Parasite Immunology, 2002, **24**, 489–492

Research note

Plasma levels of interleukin-18 and interleukin-12 in *Plasmodium falciparum* malaria

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SUMMARY

Interleukin (IL)-18 produced primarily by mononuclear phagocytes synergizes with IL-12 for interferon-γ production from T, B and natural killer cells. It has been also demonstrated that, in Plasmodium falciparum *malaria, IL-18 could have an immunoregulatory function. The aim of this study was to detect the plasma levels of IL-12 and IL-18, using an enzyme-linked immunosorbent assay, in 105 African children affected by mild and severe* Plasmodium falciparum *malaria to correlate the production of these cytokines with the severity of the disease. The levels of IL-18 and IL-12 were higher (25·7 ± 7·6 pg/ml and 17·1 ± 7·8 pg/ml, respectively) in children with mild malaria than in children with a severe form of the disease (21·5 ± 10 pg/ml and 13·2 ± 5·5 pg/ml, respectively). A positive correlation was observed between IL-18 and IL-12. This finding suggests that the production of these two cytokines (IL-18 and IL 12) may be coregulated and both have an immunoregulatory effect on the immune response in* Plasmodium falciparum *infection.*

Keywords *Il-18*, *IL-12*, Plasmodium falciparum

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Received: 5 February 2002
Accepted for publication: 11 October 2002

RESEARCH NOTE

Malaria is one of the major causes of mortality in tropical countries. In sub-Saharan Africa, severe anaemia and cerebral malaria constitute the major cause of morbidity and mortality mostly in children under the age of 5 years (1,2). The *Plasmodium falciparum* infection includes an asymptomatic extraerythrocytic hepatic (sporozoite/liver) phase, followed by the intra-erythrocytic phase when infective merozoites, responsible for all symptoms and pathologies of malaria, invade circulating erythrocytes (3). The parasite induces a specific immune response, stimulating the release of cytokines from human peripheral blood mononuclear cells (4,5), which might have an important function in activating the host's macrophages, neutrophils, T cells and natural killer (NK) cells to react to the subsequent liver and blood stage parasite (6). The inflammatory response that is required to remove parasites leads to a considerable tissue damage, and the activation of phagocytes to kill intracellular or extracellular parasites requires the production of inflammatory cytokines, such as interleukin (IL)-1 and IL-6 (7), interferon (IFN)-γ (8) and tumour necrosis factor-α (7,9), which can cause systemic effects such as severe anaemia and cerebral malaria (10). The outcome of infection depends on a delicate balance between appropriate and inappropriate induction of these mediators. It has been shown that the protective immunity in malaria is mediated by a cascade of events that also involves IL-12 (11). It appears that early events in the cell-mediated immune response required for defence against malaria are initiated by the release of IL-12 from monocytes/macrophages, B cells and other cell types (11), and reveal a prognostic significance in malaria infection (12,13). There is evidence demonstrating that IL-12 shares some of its biological activities with IL-18, although the primary structure of the two

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cytokines does not show any homology (14). This cytokine has a wide range of immunoregulatory functions, including stimulating proliferation of activated T cells, enhancing NK cells activity and inducing type I cytokine response. Analysis of the amino acid sequence and structural motifs attributes IL-18 to the IL-1 family of cytokines (15) and, similar to IL-1, IL-18 has been shown to be processed by the interleukin-1 converting enzyme and the activity of mature IL-18 is closely related to that of IL-1β (14). In terms of its biological effects, IL-18 is related to and acts synergistically with IL-12. Recently Torre *et al*. (16) showed that IL-18 may have a pro-inflammatory role in patients with uncomplicated *P. falciparum*, but it had not been previously shown that IL-18 correlates with the severity of the disease. Because IL-18 seems to be functionally related to IL-12, we hypothesized that IL-18 also is able to modulate the evolution of parasitemia and to prevent the development of severe manifestations of malaria. The main goal of this study was to detect the plasma levels of IL-18 and IL-12 in 77 children with severe malaria and in 28 children with mild malaria to correlate the production of these cytokines with the severity of the disease. The children (55 males and 50 females) with acute *P. falciparum* malaria, median age 19·5 months (range 2–144 months) were observed during October 2000 at the Pediatric Dispensary of Centre Medical Saint Camille (Ouagadougou, Burkina Faso). Severe malaria (complicated) and mild malaria (uncomplicated) were established according the WHO criteria (17). Patients with 'severe malaria' or parasitemia $> 5 \times 10^5$ parasites/mm^3 and patients with 'mild malaria' and parasitemia $< 5 \times 10^5$/mm^3 were treated according the official therapeutic protocol of the local 'Programme National de Lute contre le Paludism'. A control group of 40 healthy children of the same range of age (median 22 months, range 10–100 months) were included in the study. The determination of IL-18 and IL-12 was measured using an enzyme-linked immunosorbent assay according to the manufacturer's protocol (R&D Systems, Milan, Italy). The lower limits of detection of the assay in malaria patients were 6·3 pg/ml and 7 pg/ml for IL-18 and IL-12, respectively. The upper limits in the healthy subjects were 4·8 pg/ml and 5·2 pg/ml for IL-18 and IL-12, respectively. Statistical significance was analysed using Student's two-tailed *t*-test and correlation coefficients were calculated using the SPSS statistical package (SPSS version 10·0, SPSS Inc., Chicago, IL, USA). A two-tailed $P < 0{\cdot}05$ was considered statistically significant. The mean values of the more important haematological parameters are reported in Table 1 according to the criteria of severity of disease (age, hyperparasitemia, anaemia, neurological involvement). IL-18 and IL-12 levels were found to be significantly elevated (25·7 ± 7·6 pg/ml) and (17·1 ± 7·8 pg/ml), respectively, in all the children with 'mild malaria' (P = 0·005). The levels of

Table 1 Hematological data for 77 children with severe malaria, 28 children with mild *Plasmodium falciparum* malaria and 40 healthy controls

Patients	Age (months)	Leukocyte (mm^3)	Red cells (mil/mm^3)	Hb (g/dl)	Ht (%)	MCV (U^3)	MCH (YY)	MCHC (%)	Platelets (mm^3)	IL-18 (pg/ml)	IL-12 (pg/ml)
Severe malaria (n = 77)	13 (2–72)	17·3 ± 12·5*	1·43 ± 0·64*	3·72 ± 1·26*	11·6 ± 3·9*	84·1 ± 18·7	27·8 ± 6·04**	32·56 ± 4·31*	157·76 ± 84·65*	21·5 ± 1^{a}*	13·2 ± 5·53^{a}*
Mild malaria (n = 28)	24 (9–144)	17·8 + 7·97*	3·23 ± 0·74*	8·28 ± 1·57*	24·9 ± 5·09	73·7 ± 13·3**	25·8 ± 4·63	33·28 ± 3·43**	234·84 ± 150·67*	25·7 ± 7^{b}*	17·1 ± 7·8^{b}*
Healthy controls (n = 40)	22 (10–100)	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	9·3 ± 3·5	7·6 ± 2·5

Patients versus healthy controls. *$P < 0{\cdot}001$; **$P < 0{\cdot}01$; superscript 'a' versus 'b' IL-18, $P = 0{\cdot}044$; IL-12, $P = 0{\cdot}005$.

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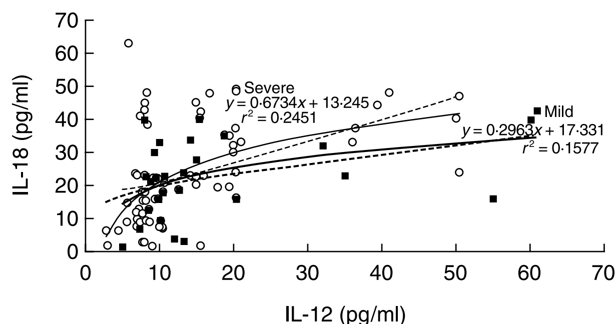

Figure 1 Correlation between plasma levels of IL-18 and IL-12 in the 105 children with*Plasmodium falciparum* malaria.

IL-18 and IL-12 were significantly lower (21·5 ± 10 pg/ml) and (13·2 ± 5·53 pg/ml), respectively, in 77 children affected by 'severe disease' (P = 0·044). The parasite density was higher (E_p 6·9 × 10^5 median, range 6–8) in children with 'severe malaria' who had lower IL-18 and IL-12 levels compared to children with 'mild malaria' (E_p 3·5 × 10^4 median, range 3–5).

Our results are consistent with a recent report that shows a significant increase in serum levels of IL-18 observed during the acute and the recovery phase of uncomplicated *P. falciparum* malaria, which reflect a pro-inflammatory role of IL-18 in these patients (16). Furthermore, in this study, we demonstrate that IL-18 correlates with the severity of the malaria disease. It is possible that in a very early phase of *P. falciparum* infection, the production of IL-12 is uncontrolled, but IL-18 balances this IL-12 increase (Figure 1). The correlation coefficients were r = 0·49 (P < 0·001) and 0·39 (P < 0·05), respectively, for severe and mild malaria This is consistent with a recent observation showing that IL-18 may be involved in the regulation of IL-12 production (18). IL-18 and IL-12 could have a critical role in the adaptive immune response to malaria through induction of IFN-γ, which has a central role in the cell-mediated immune response against blood-stage infection, inducing phagocytosis and killing. Moreover IL-12 and IL-18 synergistically induce anti-CD3 stimulated T cells or anti CD40-stimulated B cells to differentiate into highly IFN-γ producing cells (19). The molecular mechanism underlying the synergy between IL-18 and 12 may be explained in part by reciprocal modulation of cytokine receptor expression. Specifically, IL-18 has been demonstrated to up-regulate IL-12 R expression, while IL-12 has been shown to up-regulate expression of IL-18R (20). On the basis of these observations, we can conclude that, after interaction with *P. falciparum*, macrophages produce IL-12 and IL-18 that synergistically induces IFN-γ production. The amplification of the cytokine cascade may be beneficial for the host but can also be involved in the pathogenesis of malaria (6,21). This dual role is most apparent for the pro-inflammatory cytokines (6). In conclusion, these results provide evidence that IL-18 and IL-12 are up-regulated during acute malaria and play a role in the defence against *P. falciparum*, modulating the synthesis of inflammatory cytokines. Further studies are needed to establish their exact role in malaria infection.

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