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Research note

Increased levels of interleukin-12 in *Plasmodium falciparum* malaria: correlation with the severity of disease

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SUMMARY

Interleukin (IL)-12, produced by mononuclear phagocytes, activates the T-helper 1 (Th1) cells and helps, as a mediator, the innate immune response to intracellular microbes. In Plasmodium falciparum *infection, this proinflammatory cytokine has immunoregulatory functions with effects on the immune response to the blood stage of disease, but also induces protection and reduces malarial anaemia. In this study, the levels of IL-12 were determined in 73 African children, aged 2–144 months (median 19·5 months), who had severe or mild* P. falciparum *malaria. IL-12 was determined using the enzyme-linked immunosorbent assay. The levels of IL-12 were found to be significantly elevated (21·6 ± 18·3 pg/ml) in patients who suffered less severely from the disease. In contrast, the levels of IL-12 were found to be lower (13·1 ± 7·11 pg/ml) in patients who suffered more severely from the disease.*

Keywords *IL-12, malaria, macrophage activation*

RESEARCH NOTE

Malaria remains one of the major causes of mortality, mostly in children under the age of 5 years, in tropical countries and particularly sub-Saharan Africa (1,2). During the malaria crisis, it has been shown that monocytes/macrophages release large amounts of interleukin (IL)-1 (3), tumour necrosis factor (TNF) and nitric oxide (NO) (4), so that the pathogenic manifestations of malaria are mainly due to pro-inflammatory cytokines released by macrophages in response to malaria parasites and their products (3). Recent studies have shown that protective immunity in malaria is mediated by a cascade of events that also involves IL-12 (5,6), a potent immunomodulatory cytokine that has been shown to be effective in conferring protection against viral, bacterial and intracellular parasitic infections (7). This cytokine not only enhances cell-mediated immune responses, but also effects humoral immunity by inducing isotype switching through both interferon (IFN)-γ dependent and independent mechanisms (6). In fact, IL-12 seems to stimulate antibody production in B cells and it has been demonstrated that IL-12 is effective even in inducing protective immunity against blood-stage infection in the murine model (5). IL-12 acts on antigen stimulated $CD4^+$ T cells, promoting the differentiation of T cells into the Th1 subset (8), which acts on macrophages not only to stimulate their microbicidal functions, but also to increase their production of IL-12. The elevated levels of IL-12 also modulate the macrophage activity, which is associated with the increased erytrocyte destruction, bone marrow dyserythropoiesis (9), and thrombocytopenia (10). During the intraerythrocytic life cycle of *Plasmodium falciparum*, macrophages avidly phagocytize parasite specific products, leading to the impairment of macrophage functions (11) and cytokine production (12). Since IL-12 seems to have a modulating

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role on macrophage activity and on the development of anaemia (13), the main goal of this study was to measure the level of IL-12 in the plasma of groups of 73 children with severe or mild malaria and to correlate the production of this cytokine with the severity of disease. The children affected by acute malaria, median age 19·5 months (range 2–144 months) were observed during October 2000 at the Center Medical St Camille (Ouagadougou), Burkina Faso. Thirty-eight were male and 35 were female. Inclusion and classification of each case were based on the symptoms, physical signs and laboratory results of malaria at the time of first presentation. 'Severe malaria (complicated)' was established by microscopic diagnosis of *P. falciparum* parasites in the peripheral blood and clinical signs according to the WHO criteria: evidence of neurological compromission (prostration, lethargy), gastrointestinal symptoms, severe anaemia (Ht < 20%, Hb < 6 g/dl), hyperparasitaemia corresponding to $E_p > 5 \times 10^5$ or > 5%, hypoglycaemia (serum glucose less than 2·2 mmol/l corresponding to 40 mg/dl), acidosis with respiratory distress, oliguria, cardiovascular shock, jaundice, diffuse haemorrhages. 'Mild malaria (uncomplicated)' was established by parasitaemia of $E_p < 5 \times 10^5$ or < 5% with fever, headache, myalgias without any finding of severe malaria. All patients were treated according to the official therapeutic protocol of local Programme National de Lutte contre le Paludism (PNLP). On the basis of haematological parameters, hyperparasitaemia and evidence of neurological involvement, four different levels of severity were selected attributing a score from +—— to ++++. Twenty healthy Black children of the same range of age (median 20 months, range 4–150 months) were also included in the study as a control group for the determination of IL-12, which was measured by enzyme-linked immunosorbent assay, according to the manufacturer's specifications (R&D Systems, Germany). The statistical analysis of results was performed using Student's two-tailed *t* distribution test and the level of significance was set at $P < 0{\cdot}05$. The mean values of more important haematological parameters are shown in Table 1, according to the criteria of severity of disease (anaemia, hyperparasitaemia, age, neurological involvement). IL-12 levels were found to be significantly elevated (20·08 ± 17·00 pg/ml) in all children with less severe disease. In contrast, the level of IL-12 was significantly lower (13·1 ± 7·11 pg/ml) in 26 patients with more severe disease, who were younger and showed more severe anaemia (Hb 3·4 ± 098 g/dl), lower platelet count (143.38 ± 68.36 mm^{-3}) and severe signs of neurological involvement. The parasite density was higher ($E_p > 5 \times 10^5$) in patients with a lower IL-12 level. A correlation was found between IL-12 and haemoglobin concentration (Figure 1) and between IL-12 levels and age (Figure 2). In the control group, the values of IL-12 were 7·6 ± 2·5 pg/ml. It has been

Table 1 Haematological data of patients according to the clinical severity of malaria

Patient numbers	Severity degree	Age (months)	Leukocyte (mm^3)	Red cells (million/mm^3)	Hb (g/dl)	Ht (%)	MCV (U^3)	MCH (YY)	MCHC (%)	Platelets (mm^3)	Parasite density	IL-12 (pg/ml)
15	(A) +——	29·3 ± 16·6	17·0 ± 8·74**	3·02 ± 1·04*	8 ± 1·92*	24·2 ± 6·9*	73·3 ± 18·3	27·22 ± 6·59	33·85 ± 5·26*	248·91 ± 90·95	+–	21·6 ± 18·3**
19	(B) ++——	25·82 ± 12·3	15·5 ± 8·28**	3·04 ± 0·71*	7·7 ± 1·74*	23 ± 5·32*	75·3 ± 8·9	25·7 ± 5·08	33·58 ± 3·69	258·53 ± 153·53	++——	20·8 ± 17·7**
13	(C) +++–	21·9 ± 19·2	16·2 ± 9·25**	1·49 ± 0·75**	3·5 ± 1·33**	11·2 ± 4·38**	79·3 ± 12·7	25 ± 5·29	31·99 ± 3·85	170·62 ± 96·17**	+++–	18·3 ± 13·3**
26	(D) ++++	16·9 ± 17·7	19·6 ± 13·8**	1·24 ± 0·5**	3·4 ± 0·98**	10·9 ± 3·16**	88·9 ± 21·6	29·1 ± 5·15*	31·89 ± 3·67	143·38 ± 68·36**	++++	13·1 ± 7·11*
Control	———	22·08 ± 16·32	8·53 ± 2·74	3·95 ± 0·78	9·6 ± 2·28	26·4 ± 4·38	81·55 ± 11·48	24·52 ± 4·40	30·21 ± 2·66	324·12 ± 102·25	––– -	7·6 ± 2·5

Patients versus healthy controls * $P < 0{\cdot}01$, ** $P < 0{\cdot}001$; (A) versus (D), $P = 0{\cdot}040$; (B) versus (D), $P = 0{\cdot}050$.

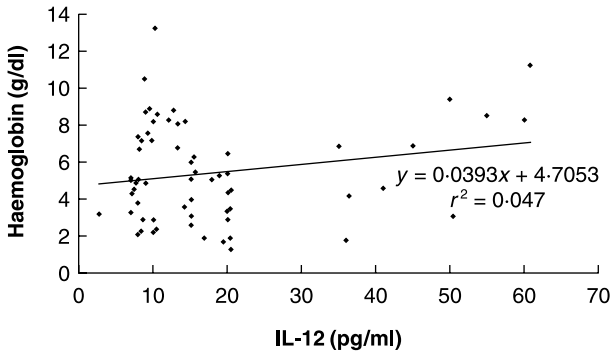

Figure 1 Correlation between IL-12 and haemoglobin in patients with acute malaria.

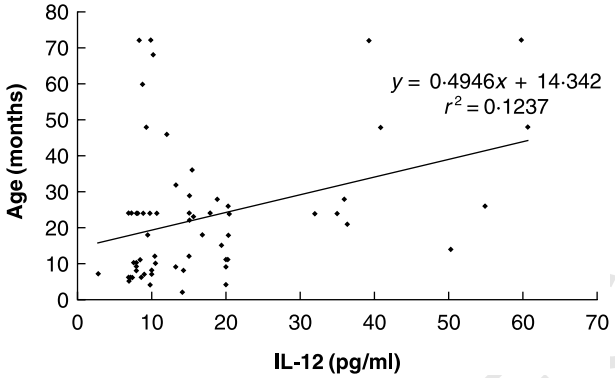

Figure 2 Correlation between IL-12 and age in patients with acute malaria.

demonstrated that early events in the cell-mediated immune response required for defense against malaria, initiate with the release of IL-12 from monocytes/macrophages, B cells, and other cell types (6) and, consequently, the level of IL-12 reveals a prognostic significance in the malaria infection. Moreover, there is the evidence that children with *P. falciparum* hyperparasitaemia have lower levels of $CD4^+$ T cell secreting IFN-γ than children with uncomplicated malaria (14). It is possible that reduced IL-12 levels in patients with hyperparasitemia and severe malaria are associated with reduced T-cell mediated IFN-γ activity. Our results establish a critical role for IL-12 in the adaptive immune response to malaria, inducing development, proliferation and activity of Th1 cells (15). The outcome of the disease, such as susceptibility to severe anaemia and other aspects of malarial pathophysiology, could depend on the response of host macrophages to parasite products and, consequently, impaired IL-12 production (14,16). Luty *et al*. (16) and Perkins *et al*. (14) demonstrated that the low levels of IL-12 play a role in exacerbation of anaemia and other clinical complications. In addition, our results provide evidence that high levels of IL-12 play an important role in the defense against *P. falciparum* infection and in protection against systemic damage induced by the presence of the parasite. Since IL-12 has an important role as the initiator of cell-mediated immunity, it could be used in therapy as a potent stimulator of the cell-mediated immune response against *P. falciparum*.

REFERENCES

1 World Health Organization. World malaria situation in 1994. *Weekly Epidemiol Rec* 1997; **72**: 269–276.
2 Miller LH, Good MF, Milon G. Malaria pathogenesis. *Science* 1994; **264**: 1878–1883.
3 Sherry BA, Alava G, Tracey KJ, Martiney J, Cerami A, Slater AFG. Malaria specific metabolite hemozoin mediates the release of several potent endogenous pyrogens (TNF, MIP1α and MIP-1β) in vitro, and altered thermoregulation in vivo. *J Inflamm* 1995; **45**: 85–96.
4 Taramelli D, Basilico N, Pagani E *et al.* The heme moiety of malaria pigment (β-hematin) mediates the inhibition of nitric oxide and tumor necrosis factor-α production by lipo polysaccharide-stimulated macrophages. *Exp Parasitol* 1995; **81**: 501–511.
5 Doolan DL, Hoffman SL. IL-12 and NK cells are required for antigen specific adaptative immunity against malaria initiated by $CD8^+$ T cells in the *plasmodium yoelii* model. *J Immunol* 1999; **163**: 884–892.
6 Crutcher JM, Stevenson M, Sedegah M, Hoffman SL. Interleukin 12 and malaria. *Res Immunol* 1995; **146**: 552–559.
7 Trinchieri G. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistence and antigen-specific adaptative immunity. *Ann Rev Immunol* 1995; **12**: 251–276.
8 O'Garra A, Arai N. The molecular basis of T helper 1 and T helper 2 cell differentiation. *Trends Cell Biol* 2000; **10**: 542–550.
9 Philips RE, Pasvol G. Anemia of *plasmodium falciparum* malaria. *Baillieres Clin Haematol* 1992; **5**: 315–330.
10 Lee SH, Looareesuwan S, Chan J *et al.* Plasma macrophage colony-stimulating factor and P-selectin levels in malaria-associated thrombocytopenia. *Thromb Haemost* 1997; **77**: 289–293.
11 Schwarzer EF, Turrini F, Ulliers G, Giribaldi HG, Ginsburg H, Arese P. Impairment of macrophage functions after ingestion of *Plasmodium falciparum* infected erythrocytes or isolated malarial pigment. *J Exp Med* 1992; **173**: 1033–1041.
12 Scholfiewd L, Hackett F. Signal transduction in host cells by glycosylphosphatidylinositol toxin of malaria parasites. *J Exp Med* 1993; **177**: 145–153.
13 Mohan K, Sam H, Stevenson MM. Therapy with a combination of low doses of interleukin-12 and cloroquine completely cures blood-stage malaria, prevents severe anemia, and induces immunity to re-infection. *Infect Immun* 1999; **67**: 513–519.
14 Perkins DJ, Weimberg JB, Kremsner PG. Reduced interleukin-12 and transforming growth factor-beta 1 in severe childhood malaria: relationship of cytokine balance with disease severity. *J Infect Dis* 2000; **182**: 988–992.
15 Trinchieri G. IL-12 and its role in the generation of Th1 cells. *Immunol Today* 1993; **14**: 335–338.
16 Luty AJF, Perkins D, Lell B *et al.* Low interleukin-12 activity in severe *Plasmodium falciparum* malaria. *Infect Imm* 2000; **68**: 3909–3915.
17 Winkler S, Willheim M, Baier K *et al.* Frequency of cytokine producing T cells in patient of different age groups with *Plasmodium falciparum* malaria. *J Infect Dis* 1999; **179**: 209–216.

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