

Differential regulation of IgG subclasses and IgE antimalarial antibody responses in complicated and uncomplicated *Plasmodium falciparum* malaria

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SUMMARY

The aim of this study was to assess the immunoglobulin (Ig)-subclass distribution of antimalarial antibody responses in 110 and 169 Thai patients with complicated and uncomplicated *Plasmodium falciparum* malaria, respectively. Antimalarial plasma IgG subclasses and IgE antibody levels against a crude malaria blood stages, and antigen preparation were determined using enzyme-linked immunosorbent assay (ELISA). On admission, the levels of anti-*P. falciparum* IgG1, IgG2 and IgG3 were significantly lower in patients with complicated malaria than uncomplicated malaria (IgG1, $P < 0.0001$; IgG2, $P < 0.0001$; IgG3, $P < 0.0001$). The levels of antimalarial IgE were slightly lower, but not statistically significant ($P = 0.389$) in the complicated malaria. After adjusting all antibody levels and age, anti-*P. falciparum* IgG3 levels remained significantly associated with complicated malaria. None of the other antibody concentrations showed statistically significant associations with complicated malaria. The anti-*P. falciparum* IgG3 levels were related to the IgG1 as well as IgG2 levels. A correlation between anti-*P. falciparum* IgG2 and IgE was observed in the complicated malaria group, and this may indicate their roles in the severity of disease. Our data suggest that anti-*P. falciparum* IgG3 is associated with a reduced risk of complicated malaria and that antimalarial Ig-subclasses are differently regulated in patients with complicated and uncomplicated malaria.

Keywords antibody, complicated malaria, IgE, IgG subclasses, *P. falciparum*, severity

INTRODUCTION

Anti-*Plasmodium falciparum* specific antibodies play a critical role in immune protection against the asexual blood stages of *P. falciparum*. Passive transfer of immunoglobulin (Ig) G from adult Africans to Gambian children (1) or to adult Thai patients (2) has been shown to protect against asexual blood stages of *P. falciparum* infection. In areas where malaria is endemic, the cytophilic IgG1 and IgG3 subclasses are considered to protect against *P. falciparum*, whereas IgG2 and IgM are not. The latter Ig classes are even suggested to block protective mechanisms (3). The correlation between isotype distribution and the clinical manifestation of malaria is controversial. Anti-*P. falciparum* merozoite surface protein (MSP) IgG3 antibodies have been shown to be associated with either lower parasitaemias or lower risk of clinical malaria (4,5). IgG1 antibodies to exoantigen are associated with clinical protection, while IgG1 antibodies to MSP2 sero group B are associated with increased risk of clinical infection (6,7). Likewise, IgG2 antibodies to exoantigen or crude schizont antigens have been shown to be positively correlated to the number of malaria attacks or clinical malaria (7,8), whereas high IgG2 and low IgG4 antibody levels have been associated with resistance to *P. falciparum* in Burkina Faso (9). Thus, the cumulative evidence of the role of different anti-*P. falciparum* Ig subclasses are contradictory and far from clear.

Another anti-*P. falciparum* Ig class, which is increased in the majority of individuals living in area of high endemicity is IgE. Anti-*P. falciparum* IgE antibodies have been shown to be significantly higher in patients with severe malaria

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Received: 9 March 2007

Accepted for publication: 11 July 2007

than in those with uncomplicated disease, suggesting a role of IgE in the pathogenesis of malaria (10–13). However, in a recent study conducted in Tanzania, anti-*P. falciparum* IgE levels in asymptomatic individuals were found to be associated with a reduced risk for subsequent malaria disease (14). In addition, in a study comprising sympatric ethnic groups, IgE levels were higher in the more malaria protected tribe, the Fulani when compared to their neighbours, the Dogon (15). Thus, these data suggest that IgE can have both protective and pathogenic roles. The reasons why people produce different anti-*P. falciparum* IgG subclasses are not fully understood. Factors such as the parasite strains, the levels of malaria transmission, or the host genetic factors have been implicated in the regulation of the production of different anti-*P. falciparum* Ig classes and subclasses. Additional factors may include the parasite-antigen preparations used in the analysis.

To understand better the natural human defence mechanism against the malaria parasite, we have investigated anti-*P. falciparum* IgG subclasses and IgE antibody responses directed against a crude *P. falciparum* extract in patients with complicated or uncomplicated malaria. We evaluated and described the isotype kinetic profile, and examined the relationship between the pattern of IgG subclasses and IgE with disease manifestation, comparing patients with complicated and uncomplicated forms of malaria.

MATERIALS AND METHODS

Malaria subjects

A total of 279 *P. falciparum* malaria patients, 110 with complicated and 169 with uncomplicated malaria, were enrolled in this study during the period between April 2002 and May 2003. They had all been living in malaria endemic areas of the Thai–Myanmar border in the west and Thai–Cambodia border in the east of Thailand. These areas are considered as having low and seasonal malaria transmission. The annual malaria incidence rates in 2001 were 2–6/1000 population (16). Clinical manifestations of complicated and uncomplicated malaria were defined according to World Health Organization criteria (17). Cerebral malaria was defined as unrousable coma with positive asexual forms of *P. falciparum* in blood smears, while other causes of coma were excluded. Severe malaria but not cerebral (noncerebral severe malaria) was defined as patients exhibiting one or more of the following signs: hyperparasitaemia ($> 250\,000$ parasites/mL); hypoglycaemia (glucose, < 22 nmol/L); severe anaemia (haematocrit, $< 20\%$ or haemoglobin, < 7.0 g/dL); or increased serum level of creatinine (> 3.0 mg/dL). Uncomplicated malaria was characterized by a positive blood smear and fever without other causes of infections

and no manifestations of severe malaria as described above. Patients diagnosed with malaria by symptoms and microscopic examination were transported for admission to the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok where all the clinical characteristics, haematological tests and biochemical tests, for example, splenomegaly, G6PD-deficiency, Hb variants blood groups, and so on, were examined and recorded. Pregnant women or those who had been given antimalarial treatment in the previous 2 weeks were excluded. The patients with complicated malaria in the present study have all forms of severe malaria including cerebral malaria. The study has been ethically approved by the Institute Review Board of the Faculty of Tropical Medicine, Mahidol University, Bangkok, and fully informed consent was obtained from all the patients.

Sample collection

Serial venous blood samples were collected in EDTA sterile tubes before and after treatment at admission (Day 0), Day 7 and Day 28. Plasma was collected, and heat inactivated at 56°C for 30 min and stored at -20°C until use.

Microscopic observation

Thick and thin films were made for microscopic examination after standard Giemsa staining. Parasite densities were determined by calculating *P. falciparum* parasites per μL of blood, obtained from the number of parasites observed per 1000 erythrocytes in thin film or per 200 leucocytes in thick film.

Parasite extract preparation

Lysates of mature stages of Percoll-enriched *P. falciparum*-infected erythrocytes of the laboratory strain F32 were prepared and used as antigen for the detection of malaria antibodies as described (18).

Detection of anti-*P. falciparum* IgG subclasses and IgE antibody levels

Levels of anti-*P. falciparum* IgG1, IgG2, IgG3 and IgE antibodies were measured by ELISA as described previously (13). Briefly, plates were coated overnight with $50\,\mu\text{L}$ /well of $10\,\mu\text{g/mL}$ of *P. falciparum* extract in sodium carbonate buffer pH 9.6, and blocked with 0.5% w/v BSA in coating buffer for 3 h at 37°C . Samples were then added at different dilutions, 1 : 400 for the determination of anti-*P. falciparum* IgG1; 1 : 100 for IgG2; 1 : 1600 for IgG3; and 1 : 100 for IgE. Samples were tested in duplicates. The sera were allowed to

react for 1 h at room temperature for all determinations, with the exception of anti-*P. falciparum* IgE antibodies, which were incubated overnight. Anti-*P. falciparum* IgG1, IgG2, IgG3 or IgE were detected with biotinylated – mouse monoclonal anti-human IgG1, 1 : 3000; IgG2, 1 : 3000; IgG3, 1 : 1000 (Pharmingen, USA), goat anti-human IgE, 1 : 8000 (Vector Laboratories, USA), and followed by ALP-conjugated streptavidin (diluted 1 : 2000) (Mabtech, Nacka, Sweden). The optical density values were read in a Vmax Microplate Reader (Molecular Devices Corporation, Sunnyvale, CA, USA) at 405 nm wavelength. The concentrations were calculated from the standard curve obtained by incubating serial dilutions of either purified myeloma IgG1, IgG2 and IgG3 isotypes (Serotec, Oxford, UK) or human serum IgE (NIBSC, UK) well coated with goat anti-human IgG1 (Pharmingen, USA) for IgG1, goat anti-human IgG (Jackson ImmunoResearch, Europe Ltd., Suffolk, UK) for IgG2, mouse anti-human IgG (Pharmingen, USA) for IgG3 or goat anti-human IgE (Vector Laboratories, USA) for IgE and assayed as described above. Plasma IgG subclasses and IgE optimum dilutions were tested before the experiments were performed. The optimum dilution for each IgG subclass and IgE was then multiplied by each IgG subclass and IgE concentrations obtained from the standard curves.

Statistical analysis

The data were analysed using SPSS or STATVIEW computer software. Antibody levels are presented as geometric mean $\pm$ 95% CI. The Mann–Whitney *U*-test was applied for comparison between complicated and uncomplicated malaria patients. The relationship between IgG subclasses and IgE antibodies was examined using linear regression analysis. Multiple logistic regressions were used to investigate whether the levels of the IgG subclasses and IgE antibodies were significantly different between complicated and uncomplicated malaria. The independent variables were anti-*P. falciparum* IgG subclasses and IgE antibodies, and age. The levels of anti-*P. falciparum* IgG subclasses and IgE antibodies were grouped into equal fifths of their distributions. Age was categorized into five groups (< 20, 20–29, 30–39, 40–49 and > 49 years). These measures were modelled as a series of binary dummy variables. The highest fifth antibody level was used as the comparison group. Thus, univariate and multivariate analyses for categorical variables were performed. A *P*-value less than 0.05 and 95% confidence intervals for odds ratios which do not cross 1.00 were considered statistically significant.

To ensure that the results are not due to co-linearity between IgG1 and IgG2, two further adjusted logistic regression models had malaria severity as the dependent

variable. The first included measures of IgG1, IgG3 and age, while the second included measures of IgG2, IgG3 and age.

RESULTS

Characteristics of patients

The characteristics of 110 adults with complicated and 169 adults with uncomplicated malaria are delineated in Table 1. No statistically significant difference was observed in the age distribution, sex ratio and nationality between the complicated and uncomplicated malaria groups. In addition, the blood group distribution, and percentages of individuals with haemoglobin variants and enzyme G6PD deficiency did not differ between the complicated and uncomplicated groups. The mean parasite density was significantly higher in the complicated group compared to the uncomplicated group ($P < 0.0001$). When the highest temperatures were compared between the uncomplicated and complicated group, a significantly higher temperature was found in the uncomplicated group ($P = 0.010$). Individuals presenting with complicated malaria were less likely to have had previous episodes of malaria, compared to those presenting with uncomplicated disease ($P = 0.0004$). Clinical examination revealed a significantly higher rate of spleen and liver enlargement in the patients with complicated malaria than those with uncomplicated malaria ($P = 0.039$ and $P < 0.0001$, for splenomegaly and hepatomegaly, respectively).

Association between anti-*P. falciparum* antibodies and complicated malaria

Anti-*P. falciparum* serum antibody levels in patients were determined before and after treatment, that is, at Days 0, 7 and 28. The geometric mean values of anti-*P. falciparum* IgG subclasses and IgE antibodies in both groups are illustrated in Figure 1. On Day 0 before treatment, the geometric means of anti-*P. falciparum* IgG1, IgG2 and IgG3 antibodies were lower in the patients with complicated malaria than in those with uncomplicated malaria (IgG1, $P = 0.0001$; IgG2, $P < 0.0001$; IgG3, $P < 0.0001$). The geometric mean of anti-*P. falciparum* IgE antibody was slightly lower, although not statistically significant ($P = 0.389$) in the patients with complicated malaria than uncomplicated malaria. On Days 7 and 28 after treatment, the geometric mean of anti-*P. falciparum* IgG1 and IgG3 was still lower in patients with complicated malaria than those with uncomplicated malaria (IgG1, $P = 0.001$ and IgG3, $P = 0.002$ for Day 7; IgG1, $P = 0.0002$ and IgG3, $P = 0.0007$ for Day 28). No significant differences were

Table 1 Characteristics of patients with complicated and uncomplicated malaria

Characteristics	Complicated malaria (110) ^a	Uncomplicated malaria (169) ^a	P-value ^b
Age ^c (range)	25·0 (14–67)	24·0 (13–65)	0·849
Sex (male/female)	75/35	133/36	0·137
Nationality ^d			
Burmese	17 (15·5)	24 (14·2)	0·614
Karen	18 (16·4)	27 (16·0)	
Mon	37 (33·6)	72 (42·6)	
Thai	38 (34·5)	45 (26·6)	
Laos	0	1 (0·6)	
Blood groups ^d			
A	21 (19·1)	27 (16·0)	0·556
AB	6 (5·5)	7 (4·1)	
B	34 (30·9)	58 (34·3)	
O	47 (42·7)	66 (39·1)	
NR	2 (8·1)	11 (6·5)	
Haemoglobin variants ^{d,e}			
Hb variants	14 (12·7)	29 (17·2)	0·825
Normal	95 (86·4)	133 (78·7)	
NR	1 (0·9)	7 (4·1)	
G6PD deficiency ^d			
Deficiency	13 (11·8)	14 (8·3)	0·597
Normal	96 (87·3)	153 (90·5)	
NR	1 (0·9)	2 (1·2)	
Parasitaemia (range) ^f	117 322 (33–1 336 900)	5641 (17–238 800)	< 0·0001
Highest fever in °C ^g	38·1 ± 1·005	38·4 ± 0·981	0·010
Previous malaria episodes ^d			
Yes	30 (27·3)	93 (55·0)	< 0·0004
No	78 (70·9)	76 (45)	
NR	2 (1·8)		
Splenomegaly ^h			
Positive	18 (16·4)	3 (8·1)	0·039
Negative	92 (83·4)	166 (98·2)	
Hepatomegaly ^h			
Positive	66 (60)	32 (18·9)	< 0·0001
Negative	44 (40)	137 (81·1)	

^aNumber of patients; ^bMann–Whitney *U*-test used for the analysis; ^cMedian; ^dPercentages (in parentheses); ^eThe Haemoglobin (Hb) variants include HbE, HbF; ^fGeometric mean/μL of blood; ^gMean ± SD; ^hPercentages of individuals with enlarged spleens and/or liver (in parentheses). NR, No report.

observed for the geometric means of anti-*P. falciparum* IgG2 or IgE between patients with complicated compared to those with uncomplicated malaria (IgG2, $P = 0·882$ and IgE, $P = 0·884$ for Day 7; IgG2, $P = 0·539$ and IgE, $P = 0·318$ for Day 28).

The results of the logistic regression analysis comparing associations of characteristics on Day 0 with risk of complicated malaria are shown in Table 2. Univariate analysis before adjustment showed that lower levels of anti-*P. falciparum* IgG3, IgG1 and IgG2 antibodies on Day 0 were associated with a statistically significant increased risk of complicated malaria (OR > 1·00, $P < 0·05$), and no association with complicated malaria was observed for the anti-*P. falciparum* IgE levels. To exclude the possibility of confounding by age and co-linearity between anti-*P. falciparum*

IgG1, IgG2, IgG3 and IgE antibodies, multiple simultaneous adjustment was performed for these factors as described in the statistical analysis section. After adjustment, only lower levels of anti-*P. falciparum* IgG3 antibodies remained significantly associated with a greater risk of complicated malaria (OR > 1·00, $P < 0·05$), whereas no significant association of anti-*P. falciparum* IgG1, IgG2 and IgE antibodies with the risk of succumbing to complicated malaria was observed.

The analysis performed for Day 0 levels was repeated for subsequent days, that is, Days 7 and 28, and the negative association of IgG3 levels in patients with complicated malaria was consistently observed and independently from the other Ig levels and age, although the magnitude of the association was reduced (data not shown).

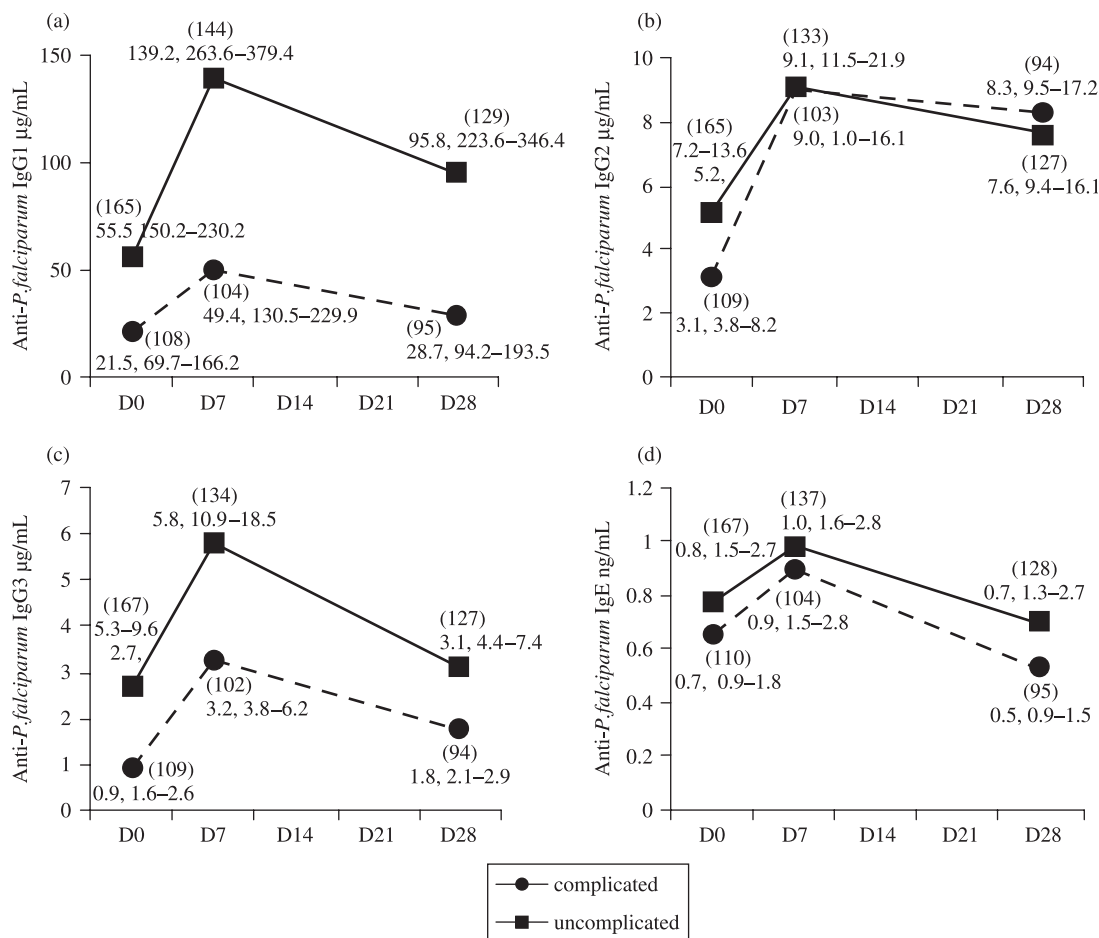


Figure 1 Geometric means of IgG1 (a), IgG2 (b), IgG3 (c) and IgE (d) antibodies in plasma from patients with complicated (circle) or uncomplicated (square) *Plasmodium falciparum* malaria before and after treatment. D0, day of admission. Values under or above each point are geometric mean and 95% CI of antibody levels. Data in the parentheses are numbers (N) in each group.

The lack of association of IgG1 and IgG2 with malaria severity in the adjusted model was not due exclusively to co-linearity between these two antibodies. When adjusted only for age and IgG3, the association of IgG1 with malaria severity was not statistically significant. Compared with the highest level, the other levels from low to high had odds ratios (and 95% CI) of: 0.48 (0.16–1.42); 0.77 (0.29–2.02); 0.85 (0.34–2.13); and 0.47 (0.18–1.23). Similarly, there was no statistically significant association of IgG2 with malaria severity after adjustment for age and IgG3, producing odds ratios of: 0.73 (0.25–2.19); 1.05 (0.38–2.85); 1.47 (0.58–3.72); and 0.78 (0.30–2.03).

Anti-*P. falciparum* IgG1, IgG2, IgG3 and IgE relationship

At admission, 110 patients with complicated and 169 with uncomplicated malaria had detectable levels of anti-

P. falciparum IgG subclasses and IgE antibodies. The levels of the different malaria-specific IgG subclasses and IgE varied greatly between individuals. The correlation between the specific IgG1 and IgG3 antibody levels ($r = 0.295$, $P < 0.001$) was similar to the correlation between IgG1 and IgG2 ($r = 0.392$, $P < 0.001$), and between IgG2 and IgG3 levels ($r = 0.279$, $P < 0.001$). A similar correlation was seen when the patients with complicated or uncomplicated malaria were analysed separately. This was in contrast to what was seen with the anti-*P. falciparum* IgE antibody concentrations where statistically significant correlation with anti-*P. falciparum* IgG2 antibody levels was found only in the patients with complicated disease ($r = 0.482$, $P < 0.001$). No statistically significant correlation was found between anti-*P. falciparum* IgE antibody levels and anti-*P. falciparum* IgG1 and IgG3 subclasses (data not shown).

Table 2 Baseline characteristics in 110 complicated and 167 uncomplicated malaria at admission (Day 0). The odds ratios estimate 'the risk of complicated malaria'

	Severity ^a		Unadjusted ^b (Day 0)		Adjusted ^c (Day 0)	
	Uncomplicated (%)	Complicated (%)	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
Age						
≤ 19	39 (24.1)	29 (27.4)	1.07 (0.58–1.99)	0.825	0.77 (0.38–1.58)	0.479
20–29	62 (38.3)	43 (40.6)	1		1	
30–39	42 (25.9)	20 (18.9)	0.69 (0.35–1.33)	0.264	0.82 (0.38–1.76)	0.607
40–49	13 (8.0)	7 (6.6)	0.77 (0.29–2.11)	0.619	0.81 (0.26–2.52)	0.717
≥ 50	6 (3.7)	7 (6.6)	1.68 (0.53–5.35)	0.379	2.41 (0.66–8.83)	0.183
Anti-Pf IgG1 ^d						
1	26 (16.0)	27 (25.5)	2.40 (1.08–5.32)	0.031	0.42 (0.13–1.29)	0.133
2	26 (16.0)	28 (26.4)	2.49 (1.12–5.50)	0.024	0.78 (0.29–2.13)	0.630
3	30 (18.5)	24 (22.6)	1.85 (0.83–4.09)	0.129	0.79 (0.30–2.05)	0.628
4	43 (26.5)	11 (10.4)	0.59 (0.24–1.43)	0.245	0.46 (0.17–1.24)	0.126
5	37 (22.8)	16 (15.1)	1		1	
Anti-Pf IgG2						
1	26 (16.0)	27 (25.5)	3.19 (1.39–7.29)	0.006	0.78 (0.25–2.44)	0.679
2	26 (16.0)	28 (26.4)	3.31 (1.45–7.54)	0.004	1.13 (0.398–3.18)	0.823
3	29 (17.9)	25 (23.6)	2.65 (1.16–6.04)	0.020	1.48 (0.57–3.88)	0.419
4	41 (25.3)	13 (12.3)	0.98 (0.403–2.36)	0.956	0.79 (0.29–2.11)	0.643
5	40 (24.7)	13 (12.3)	1		1	
Anti-Pf IgG3						
1	19 (11.7)	34 (32.1)	14.01 (5.06–38.81)	0.000	20.17 (4.64–87.63)	0.000
2	25 (15.4)	29 (27.4)	9.08 (3.33–24.80)	0.000	11.23 (3.12–40.47)	0.000
3	37 (22.8)	17 (16.0)	3.59 (1.29–10.03)	0.014	3.29 (1.03–10.45)	0.043
4	34 (21.0)	20 (18.9)	4.61 (1.67–12.69)	0.003	4.29 (1.42–12.99)	0.010
5	47 (29.0)	6 (5.7)	1		1	
Anti-Pf IgE						
1	33 (20.4)	20 (18.9)	1.40 (0.63–3.14)	0.413	1.07 (0.43–2.66)	0.879
2	31 (19.1)	23 (21.7)	1.72 (0.77–3.80)	0.184	1.33 (0.54–3.23)	0.533
3	32 (19.8)	22 (20.8)	1.59 (0.72–3.54)	0.255	1.12 (0.46–2.73)	0.799
4	29 (17.9)	25 (23.6)	1.99 (0.90–4.41)	0.088	1.88 (0.77–4.61)	0.167
5	37 (22.8)	16 (15.1)				

Antibody levels (mean) in negative controls (Swedish individuals not exposed to malaria) were: anti-*Plasmodium falciparum* IgG1, 9.52 µg/mL; anti-*P. falciparum* IgG2, 2.69 µg/mL; anti-*P. falciparum* IgG3, 0.08 µg/mL; anti-*P. falciparum* IgE, 0.40 ng/mL.

^aNumber of individuals with complicated or uncomplicated malaria; ^bUnadjusted odds ratios from univariate analysis; ^cOdds ratios adjusted for age, anti-*P. falciparum* IgG1, IgG2, IgG3 and IgE antibodies; ^dAnti-*P. falciparum* IgG subclasses and IgE antibody levels were categorized into five groups based on their antibody level distribution, where 1 is the lowest and 5 is the highest levels.

Kinetic profile of anti-*P. falciparum* IgG subclass and IgE antibodies

The patients were followed for 28 days. The number of blood samples available for testing was decreased from 110 samples at Day 0 to 94 (complicated disease), and from 167 samples to 127 (uncomplicated form) at Day 28. As in Figure 1, the levels of IgG1, IgG2, IgG3 and IgE increased on Day 7, in both uncomplicated and complicated malaria patients, then a gradual decline was seen throughout the study period. The anti-*P. falciparum* IgG subclass and IgE antibody levels were lower in complicated than in uncomplicated cases at each time points except for anti-*P. falciparum* IgG2 which showed similar levels at Days 7 and 28.

DISCUSSION

In this study, on admission, a univariate association of complicated malaria was observed with the lower levels of anti-*P. falciparum* IgG1, IgG2 and IgG3 antibodies. No significant association of severity of malaria with anti-*P. falciparum* IgE antibody was observed. After adjusting for multiple potential confounding factors, such as age and antibody Ig subclasses, only lower anti-*P. falciparum* IgG3 levels remained significantly associated with an increased risk of complicated malaria. This was in contrast to what was seen for anti-*P. falciparum* IgG1, IgG2 and IgE antibody concentrations where no such associations were seen.

An association between high antimalarial IgG3 levels and resistance to severe malaria disease was observed also on the follow-up Days 7 and 28. This suggests that anti-*P. falciparum* IgG3 antibodies may play an important role in the control of clinical manifestations during acute *P. falciparum* malaria as well as in reducing the risk of complicated malaria, which was in line with the previous studies in Africa and Asia (4,6,19). IgG3 antibodies are cytophilic and have been shown to be effective in antibody-dependent inhibition of the asexual blood stages of the malaria parasite (3,20). Indeed, IgG3 can activate complement pathways (21) and co-operate through FcR receptors (22). Thus, IgG3 could induce cell-mediated lysis via complement pathways, phagocytosis and/or ADCI mechanism (3,20) which in turn controls parasite multiplication.

In the present study, on admission day, neither associations between complicated and uncomplicated malaria nor anti-*P. falciparum* IgG1, IgG2 and IgE antibodies were observed after adjusting for anti-*P. falciparum* IgG subclasses, IgE- and age-dependent exposure to malaria. This is in agreement with the previous studies reporting that only anti-*P. falciparum* IgG3, but not IgG1, IgG2 or IgG4 antibody concentrations are associated with a significantly reduced risk of malaria attacks (4,23). Anti-IgG1, IgG2, IgG4, IgM and IgE antibodies may block the effective cytophilic IgG3 and thus may reduce parasite clearance. However, an association between the lowest IgG1 levels and an increased risk of complicated malaria during the convalescence phase, independent of the other measures was observed in this study. The elevated IgG1 levels during the convalescence may relate to a reduced risk of succumbing to complicated malaria and/or protection against re-infection, as shown in the previous findings of the correlation between anti-MSP1 IgG1 and protection in infants (24). Others have reported that high IgG2 and low IgG4 levels are associated with resistance to *P. falciparum* malaria in Burkina Faso (9). The IgG2 subclass binds to the FcγRIIα receptor carrying the H131 allele (25), and this allele is frequent in these populations. The fact that we did not see any protective role of anti-*P. falciparum* IgG2 antibodies during the acute phase might be a reflection of the H131 allele frequency in our study population. However, this still needs to be firmly established.

There was no statistically significant relationship between anti-*P. falciparum* IgE levels and severity of malaria before and after adjusting for the confounding factors in this study. However, the levels of anti-*P. falciparum* IgE were slightly lower in patients with complicated malaria than uncomplicated malaria. This is not consistent with the previous data reporting that anti-*P. falciparum* IgE antibodies were significantly lower in children with uncomplicated malaria than those with cerebral malaria (13) as well as in adults

with uncomplicated malaria compared to severe malaria in Thailand (12). Thus, our data are more compatible with those reported by others who showed high anti-*P. falciparum* IgE levels in asymptomatic individuals in Tanzania, where anti-*P. falciparum* IgE levels were associated with reduced risk for subsequent malaria disease (14). Higher antimalarial IgE levels have also been shown in the more malaria protected the Fulani tribe of West Africa (15). The reasons for these discrepancies are not known, but it could be due to many different factors, including pre-existing IgE immune complexes caused by helminthic infections and/or allergen (26–28). Thus, the protection and/or pathogenic roles of anti-*P. falciparum* IgG1, IgG2 and IgE antibodies require further investigation.

A relationship between anti-*P. falciparum* IgG subclasses and IgE was investigated in the present study. On admission, before treatment, a correlation between anti-*P. falciparum* IgG1 and IgG3 as well as between anti-*P. falciparum* IgG1 and IgG2, and between IgG2 and IgG3 levels was seen. The levels of anti-*P. falciparum* IgG2 and IgE were related to complicated malaria. No relationship between anti-*P. falciparum* IgE and IgG1, and/or IgG3 levels was observed. IgG2 is known to be a Th1 subclass Ig-dependent on IFN-γ (29,30). According to recent evidences, IL4 significantly enhances the expression of ε, γ1, γ2, γ3 and γ4 germline gene transcript (GLT) and the secretion of the corresponding proteins. Conversely, IFN-γ did not inhibit εGLT in single human B cells (31,32). This is contrary to the reported situation in murine model where IFN-γ and IL4 are antagonistic, and addition of IFN-γ inhibited the IgE induction by IL4 (33,34). Therefore, the IgG2 and IgE could be possibly co-correlated in human immune responses.

The observed co-linearity between IgG1, IgG2 and IgG3 emphasizes the importance of performing multivariate analysis to disentangle causal and non-causal associations with complicated malaria. Similarly, as functional immunity against malaria and Ig levels alter with age, it may be a confounding factor for associations of specific Ig with complicated malaria. To avoid these potential sources of confounding, we adjusted for age and all Ig using a multiple logistic regression model. The Ig levels were not normally distributed and it is unlikely that they would exhibit a linear association with the risk of complicated malaria; therefore, modelling them as untransformed continuous variables might produce spurious results. To overcome this problem, we adopted the well-recognized technique of dividing each distribution into equal fifths and modelling them as series of binary dummy variables. This technique may somewhat reduce statistical power, but cannot produce the spurious results possible using the original distributions. This approach has the added advantage of standardizing

measurement, so that the odds ratios for different Ig are more directly comparable. Multivariate modelling confirms that the association of lower levels of IgG3 with an increased complicated malaria risk is independent of the other Ig levels and age.

Patients with complicated malaria showed lower levels of anti-*P. falciparum* IgG subclasses and IgE antibodies compared to those with uncomplicated malaria. However, at each individual level, there was wide variation in their antibody response to the *Plasmodium* antigen, which could reflect the difference in host genetic and/or parasite virulent factors. The anti-*P. falciparum* antibody responses are genetically regulated (35) as shown in the recent studies in Fulani tribe in the West Africa who are more malaria protected sympatric ethnic group; the *IL4-524* T allele was found to be associated with elevated antibody levels against several malaria antigens (36). The *IL4-524* is located on chromosome 5q31–5q33, a region linked to parasite intensity and contains genes encoding cytokine involved in Ig class switching (37). In addition, specific IgG subclass responses can potentially be transmitted to the next generation (38).

In summary, we have shown that anti-*P. falciparum* antibody profiles are differentially regulated in complicated compared to uncomplicated malaria. This might indicate that the antibodies are associated with the risk of malaria severity. The predominant levels of anti-*P. falciparum* IgG3 are associated with lower risk of complicated malaria. The correlation between anti-*P. falciparum* IgG2 and IgE found only in patients with complicated malaria leads to the possibility that these two antibodies are pathogenic. These observations and the understanding of naturally acquired anti-*P. falciparum* antibody responses may help in the development of malaria control strategies, especially vaccination.

ACKNOWLEDGEMENTS

We wish to thank the patients for their kind participation in this study and staff of the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University for their assistance in blood collection. We thank late Emeritus Professor Peter Perlmann for his kind suggestions and Margareta Hagstedt of Stockholm University for her technical assistance. This study was supported by The Royal Golden Jubilee Ph.D. Program (5MMU44A1), the Basic Research Grant for the Royal Golden Jubilee Ph.D. (BGJ4580004) of the Thailand Research Fund (TRF), the Swedish Foundation for International Cooperation in Research and Higher Education (STINT), the Swedish International Development Co-operation Agency (SIDA/SAREC) and BioMalPar EU, #LSHP-CT-2004, 503578.

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