of CM. Figure 1 shows that CM occurred at similar frequencies in patients with either low or high parasite loads and that very high parasitemias were observed without signs of neurological involvement.

In *P. falciparum*-infected patients, mean serum IgG, IgM and IgA levels were increased on average compared with those in healthy controls (Table 1). No significant difference was observed between CM and NCM patients in titers of malarial antibodies of the IgG class (geometric mean of reciprocal titers of 3,206 [NCM] versus 2,504 [CM]; $P > 0.05$). IgM malarial antibody levels were, however, significantly lower in the CM group than in the NCM group (33 versus 94, respectively; $P < 0.01$).

TABLE 1. Levels of serum immunoglobulins and of specific antimalarial antibodies

Patients	Serum immunoglobulin concn (g/liter)[a]			*P. falciparum* antibody level (IFAT)[a]	
	IgG	IgM	IgA	IgG	IgM
CM (n = 40)	18.15	1.36	3.08	2,504	33
NCM (n = 57)	20.15	1.57	3.80	3,206	94
Controls (n = 20)	12.00	1.10	2.75	<100	<100

[a] Results are expressed as the geometric means of individual titers.

In 31 of 97 patients, no precipitating antibody could be detected by CIEP assay. In these CIEP-negative subjects, IgG antibody levels detectable by IFAT were also much lower than those in the group with precipitating antibodies (871 versus 4,528; $P < 0.01$), while no significant difference in IgM antibody levels was found (40 versus 59).

Forty-three patients were monitored for an interval of 8 days or more following admission and were classified on the basis of CIEP results obtained both with the initial sample and upon follow-up (Table 2). Samples from 14 patients were initially negative by CIEP, and nine remained negative. Their levels of malarial antibodies detected by IFAT were significantly lower than those of the five patients in whom CIEP results became positive and lower than those of the 29 patients with initially positive CIEP results ($P < 0.1$). IgG antibody levels, like IgM antibody levels, were markedly lower in both initial and delayed serum samples from patients of group 3 (CIEP negative) than they were in samples from patients of groups 1 and 2.

Despite large individual variations, the mean parasitemia of patients of group 3 (11.9%) was much higher than that of patients of group 1 (0.45%) ($P < 0.001$), suggesting a relationship between parasite load and antibody responses.

Circulating malarial antigens were detected in the sera of many patients of each group. They apparently persisted longer in patients with poor antibody response because their prevalence was significantly higher in delayed samples from group 2 patients (80%) and 3 patients (77%) than in those from group 1 patients (51%) ($P < 0.05$ and $P < 0.2$, respectively).

Table 2 also shows a correlation between antimalarial antibody responses and cellular reactivity. In vivo and in vitro T-cell-mediated responses were found to be altered more frequently in the group with low antibody levels. In vitro lymphocyte responses to CDD and PHA were positive in only two of the five subjects with decreased antibody production (group 3), while lymphocytes from all patients in group 1 responded to PHA and most responded to CDD. A similar difference between groups 3 and 1 was also found in cutaneous responses to the same stimulating agents, thus showing a parallelism for many patients between malarial antibody levels and non-*Plasmodium*-specific T-cell responses.

In Table 3, the data described above were correlated with clinical features other than the presence or absence of CM. Patients were classified into two groups on the basis of outcome following antimalarial treatment. Of the 12 patients who died despite intensive care, precipitating malarial antibodies were absent in all except one, while 76.5% (65 of 85) of the subjects who recovered were positive. Similarly, both classes of malarial antibody detected by IFAT were at very low levels in this group of 12 patients. Specific IgG antibody levels were 10 times lower in patients who died than in other

TABLE 2. Results of immunofluorescence assays, in vitro and in vivo responses of lymphocytes to PHA and CDD, and prevalence of SMA in 43 patients followed up

Group (time of sample)[a]	CIEP result	IFAT[b]		T-cell-mediated response (no. positive/no. tested)[c]				SMA (no. positive/ no. tested)	Parasitemia (mean %)	No. with CM (%)
				In vivo		In vitro				
		IgG	IgM	PHA	CDD	PHA	CDD			
1 (n = 29)										
Initial	+	5,065	93	16/16	10/11	11/16	9/16	21/33	0.45	16 (55)
Delayed	+	7,632	86	11/14	5/7	1/2	8/9	17/33		
2 (n = 5)										
Initial	−	6,606	86	2/3	1/3	2/4	0/3	4/5	1.88	5 (100)
Delayed	+	6,637	37	2/3	2/3	1/1	4/5	4/5		
3 (n = 9)										
Initial	−	323	19	2/5	2/5	2/5	1/4	7/9	13.4	6 (67)
Delayed	−	486	35	2/3	2/5	1/2	0/5	7/9		

[a] Initial samples were those samples collected upon admission; delayed samples were samples collected after 8 to 20 days.
[b] Results are expressed as the geometric means of individual titers.
[c] A positive response in vivo is defined in Materials and Methods. In vitro, a positive response is one higher than 2 standard deviations from the logarithmic mean value (counts per minute) for all patients (for PHA) or higher than three times the background [^{3}H]thymidine incorporation level for unstimulated cultures (for CDD).

patients, and specific IgM antibody levels were 5 times lower. As already shown, *P. falciparum*-specific immune unresponsiveness corresponded to some extent to a depression of non-*Plasmodium*-specific T-cell responses.

SMA were detected more frequently in patients who died, but the difference between these levels and those in surviving patients is not significant ($P > 0.5$).

Of the patients who died, 10 had CM and 2 had NCM. Parasitemia ranged from 0.01 to 46% of erythrocytes in the CM patients and was at 22 and 38% in the two NCM patients. The geometric mean of these patients, at 2.89%, is five times greater than that of the remaining 85 patients.

To determine whether the lower antibody levels observed in this group of 12 patients were restricted to responses to malarial antigens or whether they extended to unrelated antigens, we measured antibody to CMV. No significant difference was found between the prevalence and levels of anti-CMV antibodies in 10 patients who died despite treatment (9 of 10 positive; mean titer, 5.5; range, 2.4 to 10 units) or in levels of anti-CMV antibodies in 10 control patients randomly chosen from among the 85 patients who recovered (9 of 10 positive; mean titer, 7.6 units; range, 3.6 to 15 units) ($P > 0.5$).

DISCUSSION

In the present study, we attempted to determine whether the production of antibodies to *P. falciparum* antigens could be impaired by malaria infection and whether several factors, among them the severity of the disease, could be correlated with specific responses.

Results show that depression of the immune system associated with malaria infection, which was first described in studies of responses to nonmalarial antigens, may also extend to parasite antigens. However, depression of the immune system as a result of parasite infection is infrequent. It did not appear to be a direct function of the level of parasitemia and was not associated with the occurrence of cerebral malaria, a major complication of *P. falciparum* infection.

Depression of specific antibody responses was observed mostly in patients who died. In these patients, a correlation was found among low levels of specific IgG antibodies, low levels of IgM antibodies, the absence of production of precipitating antibodies, high frequency of SMA, frequently altered T-cell function, a mean parasitemia higher than average, and death despite administration of fast-acting drugs and intensive supportive treatment. Although not tested in the present series, the influence of rheumatoid factor or "blocking" IgG antibodies on antibody measurement was presumably very limited or not significant in our study of comparatively large groups of patients.

In *P. falciparum* infections, correlations with parasitemia are difficult to establish because most infected erythrocytes are sequestered in capillaries. Therefore, peripheral blood parasite counts do not closely reflect the total parasite load. However, in a former study we found a relationship between the peripheral blood parasite loads and the in vivo and in vitro T-cell responses to candidin antigen (4). A transient T-cell unresponsiveness was associated with increasing parasitemia and occurred in all patients with very high levels of

TABLE 3. Results of biological assays in two groups of patients classified on the basis of outcome following drug treatment

Outcome	No. CIEP positive/no. of patients (%)	IFAT[a]		No. of positive responses to CDD/no. of patients (%)		SMA (no. positive/ no. tested) (%)	Parasitemia (mean %)
		IgG	IgM	In vitro	In vivo		
Death (n = 12)	1/12 (8.3)	426	15	3/5 (60)	0/3 (0)	11/12 (91.6)	2.89
Recovery (n = 85)	65/85 (76.5)	3,840	70	45/50 (90)	22/61 (36)	59/82 (71.9)	0.49

[a] Results are expressed as the geometric means of individual titers.

parasitemia (>40% infected erythrocytes), irrespective of the presence of neurological manifestations. A similar relationship with parasitemia has been observed in antibody responses to tetanus toxoid and meningococcal antigens (6, 7).

Impairment of production of antibodies to parasite antigens did not follow the same pattern. It was only partly dependent on the number of parasites circulating in the peripheral blood and partly related to T-cell unresponsiveness. Nevertheless, levels of parasitemia were approximately 20 times greater in patients with altered antibody production than in good responders. This suggests either that the absence of specific antiparasite antibodies has allowed an increase in parasitemia or, to the contrary, that a high level of parasitemia interfered, but only in some patients, with normal antibody responses. Suppression of antibody response to foreign antigens has been reported in *P. falciparum*-infected humans (7, 12). From an immunological point of view, the response to foreign antigens introduced into already parasitized individuals and the response to malarial antigens are not similar. In the first case, priming may be impeded by the effect of a high parasite load and released parasite substances interfering with normal immune functions. In contrast, priming by malarial antigens should occur normally at an initial stage when the parasite load is still low if, as generally agreed (11), the parasite load is an essential factor. The inconsistent relationship between parasitemia and antibody response to malarial antigens suggests that only some parasite strains (or host-parasite combinations) may impede antigen recognition or antibody production.

The correlation that we found between fatality and antibody production is of interest from a therapeutic viewpoint. We first wondered whether immune functions were altered because patients were moribund or whether the low level of antiparasite immunity was a factor favoring death. The first hypothesis seemed unlikely because severe complications occur in the course of malaria infection in less time (within hours or 1 or 2 days) than the immunoglobulin half-life. The demonstration that anti-CMV antibody levels in patients who died were similar to the levels in other patients apparently rules out the hypothesis of a general suppression of antibody production by malaria infection. Thus, our study suggests that an immune response to malarial antigens may be required to achieve recovery by drug treatment. This hypothesis is supported by the experienced acquired with other parasitic diseases in immunodeficient patients. Parasites such as *Strongyloides stercoralis* in corticosteroid-treated patients or *Toxoplasma gondii*, *Pneumocystis carinii*, and *Candida albicans* in acquired immunodeficiency syndrome patients (2) are more difficult to control with drugs in immunocompromised hosts than in immunocompetent hosts. Moreover, recent data from experiments with animals also suggest that the immune system acts additively with drugs in killing plasmodia (8). Chloroquine, pyrimethemine, and quinine were all found to be much less efficient in immunocompromised mice infected by *Plasmodium chabaudi* than they were in immunocompetent mice. T-cell-deprived mice retained high levels of parasitemia during and after treatment with doses of quinine (100 mg/kg per day for 7 days) which were effective in immunologically intact animals. Since a competent immune system is known to be necessary for drug-induced control of several human parasitic infections and of animal malaria, this is very probably also the case for severe malaria infections in humans. At this stage, it can only be hypothesized that some *P. falciparum* strains are better suited than others to induce immunosuppression and that this parasite-induced immunodeficiency contributes to death despite quinine administration. This hypothesis clearly deserves further investigation, because the administration of immune IgG together with antimalarial drugs may thus improve the prognosis.

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