

CEREBRAL MALARIA II. THE ROLE OF TUMOR NECROSIS FACTOR IN ITS PATHOGENESIS

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ABSTRACT

Cerebral malaria (CM), a complication found in *Plasmodium falciparum* infection, is characterized by the presence of coma. It can be accompanied by hypoglycemia and lactic acidosis, that comprise major cause of death in 10-50% of CM cases. Conventional explanation that coma is due to obstruction of small blood vessels by trapping of parasitized red blood cells, that ends in ischemia and anoxia, does not fit to the facts that when the patients survived, only few sequels left and most of the sequels were transient. The first part of the literature review (Cerebral Malaria I) concluded that sequestration of red blood cells bearing old stages of *P. falciparum* in the vessels in the deep organs is started by cytoadherence of p-rbc to the endothelial cells lining blood vessels that involves the role of adhesion molecules. The expression of adhesion molecules on the surface of endothelial cells is stimulated by parasite antigen and up-regulated by cytokines especially TNF- α . Many reports have associated TNF- α with symptoms of malaria. Paroxysms or periodic attacks of fever in malaria was associated with schizont rupture that stimulated production of TNF- α , an endogenic pyrogen. And TNF- α overproduction was implicated with symptoms of severe malaria, including CM. The association has been confirmed by plenty of clinical data and TNF- α was also found in brain tissue from biopsy materials. CD4+T (Th1) cells, which secretes IFN- γ , is needed to amplify and optimize TNF production. The role of TNF- α , TNFR and CD4+T cells was confirmed by genetic findings and immunoepidemiologic studies. Some investigators proposed the role of NO in CM. Local NO overproduction, associated with the effects of TNF- α , IFN- γ , malaria antigen and partial hypoxia caused by sequestration, was supposed to interfere normal neurotransmission leading to altered consciousness and coma. This NO theory can explain the occurrence of transient coma and minimal sequelae when patients recovered. While severe hypoglycemia, lactic acidosis, anoxia and ischemia caused by complete occlusion of local blood vessels can be associated with fatal outcomes and permanent organ dysfunctions. Analysis of parasite antigen which stimulates TNF- α production and to use the antigen to stimulate production of blocking antibody could be one way to cope the problem of CM and other symptoms of severe malaria. Trials and research on the use of anti-TNF antibody, corticosteroids and pentoxifylline, and analysis of the effects of conventional antimalarials on TNF- α and NO production has been done, but the results were still inconclusive. The possible use of PfEMP1 and other parasite ligands involved in cytoadherence has been written in the first part of review. Further research and efforts are still needed until the results can be used in practice.

Keywords: cerebral malaria, TNF α , CD4+T, adhesion molecules, nitric oxide

INTRODUCTION

Cerebral malaria (CM) is the most severe complication of *Plasmodium falciparum* infection and one of the major causes of mortality among children in malaria endemic areas. Major clinical symptom of CM is disturbance of consciousness from mild form to deep, unrousable coma. This symptom can be accompanied by other symptoms like hypoglycemia and lactic acidosis, that comprise the major cause of death in CM patients. Fatal outcome could reach 10-50%, but if the patients survived, only few sequels left and most of the sequelae were transient (Molineux, *et al.*, 1989; Polder, *et al.*, 1989; Melancon-Kaplan, *et al.*, 1993). Conventional theory that the coma in CM is the results of obstruction of small blood vessels that leads to local anoxia and ischemia (Aikawa, 1988; Warrell, 1990), does not fit to the fact that not much residual neurological deficit could be found in patients recovered from CM.

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The first part of the literature review (Cerebral Malaria I) concluded the essential role of adhesion molecules in starting sequestration of red blood cells bearing old stages of *P. falciparum* in the vessels in the deep organs. The expression of adhesion molecules on the surface of endothelial cells is stimulated by parasite antigen and up-regulated by cytokines especially TNF- α . This second part of paper reviews and discusses further the findings and theories concerning the pathophysiology of CM that, surprisingly, mostly concerns the role of TNF- α .

THE ROLE OF CYTOKINES IN MALARIA

As already mentioned in the first part of the review (Sri Hidajati, 2003), that typical symptoms for malaria like periodic fever attacks preceded by chills and rigors can be correlated with the erythrocytic cycle of malaria parasites. The burst of schizonts at the end of the erythrocytic cycles, stimulates the adjacent phagocytic cells to produce TNF- α , IL-1, IL-6 and other inflammatory mediators (Kwiatkowski, *et al.*, 1989). It has been noted that TNF levels correlates with body

temperature and high admission levels of TNF- α dropped after 24 hours when the disease subsides (Grau, *et al.*, 1989d).

As the key molecules in cytokine network in non-adaptive immune response (Janeway, *et al.*, 1999), TNF- α has many beneficial effects in malaria. Kremsner and his colleagues reported that elevation of TNF- α level in blood shows activation of cellular immune response (Kremsner, *et al.*, 1989) and elevated capacity of TNF- α production demonstrated by patient's peripheral blood leucocytes can predict accelerated cure in *falciparum* malaria (Kremsner, *et al.*, 1995; Mordmuller, *et al.*, 1997). TNF- α and IFN- γ increase the phagocytic capacity and killing of *P. falciparum* merozoites by neutrophils (Kumaratilake, *et al.*, 1992). And long time ago, Taliafero had reported that blood smears prepared when the patients were "in crisis", showed special necrotic form of intraerythrocytic parasite, the so called "crisis form" (Taliafero, 1938 cited in Clark, 1987). It brought researchers to the idea that TNF- α , or products associated with TNF- α (possibly oxygen radical – Clark, 1988) can kill intracellular parasites; this was confirmed further by Karunaweera, *et al.*, (1992). Like in other infectious diseases, TNF- α and related molecules function in the containment of infectious agents from spreading to other part of body (Janeway, 1999). In malaria, especially in *P. falciparum* infection, this results in sequestration of p-rbc, although it gives beneficial effects to the parasites in that they can evade the immune clearance of the spleen and on the other hand gives pathological effects to the hosts as will discussed in this paper.

Numerous papers pointed that excessive production of TNF- α could be implicated in the symptoms of severe malaria. The following clinical evidence demonstrated the association of TNF- α and cerebral malaria. Kwiatkowski, *et al.*, (1990) reported in his study with a large number of patients in the Gambia, that TNF increased in all malaria patients; the mean plasma levels of TNF in children with CM were twice higher compared to uncomplicated malaria and ten times higher in CM fatal cases. Correlation of TNF- α and severe malaria, was also reported by other researchers (Grau, *et al.*, 1987; Grau, *et al.*, 1989d; Grau, 1992; Kern, *et al.*, 1992; Deloron, *et al.*, 1994; Baptista, *et al.*, 1997). And a factor which also contributes to the level of TNF- α production, is the strain of malaria parasite (Allan, *et al.*, 1993 and 1995).

As already mentioned in the first review, TNF- α and IFN- γ are involved in the expression of adhesion molecules on the endothelial cells lining small blood

vessels that serve as attachments for the p-rbc in malaria. TNF- α has also been reported to stimulate recruitment and activation of macrophages and other cells to the affected part and amplify its own production on the same macrophages and the adjacent ones (Grau, and Behr, 1995; Janeway, 1999). In situ hybridization and immuno-histochemistry in experimental mice showed that TNF- α mRNA and TNF- α was found in microglia, astrocytes, monocytes and cerebral vascular endothelium several days before cerebral symptoms were observed (Medana, 1997). TNF- α and IFN- γ were also found in brain sections of humans died from CM (Udomsangpetch, 1997). Experiments by Hanum (2003) showed early chemokine (IFN- γ and TNF- α) expression in the brain parenchyma.

Other inflammatory cytokines like IL-1, IL-2, IL-6, IL-8 showed no direct correlation with CM (Kern, *et al.*, 1989; Ringwald, *et al.*, 1993; Kossodo, 1997; Ho, *et al.*, 1998). While anti-inflammatory cytokines like IL-10 and TGF- β were reported to inhibit production of TNF, IL-1, and IL-6. The use of exogenous IL-10 in experimentally infected mice showed reduced accumulation of mRNA of those cytokines (Ho, *et al.*, 1998). Mice deficient of IL-10 exhibit more severe malaria than control mice and showed higher mortality rate (Li, *et al.*, 2003).

THE ROLE OF NITROGEN INTERMEDIATES, HYPOGLYCEMIA AND LACTIC ACIDOSIS IN CM

Clark *et al.*, (1991), learning from the minimal sequelae found when CM patients recovered, proposed the role of NO in the pathogenesis of coma. As already known, NO is produced from L-Arginine under the catalysis of NOS (nitric oxide synthase) (Moncada, *et al.*, 1991). NO has many physiological functions. In the nervous system, NO produced by the neurons catalyzed by nNOS (constitutive neuronal NO synthase) acts as a postsynaptic retrograde messenger in neurotransmission (Lancaster, 1992). This physiologic process is very well controlled, like the work of EDRF in the physiology of blood vessel. In infections like malaria, NO participate in killing the innocuous agent. TNF, IFN- γ , and IL-1, together with malaria antigen released from p-rbc at the time when schizonts burst, could induce excessive NO production from adjacent endothelial and vascular smooth muscle cells, also from recruited leucocytes through induction of iNOS (inducible NO synthase) (Clark, *et al.*, 1991 and 1995; Rockett, *et al.*, 1991; Jacobs, *et al.*, 1996). As a very small molecule, the extraneuronal-produced NO will diffuse and disturb neurotransmission, by intervening control mechanism in normal/neuronal NO production and making the neurons refractory, resulting in coma. Altered blood

flow which cause partial hypoxia as mentioned above, could also induce the activity of iNOS (Moncada, *et al.*, 1991; Lancaster, 1992; Dondorp, *et al.*, 2000).

But Clark's theory was disputed by Kremsner, *et al.*, (1993), that proved that TNF- α and IFN- γ did induce NO production, but administration of NO-synthase competitive inhibitor like L-NMMA (N^G-monomethyl-L-Arginine), did not prevent cerebral malaria in mice infected with a CM-inducing parasite. He had the opinion that NO has more protective than pathologic effects. However, Ghigo *et al.*, in 1995 stood still on Clark's opinion and proved that p-rbc posses their own NOS and have the capability to produce high level of NO, even higher than did the endothelial cells. Ghigo found that the production of NO by p-rbc works through another pathway that could not be interfered by L-NMMA. Furthermore, the increased production of NO in the brain could have resulted in the depletion of arginine in the plasma as cited by Pearson and Shaw (2003).

The NO hypothesis could explain the occurrence of transient coma and the absence or limited level of neurological sequelae found upon recovery, which could not be explained by ischemia or blood flow cessation theory. As has been reported, TNF- α could be detected in the plasma only in a few ours after schizogony (Clark and Cowden, 1990). It could be associated with its brief half-life (circa 6-20 minutes) and fast clearance (Beutler and Cerami, 1990; Waage, 1997) and no more stimulants when all merozoites had invaded new rbc.

While ischemia (if the occlusion is complete) and severe hypoglycemia that cause neuronal death associated with glutamate and cGMP accumulations, could be postulated for fatal outcomes and permanent defects (Clark and Cowden, 1999) in few other cases. Hypoglycemia can be directly stimulated by malaria antigen, as the result of TNF effect which increases cellular glucose uptake and consumption, and the results of hyper-insulinism related to Quinine and Quinidine treatment (Jakobsen, 1995; Lang, 1995). Accumulation of lactic acid caused by hypoxic anaerobic glycolysis could worsen the situation that lead to death in severe cases (Molineux, *et al.*, 1989)

THE NEED OF CD4+T CELLS AND ITS PRODUCTS IN THE DEVELOPMENT OF CM

It has been noted since more than two decades ago that the development of severe *falciparum* malaria esp. CM

needs the presence of fully functional immune system which was later identified as the presence of mature CD4+ T cells (Clark, 1987).

Grau, *et al.*, (1989b) also noted that normal mice infected with similar parasite died earlier from CM when transfused with T cells from mice with CM. And CM could not be found in athymic mice, thymectomized mice reconstituted with T-cell depleted bone marrow, mice treated with monoclonal antibody against CD4+T cells (Grau, *et al.*, 1990) and mice injected with anti-CD4+ (Hermsen, *et al.*, 1997b).

Cytokines produced by CD4+ T cells like IFN- γ , IL-3 and GM-CSF were confirmed to be involved in the development of CM. IL-3 and GM-CSF were suspected to have the role in enhancing proliferation and recruitment of monocytes from the bone marrow, while IFN- γ activates those cells to synthesize TNF- α . Antibodies against those cytokines abrogate the occurrence of CM (Grau, *et al.*, 1988 and 1989a).

IFN- γ increases the sensitivity of macrophages to antigen stimulation by increasing transcription of TNF- α gene as shown by the increase of TNF- α mRNA. It also makes TNF- α mRNA more stable by inhibiting the generation of a short-lived repressor. IFN- γ also enhances expression of TNF-receptors (Farrar and Schreiber, 1993). IFN- γ enhances the expression of ICAM-1. Those effects are related to the role of T cells because animals deficient in T cells exhibit no sequestration, neither do splenectomized mice (Berendt 1992). Grau, *et al.*, (1989a) reported that administration of neutralizing moAb to IFN- γ , prevented the rise of TNF level, the plugging of brain vessels of mice by monocytes and lymphocytes (in human CM: less leucocytes were found), and parasitized erythrocytes, and prevented mice from death. Mice deficient of IFN- γ receptor had a reduced serum TNF level, reduced IL-12 expression, and increased Th2 cytokine production (Rudin, *et al.*, 1997a). Riley (1999) showed that in primary infections, only small amount of IFN- γ was produced by NK cells stimulated by IL-12 coming from macrophages. In advanced process, activated $\alpha\beta$ T cells produce IFN- γ and stimulate $\gamma\delta$ T cells to synergize in further IFN- γ production. And IFN- γ synergizes with malaria antigen and TNF- α in the induction of NO production (Kremsner, *et al.*, 1993). Such role of TNF- α and IFN- γ on NO production in malaria is similar with their role in endotoxemia as shown by Roitt (1994).

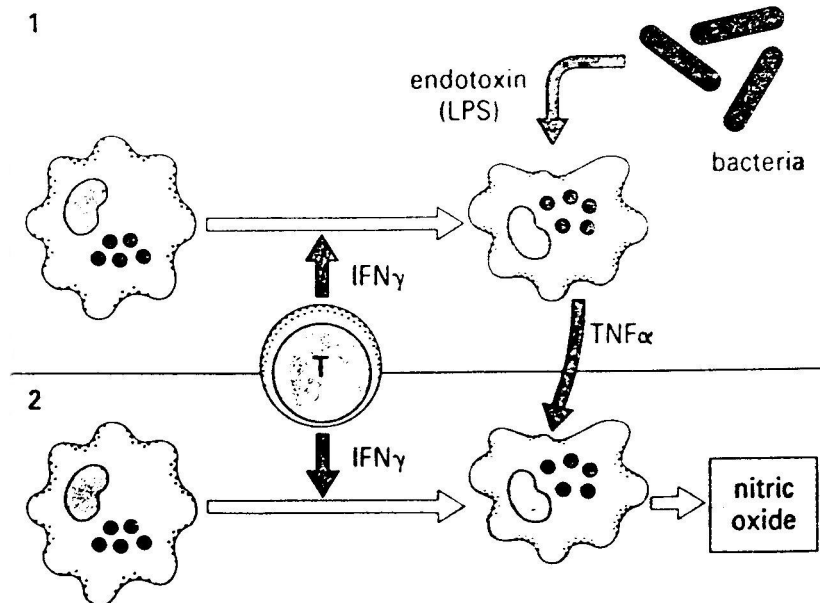


Figure 1. The role of TNF- α and IFN- γ on nitric oxide production in endotoxemia as figured out by Roitt (Roitt, 1994, Figure 9.16)

IFN- γ is not obviously increased in patient's sera (Baptista, 1997). Similar finding was noted with TNF by Hermesen, *et al.*, (1997a) in mice; they proved that the level of circulating TNF- α has no correlation with development of CM. This is parallel with Clark's review that locally produced TNF- α by sequestered macrophages was more important than systemic TNF in the development of cerebral malaria (Clark and Cowden, 1990).

THE ROLE OF TNF RECEPTORS IN THE PATHOGENESIS OF CM

TNF exerts its effects through TNF-receptors expressed on the surface of target cells. There are two kinds of TNF receptors, i.e. TNFR1 (= p55) and TNFR2 (= p75) (Higuchi and Aggarwal, 1994; Roitt, 1996). It was noted that TNFR2 is more involved in the CM syndrome (Lou, *et al.*, 2001). Mice genetically deficient of TNFR2 were protected from CM, and showed no elevation of TNF and IFN- γ , no up-regulation of ICAM-1 expression and no sequestration of p-rbc (Lucas, *et al.*, 1997a and 1997b). Receptors can be cleaved from the surface of cells and shed into the circulation as soluble receptors. High levels of serum TNF, TNFR1 and TNFR2 were found in CM and other forms of severe malaria. Hermesen, *et al.* (1997a) found that high serum level of soluble TNFR could neutralize the effects of TNF. This is in agreement with the

observation of Deloron, *et al.* (1994) that receptor levels persist longer in immune patients.

THE ROLE OF TNF IN CM WAS CONFIRMED BY GENETIC FINDINGS

A homozygous variant of the TNF- α gene promoter called TNF-2, which is characterized by high TNF- α production, was associated with susceptibility to CM (McGuire, *et al.*, 1994) and TNF2 deficient mice did not show any of the pathological signs found in CM (Wilson, *et al.*, 1997). A similar role was described for the gene for TNF receptors as mentioned previously (Lucas, *et al.*, 1997a and 1997b). Rudin, *et al.*, (1997b) also reported that genetically TNF-alpha/beta-deficient mice were completely protected from cerebral malaria. While none of HLA-B70, B50, Cw2, DRB1*13.02 and DRB1*11.01 were associated with susceptibility to CM, but HLA-B53 is protective against CM, equally strong for homozygous and heterozygous ones (McGuire, *et al.*, 1994).

IMMUNOEPIDEMIOLOGICAL STUDY ALSO SUPPORT THE ROLE OF TNF- α AND CD4+T CELLS IN CM

In highly endemic areas, the frequency of CM is comparatively higher in children than in adults. Humoral immunity which develops slowly in malaria in

general, related with declining prevalence of CM (Druilhe and Khusmith, 1987; Grau, *et al.*, 1989b). It was confirmed experimentally that TNF- α level is lower in immunized volunteers than non immunized ones (Harpaz, *et al.*, 1992). CM frequency is higher among visitors from non endemic areas than in residents of endemic areas (Grau, *et al.*, 1989b). While among visitors, CM were mostly found in adults (Baird, *et al.*, 1998), supporting hypothesis that development of CM needs maturity of immune response and pointed the role of CD4+Th1 cells (Riley, 1999). Riley mentioned

further that maturation or priming of T cells is not always associated with malaria antigen.

SCHEMATIC SUMMARY ON THE ROLE OF TNF- α IN CM

Taking all the findings and hypotheses together, a schematic summary can be drawn to illustrate the role of TNF- α in the pathophysiology of CM as shown in Figure 2.

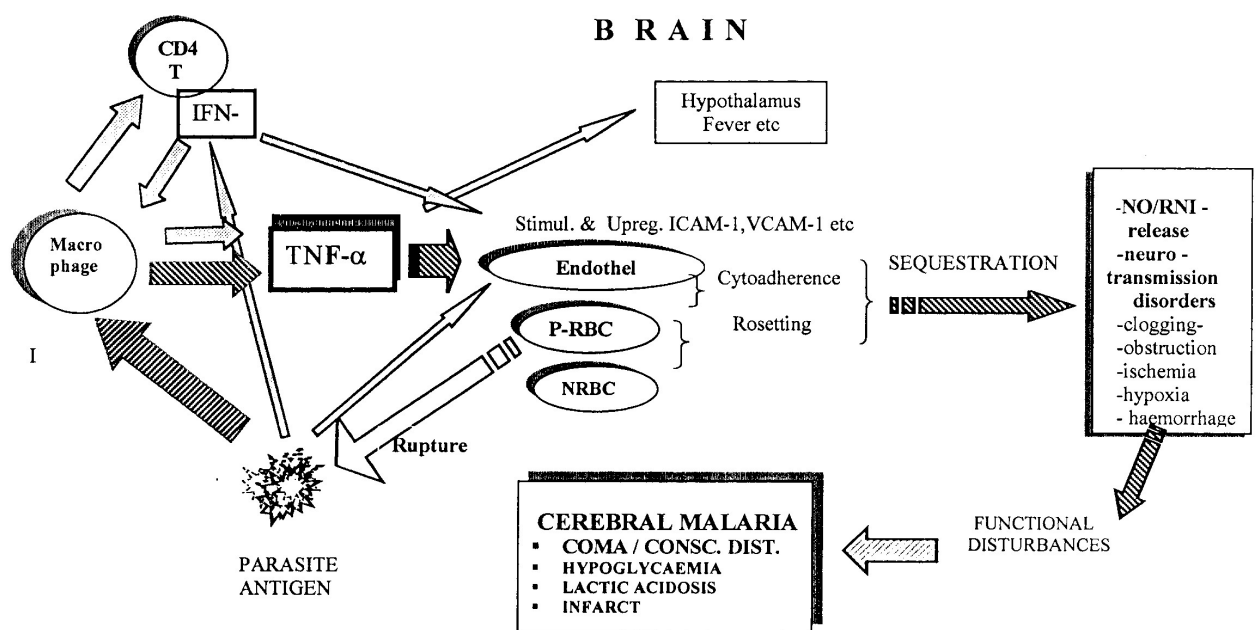


Figure 2. Schematic illustration showing the role of TNF- α in the development of pathology and clinical symptoms

Reports concerning the efforts to prevent TNF- α induction and to tame TNF effects. Aikawa (1988), learning from his electron microscopic observation on pathology of CM caused by sequestration, appealed towards development of candidate vaccine that not only reduces parasitemia, but also reduces the effects of already established infection. Later, Playfair, Bate and Taverne launched the term “antidisease vaccine” and did researches along this way. They detected the presence of a T-independent, transient IgM antibody in acute malaria patients which could prevent or inhibit TNF- α production. The “blocking” antibody is directed against a phospholipid found in the membrane of the parasites (Bate, *et al.*, 1990). They identified further the TNF-inducing molecule and tried to stimulate production of long-life antibody (IgG) that could

neutralize the epitope but still workable on second or further infections (Bate, *et al.*, 1993; Schofield, 1993). Following this line, a study has been done to search the presence of inhibitory factor in the serum of *falciparum* and *vivax* malaria patients from hypoendemic area of Lombok (Sri-Hidayati, 2001). While other researchers identified other molecules of the parasite as TNF-inducing part (Pichyangkul, *et al.*, 1994). It is still to be confirmed whether in severe patients like CM, such inhibitory capacity does not exist or is not effective to reduce TNF- α production.

The use of antibody against TNF- α (murine moAb anti-TNF antibody, CB0006) was reported in 41 cases of CM in Gambian children; the antibody showed effects in term of reduction of fever in a dose dependent matter,

but showed no reduction on fatality rate (Kwiatkowski, *et al.*, 1993). But no further reports were available.

Contradictory findings about the effects of conventional antimalarials against TNF production has been reported elsewhere before (Hoffman, *et al.*, 1984; Picot, *et al.*, 1991 and 1997; Sri Hidajati, 2002), while against production of NO, according to experiments done by Kremsner, only Quinine showed inhibitory effects in therapeutic dose (Kremsner, 1993).

In spite of the fact that corticosteroids could inhibit the production of TNF- α and inhibit the action of iNOS (Moncada, *et al.*, 1991), clinical study by Hoffman, *et al.*, (1988) proved, that high dosage of corticosteroids did not show any improvements compared to placebo. While low dosage of corticosteroids has been reported before as no use. A kind of phosphodiesterase non selective inhibitor, pentoxifylline, was proved experimentally to reduce steady-state TNF- α mRNA but had no effect on TNF- α mRNA translation in malaria (Grau and Behr, 1995). Clinically, it has been demonstrated to inhibit TNF- production in higher dosage, but it showed enhancement effects at low concentrations (Jakobsen, Koch and Bent, 1997).

CONCLUDING REMARKS

As already reviewed before, sequestration of p-rbc plays a very important role in the mechanism of CM, whereby TNF- α is essential in the up-regulation of expression of adhesion molecules on the surface of vascular endothelial cells for cytoadherence of the "knobby" p-rbc to the vascular wall. Typical periodic fever attacks of acute malaria can be correlated with TNF and TNF overproduction was implicated with symptoms of severe malaria including CM. The association of TNF and CM was confirmed by clinical data and TNF has been found in brain tissue of CM patients. While other cytokines were reported to have no direct correlation with CM.

Associated with the effects of TNF, Clark proposed the role of NO in the occurrence of transient coma and reversible sequelae found in patients recovered from CM. While more severe symptoms, permanent sequelae and fatal cases can be associated with ischemia caused by complete occlusion of local blood flow, severe hypoglycemia and lactic acidosis, which can be related to hyperparasitemia and involves the work of TNF, too. CD4+T cells are needed to optimize TNF production by secreting IFN and other cytokines. The role of TNF, TNFR and CD4 T cells was confirmed by genetic findings and immunoepidemiologic studies.

Analysis of parasite antigens which stimulate TNF production and production of antibody against the

epitope could be one way to cope the problem of CM and other symptoms of severe malaria. Trials and research on the use of anti-TNF antibody, corticosteroids and pentoxifylline and analysis of the effects of conventional antimalarials on TNF and NO production has been done with uncertain results. Possible use of PfEMP1 and other parasite ligands involved in cytoadherence in prevention of severe malaria symptoms has been written in the first review. It looks that further research is still needed to achieve realistic results.

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