



IgE low affinity receptor (CD23) expression, *Plasmodium falciparum* specific IgE and tumor necrosis factor-alpha production in Thai uncomplicated and severe falciparum malaria patients



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ARTICLE INFO

Article history:

Received 5 January 2015

Received in revised form 22 October 2015

Accepted 22 October 2015

Available online 27 October 2015

Keywords:

CD23

P. falciparum

TNF- α

IgE

ABSTRACT

Previous studies have suggested that *Plasmodium falciparum* (*P. falciparum*) specific IgE in the form of immune complexes crosslinking the low-affinity receptor (CD23) on monocyte results in tumor necrosis factor (TNF)- α and nitric oxide (NO) production. However, the roles of these parameters in severity and immune protection are still unclear. This study aimed to determine the association between CD23 expression on monocytes, plasma soluble CD23 (sCD23), total IgE, malaria-specific IgE and IgG, and TNF- α levels in *P. falciparum* infected patients. We evaluated 64 uncomplicated (UC) and 25 severe patients (S), admitted at the Hospital for Tropical Diseases, Mahidol University, and 34 healthy controls (C) enrolled in 2001. Flow cytometry and enzyme linked immunosorbent assays (ELISA) demonstrated that trends of the CD23 expression, levels of sCD23 and specific IgE were higher in the S group as compared to those in the UC and C groups. Plasma levels of *P. falciparum* specific IgE in the UC ($p=0.011$) and S groups ($p=0.025$) were significantly higher than those in C group. In contrast the TNF- α levels tended to be higher in the UC than those in the S ($p=0.343$) and significantly higher than those in C ($p=0.004$) groups. The specific IgG levels in UC were significantly higher than those in S and C ($p<0.001$) groups. At admission, a strong significant negative correlation was found between specific IgG and sCD23 ($r=-0.762$, $p=0.028$), and TNF- α and IgE-IgG complexes ($r=-0.715$, $p=0.002$). Significant positive correlations between levels of specific IgE and TNF- α ($r=0.575$, $p=0.010$); and sCD23 ($r=0.597$, $p=0.000$) were also observed. In conclusion, our data suggest that CD23 expression and malaria-specific IgE levels may be involved in the severity of the disease while TNF- α and the malaria-specific IgG may correlate with protection against falciparum malaria.

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1. Introduction

There are two IgE receptors, one is the high-affinity receptor (Fc ϵ RI) that is important in allergic reactions in type I-mediated asthma. The other receptor is the low-affinity receptor (Fc ϵ RII) also known as CD23, which is important for parasite infection (Cabrer

et al., 2003; Sutton and Gould, 1993; Vouldoukis et al., 2011). Cross-linking of CD23 contributes to the production of tumor necrosis factor (TNF)- α , interleukin (IL)-1, IL-6, H₂O₂ and nitric oxide (NO) through NF κ B and AP-1 dependent mechanism (Vouldoukis et al., 1995).

In malaria infection, elevation of IgE has been reported in areas with high malaria endemicity (Desowitz et al., 1993; Perlmann et al., 2000). Levels of total IgE and *Plasmodium falciparum* specific IgE have been shown to be higher in patients with severe malaria patients than in those with uncomplicated cases (Perlmann et al., 1994, 1997). These finding was supported in previous studies where elevated serum levels of IgE in Ivorian children (Seka-Seka et al.,

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2004) with severe falciparum malaria were seen. This is in contrast to what was seen with the levels of specific IgG, which were significantly higher in the uncomplicated malaria patients (Perlmann et al., 2000).

Previous studies have suggested that *P. falciparum* specific IgE in the form of immune complexes crosslinks the low-affinity receptor (CD23) on monocyte which results in TNF- α and NO production. These factors have been demonstrated to be controversial. Thus, some earlier studies demonstrated the involvement of IgE in the pathogenesis of severe malaria (Dugas et al., 1995; Nacher, 2004). In line with this, a previous immunohistochemical study of brain tissues from cerebral malaria (CM) patients showed IgE deposits in the capillaries sequestered with infected red blood cells. This finding indicates a role of IgE in the pathogenesis of CM as mentioned previously (Maeno et al., 2000). In contrast, induction of the CD23/NO pathway is believed to play a protective role in pathogenesis of falciparum malaria by down regulating ICAM-1 expression and decreasing cytoadherence of *P. falciparum*-infected erythrocytes (PRBC) (Pino et al., 2004). Correspondingly, Naucher suggested that severity of falciparum malaria in helminth-infected patients was decreased. Pre existing helminth infection induces IgE and IL-10 production. The IgE complexes induce NO release through the chronic activation of the CD23/NO pathway. These might lead to down regulation of cytoadherence receptor expression, that consequently reduces sequestered PRBC in the deep organs (Nacher, 2004). Earlier studies in Latin America reported IgE levels increase in patients co-infected with *P. falciparum* and *Plasmodium vivax* (Blair Trujillo et al., 2003; Carmona-Fonseca, 2013). In addition a previous study in falciparum malaria patients co-infected with *Ascaris lumbricoides* revealed higher total IgE levels and a decrease in the severity of malaria (Nacher et al., 2000). An earlier study found that specific IgE antibodies might play a role in maintaining anti-malarial immunity as revealed by a reduced risk for subsequent clinical malaria (Bereczky et al., 2004). Thus, the role of *P. falciparum* specific IgE in severity and immune protection is still unclear.

This study was designed to elucidate whether *P. falciparum* specific IgE influenced the severity of the disease, by determining the association between plasma levels of *P. falciparum* specific IgE, *P. falciparum* specific IgG, total IgE, sCD23 and TNF- α , and CD23 expression on activated monocyte and severity of disease in Thai falciparum malaria patients.

2. Materials and methods

2.1. Study design and patient population

This study was conducted at Faculty of Tropical Medicine, Mahidol University. Approval for this study was obtained from the Ethics Committees of the Faculty of Tropical Medicine, Mahidol University and the Thai Red Cross Society (Bangkok, Thailand). All participants were informed the objectives of the study, and a completed informed consent form was obtained from each one.

Eighty-nine *P. falciparum* malaria patients including 25 with severe (S) and 64 with uncomplicated malaria patients (UC), and 34 healthy controls (C) were enrolled in the study. All patients were treated at the Hospital for Tropical Diseases, Faculty of Tropical Medicine, during the transmission season from April 2001 to September 2002. All controls lived in Bangkok outside malaria endemic areas and were non-infected with normal complete blood count (CBC) examination. The age and sex distribution did not differ between the groups. The histories and clinical manifestations of the patients were recorded during admission and were classified by the specialist (SK) according to WHO criteria (World Health Organization, 2012). Giemsa stain of thick and thin

smears from finger pricks showed positive for *P. falciparum* asexual blood stage and temperature $\geq 37.5^\circ\text{C}$. Here, we focused to study on 2 groups of patients depending on their clinical outcomes. They include (1) uncomplicated patients (UC), and (2) severe patients (S). UC cases with no evidence of vital organ dysfunctions but presented conventional acute symptoms (fever, headache, muscle pain, chill, and nausea). S cases had one or more severe signs/ symptoms including impaired consciousness (including unrousable coma), severe anemia (Hb $< 7\text{ g/dL}$ or Hct $< 20\%$), metabolite acidosis, hypoglycemia, acute renal injury (serum creatinine $> 3\text{ mg/dL}$), acute pulmonary edema (radiological), jaundice (serum bilirubin $> 3\text{ mg/dL}$) plus evidence of other vital organ dysfunctions (World Health Organization, 2012) and hyperparasitemia (5% parasitemia in RBC or $\geq 200,000$ parasites/ μL) (Liu et al., 2014). Before inclusion, all volunteers and patients had not been treated with any anti-malarial drugs and had negative intestinal parasites based on feces examination.

2.2. Blood sample collection

Five milliliters of heparinized blood samples were collected from the healthy donors once and from all patients on the day of admission (D0) and then once weekly during one month from the S cases. UC cases were outpatients. They were followed up every day until fever and parasitemia was negative. Two mL of plasma were collected and kept at -70°C for detection of sCD23, TNF- α , *P. falciparum* specific IgE and IgG, and IgE-IgG complexes by enzyme linked immunosorbent assays (ELISA). Packed blood cells were used to isolate mononuclear cells for the detection of the low affinity IgE-receptor (CD23) on monocytes by flow cytometry (FACS).

2.3. Mononuclear cell isolation

Peripheral mononuclear cells (PBMC) were separated by Isoprep (Robbins Scientific Corporation, Sunnyvale, CA, Norway) gradient centrifugation according to the manufacturer's recommendation.

2.4. Detection of CD23 on monocytes

Approximately 2 million PBMC were stained with $2\mu\text{L}$ of rhodaminephycoerythrin (RPE)-conjugated mouse monoclonal anti-human CD14, (anti-CD14 RPE) (Dako, Copenhagen, Denmark) and $2\mu\text{L}$ of FITC-conjugated mouse monoclonal anti-human CD23, (anti-CD23 FITC) (Dako, Copenhagen, Denmark) at room temperature for 15 min in dark. The cells were washed with 2 mL of 0.1 M phosphate buffered saline (PBS) and centrifuged at 1300 rpm for 5 min where after the cells were fixed with $500\mu\text{L}$ of 1% paraformaldehyde in PBS. The cells stained for CD23 were detected by FACS (Becton Dickinson, San Jose, C.A., and U.S.A.). Dead cells were excluded from the analysis using propidium iodide staining. The cells were gated on CD14 $^+$ cells and the numbers of CD23 cells were calculated based on the CD14 $^+$ population. The cell analysis was conducted by CELLQUEST program (Becton Dickinson, San Jose, USA). The quantitative expression of CD23 molecule on monocyte was estimated by comparing the intensity of CD23 to the standard fluorescence intensity of standard fluorescein conjugated beads (QuantumTM 26 fluorescein microbeads standards, Flow Cytometry Standards Corp., San Juan, USA). All experimental samples were tested in duplicate. Unstained PBMC of each sample and isotype control were included as negative control.

2.5. Determination of TNF- α by ELISA

Plasma levels of TNF- α were determined by ELISA as previously described (Perlmann et al., 2000). Briefly, the ELISA plate (Costar, Cambridge, MA) were coated with $5\mu\text{g/mL}$ of mouse anti-human

TNF- α (ImmunoKontakt, AMS Biotechnology, UK) in coating buffer at 4 °C overnight. After washing, the wells were blocked nonspecific protein binding by adding 0.5% BSA in coating buffer at 37 °C for 1 h. Fifty microliter of plasma at the dilution of 1:5 in incubating buffer were added and incubated at 37 °C for 1 h. After washing, 50 μ L of biotinylated goat anti-human TNF- α (1:4000) were added and incubated for 1 h at 37 °C and followed by adding 50 μ L of streptavidin alkaline phosphatase AQ (1:2000) (Mabtech, Stockholm, Sweden) for 1 h at 37 °C. After washing, the reaction was detected by chromogen solution, *p*-nitrophenyl disodium phosphate (Sigma; Saint Louis, Missouri, USA) for 30–60 min in dark. The optical density (OD) in each well was measured at 405 nm wavelength using a Vmax microplate reader (Molecular Devices Corporation, Sunnyvale, CA). The levels of TNF- α were calculated from standard curve by incubating the coated plates with 5 serial dilution (range 10–3000 pg/mL) of recombinant TNF- α (NIBSC, Hertfordshire, UK). Positive and negative sera were included. The assay was conducted in duplicate.

2.6. Determination of total IgE, *P. falciparum*-specific IgG, and IgE, and IgE–IgG complexes by ELISA

2.6.1. Parasite extract preparation

Lysates of *P. falciparum*-infected erythrocytes of the laboratory strain F32 were prepared and used as antigen (Ag) for the detection of *P. falciparum* specific antibodies (Troye-Blomberg et al., 1983). Briefly, the *P. falciparum* strain F32 was continuously cultured as described before (Jensen and Trager, 1978). Late stage infected erythrocytes were enriched by 60% Percoll, sonicated and used as Ag in ELISA for determining the specific IgE, IgG and IgG–IgE complexes.

2.6.2. Plasma levels of total IgE, *P. falciparum* specific IgE, and *P. falciparum* specific IgG, and IgE–IgG complexes

The determination by ELISA was described by Perlmann et al. (2000). Briefly, ELISA plates were coated overnight with 50 μ L/well of affinity-purified IgG fractions of rabbit anti-human IgE (Miab, Uppsala, Sweden) at a concentration of 5 μ g/mL for total IgE, and of the parasite antigen (10 μ g/mL) for specific IgE and IgG assay. After 3 hrs of incubation, plates were blocked with 0.5% BSA in PBS at 37 °C to avoid non-specific binding. Samples were then added at the dilutions: 1:1000 for the determination of total IgE, and anti-*P. falciparum* IgG, and 1:100 for anti-*P. falciparum* IgE assay. Samples were tested in duplicate. The plasma was allowed to react for 1 h at room temperature for all determinations, with the exception of anti-*P. falciparum* IgE, which were incubated overnight. Bound IgG was then detected by adding goat-anti human IgG conjugated to alkaline phosphatase (ALP) (Mabtech, Nacka, Sweden). Total IgE and bound anti-*P. falciparum* IgE were detected with biotinylated rabbit anti-human IgE (1:1000) followed by ALP-conjugated streptavidin (1:2000) (Mabtech, Stockholm, Sweden). The OD values were read in a Vmax Microplate Reader (Molecular Devices Corporation, Sunny vale, CA). The levels of specific IgG and IgE, and total IgE were calculated from standard curves obtained by incubating the coated plates with 5 serial dilutions of either affinity-purified human serum IgE (National Institute for Biological Standards and Control, Hertfordshire, United Kingdom) or highly purified human IgG (Jackson Immuno Research Laboratories, West Grove, PA). The Ig levels are given as geometric means of the concentrations found in all samples with UC or S as indicated.

IgE–IgG complex assay in plasma was performed using IgE capture. The microplates were coated with 5 μ g/mL of monoclonal anti IgE Ab (Pharmingen, San Diego, CA, USA). Fifty microliter of plasmas obtained from patients and controls, at the dilution of 1:1000, were added and incubated overnight at 4 °C, followed by incubation with biotinylated mouse anti-Fc gamma at the dilution of 1:5000

(Sigma, Northbrook, IL, USA). The detection of reaction and amount of IgE–IgG complexes was conducted as mentioned above.

2.7. Determination of soluble (s) CD23 by ELISA

Levels of sCD23 in the plasma were determined by sCD23 ELISA kit (Pharmingen, San Diego, CA, USA.) according to the instruction of the manufacturer. Briefly, two-fold dilutions of standard sCD23 (50–3.10 ng/mL) and samples at the dilution of 1:10 were incubated in anti human CD23 pre-coated wells for 2 hrs at room temperature. After washing, the working detector solution containing CD23 Ab linked to avidin-horseradish peroxidase was added to each well and incubated for 1 h. After washing 3 times, tetramethylbenzidine (TMB) substrate solution was added and incubated for 30 min in the dark for the color reaction. Finally, the reaction was stopped by the stop solution. The plate was read OD at 450 nm using a Vmax Microplate Reader. The sCD23 concentration for each sample was derived using a standard curve for sCD23.

2.8. Statistical analysis

Quantitative data were expressed as median (range of minimum to maximum values). Difference of variables between the 2 groups were determined by Mann–Whitney U test or among 3 groups by Kruskal–Wallis test, respectively. Correlations between densities of CD23 molecules, levels of total IgE, *P. falciparum* specific IgE, *P. falciparum* specific IgG, IgE–IgG complexes, TNF- α , sCD23 and severity of the diseases, clinical manifestations including, parasite clearance times (PCT), fever clearance times (FCT), parasitemia, amount of WBC, RBC, Hb, Hct in the patient population were analyzed by using Rho–Spearman correlation analysis. The α level was set at <0.05. All statistic analysis was performed by using the statistic package SPSS for window (SPSS, Chicago, IL, USA).

3. Results

3.1. General description of the patients

The general descriptions and clinical manifestations are summarized in Table 1. There was no difference between the malaria patient groups with regard to sex, age (range 14–54 years old), WBC, and RBC. S group showed significant lower levels of Hct ($p=0.011$), Hb ($p=0.032$), and higher parasitemia ($p=0.000$). In addition, S cases had longer parasite clearance time ($p=0.045$), and a trend of longer fever clearance time ($p=0.101$) in comparing to UC group (15/64, and 24/64 cases, respectively). A total of 25 patients in S group had symptoms associated with complications including cerebral malaria 3.8% (2/25), schizotemia 23% (12/25), anemia 13.5% (7/25), acute renal injury 5.8% (3/25), jaundice 32.7% (17/25), and hyperparasitemia 21.1% (11/25).

3.2. CD23 expression and level of sCD23 in plasma

To elucidate if and how CD23 expression varied in the different study groups, the numbers of CD14⁺CD23⁺ cells were estimated by flow cytometry. The mean fluorescent intensity (MFI, indicative of numbers of molecules/cell) was compared among the study groups at admission. There was a trend of higher levels of CD23 in the S group (6572 molecules/cell) compared to the UC group (4959 molecules/cell) (Table 2, Fig. 1A). In contrast the expression was significant higher in the C groups (5995 molecules/cell) than that in the UC ($p=0.05$).

During one month of admission, the levels of CD23 in the S group were highest on Day (D) 2 of admission (8154 molecules/cell) and significantly increased compared to that seen on D0 ($p=0.017$), D14 ($p=0.014$), and D21 ($p=0.041$). On D28 the amount of sCD23 was

Table 1
General descriptions and clinical manifestations of study population.

Variables	Normal (n = 34)	UC (n = 64)	S (n = 25)
Age (years)	30 (15–50)	21 (14–54)	22 (15–53)
WBC ($\times 10^9/L$)	7.0 (5.1–10.5)	5.7 (2.4–13.1)	5.2 (2.6–12.0)
RBC ($\times 10^{12}/L$)	5.0 (4.8–6.9)	4.7 (2.9–6.2)	4.2 (3.1–5.7)
Hct (%)	44 (40.0–50.7)	38.1 (25.0–49.0)	35 (4.1–46.0)
Hb (g/dL)	14.4 (12.8–16.5)	12.6 (7.5–15.9)	11.8 (7.6–15.6)
Parasitemia (parasite/ μL)	0	15,660 (77.5–185,600)	290,880 (36,820–1,310,400)
PCT (h)	0	46 (17–67)*	57 (31–94)
FCT (h)	0	32 (4–98)**	56 (20–151)

Data is shown as median, (range lower, upper limits).

WBC—total number of leukocyte count. Normal value = $5.0\text{--}10 \times 10^9/L$.

RBC—total number of erythrocyte count. In male = $4.5\text{--}6.0 \times 10^{12}/L$, in female = $4.0\text{--}5.5 \times 10^{12}/L$.

Hb—hemoglobin, in male = 14–18 g/dL, in female = 12–16 g/dL.

Hct—hematocrit, in male 40.0–54.0%, in female = 37.0–47.0%.

PCT—parasite clearance time, FCT—fever clearance time.

*, ** 15 of 64, and 24 of 64 UC cases had parasitemia and fever, respectively during follow up.

Table 2
Levels of plasma sCD23, total IgE, *P. falciparum*-specific IgE (Pf specific IgE), Pf specific IgG, IgE–IgG complexes and TNF- α on the day of admission (day 0) in the falciparum malaria patients and healthy donors (C) and expression of CD23 molecules.

Groups	CD23 (molecules/cell) ^a	sCD23 (ng/mL)	Total IgE (ng/mL)	Pf specific IgE (pg/mL)	Pf specific IgG ($\mu g/mL$)	IgE–IgG complex ($\mu g/mL$)	TNF- α (ng/mL)
C	5,995 (3110–12,226)	62.89 (38.16–269.10)	116.00 (24.53–2662.00)	395.20 (65.61–4210.00)	1.06 (0.33–3.86)	0.43 (0.18–0.80)	2.78 (1.20–14.32)
UC	4,959 (3528–7380)	128.00 (47.04–151.50)	4,465.00 (50.36–11,954.00)	580.35 (176.50–5861.00)	9.43 (0.44–212.30)	0.76 (0.25–3.20)	6.47 (1.16–29.82)
S	6,572 (3493–10,875)	184.90 (23.90–3114.00)	3,204.50 (31.92–16,751.00)	787.80 (30.90–7039.00)	5.08 (0.07–68.41)	0.59 (0.10–1.37)	4.16 (1.17–15.27)

The data is reported as median (range lower, upper limits). UC—uncomplicated falciparum malaria patients, S—severe falciparum malaria patients, C—normal control.

^a Units of molecule represent as full number.

to lowest while the CD23 molecules was significantly increased on D28 compared with D21 ($p = 0.037$) (Fig. 1 A and B, Supplementary Table S1).

On the admission day the levels of sCD23 for the S group (184.9 ng/mL) tended to be highest when compared with those seen in UC (128.0 ng/mL) and C groups (62.9 ng/mL) (Fig. 1B, Table 2). During the study period the amount of sCD23 for S group on D2 and D28 were 292.5 (range 28.61–953.60 ng/mL) and 55.02 (range 7.43–221.90 ng/mL), respectively (Fig. 1B, Supplementary Table 1).

3.3. Level of TNF- α in plasma

At admission the plasma TNF- α concentrations were significantly higher in the UC (6.5 ng/mL) and S (4.2 ng/mL) as compared to the C (2.8 ng/mL) groups ($p = 0.004$, $p = 0.035$, respectively) (Fig. 1C, Table 2). During the study time the levels of TNF- α were significantly changed and was significantly higher on D28 compared with D14 ($p = 0.040$) (Supplementary Table S1).

3.4. Total IgE responses

At admission the total plasma IgE levels in S (3204.5 ng/mL) and UC (4465.0 ng/mL) were significantly higher when compared to those in the C groups (116.0 ng/mL) ($p < 0.001$). The level in UC tends to be higher than S groups (Fig. 1D, Table 2).

3.5. Anti-*P. falciparum* IgE responses

The amount of anti-*P. falciparum* IgE tended to be higher in the S (787.8 pg/mL) than in the UC groups (580.4 pg/mL, $p > 0.05$). While the levels for both S ($p = 0.025$) and UC ($p = 0.011$) were significantly higher than C group (395.2 pg/mL) (Fig. 1E, Table 2).

3.6. Anti-*P. falciparum* IgG responses

At admission, the levels of anti-*P. falciparum* IgG was statistically significantly higher ($p < 0.001$) in UC group (9.4 $\mu g/mL$) than in S group (5.1 $\mu g/mL$). For both groups the values were statistically significantly higher than that seen in the C group (1.1 $\mu g/mL$) ($p < 0.001$) (Fig. 1F, Table 2).

3.7. Amount of IgE–IgG complexes in plasma

Amount of IgE–IgG complexes were $< 1 \mu g/mL$ in both the patients and control groups. The levels of IgE–IgG complexes in UC tended to be higher than those seen in S groups. The amount of IgE–IgG complexes for UC ($p = 0.002$) and S ($p = 0.023$) was significantly higher than those in C groups (Fig. 1G, Table 2).

3.8. Correlation between CD23, total IgE, anti-*P. falciparum* specific IgE, anti-*P. falciparum* specific IgG, IgE–IgG complexes, TNF- α , sCD23 and clinical manifestation

All significant correlations between the parameters tested in S group only are summarized in Table 3. A positive correlation between the amount of CD23 and *P. falciparum* specific IgE ($r = 0.245$, $p = 0.050$) was seen. A significant positive correlation between *P. falciparum* specific IgE and sCD23 was also seen ($r = 0.597$, $p = 0.000$). There was strong negative correlation between TNF- α and the IgE–IgG complexes at admission ($r = -0.743$, $p = 0.035$). A negative strong correlation between sCD23 and CD23 was also seen ($r = -0.762$, $p = 0.028$). There were moderate negative significant correlations between the levels of plasma TNF- α and clinical manifestations including parasitemia ($r = -0.356$, $p = 0.042$), PCT ($r = -0.361$, $p = 0.039$), and FCT ($r = -0.388$, $p = 0.05$).

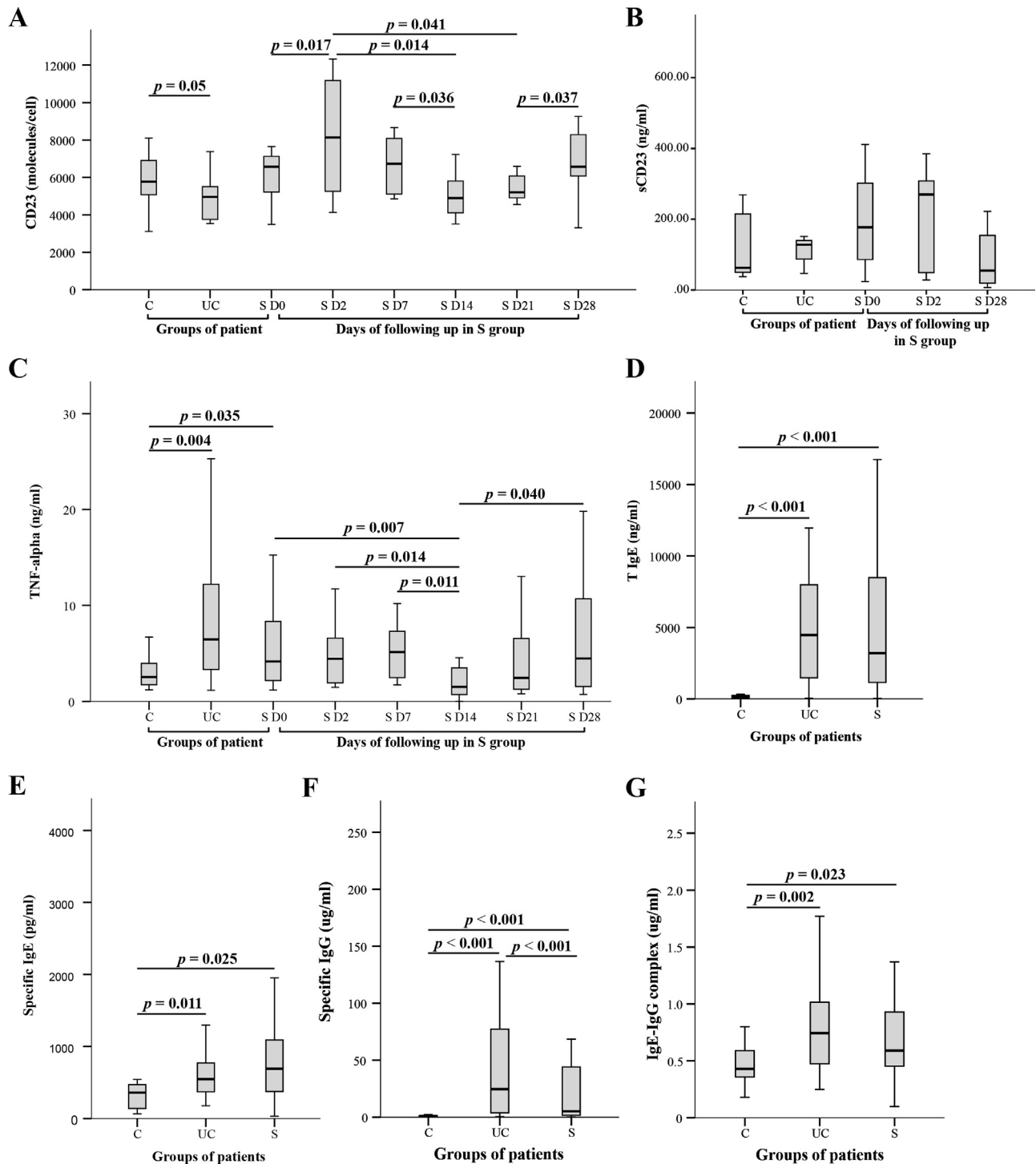


Fig. 1. Expression of CD23 molecules (A); levels of plasma sCD23 (B); TNF- α (C); Pf specific IgE (D); total IgE (E); Pf specific IgG (F); and ratio of specific IgE/IgG (G) in the uncomplicated (UC) on the day of admission (D0) and severe (S) falciparum malaria patients during a month of admission (D0, D7, D14, D21 and D28), and healthy donors (C). Data are illustrated as whisker plots, with boxes denoting the IOR and whiskers the min/max values.

4. Discussion

This study was the first to explore the association between plasma levels of *P. falciparum*-specific IgE, *P. falciparum*-specific IgG, IgE-IgG complexes, sCD23 and TNF- α , and CD23 expression on activated monocyte and severity of disease in falciparum malaria patients.

Based on our findings, specific IgE, sCD23, and CD23 expression tended to be higher in the S cases. The significantly higher

specific IgG and a trend of higher TNF- α levels were observed in the UC patients. Taken together, TNF- α and the specific IgG probably play protective roles, while specific IgE and CD23 levels are related to severity of falciparum malaria. In addition, significant moderate correlations between amounts of specific IgE, CD23, sCD23 and TNF- α were found in the S cases during admission. These may contribute in as much as partial TNF- α production in the present study was induced via *P. falciparum*-specific IgE in the form of immune complexes cross linked by CD23 on activated monocytes.

Table 3

Significant Rho-spearman correlations among expression of CD23 molecules, plasma sCD23, total IgE, *P. falciparum*-specific IgE (Pf specific IgE), Pf specific IgG, IgE–IgG complexes and TNF- α , and clinical manifestations in S group.

Correlation between variables		r	p-Value
Pf specific IgE	CD23	0.245	0.050
Pf specific IgE	sCD23 D0	0.597	0.000
Pf specific IgE	TNF- α	0.575	0.010
Pf specific IgG	sCD23 D0/D2	−0.762/−0.786	0.028/0.036
IgE–IgG complexes	TNF- α D2/D7/D14	−0.715/−0.615/−0.524	0.002/0.025/0.05
CD23 D0	sCD23 D0/D2	−0.743/−0.986	0.035/0.000
TNF- α D2	Parasitemia	−0.356	0.042
TNF- α D2	PCT	−0.361	0.039
TNF- α D2	FCT	−0.388	0.05

PCT—parasite clearance time, FCT—fever clearance time.

In the present study, the total IgE levels in UC tended to be higher than in the S groups while the levels of *P. falciparum*-specific IgE in the S patients were higher than those within the UC and C groups. This finding is in line with previous reports in humans (Desowitz et al., 1993; Maeno et al., 2000; Perlmann et al., 1997, 2000; Seka-Seka et al., 2004). Moreover, levels of *P. falciparum*-specific IgG were significantly higher in the UC patients. This might indicate a protective role for this IgG subclass and would be consistent with a previous study (Perlmann et al., 2000).

In malaria and other diseases, density of CD23 molecules have been shown to increase on activated monocytes and be associated with TNF- α production (Gosset et al., 1999; Nacher, 2004; Poole et al., 2005; Vouldoukis et al., 2011, 1995). Similarly, in our study there was a trend of higher CD23 levels for the S group than in the UC group, and significantly higher in the C than in the UC group. A small peak of CD23 expression was observed on D2 of admission in S group.

The release of CD23 from the cell surface as sCD23 is mediated by a metalloprotease (Lemieux et al., 2007; Marolewski et al., 1998). In this study, the plasma sCD23 levels tended to be higher in the S group compared with those seen in the UC and C groups. Previous studies suggested that the sCD23 level is inversely related to the density of CD23 on cell surface. (Lemieux et al., 2007; Massa et al., 1998).

Correspondingly, the S patients had strong negative correlation between the amount of sCD23 and CD23 expression ($r = -0.762$, $p = 0.028$). This finding might suggest that the S group had released their membrane-bound CD23. Our result is thus consistent with previous studies of other diseases. The amount of sCD23 is recognized as an important indicator of disease progression (Schwarzmeier et al., 2005) and correlates with disease severity (Bossolasco et al., 2001; Massa et al., 1998; Matsumoto et al., 1998; Mossalayi et al., 2009; Rujeni et al., 2013; Vouldoukis et al., 2011) probably by triggering the release of inflammatory mediators e.g., TNF- α , IL-1 β , GM-CSF, IL-8, IL-10 and PGE₂ (Armant et al., 1995; Vouldoukis et al., 2011). In agreement, we observed a marked decrease of sCD23 on D28 after admission when the S patients retrieved.

sCD23 alone is a potent inducer of TNF- α in the absence of any accessory signals. sCD23 induces TNF- α production via cell–cell contact, in an LPS-independent pathway (Armant et al., 1995). In the present study, the high level of sCD23 seen in the UC group on D0 is consistent with high levels of TNF- α production (Armant et al., 1995). In contrast, the S group (that had the highest sCD23 level) showed lower levels of TNF- α compared to the UC group. It is hypothesized that excess sCD23 levels in the S group may compete with circulating IgE cross linking with the surface CD23 of monocytes leading to decrease in TNF- α production.

The amount of CD23 expression, sCD23 and TNF- α in the S group during admission are shown in Fig. 1. This might indicate a role for sCD23 in our study. A marked decrease in the sCD23 level was

observed from D2 to D28 of the study. While the expression of CD23 molecules was significantly increased during the last week of the study. We suggest that the lower amount of sCD23 during late admission is not effective enough to interfere with the crosslinking of specific IgE and bound CD23 on monocytes. Consequently, the level of TNF- α was significantly increased on D28 compared to D14.

TNF- α is a cell signaling protein involved in systemic inflammation and is one of cytokines that make up the acute phase reaction. It is produced mainly by activated monocytes and macrophages but can also by other cell types such as CD4⁺ T cells, NK cells, neutrophils, mast cells, eosinophils and neurons (Locksley et al., 2001).

In malaria infection, a variety of malaria antigens (Ags) have been shown to induce TNF- α production, including the ring-infected erythrocyte surface Ag (RESA), the soluble antigen complex (Ag7), the merozoite surface protein (MSP)-1 and MSP-2, a glycosylphosphatidylinositol (GPI)-like structure (Kwiatkowski, 1995). In addition, sonicated normal RBC lysates also share a number of biochemical properties similar to TNF- α -inducing factors although they are much less abundant than toxins released by late stage parasitized RBCs (Kwiatkowski, 1995). Similarly, our Supplementary experiment demonstrated that TNF- α could be induced by various factors (Supplementary Fig. S1). These findings may support our suggestion that the amounts of TNF- α detected in the patient sera or plasma are the overall TNF- α production induced by various different mechanisms and pathways.

In contrast to most previous studies of TNF- α in falciparum malaria (Clark et al., 2006; Ghanchi et al., 2015; Kinra and Dutta, 2013; Perera et al., 2013; Sahu et al., 2013) and vivax malaria (Raza et al., 2013, 2014), we found that the TNF- α levels were lower in the S group compared to the UC group. The possible reason for this discrepancy could be that the S patients had higher levels of sCD23 which could bind circulating IgE and thereby prevent the cross linking of the surface CD23 on the monocyte (Daniels et al., 2005). This would then lead to a decrease in TNF- α production. During admission a trend of decreased sCD23 levels for the S group on D28 was noted. At day 28 there was significant increase of TNF- α level, which might support our suggestion about the interference of sCD23 (Fig. 1B and C, Supplementary Table S1).

TNF- α not only acts as a pathogenic agent. The lower TNF- α level in the S group found in our study was in agreement with those reported in previous studies of malaria (Brickley et al., 2015; Silverio Ade et al., 2013; Tangteerawatana et al., 2014). A recent malaria trial vaccine demonstrated that protective immunity is associated with TNF- α producing effector and central memory CD4⁺ T cells (Lumsden et al., 2012). Moreover, another study reported an inhibitory effect of TNF- α on malaria pre-erythrocytic stage development (Depinay et al., 2011). A previous study in Gabonese children found that the ability of *P. falciparum*-specific T cells to produce IL-2 and TNF- α was associated with resistance

to reinfection and thus of significance in preventing severe disease (Ramharter et al., 2004). In addition, the protective effects of TNF- α have been reported in other infectious disease e.g., typhoid (Dunstan et al., 2007; House et al., 2002), influenza A (H1N1) (Xia et al., 2014), spinal tuberculosis (Chen et al., 2014), human Chagas's disease cardiomyopathy (Drigo et al., 2006), Vietnamese patients with typhoid (Dunstan et al., 2007), pathogenic *Mycobacterium* infection (Chen et al., 2014; Roach and Schorey, 2002), influenza vaccination (Xia et al., 2014), cryptococcosis (Liu et al., 2014), mice infected with *Leishmania major* (Wilhelm et al., 2005) or with recombinant Rabies virus infection (Faber et al., 2005).

In this study, the significant moderate negative correlations between TNF- α on D2 and parasite clearance time (PCT), fever clearance time (FCT), or parasitemia suggest a protective role of TNF- α . It seems that TNF- α does not act alone, but as part of an independent synergizing network of other mediators, such as platelet-activating factor, prostaglandin and NO that actually cause illness, biochemical change and tissue damage (reviewed in Clark et al., 2006).

There was a strong negative correlation between IgE–IgG complexes and TNF- α . This finding was interpreted to suggest that large amounts of IgE–IgG complexes probably interfere with the pathway leading to TNF- α secretion. IgE–IgG complex levels were $<1 \mu\text{g/mL}$ in both the patients and control groups. Based on our findings, we could not clarify the effects of IgE–IgG complexes in the patients. Previous studies have reported associations between the complexes and cerebral malaria (Hebert et al., 1991; Mibei et al., 2008). Immune complexes are cleared from the circulation when free Fc fragments bind to the Fc receptor on mononuclear phagocytes in liver and spleen (Hebert et al., 1991).

Moreover, our results from the in vitro experiment (in Supplementary document, Fig. S1) could strengthen our hypothesis in the present study. Normal PBMC were cultured with chimeric specific IgE instead of specific IgE purified from the patients. The specific IgE (20 ng/mL)–lysate *P. falciparum* Ag complexes induced PBMC to produce TNF- α similar to those seen in PBMC activated with the lysate Ag alone (5 $\mu\text{g/mL}$). PBMC cultured with low dose IgE-complexes induced higher level of TNF- α production than in those incubated with malaria specific IgE alone, or with intact different stages such as ring form, trophozoites, and schizonts of infected RBC (iRBC). In this additional experiment, we assumed that the high and low doses of chimeric IgE represent the specific IgE levels seen in sera or plasma from the S and the UC patients, respectively. Based on these supplementary results, we might explain that the appropriate amount of specific IgE forming complexes is another regulatory factor involved in the TNF- α induction. Moreover, the findings might contribute to our observation that the amount of TNF- α produced in the UC patients is higher than that seen in S cases.

In addition, the moderate positive correlation between TNF- α production (at D0) and anti-*P. falciparum*- specific IgE was statistically significant. It is possible that TNF- α production is induced by various factors from both parasite and host immune cells (Clark et al., 2006; Kwiatkowski, 1995).

In our study no strong correlation between the amount of CD23 expression, IgE and TNF- α levels and severity of the disease was seen. In agreement, the sCD23 showed no association with total IgE observed in *Schistosoma haematobium* infected patients (Rujeni et al., 2013). In contrast, the enhancement of severity in Parkinson's disease is a consequence of CD23 activation leading to TNF- α production in glial cells (Hunot et al., 1999). Gosset et al. demonstrated IgE-dependent upregulation of CD23 on alveolar macrophages, which in turn induces production of chemokines and proinflammatory cytokines in allergic asthma and airway inflammation (Gosset et al., 1999). The association between CD23 expression and TNF- α and severity is controversial. Our study has some limitations, which include the following: (1) too small a sample size. Some

variables among our groups showed only trends of difference ($p \geq 0.05$). In this study, we focused on the roles of *P. falciparum*-specific IgE. Therefore, we had to exclude volunteers and patients with intestinal parasite infections, which are a major inducers of IgE production (reviewed in Nacher, 2004). Patients with parasitic infections have increased levels of total and parasitic specific IgE, which might have interfered with the availability of *P. falciparum*-specific IgE and CD23 crosslinking in our study. Other limitations include: (2) samples in the UC group did not completely match with those in the S group; (3) we lacked data on sCD23 levels during D14 and 21 and therefore could not follow the complete interplay of CD23 expression on activated monocytes and sCD23 in plasma; (4) we did not focus our study on the mechanisms and pathways involved in TNF- α production via CD23 crosslinking with *P. falciparum* Ag. However, our findings do provide preliminary information for future in-depth studies to clarify our hypothesis. In addition, this knowledge may be beneficial in reducing the severity of falciparum malaria.

Taken together our data show a complex interplay between CD23 molecules, malaria specific Ig and proinflammatory cytokine TNF- α . CD23 expression and malaria-specific IgE levels may be involved in the severity of the disease while TNF- α and the malaria-specific IgG may correlate with protection against falciparum malaria. To firmly establish these findings and gain an improved understanding of the pathogenesis of falciparum malaria, future sample sizes should be increased, and other related cytokines and mediators, e.g., IL-4, IL-10, IFN- γ , and nitric oxide, should be investigated.

Acknowledgements

We thank the patients, staff and Director at the Hospital for Tropical Diseases, Faculty of Tropical Medicine. We are also appreciated to Ms. Wilanee Dechkachorn for her helpful manuscript preparation. We appreciate Professor Peter Perlmann and Hedvig Perlmann for their suggestion. We also thank staff at Mabtech AB for a part of chimeric IgE preparation. This study was supported financially by the Faculty of Tropical Medicine, Mahidol University, SEAMEO-TROPED network (for RK), and The Swedish Institute, year 2000 and 2001 (for YM).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.actatropica.2015.10.017>.

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