COMPLICATED MALARIA IS ASSOCIATED WITH DIFFERENTIAL ELEVATIONS IN SERUM LEVELS OF INTERLEUKINS 10, 12, AND 15 *

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Abstract. Complicated malaria, caused by *Plasmodium falciparum*, is characterized by multiple organ dysfunction. The pathogenesis of complicated malaria involves complex host-parasite interactions that include polarized cytokine responses. Recently, correlates between Th1-like and Th2-like cytokines, especially interleukin-10 (IL), IL-12, and TNF–α, and specific types of organ dysfunction have been noted. Here, we measured IL-10, IL-12, and for the first time, IL-15, in 19 patients aged 16-55 years old with complicated malaria on days 0 (admission), 3, 7, and 14. For analysis, patients were grouped together or sub-categorized into hyperparasitemias or cerebral malaria (CM). For IL-10, a dramatic increase was noted on admission, followed by a reduction toward control values that closely paralleled parasite clearance. For IL-12, modest but persistent increases were noted over the entire 14 day period that did not correlate with parasitemia. In general, especially on days 0 and 3, hyperparasitemic patients had, in comparison with CM patients, higher IL-10 and IL-12 levels. In contrast, IL-15 was generally below detection in most samples. These results provide further insight into the pathogenesis of complicated malaria by strengthening the contention that cytokines such as IL-10 and IL-12 are involved in modulating the immune response to *P. falciparum*.

INTRODUCTION

Complicated or severe malaria, caused by *Plasmodium falciparum*, is characterized by multiple organ dysfunction, to include cerebral malaria (CM) (Warrell *et al*, 1990). Complicated malaria occurs most commonly in African children, causing up to 1.5 million deaths annually (Olliaro *et al*, 1996). The pathogenesis of complicated malaria involves cytoadherent parasitised red cells within the microvasculature, adherent normal red cells (rosetting), and massive cytokine release in response to toxic substances released by *P. falciparum* that may mediate injury (Pichyangkul *et al*, 1994; 1997a,b; Wilairatana *et al*, 1997).

Recent reports show that the ratio of some cytokines may differentiate complicated from uncomplicated malaria, and for complicated malaria, may correlate with certain clinical signs (Ho *et al*, 1998; Kurtzhals *et al*, 1998; Mohan and Stevenson, 1998; Othoro *et al*, 1999; Seder and Gazzinelli, 1999; Winkler *et al*, 1999). Indeed, the balance between Th1-like cytokines such as tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) and Th2- like cytokines such as interleukins (IL) 4 and 10 may determine the severity of falciparum malaria. For example, that IL-10:TNF-α ratios are significantly lower in children with hyperparasitemias associated with anemia suggests that higher IL-10 production reduces anemia by dampening the inflammatory effects of TNF-α (Deloron *et al*, 1994; Kurtzhals *et al*, 1998; Othoro *et al*, 1999). Indeed, *in vitro* experiments using cells from malaria patients show that IL-10 inhibits the production of the IL-1 family cytokines (TNF-α, IL-1, IL-6) (Ho *et al*, 1995, 1998; Peyron *et al*, 1994; Wenisch *et al*, 1995).

Patients with *P. falciparum* malaria are deficient in the capacity to produce IL-2, but other cytokines such as IL-12 and TNF-α are generally increased (Ho and Sexton, 1995; Ho *et al*, 1988). IL-12 has been shown to prevent or hasten clearance of parasites and may induce some degree of

* The opinions or assertions contained herein are the private views of the authors (PG, DSW) and are not to be construed as official or as reflecting the views of the US Department of the Army or the Department of Defense.

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immunity; however, IL-12 may also be harmful in some types of malaria infections (Mohan *et al*, 1999; Mohan and Stevenson, 1998; Yoshimoto *et al*, 1998a,b). IL-15, a potent pro-inflammatory T cell activator that increases the production of IL-10 and IL-12 and thus may be involved in regulating the immune response to *P. falciparum*, has not been assessed *in vivo* (Elloso *et al*, 1998; Korholz *et al*, 1997).

Here we measured serial serum levels of IL-10, IL-12, and for the first time, IL-15, in adult patients with complicated falciparum malaria. IL-10 was dramatically elevated during the acute phase of the infection, followed by a return toward healthy control values that correlated with parasite clearance. In contrast, IL-12 was persistently increased with no correlation to parasitemia and IL-15 levels were generally within or near control ranges. These data provide further insight into the immunopathogenesis of complicated malaria and strengthen the notion that IL-10 and IL-12 may function as immune modulators in *P. falciparum* infections.

MATERIALS AND METHODS

Serum samples were obtained from consenting (to include third party) male and female adult subjects aged 16-55 years old enrolled in an arteether treatment study for complicated malaria at the Bangkok Hospital for Tropical Diseases. The study was approved by the Institutional Review Board, Mahidol University. Complicated malaria, as described previously (Warrell *et al*, 1990), included the presence of asexual forms of *P. falciparum* on thick and thin smear blood films accompanied by cerebral malaria (unarousable coma), hyperparasitemia (>200,000 parasites/μl), spontaneous bleeding, convulsions, hyperpyrexia, severe anemia (hematocrit <15%), renal failure (serum creatinine >265 μmol/l) or clinically significant hypoglycemia. Thick blood films were examined every 12 hours for malaria parasites until negative, and then once daily until discharge. Parasite counts per ml were determined by counting the number of asexual parasites per 200 white blood cells in thick films or 1,000 red blood cells in thin films against total white cell count and red cell count per μl, respectively. Blood films were considered negative if no parasites were seen in 200 oil immersion fields in a thick blood film. For cytokine analysis, serum samples were collected prior to treatment on the day of admission (day 0) and on days 3, 7 and 14. Samples were stored at –70°C until analysis.

Assays for IL-10, IL-12 and IL-15

Serum levels of IL-10, IL-12 and IL-15 were measured using commercially available enzyme linked immunosorbent assays (ELISA) kits obtained from Genzyme Diagnostics (Cambridge, MA). All assays were conducted in triplicate. Results are expressed as means (± SEM) unless otherwise noted. Cytokine levels among patients and controls were compared using the student's *t*-test or the Mann-Whitney U test. P values ≤0.05 were considered significant. The limit of detection for each cytokine ELISA was 5 pg/ml for IL-10, 10 pg/ml for IL-12, and 10 pg/ml for IL-15. For analysis, values below the limit of detection were considered zero.

RESULTS

Serial serum samples were obtained from 19 patients with complicated malaria. Patient demographics, including parasitemias, are shown in Table 1. Enrollment consisted of 3 females and 16 males. Mean parasite clearance time was approximately 72 hours after the start of treatment and none of the patients failed to clear their parasitemia within 5 days of treatment.

Circulating levels of IL-10, IL-12 and IL-15 are shown Figs 1-5. Fig 1A shows that IL-10 was dramatically elevated on day 0 and then returned to baseline by day 7. Fig 1B shows that on admission, hyperparasitemic patients had, in comparison with CM patients, generally higher IL-10 levels. There was no difference by day 3. Fig 2 shows that levels of IL-12 remained modestly elevated for the entire study period. Similar to IL-10, hyperparasitemic patients had, in comparison with CM patients, generally higher IL-12 levels, especially on the day of admission (Fig 2B). Fig 3 shows that mean levels of IL-15 were generally not elevated in comparison with healthy controls. On day 7, there were a significant number of samples that were above the level of detection (10 pg/ml; Fig 4). Fig 5 shows the relationship between IL-10, IL-12 and IL-15 and parasitemia. A correlation between IL-10 levels and parasitemia was observed.

DISCUSSION

In this study, we found elevated levels of IL-10 and IL-12 in patients with complicated malaria whereas levels of IL-15 generally remained within or near healthy control ranges. IL-10 was particularly elevated early on days 0 and 3, and then de-

Table1
Patient demographics and clinical characteristics.

Pt	Age	Sex	Admission parasitemia (parasites/μl)	Clinical signs*
1	19	M	502,200	Hyp, J, OT↑, PT↑, ANE (3)
2	23	M	51,380	CM, J, ANE (1), OT↑, PT↑
3	20	M	1,018,500	Hyp
4	20	M	951,490	Hyp, J, OT↑, ANE (2)
5	29	M	393,600	Hyp
6	25	M	1,078,440	Hyp, J, OT↑, AZO, ANE (4)
7	20	M	369,200	Hyp
8	33	M	106,950	CM, J, ANE (2), ARF, Cr > 3
9	17	M	399,600	Hyp
10	16	F	213,840	Hyp, J, OT↑, PT↑
11	28	M	441,560	Hyp, J, OT↑, PT↑, ARF, ANE (2)
12	19	F	524,400	Hyp
13	39	M	38,400	CM, Cr > 3 (no HD)
14	29	M	14,640	CM, ANE (2)
15	36	M	522,460	Hyp
16	55	M	147,000	CM, Cr = 3.9, J, ANE (3)
17	22	M	49,400	CM, ET, OT↑
18	20	M	961,350	Hyp, J, ANE (2)
19	23	F	166,600	CM, J, OT↑

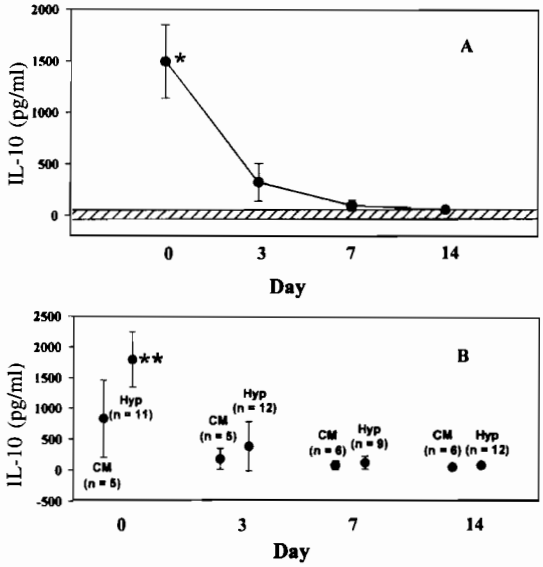
Means
Age: 25.9 ± 2.2 years old
Parasitemia: Cerebral malaria 82,053 ± 22, 062
Hyperparasitemia 614,720 ± 86,476
($p < 0.001$)

* Abbreviations
Hyp = Hyperparasitemia; CM = Cerebral malaria; J = Jaundice; AZO = Elevated creatinine or BUN; OT↑ = ≥ 2x upper limit of normal; PT↑= ≥ 2x upper limit of normal; ET = endotracheal tube with respirator; ARF = Acute renal failure (hemodialysis; HD); ANE (u) = Anemia (units of blood administered)

creased in parallel with parasite clearance. In contrast, increased IL-12 levels were noted throughout the study period but did not correlate with parasitemia. These observations are in agreement with previous reports indicating that the immune response in falciparum malaria is characterized by polarized Th1-like versus Th2-like cytokine responses whereby the acute, untreated phase is associated with a strong Th2-like response (increased IL-10:IL-12 ratio), followed by a shift toward a Th1-like predominant response (reduced IL-10:IL-12 ratio) as parasite clearance proceeds (Winkler *et al*, 1998, 1999). The role of IL-15 in falciparum malaria remains unclear.

IL-10, an anti-inflammatory cytokine secreted by Th2-like lymphocytes, suppresses immune responsiveness by downregulating some Th1-like cytokines, in particular TNF-α and IL-12 (van der Poll *et al*, 1994). In malaria, increased IL-10 levels may reduce parasite killing and prolong blood-stage infection in some animal models, but also beneficially dampens the effects of the pro-inflammatory IL-1 family of cytokines (especially TNF-α) and alters macrophage and T helper cell function (Ho *et al*, 1998; Peyron *et al*, 1994; Wenisch *et al*, 1995). Indeed, in pediatric patients, the IL-10:TNF-α ratio is highly correlative with the development of malaria anemia (Kurtzhals *et al*, 1998; Othoro *et al*, 1999), and for IL-10, levels generally correlate with parasitemia (Kurtzhals *et al*, 1998).

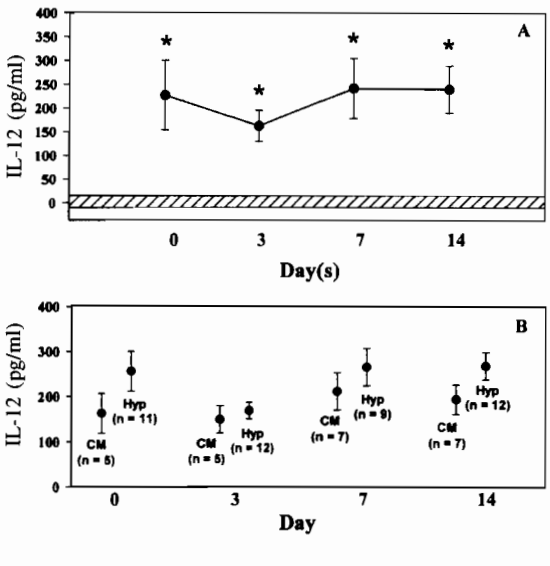
IL-12 (also called natural killer cell stimulatory factor) is produced by activated monocytes and macrophages. In the absence of IL-10, the production of IL-12 is increased and more macrophages are activated (Gazzinelli, 1996). Here, elevated IL-12 levels indicated that a specific (adaptive) immune response was probably triggered early (Crutcher



Healthy control; CM = Cerebral malaria; Hyp = Hyperparasitaemia

Fig 1–Serial concentrations of serum IL-10 in complicated malaria patients. (●) represents mean (±SEM) for patients and hatched bar represents 95% confidence interval for control values. (A) represents all patients and (B) is a comparison of IL-10 among cerebral malaria and hyperparasitemic patients.

* p=0.003 versus healthy controls; ** p=0.007, hyperparasitemia versus cerebral malaria, Mann Whitney Rank Sum Test.



Healthy control; CM = Cerebral malaria; Hyp = Hyperparasitaemia

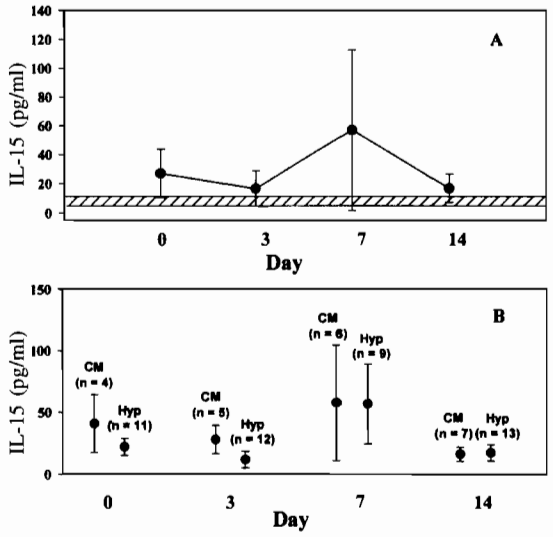
Fig 2–Serial concentrations of serum IL-12 in complicated malaria patients. (●) represents mean (±SEM) for patients and hatched bar represents 95% confidence interval for control values. (A) represents all patients and (B) is a comparison of IL-10 among cerebral malaria and hyperparasitemic patients.

* p=<0.001 versus healthy controls; Mann Whitney Rank Sum Test.

et al, 1995; Yoshimoto *et al*, 1998b). In general, most of the patients were experiencing their first malaria attack, reducing the possibility that IL-12 was elevated prior to admission. In some models, IL-12, together with IFN-γ, enhances parasitemia clearance and induces protective immunity (Mohan and Stevenson, 1998; van der Poll *et al*, 1994; Yoshimoto *et al*, 1998b). This may be related to the persistence of IL-12 beyond the period of parasite clearance. Interestingly, however, IL-10 may inhibit IFN-γ production in blood mononuclear cells by suppressing IL-12 production, suggesting one mechanism for an early Th2-like response (D'Andrea *et al*, 1993).

Because IL-15 triggers monocytes to release IL-12 and natural killer cells to release IFN-γ (both elevated in malaria infections), the lack of detectable IL-15 in most samples was unexpected. In addition, IL-10 is thought to play a role in modulating IL-15 mediated T cell responses and IL-12 together with IL-15 are thought to be necessary to induce IFN-γ production, deemed essential for the development of CM (Ho and Sexton, 1995; Korholz *et al*, 1997). Although there is no structural homology with IL-2, the biological effects of IL-15 and IL-2 are similar in many respects and both bind to the same sets of cell receptors (Elloso *et al*, 1998). In general, however, IL-15 must be present in larger concentrations to elicit the same response (Korholz *et al*, 1997). Presuming that our detection method was sensitive enough to measure biologically important levels, our data suggest that *P. falciparum* infections may not necessarily be associated with the release of clinically significant amounts of IL-15 or alternatively, may inhibit the production of IL-15 in a similar manner to IL-2. Indeed, other substances such as parasite toxins may also trigger IL-12 (Pichyangkul *et al*, 1994, 1997a).

In conclusion, our data confirm that IL-10, IL-12, and IL-15 are differentially elevated in complicated malaria patients. The ratio of IL-10 to IL-12, higher during the untreated phase, gradually is reduced as parasitemias clear. Further work to understand the divergent kinetics, biological significance, and relationship to parasitemia, especially for IL-10 and IL-12 is warranted.



Healthy control; CM = Cerebral malaria; Hyp = Hyperparasitaemia

Fig 3–Serial concentrations of serum IL-15 in complicated malaria patients. (●) represents mean (±SEM) for patients and hatched bar represents 95% confidence interval for control values. (A) represents all patients and (B) is a comparison of IL-10 among cerebral malaria and hyperparasitemic patients.

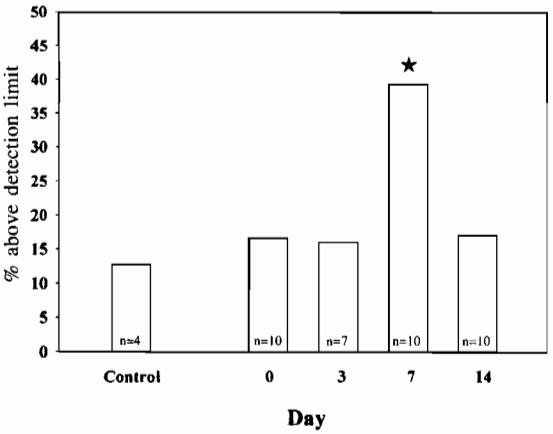


Fig 4–The proportion of serum samples wiht levels of IL-15 above the detection limit of the ELISA assay (10 pg/ml) among complicated malaria patients and healthy controls.

* $p \leq 0.05$ versus controls, Mann Whitney Rank Sum Test.

ACKNOWLEDGEMENTS

We thank Prapatsorn Ngaowichit for administrative assistance.

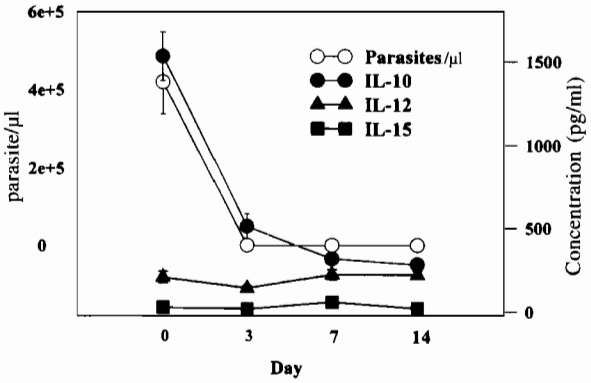


Fig 5–Serial comparison of serum IL-10, IL-12 and IL-15 levels with parasitemia.

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