Endogenous Interleukin-10 Modulates Proinflammatory Response in *Plasmodium falciparum* Malaria

May Ho, Tineke Schollaardt, Susan Snape, Sornchai Looareesuwan, P. Suntharasamai, and Nicholas J. White

Department of Microbiology & Infectious Diseases, University of Calgary, Calgary, Canada; Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom

Tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6 are implicated in the pathogenesis of severe *Plasmodium falciparum* malaria. In this study, the effect of IL-10 on their production by peripheral blood mononuclear cells (PBMC) from acutely infected patients was examined. Exogenous IL-10 inhibited malarial antigen–induced cytokine production by reducing mRNA accumulation. Maximal inhibition occurred when IL-10 was added in the first 2 h of stimulation. Conversely, the addition of anti–IL-10 markedly enhanced TNF-α, IL-1β, and IL-6 production. The effect was significantly greater on PBMC from patients with uncomplicated infection than PBMC from patients with severe disease. Kinetics studies showed that TNF-α, IL-6, and IL-1β were produced within 2–4 h of stimulation, while IL-10 was first detectable after 8 h. These findings suggest that IL-10 counter-regulates the proinflammatory response to *P. falciparum*. Severe falciparum malaria may be associated with an inadequate negative feedback response by IL-10.

Severe *Plasmodium falciparum* infection is associated with markedly elevated circulating levels of the proinflammatory cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 [1–3]. Some of the clinical manifestations, such as fever [4], hypoglycemia [5], and hematopoietic suppression [6], have been attributed to the direct effects of these cytokines. The proinflammatory cytokines can also exacerbate pathologic processes in falciparum malaria through the up-regulation of endothelial receptors of cytoadherence [7].

The presence of high levels of circulating proinflammatory cytokines in *P. falciparum* malaria raises the possibility that anticytokine therapy in the form of antibodies, soluble receptors, or counter-regulatory mediators might benefit patients in the period before the parasite burden can be reduced significantly by antimalarial therapy. However, the measurable effects of the intravenous administration of anti–TNF-α monoclonal antibodies (MAbs) in children with cerebral malaria have been confined to fever reduction [8]. One explanation for the disappointing results with anti–TNF-α antibodies is that by the time the patients present to hospital, the cytokine cascade has already been activated. It may therefore be necessary to target multiple steps in the complex sequence of cytokine activation or to use an agent which switches off the whole chain of events.

IL-10 is an 18-kDa peptide, with a number of antiinflammatory and immunosuppressive properties, that is produced by T cells, monocytes, and B cells. In animal models of sepsis, IL-10 given at the time of challenge has been shown to inhibit the development of septic shock and other severe manifestations [9–11]. IL-10 given at the time of infection can protect mice against the neurologic complications of *Plasmodium berghei* ANKA malaria [12]. It has also been shown that gene-targeted mice deficient in IL-10 infected with *Plasmodium chabaudi chabaudi* had more severe signs of disease than did +/+ or heterozygous litter mates [13]. Although there are considerable pathologic differences between these murine models of malaria and the infection in man, the above observations prompted us to assess the effects of IL-10 on the proinflammatory response of peripheral blood mononuclear cells (PBMC) from acutely infected patients with *P. falciparum* malaria.

Materials and Methods

Study population. Adult patients admitted with smear-positive *P. falciparum* malaria to the Hospital for Tropical Diseases, Bangkok, were studied. Parasite density was determined by counting the number of asexual forms of *P. falciparum* per 1000 erythrocytes and was expressed as the number of parasites per microliter. Clinical laboratory investigations included complete blood cell counts and measurements of plasma glucose, serum electrolytes, blood urea nitrogen, serum creatinine, total bilirubin, and alanine aminotransferase. Arterial blood gases and pH were determined in patients with severe malaria (see below).

Patients were considered to have severe malaria if they developed at least one of the following complications [14]: impaired levels of consciousness (Glasgow coma score <15), pulmonary edema, serum creatinine >250 μmol/L, total bilirubin >50 μmol/

Received 20 January 1998; revised 23 March 1998.

Informed consent was obtained from all patients or their relatives. The research protocol was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University.

Financial support: Schering Plough Research Institute (Kenilworth, NJ) and Wellcome–Mahidol University Oxford Tropical Medicine Research Programme (funded by Wellcome Trust of Great Britain). M.H. is a Medical Scholar of the Alberta Heritage Foundation for Medical Research, Canada.

Reprints or correspondence: Dr. May Ho, Dept. of Microbiology & Infectious Diseases, Health Sciences Centre, 3330 Hospital Dr. N.W., Calgary, Alberta, Canada T2N 4N1 (mho@acs.ucalgary.ca).

The Journal of Infectious Diseases 1998; 178:520–5

0022–1899/98/7802–0029$02.00

L with parasitemia of >100,000/μL, or hyperparasitemia (>500,000/μL) alone. Anemia was not used in this classification because of its multifactorial etiology, especially in female patients. All other patients with none of the above abnormalities were considered to have uncomplicated falciparum malaria.

Malaria antigen preparation. A *P. falciparum* schizont lysate (ML) was prepared from overnight culture of a clinical *P. falciparum* isolate. Briefly, erythrocytes with parasitemia of 9.2% were washed twice in Hanks' balanced salt solution. Washed cells were resuspended at 1.5% hematocrit in RPMI medium (Gibco-BRL, Gaithersburg, MD) supplemented with 150 μg/mL gentamicin (Gibco-BRL), 2 m*M* L-glutamine (Sigma, St. Louis), and 10% AB serum from a healthy Thai donor. The medium was changed every 8 h, and thin blood smears were examined to monitor parasite growth. Infected erythrocytes containing mature trophozoites and schizonts were lysed by 0.01% saponin (Sigma) after being washed in PBS. The freed parasites were washed three times in ice-cold PBS and resuspended at 1×10^8 parasites/mL of PBS. The antigen preparation was negative for mycoplasma by a commercial polymerase chain reaction (PCR) kit (Stratagene, La Jolla, CA). Aliquots were stored at −70°C. Antigen stimulation was carried out by adding 1×10^6 parasites/mL (i.e., 1:100 dilution).

PBMC culture and cytokine production. PBMC were obtained from 15 mL of heparinized venous blood by Ficoll-Paque (Pharmacia, Piscataway, NJ) sedimentation. Cells were washed twice in Hanks' balanced salt solution and resuspended in RPMI (Gibco-BRL) supplemented with 100 U/mL penicillin plus 100 μg/mL streptomycin (Gibco-BRL), 2 m*M* L-glutamine (Sigma), and 5% fetal calf serum (Gibco-BRL). Polymyxin B (Sigma) at 20 μg/mL was also added to the cultures to inhibit the activity of any contaminating bacterial lipopolysaccharide (LPS).

PBMC production of TNF-α, IL-1β, IL-6, and IL-10 in response to malarial antigens was measured by culturing cells at 2×10^6/mL in 24-well plates with or without ML. After 48 h of incubation at 37°C in 5% CO_2, the supernatants were aspirated for cytokine determination. In some experiments, supernatants were also harvested after 1, 2, 4, 8, 12, and 24 h of incubation in order to characterize the kinetics of cytokine production in response to malarial antigens.

To study the inhibitory effect of exogenous IL-10 on TNF-α, IL-1β, and IL-6 production by PBMC, cells were cultured with ML in the presence or absence of 10 ng/mL recombinant human IL-10 (gift of K. Moore, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA). In other experiments, IL-10 was added to the cultures 1, 2, 4, 8, 12, and 24 h after the start of stimulation with antigen. The cells were cultured for a total of 48 h before the supernatants were harvested for cytokine determination.

To determine the effect of endogenous IL-10 on cytokine production by PBMC in response to parasite lysate, PBMC were cultured for 48 h with ML in the presence or absence of a neutralizing MAb to human IL-10 at a concentration of 25 μg/mL (clone JES3-9D7; Schering Plough Research Institute, Kenilworth, NJ). Control cultures contained an isotype-matched mouse IgG at the same concentration.

Cytokine measurement. IL-1β, IL-6, and IL-10 were measured by ELISA using pairs of cytokine-specific MAbs (Endogen, Cambridge, Mass) according to the manufacturer's instructions. TNF-α was measured by ELISA kits (BioSource International, Camarillo, CA) that included a polyclonal anti–TNF-α antibody for capture and a biotinylated anti–TNF-α MAb for detection. Each plate included a standard curve of recombinant human cytokine and known positive and negative controls. The values of the controls showed <15% interassay variation over a 6-month period. The lower limit of detection of the cytokine assays was 15 pg/mL.

Cytokine mRNA detection. Cytokine transcript production by PBMC was determined using a reverse transcription–PCR technique, as described [15]. Briefly, total cytoplasmic RNA was extracted from PBMC by using guanidinium thiocyanate (Trizol; Gibco-BRL) according to the manufacturer's instructions 4 h after incubation with or without ML and in the presence or absence of IL-10 (10 ng/mL). Cytoplasmic RNA (1 μg) was reverse-transcribed using Moloney murine leukemic virus reverse transcriptase (Gibco-BRL) in a 25-μL reaction. Human placenta ribonuclease inhibitor (Gibco-BRL) was included in the reaction mixture, and oligo-dT (Gibco-BRL) was used to prime the reaction.

The transcribed product (2–7 μL) was used for specific amplification of cytokine mRNA using Taq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT) and cytokine-specific primers (Clontech, Palo Alto, CA). There were 22 cycles of PCR, each consisting of denaturation at 94°C for 1 min, annealing at 60°C for 2 min, and extension at 72°C for 3 min. A 7-min extension at 72°C was added to the end of each PCR run. To control for the relative amount of mRNA reverse-transcribed in each reaction, the housekeeping gene β-actin was amplified in the same manner. Positive controls were provided by the simultaneous amplification of control templates provided by Clontech. The PCR products were separated on 1.5% agarose gels containing ethidium bromide.

Statistical analysis. Values are presented as mean and SD or SE if normally distributed and as geometric mean and 95% confidence interval (CI) if log-normally distributed. In vitro cytokine production by PBMC from patients with severe and uncomplicated *P. falciparum* malaria was compared using the two-tailed Student's *t* test. $P > .05$ was considered statistically significant.

Results

Effect of exogenous IL-10 on TNF-α, IL-1β, and IL-6 production induced by P. falciparum. The effect of exogenous IL-10 on the production of the proinflammatory cytokines TNF-α, IL-6, and IL-1β was studied using PBMC from 30 acutely infected patients. All three cytokines were produced when PBMC were stimulated by ML (figure 1). The geometric mean levels (95% CI) were 1244 (906–1710) pg/mL for TNF-α, 1498 (1183–1897) pg/mL for IL-1β, and 724 (382–1175) pg/mL for IL-6. The production of all three cytokines was reduced by >90% in the presence of 10 ng/mL IL-10. The inhibition was associated with markedly reduced cytokine mRNA accumulation (figure 2). The results shown are representative of 5 patients studied.

Time course of IL-10 action. To further define the mechanism of IL-10 inhibition, IL-10 was added to cultures of PBMC from 5 patients at various times relative to stimulation with ML. The supernatants were harvested for cytokine determination after 48 h. Results are expressed as mean percent inhibition

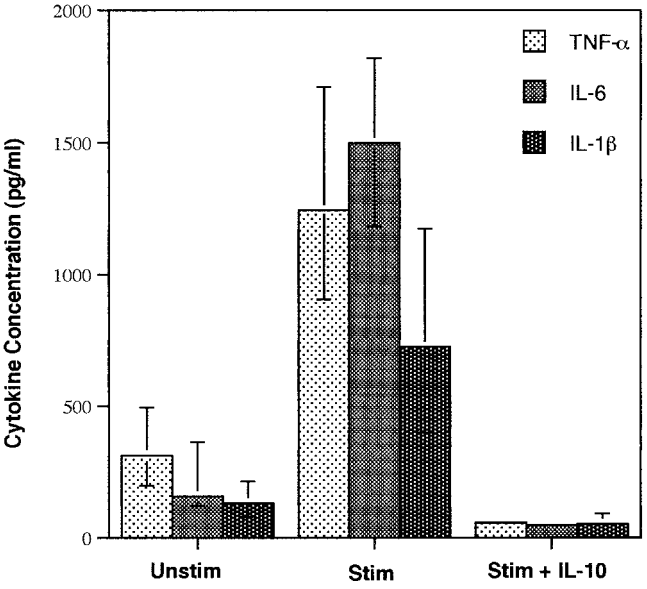

Figure 1. Effect of exogenous IL-10 on TNF-α, IL-1β, and IL-6 production induced by *P. falciparum* antigen. Values represent geometric mean ± 95% confidence interval. Stim, stimulated; Unstim, unstimulated.

(±SD) compared with control values. Simultaneous addition of ML and IL-10 resulted in almost total inhibition of cytokine release (figure 3). The same degree of inhibition was seen when PBMC were pretreated for 2 h with IL-10 (data not shown). The addition of IL-10 at 2 h after that of ML was as effective as simultaneous addition. For TNF-α and IL-1β, inhibition was greatly reduced when IL-10 was added 4 h after ML. No inhibition was observed when IL-10 was added 8 h after ML. In comparison, the inhibitory effect on IL-6 release was still evident when IL-10 was added 12 h (50%) and 24 h (31%) after ML.

Effect of anti–IL-10 on cytokine production. To determine if IL-10 was produced endogenously in response to ML, and whether the endogenous production of IL-10 was sufficient to down-regulate the proinflammatory response, a neutralizing MAb (25 mg/mL) to IL-10 was added to cultures of PBMC from 23 acutely infected patients. This antibody concentration has been shown to neutralize the effect of 10 ng/mL IL-10 (Ho M, unpublished observation). The results in figure 4 are expressed as the geometric mean fold increase in cytokine release. There was a marked increase in cytokine production in response to ML in the presence of the anti–IL-10 antibody. Furthermore, the enhancing effect on TNF-α and IL-6 production by PBMC from 10 patients with uncomplicated falciparum malaria was significantly greater than the effect on PBMC from 13 patients with severe infection ($P = .028$ and $< .036$, respectively).

Cytokine production by PBMC from patients with uncomplicated and severe P. falciparum malaria. The lesser effect of anti–IL-10 (described above) on the PBMC from patients with severe falciparum malaria may be due to at least two factors. Either the PBMC were unable to respond to ML, even when IL-10 was neutralized, or the PBMC did not produce as much IL-10 as did the PBMC from patients with uncomplicated infection. To determine if there was indeed a difference in IL-10 production between the 2 patient groups, IL-10 production by PBMC from 34 patients with severe falciparum malaria and 35 patients with uncomplicated infection was measured. The results are summarized in table 1. There was considerable overlap of cytokine levels in the supernatants between the 2 patient groups. In this relatively small study population, a trend for greater production of IL-10 in patients with uncomplicated infection was seen, but the difference between the geometric mean IL-10 levels did not reach statistical significance ($P = .088$). Conversely, the corresponding TNF-α levels showed that there was a trend for greater TNF-α production by patients with severe disease ($P = .128$)

Kinetics of cytokine production in response to ML. To serve a counter-regulatory role in *P. falciparum* malaria, IL-10 would be expected to be produced after the proinflammatory response has been established. To determine the kinetics of cytokine production following stimulation by ML, levels of TNF-α, IL-1β, IL-6, and IL-10 were determined in supernatants of cultures of PBMC from 10 patients; the PBMC had been harvested at 1, 2, 4, 8, 12, and 24 h after the start of stimulation (figure 5). TNF-α, IL-6, and IL-1β were detected

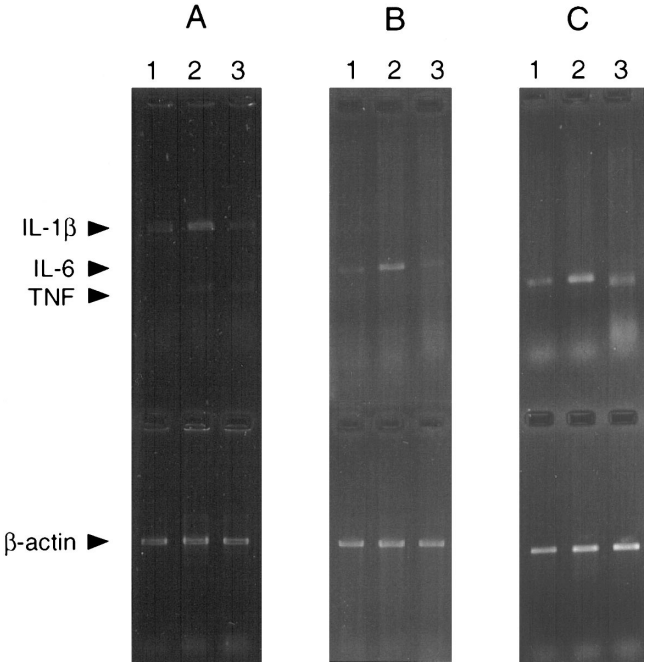

Figure 2. *P. falciparum* antigen–induced cytokine mRNA expression in presence or absence of IL-10: lanes 1, unstimulated; lanes 2, stimulated; lanes 3, stimulated + IL-10 for each of IL-1β (**A**), IL-6 (**B**), and TNF-α (**C**). Results shown are representative of 5 patients studied.

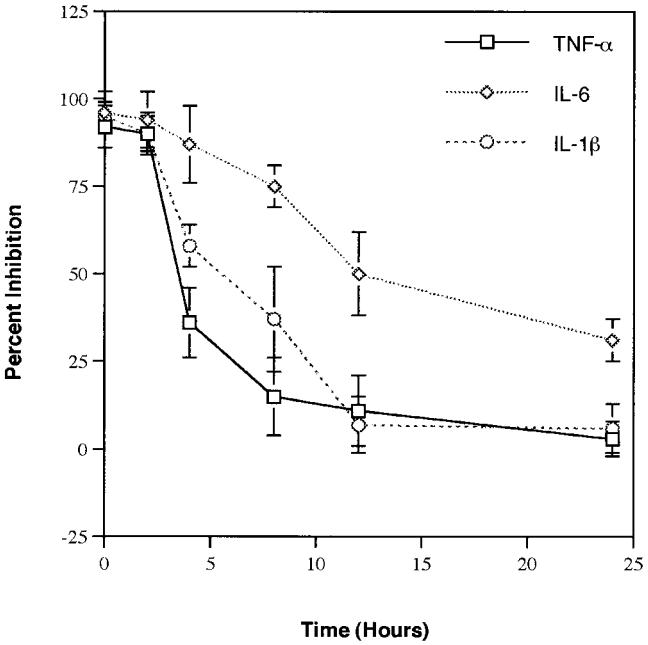

Figure 3. Kinetics of inhibitory effect of IL-10 on cytokine production induced by *P. falciparum* antigen. Suppression of cytokine production by IL-10 added at various times after start of antigen stimulation is expressed as % inhibition in relation to time 0 values. Values represent mean % inhibition ± SE.

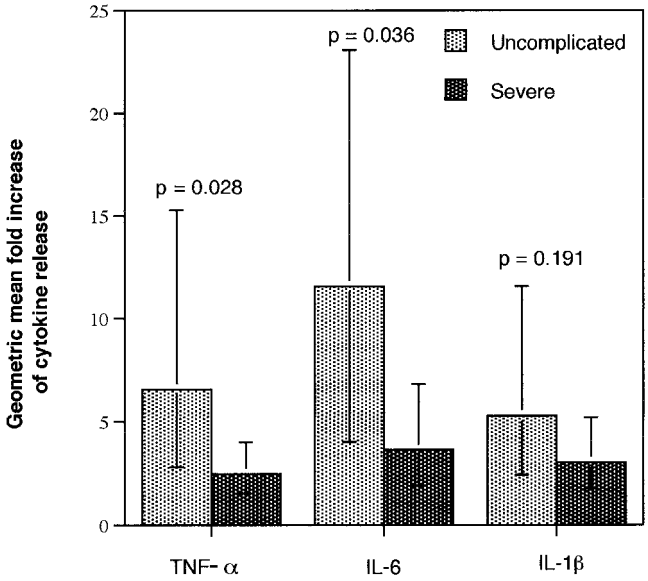

Figure 4. Effect of anti–IL-10 on cytokine production by peripheral blood mononuclear cells of 13 patients with severe falciparum malaria and 10 patients with uncomplicated infection. Results were expressed as geometric mean fold increase in cytokine release ± 95% confidence interval in presence of anti–IL-10.

as early as 2–4 h after the start of stimulation and reached maximal levels by 4–8 h. The geometric mean peak concentrations (95% CI) were 871 (491–1545) pg/mL for TNF-α, 3724 (2080–6668) pg/mL for IL-6, and 492 (275–882) pg/mL for IL-1β. In contrast, IL-10 was essentially undetectable until 8–12 h, with a peak concentration of 331 (154–679) pg/mL at 24 h. Similar kinetics of cytokine production were observed when 7 of 10 patients were restudied during convalescence (data not shown). A dose-response curve for IL-10 confirmed that the relatively low concentration of endogenously produced IL-10 was sufficient to inhibit the production of all three cytokines (figure 6).

Discussion

Much of the current knowledge on the regulatory role of IL-10 during an infection is based on studies involving cytokine responses to bacterial LPS. IL-10 has been shown to have a suppressive effect on the synthesis of the proinflammatory cytokines TNF-α, IL-1β, and IL-6 by inhibiting LPS-induced transcription of all three cytokine genes [16, 17] and enhancing the degradation of LPS-induced IL-6 mRNA [17]. Whether IL-10 has similar effects on the proinflammatory response of PBMC from acutely infected patients to other microbial antigens is less well established.

The proinflammatory cytokines TNF-α, IL-1β, and IL-6 have been implicated in the pathogenesis of *P. falciparum* malaria since reports from several centers showed that markedly elevated concentrations were present in the plasma of acutely infected patients, and these concentrations were higher in severely ill patients and highest in fatal cases. However, circulating cytokine levels provide, at best, an estimation of production and do not shed any light on the regulation of their production in this infection. We have shown previously that the human host produces both a Th1-like and a Th2-like response to *P. falciparum* [15] and that severe infection is associated with an intense Th1-like response. Since both Th1 and

Table 1. TNF-α and IL-10 production in response to malarial antigens by peripheral blood mononuclear cells from 34 patients with severe falciparum malaria and 35 patients with uncomplicated infection.

	Cytokine concentration (pg/mL)	
	TNF-α	IL-10
Unstimulated		
Uncomplicated	243 (151–390)	238 (167–337)
Severe	600 (280–1284)	203 (139–298)
*P**	0.065	0.541
Stimulated		
Uncomplicated	1114 (769–1614)	416 (311–557)
Severe	1726 (1188–2504)	299 (231–385)
*P**	0.128	0.088

NOTE. Data are geometric mean (95% confidence interval).
* Statistical analysis was performed using two-tailed Student's *t* test.

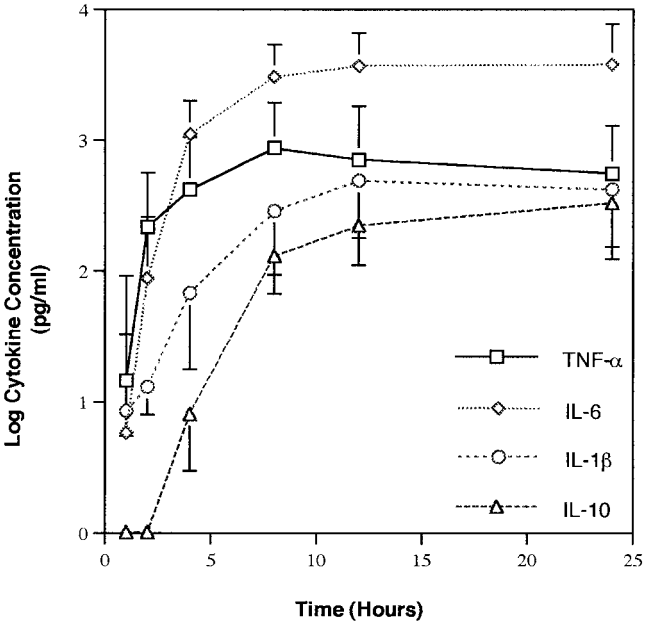

Figure 5. Kinetics of cytokine production by peripheral blood mononuclear cells of 10 acutely infected patients in response to malarial antigens. Values represent mean log cytokine concentration ± 95% confidence interval.

Th2 cytokines are produced, the relative magnitude and kinetics of cytokine production, rather than the selective activation of a specific subset of $CD4^+$ T cells as seen in animal models, appear to be more important determinants of outcome. Few studies to date have examined the kinetics of cytokine production and its regulation in human *P. falciparum* malaria.

In this study, we have explored the immunoregulatory role of IL-10 on the proinflammatory response during human *P. falciparum* infection. The investigations were conducted in vitro, as there is no good animal model of the human infection. We showed that exogenous IL-10 inhibited TNF-α, IL-1β, and IL-6 production in response to malarial antigens by PBMC from patients with acute falciparum malaria. The inhibition of all three cytokines occurred rapidly and was accompanied by reduced cytokine mRNA accumulation, which suggests that IL-10 acts primarily by inhibiting ML-induced cytokine gene transcription, as in the case of LPS [16, 17]. The effect on IL-6 release when IL-10 was added 12 and 24 h after ML may reflect enhanced degradation of IL-6 mRNA. Further molecular studies to clarify the mechanisms of IL-10 action are warranted.

The next question addressed was whether there is sufficient endogenous IL-10 production in response to ML that would modulate the production of TNF-α, IL-1β, and IL-6. Here we showed that the addition of a neutralizing anti–IL-10 MAb to PBMC cultures resulted in marked enhancement of cytokine production, thus confirming a critical role of IL-10 in controlling the ML-induced cytokine cascade. We further demonstrated that the neutralization of the endogenous IL-10 had a significantly greater effect on TNF-α and IL-6 production by PBMC from patients with uncomplicated infection than by PBMC from patients with severe falciparum malaria. Together with a trend for greater production of IL-10 by PBMC from patients with uncomplicated infection, these observations suggest that patients with uncomplicated infection may produce more IL-10, which in turn may lead to a greater ability on their part in limiting the proinflammatory response. Thus the pathogenesis of severe falciparum malaria may include not only an excessive proinflammatory response, but also a defective negative feedback mechanism.

Further evidence for a counter-regulatory role of IL-10 comes from analysis of the temporal sequence of cytokine production. TNF-α production increased rapidly after ML stimulation, followed by production of IL-6 and IL-1β. IL-10 production lags behind by a few hours and coincided with a cessation of further TNF-α, IL-6, and IL-1β production. Similar kinetics of cytokine production have been shown for the stimulation of human monocytes by LPS [16, 18] and after in vivo challenge with gram-negative bacteria or purified endotoxin [19, 20]. The delayed production of IL-10 provides a mechanism by which it can act as negative feedback to dampen the proinflammatory response elicited by parasite antigens.

IL-10 administration could potentially be beneficial to patients with severe falciparum malaria. It is well tolerated in human volunteers [21] and has promising efficacy in chronic inflammatory conditions, such as inflammatory bowel disease [22]. Our results indicate that to be therapeutically useful in halting the development of severe falciparum malaria, IL-10 needs to be given within the first few hours of antigen stimulation, which would be difficult to accomplish in the clinical

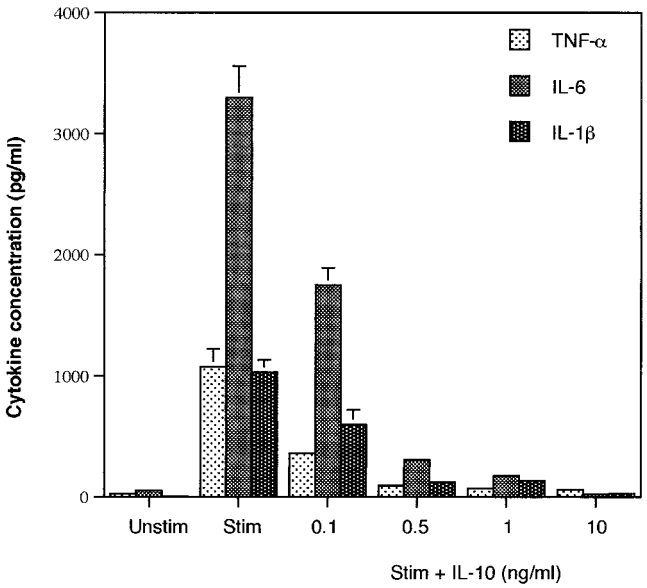

Figure 6. Dose-response curve of inhibitory effect of IL-10 on cytokine production. Values represent mean ± SD of triplicate wells using peripheral blood mononuclear cells from 1 convalescent patient.

setting. However, thorough understanding of the mechanism of action of IL-10 in this infection may reveal other steps in the inhibition of the proinflammatory response in a less time-dependent fashion.

Acknowledgments

We thank the patients and nursing staff of the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, for their kind cooperation and Paul Grint, Lee Schwocho, and the Schering Plough Research Institute for their interest and support.

References

1. Kern P, Hemmer CJ, Damme JV, Gruss HJ, Dietrich M. Elevated tumor necrosis factor alpha and interleukin-6 serum levels as markers for complicated *Plasmodium falciparum* malaria. Am J Med **1989**;87:139–43.
2. Grau GE, Taylor TE, Molyneux ME, et al. Tumor necrosis factor and disease severity in children with falciparum malaria. N Engl J Med **1989**;320:1586–91.
3. Kwiatkowski D, Hill AVS, Sambou I, et al. TNF concentration in fatal cerebral, non-fatal cerebral, and uncomplicated *Plasmodium falciparum* malaria. Lancet **1990**;336:1201–4.
4. Kwiatkowski D, Cannon JG, Manogue KR, Cerami A, Dinarello CA, Greenwood BM. Tumour necrosis factor production in falciparum malaria and its association with schizont rupture. Clin Exp Immunol **1989**;77:361–6.
5. Evans RD, Argiles JM, Williamson DH. Metabolic effects of tumour necrosis factor (cachectin) and interleukin 1. Clin Sci **1989**;77:357–64.
6. Miller KL, Silverman PH, Kullgren B, Mahlmann LJ. Tumor necrosis factor alpha and the anaemia associated with murine malaria. Infect Immun **1989**;57:1542–6.
7. Berendt AR, Simmons DL, Tansey J, Newbold CI, Marsh K. Intercellular adhesion molecule-1 is an endothelial cell adhesion receptor for *Plasmodium falciparum.* Nature **1989**;341:57–9.
8. Kwiatkowski D, Molyneux ME, Stephens S, et al. Anti-TNF therapy inhibits fever in cerebral malaria. Q J Med **1993**;86:91–8.
9. Gerard C, Bruyns C, Marchant A, et al. Interleukin 10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia. J Exp Med **1993**;177:547–50.
10. Howard M, Muchamuel T, Andrade S, Menon S. Interleukin 10 protects mice from lethal endotoxemia. J Exp Med **1993**;177:1205–8.
11. Rogy MA, Auffenberg T, Espat NJ, et al. Human tumor necrosis factor receptor (p55) and interleukin 10 gene transfer in the mouse reduces mortality to lethal endotoxemia and also attenuates local inflammatory responses. J Exp Med **1995**;181:2289–93.
12. Eckwalanga M, Marussig M, Tavares MD, et al. Murine AIDS protects mice against experimental cerebral malaria: down-regulation by interleukin 10 of a T-helper type 1 $CD4^+$ cell-mediated pathology. Proc Natl Acad Sci USA **1994**;91:8097–101.
13. Linke L, Kuhn R, Muller W, Honarvar N, Li C, Langhorne J. *Plasmodium chabaudi chabaudi:* differential susceptibility of gene-targeted mice deficient in IL-10 to an erythrocytic-stage infection. Exp Parasitol **1996**;84:253–63.
14. World Health Organization, Division of Control of Tropical Diseases. Severe and complicated malaria. Trans R Soc Trop Med Hyg **1990**;84(suppl 2):1–65.
15. Ho M, Sexton MM, Tongtawe P, Looareesuwan S, Suntharasamai P, Webster HK. Interleukin-10 inhibits tumor necrosis factor production but not antigen-specific lymphoproliferation in acute *Plasmodium falciparum* malaria. J Infect Dis **1995**;172:838–44.
16. de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Exp Med **1991**;11209–20.
17. Wang P, Wu P, Siegel MI, Egan RW, Billah MM. IL-10 inhibits transcription of cytokine genes in human peripheral blood mononuclear cells. J Immunol **1994**;153:811–6.
18. Donnelly RP, Freeman SL, Hayes MP. Inhibition of IL-10 expression by IFN-γ up-regulates transcription of TNF-α in human monocytes. J Immunol **1995**;155:1420–7.
19. Fong Y, Tracey KJ, Moldawer LL, et al. Antibodies to cachectin/tumor necrosis factor reduce interleukin 1β and interleukin 6 appearance during lethal bacteremia. J Exp Med **1989**;170:1627–33.
20. Martich GD, Danner RL, Ceska M, Suffredini AF. Detection of interleukin-8 and tumor necrosis factor in normal humans after intravenous endotoxin: the effect of antiinflammatory agents. J Exp Med **1991**;173:1021–4.
21. Chernoff AE, Granowitz EV, Shapiro L, et al. A randomized, controlled trial of IL-10 in humans—Inhibition of inflammatory cytokine production and immune responses. J Immunol **1995**;154:5492–9.
22. Schreiber S, Heinig T, Thiele HG, Raedler A. Immunoregulatory role of interleukin 10 in patients with inflammatory bowel disease. Gastroenterology **1995**;108:1434–44.