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# Levels of Stem Cell Factor and Interleukin-3 in Serum in Acute *Plasmodium falciparum* Malaria

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**The purpose of the present study was to measure serum concentrations of stem cell factor (SCF) and interleukin-3 (IL-3) in patients with acute *Plasmodium falciparum* malaria. Serum samples from 15 patients were taken on day of admission and days 7, 14, 21, and 28. Anemia developed in 80% of patients. A transient increase in IL-3 could be observed at the beginning of the disease. It remains controversial whether the measured concentrations of IL-3 and SCF correlate with the grade of anemia. The possibly suppressed IL-3 and SCF production may contribute to the prolonged anemia in *P. falciparum* malaria, as has been shown for erythropoietin.**

The pathophysiologic backgrounds of anemia in malaria are complex and multifactorial (6, 7). Previous studies suggest that a depressed erythropoietic response contributes to the development of anemia (10). In a previous study we found inadequate erythropoietin production in patients with acute severe *Plasmodium falciparum* malaria (2).

Stem cell factor (SCF), also known as c-kit ligand, mast cell growth factor, and steel factor, is a recently identified pleiotropic growth factor that may participate in hematopoiesis (4). Interleukin-3 (IL-3) is a T-lymphocyte-derived, 28-kDa glycoprotein that supports the viability and differentiation of hematopoietic progenitor cells (8). It works as a hematopoietic growth factor, acting on pluripotent stem cells and leading to the differentiation and proliferation of early erythrocytes, neutrophils, eosinophils, basophils, macrophages, and megacaryocytes. It was the aim of this study to measure SCF and IL-3 concentrations in patients with acute *P. falciparum* infections and to evaluate the impact of both growth factors in the pathogenesis of anemia in these patients.

**Location of the study.** The study was performed at the Hospital for Tropical Diseases in Bangkok, Thailand. Patients with malaria met the following criteria: (i) they were between the ages of 15 and 65 years, (ii) their infection with *P. falciparum* was only during the study period, (iii) no application of chemotherapeutic drugs during the preceding 14 days had been made, and (iv) they had symptoms of severe and complicated malaria as defined by World Health Organization criteria (9). The study was approved by the Ethical Board of the Mahidol University, and all patients gave informed consent before enrolling in the study. Fifteen age- and sex-matched healthy volunteers were used as the negative control group.

**Treatment.** Malaria was treated with intravenously applied artesunate or artemisinin derivatives followed by mefloquine. The control group received no treatment.

**Investigations.** For routine examination of malaria patients prior to treatment, a medical history was obtained, a physical examination was performed, and laboratory studies were conducted. Daily clinical examinations were performed during the acute phase of the disease. All patients were observed for 28 days. Blood samples for determination of SCF and IL-3 concentrations were taken on days 0, 7, 14, 21, and 28.

**Laboratory examinations.** Routine examinations included erythrocyte count, hematocrit, leukocyte count, platelet count, serum electrolytes, total bilirubin, serum creatinine, and liver enzymes. Blood smears (thick and thin films) were obtained from finger pricks and stained with Giemsa stain, and parasite counts were performed (2). Blood smears were performed every 6 h from initiation of treatment until blood films were negative for two consecutive examinations; then smears were done daily.

Serum concentrations of SCF and IL-3 were measured by an enzyme-linked immunosorbent assay (ELISA) technique using commercially available kits (Quantikine; R&D Systems). In brief, these assays employ the quantitative sandwich enzyme immunoassay technique using monoclonal antibodies specific for SCF and IL-3.

**Statistical analysis.** Data were expressed as means and standard deviations and analyzed by analysis of variance (ANOVA) and the Tukey test. Spearmen correlation coefficients between different parameters were performed. $P$ values of $<0.05$ were considered statistically significant.

**Clinical findings.** The estimated mean duration of fever before admission into the hospital was 3.7 ± 1.8 days. The highest recorded temperature prior to treatment was 40.2°C. The median parasite count prior to treatment was 338,670 asexual parasites per μl (range, 533 to 1,780,020). The mean parasite clearance was 48.2 ± 19 h.

**Course of anemia.** Anemia, defined as a hematocrit of less than 35% (6), developed in 80% of patients. The mean value of hematocrit was 32.8 ± 9.65% on the day of admission, 31.85 ± 8.5% on day 7, 29 ± 3.29% on day 14, 31.14 ± 3.69% on day 21, and 33.3 ± 5.18% on day 28 (Table 1). The mean lowest hematocrit was 24.75 ± 6%, and the mean maximum fall was 10.18 ± 6.44%.

Serum SCF concentrations decreased from 1,571 ± 588 pg/ml on the day of admission to 1,443 ± 600 pg/ml on day 7 and 1,401 ± 464 pg/ml on day 14 and increased to 1,488 ± 414 pg/ml on day 21 and finally to 1,523 ± 330 pg/ml on day 28 (Table 1). No significance was determined in comparison with the control group (1,507 ± 360 pg/ml).

Serum IL-3 concentrations were highest on the day of ad-

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TABLE 1. Serum SCF,[a] IL-3,[a] and hematocrit of patients suffering from acute *P. falciparum* malaria[b]

| Patient | Day 0 | | | Day 7 | | | Day 14 | | | Day 21 | | | Day 28 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hct | SCF | IL-3 | Hct | SCF | IL-3 | Hct | SCF | IL-3 | Hct | SCF | IL-3 | Hct | SCF | IL-3 |
| 1 | 44 | 958 | 16.1 | 33 | 1,232 | ND[b] | 32 | 1,447 | ND | 28 | 1,538 | ND | 31 | 1,652 | 2.25 |
| 2 | 26 | 1,712 | 67.3 | 30 | 1,691 | 14.4 | 28 | 2,096 | 23.8 | 28 | 1,870 | 28 | 24 | 1,752 | ND |
| 3 | 37 | 2,624 | 12.8 | 38 | 2,720 | 6.0 | 23 | 1,973 | 4.2 | 38 | 1,262 | 31.2 | 29 | 1,442 | 14.4 |
| 4 | 31 | 1,279 | 42.7 | 28 | 1,473 | 41.3 | 30 | 1,963 | ND | 31 | 1,674 | 25.3 | 36 | 1,679 | 28.3 |
| 5 | 25 | 1,185 | 26.8 | 30 | 1,307 | 49.7 | 33 | 1,236 | 32.7 | 34 | 1,352 | 52.4 | 38 | 1,513 | 44.1 |
| 6 | 26 | 1,216 | ND | 30 | 1,125 | ND | 30 | 1,614 | ND | 29 | 1,645 | 22.2 | 36 | 1,090 | 32.7 |
| 7 | 15 | 1,151 | 17.6 | 32 | 918 | 23.8 | 30 | 900 | 26.8 | 30 | 1,199 | ND | 34 | 1,284 | 11.2 |
| 8 | 34 | 1,079 | ND | 30 | 855 | 21 | 31 | 834 | 20.7 | 34 | 894 | 16 | 40 | 1,183 | 16 |
| 9 | 46 | 1,096 | 39.8 | 40 | 791 | ND | 34 | 796 | ND | 32 | 1,048 | ND | 37 | 859 | ND |
| 10 | 47 | 1,267 | 21 | 37 | 1,267 | 4.2 | 33 | 1,342 | 20.7 | 36 | 1,537 | 29.7 | 40 | 1,406 | 2.3 |
| 11 | 31 | 2,860 | ND | 32 | 2,440 | ND | 28 | 1,958 | 6.1 | 24 | 1,924 | 11.2 | 29 | 1,904 | 4.2 |
| 12 | 40 | 1,792 | 116 | 32 | 2,150 | 38.4 | 30 | 809 | ND | 28 | 2,430 | 6 | 26 | NA[c] | NA |
| 13 | 18 | 1,470 | 23.8 | 34 | 962 | ND | 26 | 900 | 31.2 | 31 | 996 | 4.2 | 33 | 1,242 | 21 |
| 14 | 33 | 2,300 | 9.5 | 20 | NA | NA | 23 | 1,698 | 9.5 | 33 | 1,568 | ND | 29 | 1,985 | 4.2 |
| 15 | 39 | 1,585 | 12.8 | 35 | 1,274 | 6 | 31 | 1,302 | ND | 33 | 1,483 | ND | NA | NA | NA |

[a] In picograms per milliliter.
[b] Hct, hematocrit (percent); ND, not detectable; NA, not available.

mission (27.07 ± 30 pg/ml), decreased to 15.05 ± 17.11 pg/ml on day 7 and 10 ± 11.59 pg/ml on day 14, increased to 16.16 ± 15 pg/ml on day 21 and finally decreased to 13.68 ± 14 pg/ml on day 28 (Table 1). A significant increase was only calculated for the serum concentrations on the day of admission in comparison to control values (10 ± 5 pg/ml; $P < 0.05$).

A negative correlation was found between SCF and hematocrit on day 14 ($r = 0.5876$; $P = 0.0279$) and day 21 ($r = 0.6482$; $P = 0.0194$). No correlation was calculated for the following parameters (on day of admission): IL-3 versus SCF, IL-3 versus hematocrit, SCF versus hematocrit, IL-3 versus parasite counts, and SCF versus parasite counts.

The hemopoietic and immune systems are known to be interrelated via a network of growth factors and cytokines regulating the proliferation and differentiation of cells. Several of these factors have been shown to inhibit erythropoiesis in vitro. These include IL-1, gamma interferon, macrophage inflammatory protein-1-alpha (MIP-1-alpha) (2), and tumor necrosis factor alpha (TNF-α). TNF-α has also been shown to inhibit erythropoiesis in vivo.

We wanted to study whether SCF and IL-3, both growth factors, could be involved in the pathogenesis of anemia in patients infected with *P. falciparum* malaria. SCF, also known as c-kit ligand, mast cell growth factor, and steel factor, is a recently identified pleiotropic growth factor that may participate in the development of early stages of hematopoiesis (4). It has been demonstrated to be a potent regulator of hematopoietic progenitor cell proliferation. SCF is produced by bone marrow stromal cells, fibroblasts, endothelial cells, and hepatocytes. In both human and mouse hematopoiesis, SCF acts in a synergistic manner with various growth factors such as IL-3 and erythropoietin (5).

SCF has the broadest target specificity of any of the haematopoietic growth factors. This supports the hypothesis of hierarchy of action on growth factors in hematopoietic development with IL-3 activity on early multipotential progenitors and erythropoietin on later progenitors. Recent publications have reported the potential use of IL-3 in the treatment of aplastic or other anemia. IL-3 may also act as a functional regulator of mature eosinophils and monocytes. Normally IL-3 is undetectable in the blood of healthy animals. In addition, it could be demonstrated that IL-3 induces antimicrobial and tumoricidal activities (3).

In the present study we found no significant difference in serum levels of SCF measured in patients infected with *P. falciparum* malaria and those of the control group. A negative correlation was calculated for SCF and hematocrit on days 14 and 21. However, it seems that serum SCF concentrations are not mainly influenced during and after *P. falciparum* infection. With regard to IL-3, significantly higher concentrations were determined on the day of admission for infected patients than were determined for the control group. This may be considered a response to anemia. Because SCF and IL-3 are growth factors involved in hematopoiesis, we would expect an anemia-induced increase of both factors. However, reviewing the recent literature, we could not find any data demonstrating increased serum concentrations of both factors in anemia. Additionally, interpretation of these values is difficult because serum concentrations of SCF and IL-3 prior to *P. falciparum* infection were not available.

In conclusion, we found transiently elevated IL-3 serum levels in patients with anemia suffering from acute *P. falciparum* malaria. Because of the prolonged anemia observed in these patients, it is not clear whether this IL-3 increase might be interpreted as an inadequate response to anemia, as with erythropoietin production. The therapeutical effectiveness of both growth factors in the treatment of prolonged anemia in patients suffering from acute *P. falciparum* malaria remains to be the subject of further clinical studies. The high costs of such treatment will limit its use in developing countries.

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## REFERENCES

1. **Burgmann, H., U. Hollenstein, C. Wenisch, F. Thalhammer, S. Looareesuwan, and W. Graninger.** 1995. Serum concentrations of MIP-1 alpha and interleukin-8 in patients suffering from acute *Plasmodium falciparum* malaria. Clin. Immunol. Immunopathol. **76:**32–36.
2. **Burgmann, H., S. Looareesuwan, S. Kapiotis, C. Viravan, S. Vanijanonta, U. Hollenstein, E. Wiesinger, E. Presterl, S. Winkler, and W. Graninger.** 1996. Serum levels of erythropoietin in acute Plasmodium falciparum malaria. Am. J. Trop. Med. Hyg. **54:**280–283.
3. **Hatzigeorgiou, D. E., S. He, J. Sobel, K. H. Grabstein, A. Hafner, and J. L. Ho.** 1993. IL-6 down-modulates the cytokine-enhanced antileishmanial activity in human macrophages. J. Immunol. **151:**3682–3692.
4. **Langley, K. E., L. G. Bennett, J. Wypych, S. A. Yancik, X. D. Liu, K. R. Westcott, D. G. Chang, K. A. Smith, and Z. M. Zsebo.** 1993. Soluble stem cell

factor in human serum. Blood **81:**656–660.
5. **McNiece, I. K., K. E. Langley, and K. M. Zsebo.** 1991. Recombinant human stem cell factor synergises with GM-CSF, G-CSF, IL-3 and epo to stimulate human progenitor cells of the myeloid and erythroid lineages. Exp. Hematol. **19:**226–231.
6. **Phillips, R. E., S. Looareesuwan, D. A. Warrell, S. H. Lee, J. Karbwang, M. J. Warrell, N. J. White, C. Swasdichai, and D. J. Weatherall.** 1986. The importance of anaemia in cerebral and uncomplicated falciparum malaria: role of complications, dyserythropoiesis and iron sequestration. Q. J. Med. **58:** 305–323.
7. **Phillips, R. E., and G. Pasvol.** 1992. Anaemia of Plasmodium falciparum malaria. Bailliere's Clin. Haematol. **5:**315–330.
8. **Sieff, C., and E. Guinan.** 1993. In vitro enhancement of erythropoiesis by steel factor in Diamond-Blackfan anemia and treatment of other congenital cytopenias with recombinant interleukin 3/granulocyte-macrophage colony stimulating factor. Stem Cells (Dayton) **11**(Suppl. 2)**:**113–122.
9. **Warrell, D. A., M. E. Molyneux, and P. F. Beales.** 1990. Severe and complicated malaria. Trans. R. Soc. Trop. Med. Hyg. **84**(Suppl. 2)**:**1–65.
10. **Woodruff, A. W., V. E. Ansdell, and L. E. Pettitt.** 1979. Cause of anaemia in malaria. Lancet **i:**1055–1057.