

## Increased Levels of Soluble Fas Ligand in Serum in *Plasmodium falciparum* Malaria

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**Levels of soluble Fas ligand (sFasL) in serum were elevated in patients with *Plasmodium falciparum* malaria and showed a significant decline during disease course. sFasL levels that were elevated before antimalarial treatment began correlated significantly with depressed total lymphocyte and T-cell counts. These data suggest that Fas-induced apoptosis might play a role in malaria-associated lymphopenia.**

In human malaria, a decrease in the number of circulating lymphocytes, particularly of the T-cell population, is frequent (7, 17). However, the pathophysiology of this lymphopenia is still unclear. Recent studies have shown that the level of spontaneous apoptosis in peripheral blood mononuclear cells is increased during acute malaria attacks (2). This could be attributed to the parasite itself, since *Plasmodium falciparum* schizont-rich extract induces ex vivo apoptosis in peripheral blood mononuclear cells of affected patients (14). It is, therefore, assumed that decreased lymphocyte counts in malaria are caused by *P. falciparum*-induced apoptosis.

In this study, we investigated the possible involvement of the Fas-Fas ligand system in malaria-associated lymphopenia. We determined the level of soluble Fas ligand (sFasL) in the serum of 17 patients with acute *P. falciparum* malaria before the start of treatment and subsequently during disease course and compared it to that of age- and sex-matched healthy controls.

Serum samples of 17 patients with *P. falciparum* malaria (16 Europeans and 1 African immigrant who had lived in Germany for  $\geq 2$  years; 13 males and 4 females; mean age, 40 years; range, 16 to 61 years) who had already been subjects of a former study of tumor necrosis factor (TNF) receptors (6) were obtained at the Tropical Institute, Hamburg, Germany, in 1990. All subjects had contracted malaria while traveling in Africa. Malaria was diagnosed by examination of thick and thin blood films. Parasites in 2,000 erythrocytes were counted for the calculation of parasitemia. All patients received adequate antimalarial treatment. Five patients had to be classified as having severe malaria due to occurring complications: three suffered from impaired cerebral function (disorientation, drowsiness, and unconsciousness), one showed abnormal clotting tests (prothrombin time was  $< 50\%$  of the normal value and partial thromboplastin time was  $> 45$  s), and one showed severe anemia (hemoglobin was  $< 7.5$  g/dl).

Blood samples were taken on day 0 (before the onset of treatment), day 2, and day 7 of the disease. Serum samples were prepared immediately by centrifugation ( $2,000 \times g$ ) for 10 min, and aliquots were stored at  $-80^\circ\text{C}$  until quantification of sFasL by enzyme-linked immunosorbent assay (Diaclone Research, Besançon, France). Lymphocyte count and subpopulations in EDTA-anticoagulated blood were determined by two-color flow cytometry (FACStar; Becton Dickinson, Hei-

delberg, Germany). Samples of blood from 17 age- and sex-matched healthy donors (13 males and 4 females; mean age, 42 years; range, 18 to 61 years), obtained in 1999 from the German Red Cross Blood Center in Ulm, Germany, served as controls. Samples from healthy donors were handled similarly to the patients' samples, but four-color flow cytometry analysis was performed with FACS Calibur (Becton Dickinson). For

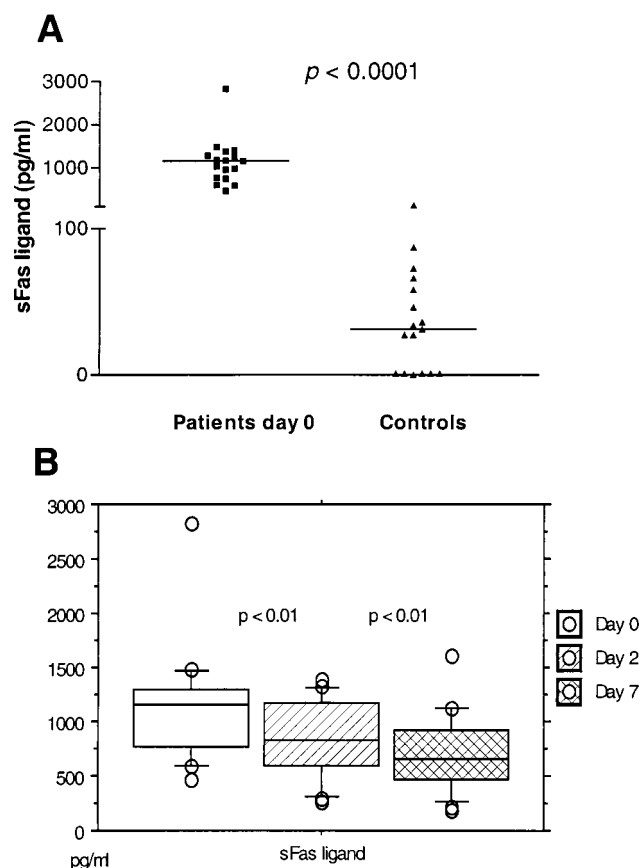


FIG. 1. (A) Levels of sFasL in serum (medians are indicated with horizontal lines) in 17 patients with acute malaria (day 0, before antimalarial treatment) in comparison to those in 17 healthy controls. (B) Levels of sFasL in serum during patients' disease course are shown. Box plots display the median and the 10th, 25th, 75th, and 90th percentiles as horizontal lines. Values outside the 10th and 90th percentiles are plotted as points (open circles).

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TABLE 1. Cell populations of the malaria patients during disease course

Cell type	Cells/ $\mu$ l (mean $\pm$ SD) on day <sup>a</sup> :			Correlation with sFasL level (day 0 only)	
	0	2	7	$\rho$	$P^b$
Leukocytes	5,965 $\pm$ 2,556	6,665 $\pm$ 3,974	7,047 $\pm$ 2,790	-0.41	NS
Total lymphocytes	830 $\pm$ 650 (15.6)	1,539 $\pm$ 696 (26.1)	2,185 $\pm$ 868 (32.2)	-0.59	0.02
T cells	604 $\pm$ 565 (11.4)	918 $\pm$ 496 (16.3)	1,490 $\pm$ 614 (22.1)	-0.62	0.01
B cells	71 $\pm$ 74 (1.2)	158 $\pm$ 265 (1.8)	186 $\pm$ 146 (2.6)	-0.41	NS
Monocytes	503 $\pm$ 319 (9.1)	600 $\pm$ 227 (10.2)	694 $\pm$ 372 (10.4)	-0.43	NS

<sup>a</sup> Values in parentheses represent mean percentages of total leukocyte counts.

<sup>b</sup> NS, not significant.

statistical interpretation, levels of significance were determined by nonparametric tests (Mann-Whitney rank sum U test and Wilcoxon signed-rank test, respectively). Spearman rank correlation coefficients ( $\rho$ ) between different parameters were calculated.

Samples from patients with acute *P. falciparum* malaria had significantly increased levels of sFasL in serum as compared to controls (mean, 1,132.8 pg/ml [day 0] versus 36.5 pg/ml;  $P < 0.0001$ ). During the disease course, sFasL levels significantly declined to a mean of 701.7 pg/ml on day 7 (day 0 versus day 2 and day 0 versus day 7, both  $P < 0.01$ ) (Fig. 1). This decline was associated with a normalization of the initially depressed total lymphocyte count, including T- and B-cell counts (Table 1). A significant negative correlation was found between initial sFasL levels on day 0 and both total lymphocyte and T-cell counts ( $\rho = -0.59$  and  $P = 0.02$  and  $\rho = -0.62$  and  $P = 0.01$ , respectively) but not the B-cell count (Table 1). The CD4/CD8 cell ratio did not change significantly during disease course, and there was no difference in the cell ratio between samples from the malaria patients and controls (data not shown). In contrast to lymphocyte count, total leukocyte and monocyte counts remained more or less stable during disease course (Table 1).

Lymphocyte apoptosis is a regulatory mechanism of the immune system used to eliminate expanded lymphocyte populations in order to maintain cellular homeostasis. On the other hand, apoptosis may also be triggered by infectious agents in order to weaken the immune response (1). Activation of Fas, a so-called death receptor belonging to the same family as TNF, by FasL plays a major role in the induction of lymphocyte apoptosis (10, 16). While Fas is expressed in a variety of epithelial and hematopoietic cells, FasL is predominantly found on activated T cells and natural killer cells. Membrane-bound FasL can be cleaved by metalloproteinases to produce the soluble form (13). Our study showed significantly increased levels of sFasL in the serum of patients suffering from acute malaria as compared to samples from healthy donors. The origin of the detected increased levels of sFasL is, however, not clear. On the one hand, it might have been shed by activated FasL-bearing lymphocytes. On the other hand, it could also originate from endothelial cells, since FasL expression on vascular endothelial cells has been reported recently (8). Since systemic endothelial activation is observed in both mild and severe malaria (15), enhanced shedding of FasL from activated vascular endothelium may also be assumed.

In the present study, a significant correlation between enhanced sFasL levels and a depressed lymphocyte count was found. In accordance with earlier studies reporting T-cell depletion but normal levels of B cells in the serum of patients with malaria (17, 18), a significant correlation of sFasL levels was found in our study only with T- but not B-cell counts.

These results suggest that Fas-induced apoptosis plays a role in the observed lymphopenia. However, although sFasL has been reported to induce apoptosis, there are also data reporting inhibition of membrane-bound FasL by sFasL (12, 13). Thus, by an increased shedding of FasL the apoptosis-inducing effect of membrane-bound FasL might be regulated and lymphopenia could even be limited. Interestingly, recent studies have shown that the activity of FasL is regulated by intracellular adhesion molecule 1 (ICAM-1) (11), which is also upregulated in malaria (3).

Since FasL has immunoregulatory functions and may even cause systemic tissue damage, like TNF (9, 13), it would be valuable to investigate a possible association between sFasL levels and the clinical status of the patients. However, we could include only three patients with severe malaria in our study and, therefore, significance of the data is very limited. Nevertheless, unlike TNF (4, 5) no correlation either with disease severity or with parasitemia was found (data not shown). This may be explained by the observation that, in contrast to TNF, Fas does not induce the activation of proinflammatory transcription factors such as NF- $\kappa$ B (10).

In summary, this study revealed increased levels of sFasL in the serum of patients with *P. falciparum* malaria which correlated significantly with depressed total lymphocyte and T-cell (CD3<sup>+</sup>) counts, suggesting that the Fas-FasL system plays a role in malaria-associated lymphopenia. Although functional data are still missing, these results provide the basis for further experimental and clinical studies addressing the mechanisms of lymphopenia in malaria.

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