

Possible role of granulocyte macrophage colony stimulating factor receptor (GM-CSF R) in malaria

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Malaria has been reportedly increasing in incidence on the globe. Evidence from clinical studies supports a role for cytokines in the pathogenesis of cerebral malaria. Given the stimulatory effect of the ligand GM-CSF on the synthesis and release of the pyrogenic cytokine TNF alpha, the present study has been undertaken to investigate a possible role of GM-CSF receptor in the pathogenesis of both *Plasmodium vivax* and *Plasmodium falciparum* malaria. An enzyme immunoassay developed by us at our laboratory for the quantitation of GM-CSF receptor has been used. No changes in the concentration of the receptor have been indicated either at the time of diagnosis or after treatment. In addition, an intercomparison of the receptor concentration between the *P. vivax* and *P. falciparum* groups does not show any significant difference. The results suggest that GM-CSF receptor has no significant role in the pathogenesis of either type of malaria.

Malaria has been steadily increasing worldwide in the recent years. About 40% of the world's population is at a risk of acquiring malaria. The epidemiology of *Plasmodium vivax* and *Plasmodium falciparum* has been well documented. It is estimated that 2.7 million deaths occur every year due to malaria¹. The two forms of malaria that commonly occur in the coastal Karnataka, India are those caused by *Plasmodium vivax* and *Plasmodium falciparum*. A number of factors contribute to the pathophysiology and ensuing complications in malaria. Many host receptors have now been identified which allow parasite binding *in vitro*. Cytokines receptors have been inducted as those, which allow the sequestration of the parasite leading to cerebral malaria, a largely fatal condition, particularly in the pediatric age group. The initial clinical picture of vivax and falciparum malaria is almost identical, but if left untreated, falciparum may become life threatening with multiple organ involvement, while vivax runs a more benign course². In falciparum (but not vivax) malaria, sequestered parasitised red blood cells accumulate in small blood vessels, reducing local blood flow and so cause ischaemic hypoxia^{3,4}. A number of different host receptors have now been identified which allow parasite binding *in vitro*. It has been shown that several of these receptors, especially intercellular

adhesion molecule-1 (ICAM-1), are involved in mediating sequestration in the brain during fatal cerebral malaria⁵. It has been shown that although sequestration always occurs in the brain in cerebral malaria, it can happen in patients who do not suffer coma, and is thus necessary, but not solely sufficient, to cause coma in malaria. Various groups are now looking at other pathophysiological mechanisms, which may contribute to coma and brain dysfunction during cerebral malaria, including disruption of the blood brain barrier, local cytokine release and neurotoxicity. Simultaneous release of the pyrogenic cytokines TNF alpha and consequently IL-1, IL-6 and IL-8 have been reported as the cause of the rise in body temperature⁶. Of particular interest has been the increase in TNF alpha⁷. Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) is a cytokine involved in promotion of proliferation among haemopoietic progenitors⁸. Increased serum levels of GM-CSF have been associated with a rise in body temperature. The molecule has been shown to induce the synthesis of TNF alpha and IL-1⁹. The molecule is known to mediate its effects through a distinct receptor known as the GM-CSF receptor (GM-CSF R), which occurs on monocytes, neutrophils and eosinophils^{10,12}. The role of the GM-CSF receptor (CD 116) has not yet been fully understood. Keeping in mind the stimulatory effect of GM-CSF on the synthesis of TNF alpha we embarked to investigate the role of GM-CSF receptor in the pathophysiology of malaria.

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Fifty patients, 25 each suffering from *Plasmodium vivax* and *Plasmodium falciparum* malaria who tested positive for the respective parasite on both the fluorescent quantitative buffy coat (QBC) and smears demonstrating the presence of asexual forms at our hospital were selected as the study group. The age group selected for the study was in the range 5-30 years. None of our subjects acquired complications owing to voluntarily prompted early detection, given the endemicity of malaria in the population here. Twenty five subjects visiting the hospital with fever but negative for malarial parasite by smear and QBC were selected as controls for the study. Ten normal subjects were selected as normal controls. The fever controls received standard anti-pyretic therapy. Regression of fever was taken as the point of cessation of treatment in this group. The malaria affected subjects received standard treatment following National Anti-Malarial Programme. The treatment schedule for *P. vivax* infection consisted of 1500 mg chloroquine (600 mg day 1, 600 mg day 2 and 300 mg day 3) and 15 mg primaquine for 5 days starting day 1 along with Chloroquine. For *P. falciparum* infection, the same dosage of chloroquine was followed, with primaquine at 45 mg on day 1. After the anti-malarial therapy, the subjects were subjected to QBC and smear examinations and the absence of asexual forms of the parasite was ascertained. Blood samples drawn at this stage were used as the samples to assess the concentration of the GM-CSF receptor after treatment. Ten ml blood collected under strict aseptic conditions was centrifuged and the buffy coat used as a source of neutrophils. Neutrophils were isolated by hypotonic lysis of contaminating erythrocytes and centrifugation at 4°C and stored frozen in a modified Dulbecco's Phosphate buffered saline (DPBS). The cell suspension was then thawed and cell count determined by method of turbidimetry and fixed at 10^2 cells per ml. The standardized cell suspensions were then subjected to membrane solubilization of the receptor¹³. The supernatant thus obtained was then used for enzyme immunoassay (EIA) for quantification of GM-CSF R. We developed the EIA at our laboratory using Monoclonal antibody against GM-CSF R (Serotec, UK). The microwell plates were procured from NUNC-Nalgen International. The developed EIA was standardized against a commercial kit procured from Immunotec, France. Specificity of the developed Enzyme immunoassay was determined to be 96.3 % and Sensitivity 92%.

Statistical analysis was performed using the Student's *t* test with the SPSS software Version 7.5.

The results (Table 1) indicate a minor increase in the receptor concentration in the fever controls when compared with the normal subjects. However, no significant increase has been noted in the comparison between the fever controls and either type of malaria. Also an inter-comparison between the *P. vivax* and *P. falciparum* groups indicates no significant change. A comparison between the fever controls after treatment and normal subjects indicates a small decline in the receptor concentration. However, no significant difference was observed between the *P. vivax* and *P. falciparum* groups.

There has been compelling evidence that pro-inflammatory cytokines mediate cerebral dysfunction in murine cerebral malaria, although it has been accepted that this model differs somewhat from the human disease⁷. In addition to systemic production, local cytokine release could contribute to organ specific pathophysiology¹⁴. Many studies have concentrated on determination of cytokine levels in the cerebrospinal fluid or have used animal models¹⁴. TGF beta protein found in hemorrhagic white matter lesions is associated with neurodegenerative lesions in the CNS, where it acts as an anti-inflammatory and neuroprotective cytokine¹⁵. TNF alpha protein has been detected within the brain parenchyma in meningitis and cerebral malaria¹⁶. Immunohistochemistry shows that a large number of possible sequestration receptors are expressed on brain endothelium during cerebral malaria. However, these receptors are also expressed in other tissues, and not just in severe malaria but mild malaria and other septic conditions. This implies that other factors influence receptor mediated parasite sequestration, not just the pattern of expression of

Table 1 — GM-CSF receptor concentrations at diagnosis and after treatment
[Values, expressed in pg/ml, are mean \pm SD]

Group	
At diagnosis	
Normals (n=10)	50.18 \pm 6.16
Fever (n=25)	51.84 \pm 5.73
<i>P. vivax</i> (n=25)	51.19 \pm 5.05
<i>P. falciparum</i> (n=25)	52.07 \pm 4.86
After treatment	
Normals (n=10)	50.18 \pm 5.86
Fever (n=25)	48.71 \pm 5.13
<i>P. vivax</i> (n=25)	50.08 \pm 5.05
<i>P. falciparum</i> (n=25)	51.27 \pm 4.67

these receptors in the host microvasculature. GM-CSF has been shown to induce the synthesis and release of cytokines such as IL-1 and TNF⁹. The rise in the body temperature on an increase in the serum levels of GM-CSF may be the direct effect or due to the release of mediators such as IL-1 or TNF alpha.

The results of the present study do not support any significant role for the receptor in either form of malaria. However, similar studies on the cerebrospinal spinal fluid may provide valuable information about the role of the receptor, if any. In the wake of GM-CSF being attempted as an adjuvant to malaria vaccines the study assumes more significance.

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