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Short Report

Interleukin-8 and *Plasmodium falciparum* malaria in Thailand

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The role of interleukin 8 (IL-8) in *Plasmodium falciparum* malaria is unknown, although activation of the cytokine network and secretion of pro-inflammatory cytokines such as tumour necrosis factor (TNF) and interleukin 6 (IL-6) does occur. IL-8 is secreted by many cells including monocytes, macrophages and endothelial cells. It is a chemoattractant and activator of neutrophils and is also chemotactic for T cells (BAGGIOLINI *et al.*, 1989). However, there are few *in vivo* data on IL-8 in clinical infections. We have investigated plasma IL-8 concentrations in a pilot study of 6 patients with relatively severe, but non-fatal, non-cerebral, *P. falciparum* malaria admitted consecutively to the Hospital for Tropical Diseases, Bangkok, Thailand. The study was approved by the ethics committee of the Faculty of Tropical Medicine, Mahidol University, Thailand.

Following diagnosis of malaria by microscopical examination of thick and thin blood films, 5 ml blood samples were taken immediately before initiation of treatment (artemether or artesunate and appropriate supportive therapy), then daily for 7 d and weekly thereafter until discharge at 1 month. The prolonged admission policy at the Hospital for Tropical Diseases ensures that there is no second episode of infection in patients and allows insight into the natural history of a single treated episode of malaria. Samples were collected into endotoxin-free tubes containing the anticoagulant potassium ethylene diaminetetraacetate and 100 µl trasylol, a protease inhibitor. They were then centrifuged (1000 *g* for 5 min at 4°C) and plasma was stored at −70°C for a maximum of 2 months. IL-8 was measured with a previously described enzyme-linked immunosorbent assay (ELISA) (DEFORGE & REMICK, 1991). Plasma TNF and IL-6 concentrations were measured using established bioassays (ESPEVIK & NISSEN-MEYER, 1986; AARDEN *et al.*, 1987). The lower limit of sensitivity of the TNF assay was 23 pg/ml, of the IL-6 assay 1 pg/ml, and of the IL-8 ELISA 95 pg/ml. Standard curves were determined for all assays, which were performed with appropriate control samples and carried out in a 'blinded' fashion by a laboratory unaware of the clinical details.

The 6 patients were all male, aged between 16 and 45 years; their initial peripheral parasitaemias were 2·1%, 5·6%, 7·0%, 7·6%, 7·8% and 13%. None had evidence of cerebral malaria and all recovered following treatment. Five patients had abnormal liver function tests with raised serum bilirubin and transaminases. One patient required haemodialysis and cytokine analyses were discontinued as renal replacement therapy may activate the cytokine network. A neutrophil leucocytosis ($11{\cdot}9\times 10^9$/L, 84% neutrophils) was documented in only one patient. Malarial parasites were cleared from the circulation within 3 d of starting specific treatment and all patients were afebrile within one week.

The initial mean plasma IL-8 concentration was raised at 216±9·5 pg/ml, which is similar to levels we have previously recorded in patients with fatal Gram-negative sepsis (97–362 pg/ml.). In normal healthy subjects without malaria no plasma IL-8 is detectable using this ELISA (DEFORGE & REMICK, 1991), and in control experiments we have found that the freezing and storage protocol used in this study does not affect plasma measurements. The prognostic significance of raised plasma IL-8 concentration cannot be deduced from this small pilot study. IL-8 may be the stimulus for the leucocytosis found in some patients with severe malaria, although a raised neutrophil count was found in only one patient in our study. The current data suggest that IL-8 has as yet uncharacterized functions in host defence to this protozoal pathogen, unrelated to activation of neutrophils. Mononuclear cells express the IL-8 gene following adhesion (KASAHARA *et al.*, 1991), suggesting a relationship between IL-8 and the regulation of adhesion molecules, such as ICAM-1. Such an interaction may be important if endothelial expression of intercellular adhesion molecule 1 (ICAM-1), an adhesion receptor for *P. falciparum in vitro* (BERENDT *et al.*, 1989), is involved in capillary sequestration of the parasite with consequent organ dysfunction *in vivo*.

Admission plasma IL-6 concentrations detected by bioassay in these patients were slightly elevated at 44±21·6 pg/ml. This is similar to levels we have detected in relatively trivial bacterial infections but lower than the concentrations measured in African children with cerebral malaria (MOLYNEUX *et al.*, 1991). This difference may be important, as high plasma IL-6 concentrations have been associated with poor prognosis in the African study and with organ damage in severe malaria in non-immune European adults (KERN *et al.*, 1989). However, this discrepancy might reflect the fact that we have used a biological rather than an immunoradiometric assay. Bioactive TNF was detectable in the plasma of 4 patients on admission to the study (120±65 pg/ml), at concentrations similar to those previously reported (GRAU *et al.*, 1989).

An unexpected and interesting finding of the current study was that plasma IL-8 and IL-6 levels remained elevated throughout the period of investigation (Figure). Patients were studied for at least 3 weeks after they had become afebrile and recovered clinically from their illness. In only one patient was plasma TNF bioactivity detected beyond day 7, the period during which fever set-

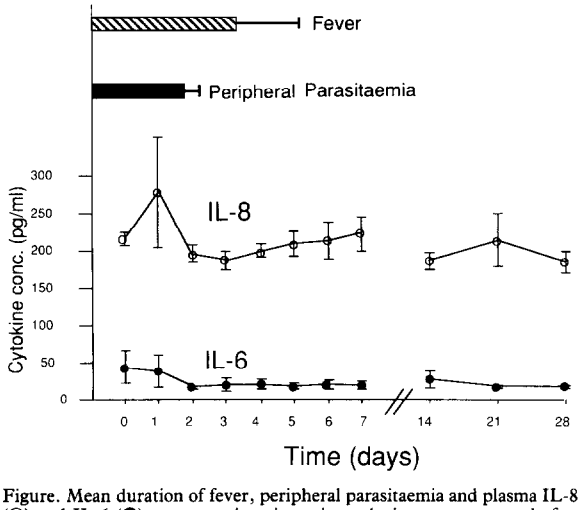

Figure. Mean duration of fever, peripheral parasitaemia and plasma IL-8 (○) and IL-6 (●) concentrations in patients during treatment and after recovery from *P. falciparum* malaria; vertical bars indicate standard error of the means.

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tled. Continued production of bioactive IL-6 and of IL-8 during and after clinical recovery from *P. falciparum* infection may indicate that these cytokines are involved in the healing process.

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Short Report

Pneumocystis carinii pneumonia and tuberculosis in Tanzanian patients infected with HIV

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Although *Pneumocystis carinii* is clearly recognized in North America and Europe as a leading cause of fatal pneumonia in malnourished children and immunocompromised patients such as those with the acquired immunodeficiency syndrome (AIDS) (BARTLETT & SMITH, 1991), overt pneumocystosis has rarely been recognized in AIDS patients living in African countries (LUCAS, 1988; FLEMING, 1990). On the contrary, tuberculosis appears to be prevalent among African populations infected with human immunodeficiency virus (HIV) (LUCAS, 1988; FLEMING, 1990).

Eighty-three adult patients (38 male, 45 female, mean age 25·3 years, range 18–38 years) infected with HIV and with respiratory symptoms, dwelling in Malenga Makali rural area, Iringa district, Tanzania, were studied to determine the prevalence of *P. carinii* infection and tuberculosis during 5 months ending 29 December 1991. Patients were enrolled if they were HIV seropositive and presented with dyspnoea, cough, fever of at least two weeks duration, or overt pneumonia. HIV infection was assessed by positive serological results in 2 different commercially available tests, using freshly collected sera (HIV Check 1–2®, Dupont, Wilmington, Delaware, USA and Recombinant ELISA®, Abbott, North Chicago, Illinois, USA) and positive results confirmed by a commercial Western blot using sera stored at -20°C (Biotech®, Dupont). Sputum was collected after inhalation of hypertonic solution (NaCl 10%) from an ultrasonic nebulizer (Fisoneb®, Fisons, Rochester, New York, USA) for 10–15 min. About 1 ml of each sample was treated with a solution of dithiothreitol (Sputasol®, Oxoid, Basingstoke, Hampshire, England) for 15 min and centrifuged: the supernatant was discarded and smears were prepared from the pellet. Slides were stained with toluidine blue O and Diff-Quik® (Baxter, Switzerland). To detect mycobacteria, smears were stained with Ziehl-Neelsen to identify acid-alcohol resistant bacilli after 3-step decontamination using L-cysteine, benzalkonium chloride and albumin (Biomérieux, Italy) followed by centrifugation. Using a sterile wire loop, samples of the pellet were inoculated into tubes containing Lowenstein-Jensen medium (Biomérieux) and incubated at 37°C.

Three of 83 induced sputum specimens (3·6%) were positive for *P. carinii*, in both Diff-Quik and toluidine blue O stained smears. The infection was confirmed by Giemsa staining. In addition, *P. carinii* cysts were observed in 2 other smears stained with toluidine blue O, but these could not be confirmed by Giemsa staining either because only empty cysts were present or because the infection was very scanty (both preparations were heavily contaminated with fungi). Mycobacteria were found in 32/83 (38·5%) preparations, and 11 cultures (13·2%) were positive; 21 other cultures were contaminated. The isolates were all *Mycobacterium tuberculosis*, fully sensitive to standard drugs. Two of the 3 patients infected with *P. carinii* also had concomitant pulmonary tuberculosis, as reported by MCLEOD *et al.* (1990) in Zimbabwe, and one of them died during therapy.

As expected, *M. tuberculosis* appears to be a dominant pathogen in Tanzanian HIV-infected patients, by virtue of its high prevalence and pathogenicity also in immunocompetent subjects in the same area (personal observations). Our study confirmed that tuberculosis among AIDS pat-ients often results in the sputum being very rich in mycobacteria. As emphasized in many studies in several African countries, tuberculosis remains one of the most common severe diseases associated with HIV infection, probably because of its occurrence among populations with low socioeconomic indices, gross exposure to the pathogen without previous vaccination, and failure of tuberculosis control measures (LUCAS, 1988; CHAULET, 1990; MCLEOD *et al.*, 1990).

Even if *P. carinii* pneumonia seems to be of minor importance in tropical countries, our results confirmed that it occurs there, even if less frequently than in Europe and the USA (GRIFFIN & LUCAS, 1982; AIOYE, 1987; LUCAS, 1988, 1990; LUCAS *et al.*, 1989; ELVIN *et al.*, 1989; MCLEOD *et al.*, 1989; CARME *et al.*, 1991; FLEMING 1990; M'BOUSSA *et al.*, 1991). As it has been also serologically demonstrated that *P. carinii* is present in healthy Gambian children (WAKEFIELD *et al.*, 1990), it is probable that the parasite does not appear to be an im-

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