Plasma Levels of the Interleukin-6 Cytokine Family in Persons with Severe
Plasmodium falciparum Malaria

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Plasma levels of interleukin (IL)-6, soluble IL-6 receptor, soluble gp130, leukemia inhibitory
factor (LIF), and ciliary neutrophic factor (CNTF) were analyzed in 32 patients with severe
malaria. Ten had renal failure, 8 had cerebral malaria, and 14 had other causes of severity.
Before treatment, the IL-6 and soluble IL-6 receptor plasma levels were significantly higher
in persons with cerebral malaria or renal failure than in other groups (P < .01 for both). After
initiation of therapy, IL-6 levels dropped within 24 h, but soluble IL-6 receptor levels increased.
CNTF levels were significantly reduced in persons with cerebral malaria or renal failure but
normalized within 24 h. Plasma concentrations of gp130 and LIF did not differ between
the malaria groups or normal controls. Excessive levels of IL-6 could be controlled by a subsequent
shedding of the soluble IL-6 receptor, and low-level CNTF expression could contribute to or
even result from cerebral malaria or renal failure.

In severe malaria, cytokines appear to play a pivotal role in
the activation of the immune response, and their levels correlate
with disease severity [1]. Serum levels of interleukin (IL)-6 and
tumor necrosis factor (TNF) correlate with various indices of
disease severity [1]. IL-6 is a member of the IL-6 cytokine family,
which includes IL-6, leukemia inhibitory factor (LIF), on-
costatin M, ciliary neutrophic factor (CNTF), IL-11, and car-
diotrophin-1, which exhibits pleiotropy and redundancy in
biologic activities [2]. LIF has been implicated in the regulation
of acute-phase protein synthesis, bone resorption, neuronal and
myeloid cell differentiation, lipid metabolism, early embry-
genesis, and megakaryocytes [3]. In persons with septic
shock, serum levels of LIF correlate with disease severity and
mortality [4]. CNTF has activity as a survival and differenti-
ation factor for cells of the nervous system, and an increase in
CNTF expression is an early marker of neural injury [5, 6]. IL-
6, LIF, and CNTF possess a similar helical structure [7]. The
IL-6 receptor and other cytokine receptors are structurally sim-
ilar and constitute the cytokine receptor superfamily [7]. In
addition, the cytokine receptor subunit gp130 is shared among
the receptors for IL-6, LIF, CNTF, and other members of the
IL-6 family [8]. This sharing is consistent with functional re-
dundancy among the IL-6-related cytokine family.

In this study, we determined plasma concentrations of mem-
ers of the IL-6 cytokine family (IL-6, LIF, CNTF), the soluble
IL-6 receptor (sIL-6R), and the gp130 receptor subunit in 32
patients with severe Plasmodium falciparum malaria in Bang-
kok. The relation of these parameters to disease severity and
plasma levels of TNF and of the TNF receptor were
determined.

Methods

Patients and controls. We studied 32 patients who met World
Health Organization criteria for severe P. falciparum malaria. Ce-
bral malaria (CM) was defined as unrousable coma not attrib-
utable to any other cause in a patient with P. falciparum malaria.
Renal failure (RF) was defined as urine output of <400 mL/24 h
or serum creatinine >3.0 mg/dL [9]. All patients were of Thai origin
and from a population in which malaria is not endemic. Clinical
disease severity was estimated by APACHE (acute physiology
and chronic healthy evolution) II score. Local laboratory personnel
were the healthy controls (n = 16).

Antimalarial treatment. All patients received standard anti-
malarial treatment with artesunate (120 mg statim intravenously
and 60 mg every 12 h to 600 mg total). Parasite clearance times
were calculated from initiation of treatment until the first time that
peripheral blood films were negative for asexual parasites. Fever
clearance times were calculated from initiation of treatment until
normalization of body temperature.

Laboratory assessments. For routine patient care, parasite
counts were done 4 times a day (6-h intervals) until blood films
were negative on 3 consecutive examinations by Giemsa-stained
thick smears. Blood samples for the determination of cytokines
and receptors were obtained before and after initiation of treatment
at 6, 12, 24, 48, and 96 h after therapy. The blood specimens were
centrifuged, and EDTA-plasma was separated and immediately frozen at $-70^\circ$C. The samples were shipped to Austria on dry ice. Plasma levels of IL-6, IL-6 receptor, TNF, soluble TNF receptor (sTNFR; 55 kDa), LIF, CNTF, and gp130 were determined by ELISA (R&D Systems, Minneapolis). All assays were performed according to manufacturers’ instructions. In normal controls, the median (range) plasma levels were as follows: TNF, 4.4 (0.1–8.3) pg/mL; sTNFR (55 kDa), 1014 (678–1546) pg/mL; IL-6, 3.0 (1.1–6.4) pg/mL; IL-6 receptor, 11.4 (6.9–18.2) pg/mL; and gp130, 2.6 (1.3–4.5) ng/mL. In the 16 healthy subjects, LIF was measured in 4, and CNTF was measured in 13 (81%). The median plasma levels of CNTF and LIF in normal subjects were 5.7 (0–13) and 4.3 (3–7.2) pg/mL, respectively.

**Statistical analysis.** For comparisons of patient groups and healthy controls and serum levels at different time points, Wilcoxon rank sum tests and Kruskall-Wallis tests were used. For correlation analysis, we used Spearman’s test. All analyses were two-sided, and differences with $P < .01$ were considered significant.

**Results**

**Patients.** Of patients with malaria, 10 had RF, 8 had CM, and 15 had various types of severe malaria. Of the latter, 5 had hyperparasitemia, 4 had jaundice (bilirubin $>3.0$ mg/dL), 2 had hyperpyrexia (rectal temperature $>40^\circ$C), 2 had hypoglycemia (blood sugar $<40$ mg/dL), and 1 had circulatory collapse (systolic blood pressure $<70$ mm Hg). These patients (noncerebral, nonrenal malaria) were grouped together as representing “moderate severe malaria” (MSM). The APACHE II score was significantly higher in RF (median [range], 14 [3–21]) and CM (20

**Figure 1.** Median and ranges of plasma levels of IL-6, IL-6 receptor (IL-6R), tumor necrosis factor (TNF), and soluble TNF receptor (sTNFR) in patients with severe complicated (○, renal failure or cerebral malaria) and severe moderate malaria (■, other indices of severe malaria). In normal controls, median (range) plasma levels (pg/mL) were as follows: TNF, 4.4 (0.1–8.3); sTNFR (55 kDa), 1014 (678–1546); IL-6, 3.0 (1.1–6.4); and IL-6R, 11.4 (6.9–18.2). Median plasma levels of CNTF and LIF in normal subjects are shown in table 1.
positive LIF or in LIF plasma levels were seen in patients with RF, CM, and MSM and in normal controls (table 1). In addition, no differences in LIF detection or in LIF levels during and after therapy were seen for all groups (data not shown).

Discussion

We determined plasma levels of TNF-α, sTNFR, IL-6, sIL-6R, LIF, CNTF, and gp-130 in nonimmune patients with severe P. falciparum malaria to elucidate possible interactions and/or synergism of different members of the IL-6-cytokine family with indices of disease severity. As expected, plasma concentrations of TNF and sTNFR were significantly elevated in all 3 patient groups (CM, RF, MSM). The highest concentrations were in patients with CM or RF, which is similar to previous findings [1, 10].

IL-6 is also a sensitive marker of severity, the time course of raised concentrations perhaps being of greater significance than

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**Table 1.** Characteristics and plasma levels (median, range) of leukemia inhibitory factor (LIF) and ciliary neutrophic factor (CNTF) of 32 patients with severe *P. falciparum* infection.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Moderate severe malaria (n = 14)</th>
<th>Renal failure (n = 10)</th>
<th>Cerebral malaria (n = 8)</th>
<th>P* for RF/MSM</th>
<th>CM/MSM</th>
<th>RF/CM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>20 (16–45)</td>
<td>23 (16–60)</td>
<td>22 (16–60)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>6/4</td>
<td>4/4</td>
<td>9/5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>159 (151–169)</td>
<td>163 (150–173)</td>
<td>160 (150–170)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>49 (42–72)</td>
<td>50 (46–76)</td>
<td>50 (45–77)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days with fever before treatment</td>
<td>4 (2–10)</td>
<td>3 (2–7)</td>
<td>4 (2–7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous malaria attacks (no.)</td>
<td>0 (0–5)</td>
<td>0 (0–2)</td>
<td>0 (0–1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highest temperature before therapy (°C)</td>
<td>38.0 (37–40.5)</td>
<td>39.1 (36.5–40)</td>
<td>38.7 (36.5–40)</td>
<td>.918</td>
<td>.127</td>
<td>.232</td>
</tr>
<tr>
<td>Fever clearance time (h)</td>
<td>60 (10–194)</td>
<td>64 (18–148)</td>
<td>110 (50–264)</td>
<td>.046</td>
<td>.050</td>
<td>.978</td>
</tr>
<tr>
<td>Parasite clearance time (h)</td>
<td>51 (33–69)</td>
<td>76 (28–94)</td>
<td>71 (28–89)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>APACHE score</td>
<td>4 (0–11)</td>
<td>14 (3–21)</td>
<td>20 (14–23)</td>
<td>.006</td>
<td>.003</td>
<td>.794</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>37 (24–47)</td>
<td>33 (22–46)</td>
<td>33 (20–47)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White blood cells (g/L)</td>
<td>8.1 (4–17)</td>
<td>6.3 (4–12)</td>
<td>6.5 (5–20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets (g/L)</td>
<td>52 (13–142)</td>
<td>38 (12–106)</td>
<td>32 (12–62)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilirubin (μL)</td>
<td>2.3 (1–4.5)</td>
<td>2.9 (0.5–11)</td>
<td>3.8 (0.5–19)</td>
<td>.001</td>
<td>.001</td>
<td>.119</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.1 (0.9–2.0)</td>
<td>4.0 (2.6–5.8)</td>
<td>1.7 (1.3–6)</td>
<td>.001</td>
<td>.001</td>
<td>.706</td>
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<tr>
<td>Blood urea nitrogen (mg/dL)</td>
<td>20 (6–60)</td>
<td>84 (34–143)</td>
<td>52 (35–87)</td>
<td>.003</td>
<td>.023</td>
<td>.126</td>
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<tr>
<td>Initial mean parasitemia (μL)</td>
<td>127,500 (11,340–1,056,000)</td>
<td>225,280 (429–707,500)</td>
<td>157,740 (1134–1,017,600)</td>
<td></td>
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<tr>
<td>LIF (no. of positive detection)</td>
<td>4 (29%)</td>
<td>3 (30%)</td>
<td>2 (25%)</td>
<td></td>
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<td></td>
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<tr>
<td>LIF (plasma levels, pg/mL)</td>
<td>6.7 (3–13)</td>
<td>3.3 (2.4–6)</td>
<td>4.7 (3.3–6)</td>
<td>.260</td>
<td>.558</td>
<td>.825</td>
</tr>
<tr>
<td>CNTF (no. of positive detection)</td>
<td>11 (69%)</td>
<td>4 (40%)</td>
<td>4 (50%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNTF (plasma levels, pg/mL)</td>
<td>5.2 (0–11)</td>
<td>0 (0–4.7)</td>
<td>0.7 (0–5)</td>
<td>.009</td>
<td>.007</td>
<td>.612</td>
</tr>
</tbody>
</table>

NOTE. RF, renal failure; CM, cerebral malaria; MSM, moderate severe malaria.

*a* P < .05 for all comparisons.
a single value [1]. In addition, although TNF can initiate the synthesis of IL-6, experimental inhibition of TNF by pentoxifylline suggests that some IL-6 production can occur independently of TNF [11]. In the current study, the half life for both TNF and IL-6 plasma concentrations were similar (figure 1). However, in contrast to TNF, no differences in IL-6 concentrations were seen in the 3 patient groups before therapy.

Soluble forms of both the IL-6 receptor and gp130 can inhibit sIL-6R or gp130-mediated cytokine activities in vitro [2]. In our patients, levels of sIL-6R were significantly elevated in all patients before therapy and increased further after the decline in plasma IL-6 levels and clinical improvement. This suggests, similar to the relation of TNF and sTNFR [10], a naturally occurring control for the excessive release of IL-6. On the other hand, the correlation between serum levels of sIL-6R and parasite clearance time could underline the pivotal role for IL-6 in effective parasite clearance.

In severe malaria, serum levels of the gp130 receptor subunit were not different from those of normal subjects. Thus, it appears that this mechanism is not involved in the control of the effects of excessive IL-6 concentrations, possibly because sIL-6R can specifically bind to circulating IL-6, whereas the gp130 subunit is shared among other receptors [2, 3, 6–8]. LIF has been detected in several inflammatory conditions, including kidney allograft rejection, giant cell arteritis, and septicemia [4, 12]. In two studies of septicemia, LIF was detected in 11 of 31 patients [4] and in 8 of 90 patients [12]: the two studies used different ELISAs. Low levels of LIF were detected in 9 of 32 patients with severe malaria, but also in 4 of 16 control subjects in this study. The detection limit of our assay was 2 pg/mL, as opposed to 56 pg/mL in the assays used in the sepsis studies, which could explain the positive detection in healthy controls in our study. In septicemia, high LIF concentrations (100–37,000 pg/mL) were related to higher mortality and shorter survival [4]. In contrast, in malaria, an inverse correlation to indices of severity, such as parasite counts and thrombocytopenia, suggests a benefit. This difference could be related to the pleiotropic activity of this cytokine, including not only acute-phase protein synthesis, but also bone resorption, lipid metabolism, early embryogenesis, and megakaryopoiesis [3]. Since mice with endotoxic shock had no circulating LIF, but LIF mRNA was detectable in tissues by polymerase chain reaction [13], the difference in LIF detection between patients with septicemia and malaria remains to be elucidated.

CM results in multiple substantially unpredictable neuropsychiatric sequelae [14, 15]. CNTF has demonstrated activity as a survival and differentiation factor for cells of the nervous system [6]. In CM and in RF, CNTF levels were significantly lower than in persons with MSM or healthy subjects and normalized within 24–48 h. Normal CNTF expression could prevent neuron damage in malaria, and low CNTF expression could predispose for CM. However, since reduced CNTF levels were seen in both patients with CM and RF, its down-regulation may be systemic rather than organ-specific.

We conclude that the high expression of IL-6 could be controlled by a subsequent shedding of the sIL-6R but not the gp130 subunit, which is shared among other cytokines of the IL-6 family. In contrast to septicemia, plasma LIF levels were not affected by severe malaria. Whether low-level expression of CNTF in CM or RF affects disease outcome remains to be determined.

Acknowledgments

We thank Tan Chongsuphajasiddhi (Faculty of Tropical Medicine, Mahidol University) for support, the Hospital for Tropical Diseases staff for help, Eva Schönthal for laboratory assistance, and Wolfgang Graninger for manuscript preparation support.

References