

CONTRASTING FUNCTIONS OF IGG AND IGE ANTIMALARIAL ANTIBODIES IN UNCOMPLICATED AND SEVERE *PLASMODIUM FALCIPARUM* MALARIA

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Abstract. Plasmodial infection results in a significant elevation of the blood concentrations of immunoglobulins including IgE. Two well-characterized groups of adult Thai patients with either uncomplicated or severe *Plasmodium falciparum* malaria were studied over a period of four weeks. The mean parasitemias were approximately three-fold higher in patients with severe malaria than in those with uncomplicated disease. The mean concentrations of both total IgG and IgG antiplasmodial antibodies tended to be highest in the group with uncomplicated disease while total IgE and IgE antibodies were higher in the group with severe disease. The IgE antibodies detected in approximately 65% of the patients were positively correlated to parasitemia. These results suggest that antiplasmodial IgG antibodies are involved in reducing the severity of *P. falciparum* malaria, while IgE antibodies may contribute to the pathogenesis of this infection.

INTRODUCTION

Elevated levels of IgE, the immunoglobulin instrumental in type I allergy, may also be found in many infections. In malaria, IgE is increased in the majority of individuals living in areas of high endemicity.^{1,2} Evidence from experimental malaria indicates that *Plasmodium* can give rise to IgE in the absence of other pathogens such as helminths³ which are known to induce IgE elevation.⁴

IgE in association with IgE-receptor-bearing effector cells such as monocytes or platelets may give rise to reactions that are protective and/or pathogenic to the responder. Cross-linking of these receptors by IgE-containing immune complexes will lead to cellular activation, resulting in the production of nitric oxide (NO) and tumor necrosis factor- α (TNF- α).⁵ In severe *P. falciparum* malaria, TNF- α is known to be elevated and in some studies to correlate with mortality from cerebral malaria.^{6,7} Malaria pigment and, in particular, malaria “toxins” containing GPI (glycosyl-phosphatidylinositol) are usually considered to be responsible for the overproduction of TNF- α .⁸

We became interested in the role of IgE in the pathogenesis of *P. falciparum* malaria when we observed that total IgE and IgE-antimalarial antibodies were most elevated in patients with cerebral malaria.² We subsequently reported similar findings in 6 African patients with severe disease, and showed that sera from malaria patients can induce monocytes to produce TNF- α *in vitro* in an IgE-dependent reaction.⁹ To elucidate further the possible association of IgE with malaria severity, we analyzed sera from two well-characterized groups of Thai patients which had either uncomplicated or severe *P. falciparum* malaria exclusive of cerebral disease.¹⁰ Total IgE and antiplasmodial IgE antibodies were measured weekly for four weeks and compared with analogous IgG levels, parasitemias, and clinical disease severity.

MATERIALS AND METHODS

The study subjects were of Thai (20%) or of Mon and Karen (80%) origin. They lived at the Thai-Myanmar border

in a malaria-endemic region with seasonal transmission. Patients who were non- or semi-immune were admitted to the Bangkok Hospital for Tropical Diseases. Most of these subjects, (59 out of 70) were males, age 16–64 years, (mean age 24). One group of 35 patients was diagnosed as having severe (but not cerebral) malaria defined according to World Health Organization (WHO) criteria.¹⁰ These subjects had severe anemia and/or jaundice, acute renal failure, and hyperparasitemia.¹¹ Patients in the other group had acute uncomplicated *P. falciparum* malaria with only acute symptoms (fever, headache, muscle pain, chills, and nausea).¹² Age, sex, and geographic origin were identical. There was no difference between the groups in duration of illness before admission (mean for uncomplicated cases, 3.9 days of fever; and for severe disease, 4.4 days). Pregnant women, subjects with acute diarrhoea, or those given antimalarial treatment in the previous 2 weeks were excluded. Uncomplicated cases were treated with artesunate orally (a total of 600 mg over 5 days) followed by oral mefloquine (25 mg/kg in 2 doses, 6 hours apart). Subjects with severe malaria were treated with artesunate intravenously (a total of 600 mg over 5 days).¹² All patients gave informed consent. The study was approved by the Ethical Committee of Mahidol University.

Parasitologic and hematologic examination. Blood samples were taken at admission and then once a week for four weeks. Sera were kept on ice immediately following collection up to 28 days and were then stored at -70°C . Hematologic evaluation and parasitologic examination of thick blood films were done as described elsewhere.¹¹

Immunoglobulin and antimalarial-antibody levels. Lysates of infected erythrocytes (*P. falciparum* laboratory strain F32) were prepared as previously described¹³ and used for determination of antimalarial antibodies. Total IgE and IgG, and antimalarial IgE and IgG antibodies were determined by ELISA as described.^{2,14} Sera containing antigen-binding IgE or IgG above the background of Swedish controls (IgE 0.40 ng/ml, IgG 15.49 $\mu\text{g/ml}$; means ± 2 SD) who had not encountered malaria were defined as antibody positive. All results are given as geometric means.

TABLE 1
Analysis at admission (day 0) of sera from patients with uncomplicated or severe *Plasmodium falciparum* malaria*

	Uncomplicated	Severe	P
Parasites/ μ l of serum	28,443 (1,220–377,300)	99,198 (49–818,400)	0.014
Total IgG (mg/ml)	55.19	33.00	0.002
Range	14.38–218.00	8.46–120.00	
Total IgE (μ g/ml)	0.52	0.64	NS
Range	0.03–2.36	0.05–3.59	
IgG-anti- <i>P. falciparum</i> (μ g/ml)			
All patients	29.30	23.21	NS
Antibody-positive patients	36.46	28.48	0.018
Range	17.16–239.20	16.58–136.20	
IgE anti- <i>P. falciparum</i> (ng/ml)			
All patients	0.45	0.57	NS
Antibody-positive patients	0.53	0.66	0.018
Range	0.40–1.29	0.40–4.43	
IgG/IgE antibody ratio ($\times 10^{-3}$)	64.9	40.7	0.018

* Values are geometric means of parasites, total immunoglobulin (IgG, IgE), anti-*P. falciparum* antibody concentrations, of IgG/IgE antibody ratios in the sera from 35 patients with uncomplicated disease or 35 with severe disease. NS = not significant. For additional data and comments, see Results.

Statistics. Statistical evaluation of differences between groups was by unpaired t-test and single factor ANOVA. The correlation between immunoglobulin levels and parasitemias was assessed by Spearman's rank-order correlation coefficient.

RESULTS

Parasitemia, IgG, and IgE levels on the day of admission (Day 0) for the groups of subjects with uncomplicated or severe malaria are summarized in Table 1. Mean parasitemia for patients with severe disease was approximately three-fold higher than that for patients with uncomplicated disease. In both instances, treatment rapidly brought down parasitemia and no parasites were observed in any of the blood samples obtained from 7 to 28 days after admission (data not shown).

Compared with healthy Thai donors living in Chantaburi, East Thailand, total IgG levels for patients with uncomplicated disease increased five-fold, and for severe disease, three-fold.¹⁵ The difference in IgG concentrations between the two study groups was significant. The concentration of total IgE was also increased in both patient groups. However, in contrast to IgG, the IgE levels tended to be higher for

patients with severe disease, although the value was not significantly different from that of persons with uncomplicated malaria (Table 1). The highest total IgE concentrations were generally observed in the severe disease group at every time point except for the last, Day 28 (Figure 1).

IgG and IgE antibodies to *P. falciparum* blood-stage antigens were determined using lysates of infected erythrocytes. At admission, 22 of the 35 patients with uncomplicated malaria and 23 of the 35 subjects with severe disease had detectable IgG antibodies. A significant negative correlation was seen ($r = -0.399$, $P = 0.005$) between IgG antibody levels and parasitemia. This correlation tended to be negative also when calculated separately for patients whether their disease was uncomplicated or severe. IgE antibodies at admission were detectable in 24 out of 35 patients with uncomplicated malaria and in 22 out of 35 subjects with severe disease. IgE antibody concentrations were positively correlated with parasitemia ($r = +0.387$, $P = 0.013$). A positive correlation was seen as well when patients with either uncomplicated or severe disease were analysed separately.

Mean IgG antibody concentrations at admission were higher for patients with uncomplicated malaria than for those with severe disease. In contrast, IgE antibody concentrations were highest in patients with severe disease. The differences between the groups were statistically significant for the antibody-positive patients. The ratio of IgG to IgE antibodies was significantly higher for the group with uncomplicated malaria than for that with severe disease (Table 1).

The patients were followed for one month; blood samples were taken on 5 occasions: Days 0, 7, 14, 21, and 28. The number of blood samples available for testing decreased from 35 samples for each group at Day 0 to 16 (uncomplicated disease) and 19 (severe disease) at Day 28. Beginning at Day 7 when the patients were free of parasites and throughout the rest of the 28 day period, all subjects were positive for IgG antibodies. The level of IgG antibodies was maximal at Day 7 or earlier, and then declined slowly. IgG antibody levels were higher in the uncomplicated than in the severe cases at four out of five time points (Figure 2A). IgE

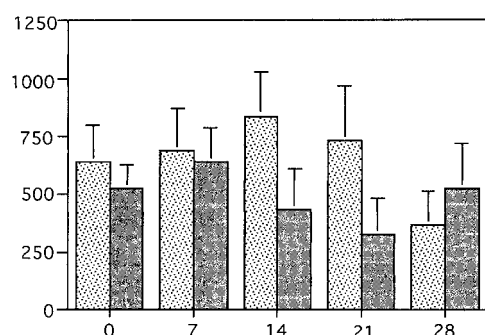


FIGURE 1. Geometric means of total IgE ng/ml in sera from patients with severe (dotted columns) or uncomplicated *Plasmodium falciparum* malaria (gray columns). Vertical lines represent standard errors of the mean. Day 0 = day of admission.

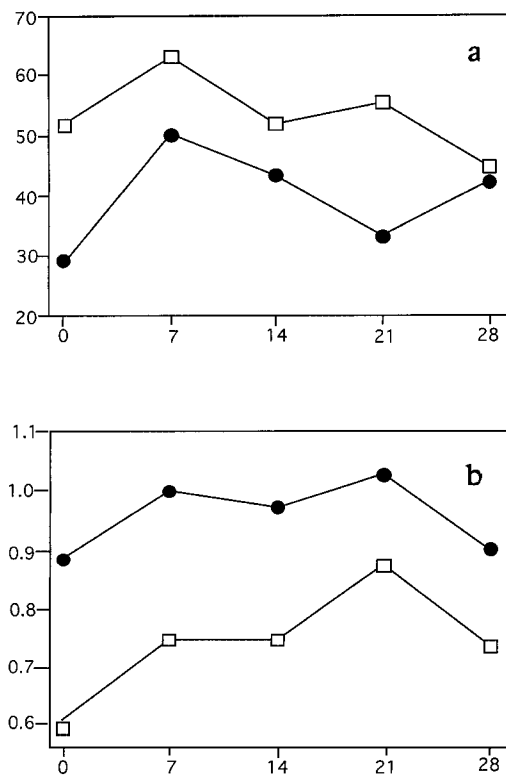


FIGURE 2. Geometric means of IgG $\mu\text{g/ml}$ (a) and IgE ng/ml (b) antibodies in sera from patients with severe (black circles) or uncomplicated (empty squares) *Plasmodium falciparum* malaria. Day 0 = day of admission.

antibody levels were also maximal for both groups at Day 7, but remained highest in the severe cases throughout the whole month (Figure 2B).

DISCUSSION

Although disease is the result of infection of erythrocytes by the blood stages of the malaria parasite, the correlation between disease severity and parasitemia is not simple.^{16,17} Nevertheless, in the current report, the mean parasitemias at the time of the initial blood sampling were approximately three times higher for subjects with severe disease than for those with uncomplicated *P. falciparum* malaria. Subjects in both groups had elevated total IgG; however, patients with uncomplicated disease had higher levels than those with severe malaria. The basis for this difference is not known. Malaria may variably suppress both humoral and cellular responses to third party antigens^{18,19} and immune response to *P. falciparum* itself may be reduced in patients with complicated cerebral disease,^{15,20–22} or repeated malaria attacks.²³

Differences in the immune response between patient groups may have several explanations, including differences in parasite virulence,^{24,25} genetics of the human host, and differences in immunity related to variations in malaria exposure.^{26–28} Lower antibody levels in patients with severe disease may also reflect increased consumption due to the presence of larger amounts of parasite-derived material.²⁹ This explanation, however, does not account for lower levels of total IgG without demonstrable anti-parasite activity in

severe malaria; this probably reflects variable levels of polyclonal B-cell activation.

The negative correlation between IgG antibody concentration and parasitemia described here supports well-established findings that antibodies are important for protection by reducing parasitemia and alleviating clinical illness in *P. falciparum* malaria.^{30,31} On the other hand, elevated concentrations of antimalarial IgG antibodies may reflect not only recent exposure, but also previous exposure to the parasite and thus not necessarily correlate with protection^{27,32} which depends on both quality (specificity, affinity) and concentration of the relevant antibodies and the balance between different IgG subclasses.^{32–34} We show here that the concentrations of the IgG antibodies early in the course of the infection are higher in patients with uncomplicated malaria than in those with severe disease. This finding may be due in part to previous exposure because 16 of the 35 patients with uncomplicated disease compared with only 6 of the 35 with severe disease were known to have had malaria attacks before admission to the hospital (Looareesuwan S and others, unpublished data).

The Thai patients investigated in this study showed elevated total IgE and anti-parasite IgE antibodies similar to levels observed in Africa, other areas of Thailand,² and Papua New Guinea.¹ As in our previous studies of African patients where we compared uncomplicated disease with cerebral malaria,² IgE levels were highest in patients with severe disease. There is no evidence that IgE antibodies to a specific parasite antigen are associated with severe disease. Furthermore, although severe malaria represents a heterogeneous group,¹¹ there was no obvious relationship between the degree of IgE elevation and patient symptoms (severe anaemia, hypoglycemia, and jaundice; data not shown). Since the half life of IgE is short (approximately 2 days), the current results suggest that the difference between the disease groups, assessed at 7-day intervals, reflects a more pronounced and ongoing production of IgE for 3 to 4 weeks in persons with severe malaria. The pattern of IgE elevation was distinctive in comparison with that of IgG: both total IgG and IgG-antimalarial antibodies tended to be higher in patients with uncomplicated disease than in those with severe malaria. This is unlikely to be a reflection of ongoing IgG synthesis, however, since the half-life of IgG (25 days) is much longer than that of IgE (2 days).

A likely explanation for the lack of significant differences in antibody concentrations between the two groups (uncomplicated disease or severe disease, and including all subjects) was the large natural variation in individual immune responses (Table 1). Because IgG and IgE antibodies react with the same plasmodial antigens,² but apparently affect the course of the infection differently, an appropriate way to estimate antibody activity was to calculate IgG/IgE antibody ratios. The increased IgG/IgE antibody ratios in uncomplicated disease were significant and most pronounced at admission. This is important because it is during the early, acute phase of infection—the period when patients are parasitemic—that the antibodies are most likely to exert their effects.³¹

In summary, these results support the conclusion that IgG contributes to the reduction in the severity of *P. falciparum* malaria.^{30,31} The data also suggest a pathogenic role for IgE.

As previously reported,^{9,35} a pathogenic role for IgE is likely related to the capacity of IgE-containing immune complexes or aggregates to induce TNF- α production by monocytes and possibly other effector cells through cross-linking of cellular Fc ϵ receptors (CD23).⁵ An IgE-dependent TNF- α production by monocytes induced by malaria sera has recently been demonstrated *in vitro*.^{9,36} Thus, a local overproduction of TNF- α may be initiated by IgE deposition in small vessels of the brain or elsewhere as a consequence of endothelial cytoadherence, a capacity that is unique for *P. falciparum* among the human malaria parasites.³⁷ Because of the great excess of IgG antibodies over those of IgE, however, IgE-dependent TNF- α induction also requires that the latter antibodies successfully compete with IgG for malaria antigen, e.g., by means of higher avidity. Since the parasite-induced switch from IgM and IgG to IgE production appears to occur relatively late during the immune response,^{3,38} it may well be associated with an increase in avidity. In any event, the binding of IgE antibody to many parasite antigens is easily detectable *in vitro* despite the large excess of IgG.² Deposition of IgE-containing immune complexes in the placentas of *P. falciparum*-infected pregnant women has also been reported.³⁹

Because IgE elevations are the expression of the activity of CD4+ T-helper-2 (Th2) cells,^{40,41} our results also suggest that increased numbers of *P. falciparum* parasites preferentially stimulate Th2 rather than T-helper-1 (Th1) cells. Alternatively, by directly suppressing the Th1 responses, some parasite-derived factors may affect the Th1/Th2 balance which is decisive for the outcome of infection. An elucidation of the molecular mechanisms of the switch toward Th2 differentiation in malaria infection will be of considerable interest for vaccine development and pharmacological immunomodulation.

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