CMLS, Cell. Mol. Life Sci. 60 (2003) 1623–1635
1420-682X/03/081623-13
DOI 10.1007/s00018-003-2347-x

CMLS Cellular and Molecular Life Sciences

Review

Tumor necrosis factor α in the pathogenesis of cerebral malaria

F. Gimenez[a,*], S. Barraud de Lagerie[a], C. Fernandez[a], P. Pino[b] and D. Mazier[b]

[a] UPRES 2706, Pharmacie Clinique, Faculté de Pharmacie, Université Paris XI, 5, rue Jean Baptiste Clément, 92296 Châtenay-Malabry (France), Fax: + 33 1 46 83 56 18, e-mail: francois.gimenez@nck.ap-hop-paris.fr
[b] INSERM U511 Immunobiologie Cellulaire et Moléculaire des Infections Parasitaires. CHU Pitié-Salpêtrière, Université Paris 6, 91 Boulevard de l'hôpital, 75013 Paris (France)

Received 4 December 2002; received after revision 7 February 2003; accepted 14 February 2003

Abstract. Physiologically in the brain, cytokines such as tumor necrosis factor-alpha (TNFα) are released by the immune system and can modulate neurological responses. Conversely, the central nervous system (CNS) is also able to modulate cytokine production. In the case of CNS disorders, cytokine release may be modified. Cerebral malaria (CM) is a complication of *Plasmodium falciparum* infection in humans and is characterized by a reversible encephalopathy with seizures and loss of consciousness. Central clinical signs are partly due to sequestration of parasitized red blood cells in the brain microvasculature due to interactions between parasite proteins and adhesion molecules. TNFα is produced and released by host cells following exposure to various malarial antigens. The increase of TNFα release is responsible for the overexpression of adhesion molecules. This article reviews the involvement of TNFα in cerebral malaria and the relation with all the processes involved in this pathology. It shows that (i) TNFα levels are increased in plasma and brain but with no clear correlation between TNFα levels and occurrence and severity of CM; (ii) TNFα is responsible for intercellular adhesion molecule-1 upregulation in CM, the relation being less clear for other adhesion molecules; (iii) TNFα receptors are upregulated in CM, with TNF receptor 2 (TNFR2) showing a higher upregulation than TNFR1 in vivo; (iv) in murine CM, low doses of TNFα seem to protect from CM, whereas excess TNFα induces CM and anti-TNFα therapies (antibodies, pentoxifylline) did not show any efficiency in protection from CM. Moreover, the involvement of lymphotoxin α, which shares with TNFα the same receptors with similar affinity, appears to be an interesting target for further investigation.

Key words. Cerebral malaria; tumor necrosis factor-α; TNF receptor; cytokine; adhesion molecules; blood-brain barrier.

Introduction

Cerebral malaria (CM) is a major life-threatening complication of *Plasmodium falciparum* infection in humans. Although the physiopathology has been extensively investigated, cellular and molecular bases of the neurological pathology are still unclear, particularly the intricacy of the different factors involved in pathogenesis: secretion of cytokines; sequestration of parasitised red blood cells (PRBCs) leading to mechanical blockage of microvessels; modifications of the T cell repertoire; immune status and genetic background of the host; parasite factor [1]. Sequestration of PRBCs to the surface of the microvasculature of various organs including the brain and the lungs is mediated by different endothelial cell surface receptors, including thrombospondin (TSP), CD36,

* Corresponding author.

intercellular adhesion molecule-1 (ICAM-1), E-selectin, vascular cellular adhesion molecule-1 (VCAM-1), CD31, $\alpha_v\beta_3$ integrin and hyaluronic acid [2]. The expression of part of these adhesion molecules is reportedly modulated by cytokines [3], such as tumor necrosis factor-α (TNFα), a molecule known to be involved in the pathogenesis of cerebral malaria. In humans, it upregulates endothelial adhesion molecules ICAM-1 and VCAM-1 [3], and therefore increases sequestration of PRBCs within the microvasculature of the brain and other organs such as lungs, kidneys and so on. In murine models, blockage of cerebral vessels is also associated with the accumulation of activated leucocytes as well as with infected red blood cells [4].

Various studies reported the critical role of TNFα in human CM [5], while at the same time this role was seriously questioned by the failure of TNFα-neutralising agents to decrease the incidence of CM.

Whatever the precise role of TNFα in CM, it is clear that this cytokine acts in concert with other cytokines and mediators [1]. The objective of this article is to review the data available in the literature regarding TNFα and CM in order to clarify the place of this cytokine in the pathogenesis of CM.

TNFα

TNFα is a pro-inflammatory cytokine principally produced by activated immune cells, such as macrophages, T and B cells, and mast cells. TNFα is produced mainly as a soluble 17-kDa secreted protein and also in transmembrane form at the surface of macrophages and activated T cells [6–8]. TNFα may have both beneficial and detrimental functions. It can activate host defense and promote resistance to infectious diseases, and it can also be involved in toxicity and inflammatory processes [9, 10].

It plays an important role in inflammatory reactions by:

- promoting extravasation of neutrophils, lymphocytes and monocytes, and enhancing their adhesion to endothelial cells [11]
- affecting immune responses by controlling T cell activation, which stimulates cell surface expression of major histocompatibility complex (MHC) class I and II molecules on a variety of cell types [12]
- inducing the synthesis of numerous pro-inflammatory cytokines [interleukin (Il)-1, Il-6, …] and TNFα itself [13]
- inducing apoptosis of different cell types, including endothelial cells [14].

Contradictory data indicate that TNFα has multiple physiological effects in the brain:

- in the embryonic development of the brain, the role of TNFα is controversial. High levels of TNFα have been reported in the embryonic brain [15], while no alteration of the brain has been described in mutant mice lacking TNFα receptors (TNFR1 and TNFR2) or in TNFα knockout mice [16]
- TNFα is implicated in CNS homeostasis and is involved in the proliferation and survival of CNS cells. It induces proliferation of astrocytes and glioma cells [17]
- it also has pro-inflammatory effects through modulation of MHC class I expression in astrocytes and neurons [17].

TNFα binds to two types of TNF receptors: TNFR1 (55–60 kDa) and TNFR2 (75–80 kDa) with similar affinity. TNFα actions mediated by binding to TNFR1 are mainly cytotoxic, while those mediated by binding to TNFR2 are proliferative. In most cells, both receptors are coexpressed, and some data suggest that TNFR1 mediates most of the actions of soluble TNFα, while TNFR2 mediates most of those of transmembrane TNFα [7].

Astrocytes, microglia and neurons are able to locally produce TNFα after physiological and pathological stimuli [18].

The overproduction of TNFα is related to brain damage in pathological situations, such as bacterial meningitis, multiple sclerosis, Alzheimer's disease and CM. Brain damage has been evidenced in transgenic mice overexpressing TNFα and has been prevented by pretreatment with antimurine TNFα antibody [19].

Different data suggest that the toxic role of TNFα in inflammatory pathologies is more targeted at the blood-brain barrier (BBB) (astrocytes) rather than the central nervous system (CNS) (neurons) itself. Transgenic mice overexpressing noncleavable transmembrane human TNFα in astrocytes develop CNS inflammation, while transgenic mice overexpressing transmembrane TNFα in their neurons do not [17].

Production of TNFα in malaria and CM

In humans, TNFα is synthesised as a transmembrane molecule and can be released by metalloproteinases from the immune cells into biological fluids. TNFα is produced and released by immune host cells following exposure to various malarial antigens at different steps of the life cycle of *Plasmodium* species [20, 21]:

- At the preerythrocytic phase, when parasites enter the host via the saliva of an infected female *Anopheles* mosquito during feeding, the sporozoite circulates in the blood stream before invading the liver. During this brief sporozoite phase, sporozoite antigens stimulate TNFα release in a short period of time [21].
- At the liver stage, parasites invade the host's hepatocytes, where they multiply. Very few studies have reported the involvement of TNFα at this stage [22, 23].
- After multiplication in the liver, merozoites are released in the bloodstream and infect red blood cells by

receptor-mediated endocytosis. At this erythrocytic stage after asexual reproduction, mature schizonts are produced. Severe clinical manifestations of malaria and especially CM, occur at this erythrocytic stage. After rupture of schizonts, toxins are released. Among malaria toxins, *P. falciparum* glycosylphosphatidyl inositol (GPI) has been shown to induce TNFα release in mice [24] and to stimulate TNFα production in vitro by macrophages [25, 26]. The recent observation [27] that anti-GPI vaccination could prevent pathology and fatalities in the *Plasmodium berghei* rodent model of severe malaria demonstrates the deleterious role of GPI and TNFα. The involvement of TNFα in CM at this erythrocytic stage constitutes the focus of this review.

- After asexual reproduction, infective male and female gametocytes are produced from a subpopulation of infected red blood cells and are ingested by *Anopheles* during feeding to complete the life cycle of the parasite. At this sexual stage, the production of TNFα by gametocytes and the role of TNFα in killing them is unknown in vivo. The ability of TNFα to kill gametocytes has only been demonstrated in vitro [28].

CM

CM is one of the most severe complications of *P. falciparum* infection in humans, characterised by a reversible encephalopathy with seizures and loss of consciousness. CM is diagnosed in a patient who is unable to localise a painful stimulus, has peripheral asexual falciparum parasitaemia and has no other causes of encephalopathy.

Very little is understood about the mechanism by which the parasite, which remains intravascular and does not enter the brain parenchyma, may induce a neurological syndrome.

Early studies have reported multiple pathologic changes in CM such as (i) sequestration of parasitised red blood cells in small cerebral vessels, endothelial cell damage, haemorrhage, thrombosis and mononuclear cell inflammation [29] and (ii) schizont rupture releasing toxins that stimulate monocytes and macrophages to produce TNFα and other cytokines [30].

In humans, cerebral symptoms in CM may be explained by the concomitant phenomena:

- schizont rupture releases toxins that stimulate monocytes and macrophages to produce TNFα and other cytokines [30]
- TNFα increases the expression of adhesion molecules [3]
- parasitised erythrocytes form ‘rosettes’ with several unparasitised red blood cells and express at their surface a parasite protein PfEMP-1 (*Plasmodium falciparum* erythrocyte membrane protein-1) [31]
- interactions occur between PfEMP-1 and adhesion molecule receptors, followed by cytoadherence [20].

The occurrence and severity of CM depends upon the ability of parasitised erythrocytes both to adhere to the endothelium of microvessels in the brain and to form rosettes with unparasitised red blood cells.

In this cascade of reactions, the correlation between cerebral symptoms and TNFα levels in the plasma or the brain, the existence of BBB disruption and the role of TNFα in this disruption, the involvement and correlation with TNFα of nitric oxide, and the efficacy of anti-TNFα therapies in CM all remain to be clarified.

As studies of CM are difficult to conduct in humans, experimental animal models have been developed in order to find one bearing close similarity to human CM. Primate models in rhesus monkeys infected with *Plasmodium caotneyi* have been reported but have limited application due to practical and cost problems [32]. For these reasons, murine models are more accessible by infection with either *Plasmodium berghei* ANKA (PbA) or *P. berghei* K173. As in humans, an increase of cerebral TNFα has been evidenced in mice, which results in upregulation of adhesion molecules. In contrast, differences between both species have been reported. For example, a predominant sequestration of leukocytes in cerebral capillaries in the murine model has been reported, while sequestration of parasitised red blood cells is responsible for cerebral symptoms in humans [13]. Hearn at al. [33], however, suggested that both parasitised erythrocytes and leukocytes contribute to the microvascular lesion during murine CM as well as in humans, and Lou et al. [34] described similarities between the murine model and human CM, such as the presence of mononuclear cells in brain venules and upregulation of TNFR2.

Correlation between TNFα levels and CM in human

Levels of TNFα in human cerebral malaria

Plasma TNFα levels

Increased levels of cytokines have been demonstrated in human CM, such as interferon-γ (IFN-γ), IL-1, IL-6, IL-8 and TNFα [35]. The plasma level of TNFα seems to be related to the occurrence of fever and complications but is not clearly correlated with CM [36].

In children, TNFα plasma levels are higher in cases of fatal malaria compared with nonfatal malaria and in CM compared with noncomplicated malaria [37–39]. In *Plasmodium vivax* malaria, Karunaweera et al. [40] have demonstrated that changes in TNF plasma levels paralleled the rise and fall in temperature preceding them by 30–60 min during paroxysms, which suggests that fever paroxysm in malaria could be regulated by TNFα plasma levels.

Renal failure in severe malaria is associated with high levels of plasma TNFα. Severe falciparum malaria is associated with acute renal failure with acute tubular necrosis.

In Vietnamese patients, serum creatinine levels were strongly correlated with TNFα and poorly with IL-10 and IFN-γ [41]. In contrast, in these same patients, Day et al. [41] demonstrated that CM patients had significantly lower plasma levels of IL-1β, IL-10, IL-6, IFN-γ and TNFα when compared with conscious patients. Within the CM patients, associated multiple vital organ dysfunction was associated with higher levels of cytokines than in patients with coma alone. This suggests that previously reported correlations between high TNFα plasma levels and CM [38, 39, 42] better reflect associated noncerebral complications [41].

Data suggest that the timing and amounts of TNFα released may be a determinant of pathological events and perhaps mortality [43].

Brain levels of TNFα

Brown et al. [5] observed an increase in proinflammatory cytokines and especially in TNFα, but TNFα messenger RNA (mRNA) induction was not specific to CM (when compared with other infectious meningitis), did not correlate with parasite sequestration and was also seen in the spleen.

Polymorphisms of TNFα and CM

Genetic variation in cytokine promoter regions has been postulated to influence susceptibility to infection.

The TNFα gene lies in the class III region of the MHC. In West African children, it has been shown that a human leucocyte class I antigen and an histocompatibility leukocyte antigen (HLA) class II haplotype, common in West Africans but rare in other racial groups, are independently associated with protection from severe malaria [44]. Single-nucleotide polymorphisms of the TNF promoter have been reported to be associated with susceptibility to or protection from severe malaria. Four mutations are transitions of adenosine to guanine and are located at nucleotide positions –238, –244, –308 and –376, relative to the transcriptional site [10, 45]. Various studies have investigated the relation existing between TNF promoter alleles and severity of malaria.

Two studies performed on Gambian children demonstrated that the TNF_{-238} A allele was associated with susceptibility to severe malarial anaemia, but not to CM [46], whereas the neighboring TNF_{-308} A allele was associated with CM but not with severe malarial anaemia [47].

In Kenyan children, the TNF_{-308} A allele was significantly associated with high *P. falciparum* parasitaemia. Among low birth weight children, the TNF_{-308} A allele was associated with severe anaemia and showed a trend toward a risk for severe malarial anaemia [48].

Knight et al. [49] demonstrated that the TNF_{-376} polymorphism is located in a region of multiple DNA-protein interactions, and the less common allele acts to recruit OCT-1 to this region. OCT-1 is a transcription factor whose interactions with other proteins lead to diverse effects on gene regulation. The OCT-1-binding genotype is found in ~5% of Africans and is associated with a fourfold increase in susceptibility to CM.

In Myanmar, patients with falciparum malaria (200 with uncomplicated malaria and 43 with CM), the TNFα promoter allele, TNFP-D, was significantly associated with CM. TNFP-D showed no significant linkage disequilibrium with any alleles of HLA-B or HLA-DRB1, suggesting that TNFP-D was primarily associated with CM in Myanmar [50].

In contrast with other studies, in a work conducted in Gabon and comparing malaria severity and TNF promoter variants, May et al. [10] observed that the frequencies of carriers of distinct TNF variants did not differ significantly in the groups with mild and severe malaria. Moreover, TNF plasma levels were not significantly associated with any of the TNF variants.

***P. falciparum* strains and levels of TNFα**

Allan et al. [51] have raised an interesting question of whether CM could be associated with strains of *P. falciparum* that induce an abnormally high TNFα response. To investigate that hypothesis, they collected parasite isolates from Gambian children with uncomplicated malaria fever or CM and tested the ability of parasitised erythrocytes to induce TNF production in human PBMCs. Even if CM isolates tended to stimulate more TNF production than uncomplicated malaria isolates, a huge overlap was observed between the two groups, which provides limited support for the hypothesis that CM was caused by *P. falciparum* strains inducing high levels of TNFα.

TNFα in murine CM model

If controversies remain concerning the fact that in humans, neurological symptoms are due to sequestration of parasitised erythrocytes within the microvasculature, whereas in the murine model of CM they are related to leukocyte sequestration, there is no discussion about the involvement of TNFα-induced upregulation of endothelial adhesion molecules in the sequestration of cells within the cerebral vasculature [52, 53].

The development of an animal model of CM gave the opportunity to investigate relations between CM and TNF brain or systemic levels.

Mouse CM is associated with high levels of TNFα; however, systemic injections of TNFα did not induce cerebral symptoms, suggesting that TNFα might be produced locally in the brain in the case of CM [54, 55].

When measured in the brain, spleen and liver of mice developing CM, levels of TNFα mRNAs paralleled parasite mRNA levels in the brain but not in the spleen or liver,

suggesting that CM in mice is an encephalitis [56]. In the brain, messages for IFN-γ also paralleled parasite RNA levels but to a lesser degree than TNF and earlier. In contrast, no significant induction of message for IL-2, IL-4, IL-5, IL-6, IL-10 or IL-12 was detected.

In a study comparing TNF expression in normal brains of uninfected mice, in CM-negative mice infected with PbA and in CM-positive mice infected with PbA, Hearn et al. [33] demonstrated that TNF was weakly detected in normal brains and in CM-negative brains, while strong levels of TNFα were observed in CM-positive brains.

Rudin et al. [57] reported that PbA infection induces fatal CM in wild-type mice, whereas TNFα/β-deficient mice are completely resistant to PbA-induced CM.

Various articles have reported the role of IFN-γ in producing TNFα and in inducing CM. In CBA/Ca mice infected with PbA, the administration of antibodies to IFN-γ has shown that IFN-γ is responsible for the release of TNFα and the induction of CM [37], and use of IFN-γ-deficient mice has demonstrated that these mice do not develop CM and show lower levels of cerebral TNFα when compared with wild-type mice developing CM [58, 59]. However, recent data [4] show that more important than the level of IFN-γ, cytotoxicity of $CD8^+$ lymphocytes having migrated to the brain is a key player in CM pathogenesis.

A recent article from Engwerda et al. [60] discussed the primordial role of TNFα in the pathogenesis of CM in experimental murine models and suggested that lymphotoxin α (LTα) would be the principal mediator of murine CM. LTα is a related member of the TNF family and like TNFα is able to bind to both TNFR1 and TNFR2. It can also form a heterotrimer with two LTβ molecules, and binds to the LTβ receptor. Engwerda et al. [60] investigated the susceptibility to CM of TNFα-deficient, LTα-deficient and TNFα/LTα-deficient C57BL/6 mice. They observed that TNFα-deficient mice developed neurological symptoms of CM 6–8 days after infection with PbA, whereas LTα- and TNFα/LTα-deficient mice did not. All three types of mice presented similar blood parasitaemia.

In summary, both in human and mouse CM, TNFα levels are increased in plasma and in the brain. However, the correlation between TNFα levels in plasma and in the brain and the occurrence of CM has never been clearly demonstrated. In humans, TNF promoter variants seem to play a role in the severity of malaria, but controversies remain regarding a potential correlation between the two major TNF_{-308} A and TNF_{-238} A alleles and the severity in terms of anaemia and CM.

TNFα and adhesion molecules in CM

Adhesion molecules are expressed on the surface of cerebral endothelium and are implicated in a wide range of neurological disorders, such as multiple sclerosis and CM. Various adhesion molecules have been identified: ICAM-1, VCAM-1 or CD54, thrombospondin (TSP), CD36, PECAM (CD31), E-selectin and so on.

In CM, TNFα and other cytokines such as IFN-γ are known to act by increasing the expression of adhesion molecules. Among all the adhesion molecules identified, ICAM-1 seems to be the most important [61]. TNFα binding to its receptors TNFR1 and TNFR2 induces a recruitment of signal transducers that activates effectors and transcription factors, leading to a strong increase in the level of expression of ICAM-1 [62]. Adhesion molecules are implicated in the adherence of parasitised erythrocytes to cerebral endothelial cells within the microvasculature, which is responsible for pathogenesis of CM. Other adhesion molecules are involved, such as thrombospondin, VCAM, E-selectin (ELAM-1), CD36, chondroitin sulfate A, P-selectin, integrin $\alpha v\beta 3$, PECAM-1 (CD31) [1, 61]. The binding of parasitised red blood cells to these adhesion receptors on the cerebral endothelium modifies the integrity of the BBB by altering cell junction proteins occludin, vinculin and ZO-1 [63].

The involvement of TNFα in the upregulation of adhesion molecules has been clearly reported in different in vitro and in vivo experiments.

In vitro correlation between TNFα and adhesion molecules

The treatment of immortalised human brain capillary endothelial BB19 cells with TNFα and other cytokines resulted in an increase in expression of ICAM-1, VCAM-1, E-selectin and CD36 after 6–8 h with a return to normal at 24 h. The stimulation observed with cytokine treatment was greater for ICAM-1 [64].

In coculture of immortalised human umbilical vein endothelial cells with rat glioma cells, the treatment with TNFα (400 ng/ml) for 6 or 18 h resulted in an increase in ICAM-1 expression, while that of CD36 remained unchanged [65].

Lou et al. [66] investigated whether the genetic susceptibility to CM was associated with a differential reactivity of these cells to TNFα and compared the responsiveness of brain microvascular endothelial cells (BMVECs) from CM-sensitive and CM-resistant mice to TNFα. At basal conditions, adhesion molecule expression was similar in the two types of mice. After TNFα treatment of the cells, upregulation of ICAM-1 and VCAM-1 was specifically observed with BMVEC from CM-sensitive mice.

In vivo correlation between TNFα and adhesion molecules

In TNF-α/β-deficient mice, Rudin et al. [57] demonstrated that PbA did not induce the upregulation of en-

dothelial ICAM-1 expression required for development of CM.

In a study conducted on Gambian children, McGuire et al. [67] observed a correlation between circulating ICAM-1 and TNFα plasma levels in acute malaria, but this correlation was not investigated in CM.

Favre et al. [68] investigated the role of ICAM-1 in CM by studying the secretion of TNFα and the extent of sequestration of macrophages and parasitised erythrocytes in the brain in ICAM-1-deficient (–/–) mice and in wild-type ICAM-1 (+/+) mice. Although parasitaemia was similar in both (–/–) and (+/+) mice, neurological symptoms and death within 6–8 days with BBB disruption were observed only in (+/+) mice. They observed that in ICAM-1 (–/–) infected mice, plasma levels of TNFα were above those of noninfected mice but were significantly lower than those of (+/+) infected mice, while IFN-γ levels were similar in both (–/–) and (+/+) mice. Surprisingly, TNFα mRNA levels in the brain were higher in (–/–) mice compared with (+/+), suggesting that brain-produced TNFα would not be directly responsible for the neurological symptoms observed in CM [68].

The authors suggested that the lower plasma levels of TNFα in ICAM-1 (–/–) mice could be due to a decrease of balance between production and elimination of the cytokine, production being similar and elimination being increased in ICAM-1(–/–) when compared with wild-type mice. This more rapid elimination could also be explained by the leukocytosis associated with CM, with the absence of adhesion molecules increasing the number of TNF-bearing leukocytes in circulation.

In summary, in vitro models strongly demonstrate in CM that TNFα increases expression of adhesion molecules. All in vivo reports suggest a strong correlation between TNFα and ICAM-1 in CM, with a clear increase of ICAM-1 expression with TNFα and modulation of TNFα expression in the absence of ICAM-1 in ICAM-1-deficient mice. The correlation between TNFα and other adhesion molecules is less clear.

TNFα and BBB disruption in CM

Many in vivo and in vitro studies have reported the implication of TNFα in modulation of permeability in the BBB, but data are very controversial [69]. TNFα either does not alter the transport of molecules across the BBB in vitro [70] or in vivo [71, 72], produces an increase of this transport in vitro [73, 74] or in vivo [75, 76], or decreases this transport [77].

Early investigations of malaria-infected *Macaca mulatta* monkeys demonstrated an increase in the cerebrospinal fluid (CSF) blood ratio of albumin, suggesting that as albumin is responsible for oncotic pressure, it would draw water into the brain interstitium and cause cerebral oedema [78].

In his study comparing CM in ICAM (–/–) mice and wild-type ICAM-1 (+/+) mice, Favre et al. [68] observed a post-mortem breakdown of the BBB in CM-positive ICAM-1 (+/+) only, evidenced by blue staining of the brain after intravenous (IV) injection of Evans blue. As reported above, TNFα mRNA levels in the brain were higher in (–/–) mice compared with (+/+), suggesting that brain-produced TNFα would not be directly responsible for the neurological symptoms observed in CM and the breakdown of the BBB.

In an in vitro coculture BBB model with human umbilical vein endothelial cell (HUVEC) and rat glioma cells, Dobbie et al. [65] observed a decrease in barrier integrity evidenced by a 47% decrease in transendothelial electrical resistance after treatment of the coculture with TNFα for 18 h in comparison with nontreated cells.

Using macroarrays to screen gene specific expression of human endothelial cells cocultivated with the 3D7 clone of *P. falciparum*, Pino et al. [79] found an upregulation of pro-inflammatory and pro-apoptotic genes, with a significant increase in members of the TNF superfamily, including Fas, Fas ligand, VEGI and TRAIL R2. The observed endothelial cell apoptosis may contribute to the dysfunction of BBB.

In humans, Brown et al. [63] compared Vietnamese brains collected post portem after CM with brains collected post mortem after nonmalarial causes of death (control brains). By immunohistochemistry, although the cell junction proteins ZO-1, occludin and vinculin were constitutively high in control brains, they observed a generalised reduction in expression of the corresponding antigens with even, in some cases, a focal loss of staining. Brown et al. [63] hypothesised that sequestration of parasitised red blood cells in the brain via adhesion to receptors such as ICAM-1 and others could result in activation of cerebral endothelial cells and that a potential consequence of this activation could be disruption of the BBB, resulting in exposure of the brain parenchyma to plasma proteins. Perivascular macrophages would then encounter proteins leaking across the disrupted BBB and, as a consequence of activation or phagocytosis, could secrete pro-inflammatory and neuroactive mediators, such as TNFα, which could influence local neuronal function [63]. BBB disruption was further evidenced by an increase in immunoreactivity for fibrinogen in cases of CM, indicating widespread leakage of this plasma protein across the BBB. However, correlation between this BBB disruption and the level of TNFα in the brain was not investigated.

In summary, investigations of the BBB in CM are difficult to interprete but suggest that if BBB disruption is involved in CM, it cannot by itself explain the neurological symptoms of this disease. Most of the studies investigat-

ing BBB integrity in CM did not correlate their results with TNFα levels in brain.

TNFα receptors and CM

TNFα sends signals to target cells through two homologous homodimeric receptors: TNFR1, also known as p55, CD120a, p60, and TNFR2, also known as p75, p80, CD120b [80]. TNFR1 and TNFR2 are membrane glycoproteins present on almost all types of cells except erythrocytes. Cell surface expression of these receptors is controlled by various factors, such as cytokines, protein kinases, phosphatases and so on. They also exist as soluble forms following cleavage from membrane and circulate in serum, urine and other biological fluids under normal and pathological situations. These soluble forms of TNFRs are able to bind TNFα and to act either as agonists at low concentrations (stabilising the trimeric structure of TNFα) or as inhibitors at high concentrations (by competing with cell membrane receptors) of TNFα activity.

Transgenic mice deficient in one of the two TNF receptors have been used to investigate their respective role in infectious diseases. These experiments have shown that TNFR1 was more important than TNFR2 for resistance against most infections with fungi (*Candida*), with intracellular pathogens (*Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Toxoplasma gondii*, *Leishmania major*) and with viruses [80]. In CM, things appear to be different [34, 81, 82].

Lucas et al. [81, 83] have investigated the in vivo expression of both TNFR1 and TNFR2 in CM-susceptible CBA/CA mice in comparison with CM-resistant BALB/c mice after PbA infection. They observed that TNFR2 expression was increased in brain microvessels at the onset of neurological signs in CM-susceptible CBA/CA mice, whereas TNFR1 expression was not modified. This upregulation of TNFR2 was not detected in the lungs. In CM-resistant BALB/c mice, levels of both receptors remained unchanged after infection with PbA.

This difference in modulation of TNF receptors in CM was confirmed by using TNFR1-deficient mice and TNFR2-deficient mice. While the evolution of parasitaemia was similar in both types of deficient mice, TNFR2-deficient mice but not TNFR1-deficient mice were protected from PbA-induced CM. Lucas et al. [81] also demonstrated that protection of TNFR2-deficient mice from CM was due to an absence in the brain of ICAM-1 upregulation required to induce neurological signs in CM. In contrast, upregulation of ICAM-1 was observed in the brains of wild-type and TNFR1-deficient mice.

Piguet et al. [82], using TNFR1- and TNFR2-deficient mice, confirmed the essential role of TNFR2 in CM but did not find upregulation of ICAM-1 in TNFR2-deficient mice as described by Lucas et al. [81]. Moreover, they observed the pleiotropic changes in glucose metabolism seen in CM in wild-type and TNFR1-deficient mice, but not in TNFR2-deficient mice, and concluded that coma occurring in CM could be mediated by metabolic perturbations under the control of TNFR2.

As (i) TNFR2-deficient mice were protected from PbA-induced CM [81, 82], (ii) TNFα-deficient mice are sensitive to CM, whereas LTα and TNFα/LTα-deficient are protected from CM [60, see also above], and (iii) TNFα and LTα share the same two receptors with similar affinity [6], LTα, but not TNFα, could be the primary mediator of murine CM, as suggested by Engwerda et al. [60].

In contrast, in vitro, Lou et al. [66] did not find such differences between TNFR1 and TNFR2. On BMVECs from CM-sensitive and CM-resistant mice, they investigated a differential reactivity to TNFα by comparing the expression of the two specific cell surface membrane receptors TNFR1 and TNFR2. At basal conditions without TNFα stimulation, the constitutive expression of both receptors was similar. In contrast, after 12 h of TNFα treatment, TNFR1 and TNFR2 were upregulated on BMVECs from CM-sensitive mice but not from CM-resistant mice. No difference in upregulation was observed between the two types of receptors. This upregulation on BMVECs of CM-sensitive mice was dependent on time of TNFα treatment but not on TNFα concentration.

Using focal intrastriatal injection of TNFα, Sibson et al. [84] demonstrated that acute administration provoked a dose-dependent reduction in cerebral blood volume, mediated by endothelin and coupled to activation of the TNFR2 pathway, and suggested that this increase in cerebral blood volume could be a contributing factor to neuronal dysfunction or degeneration of CM.

A hypothesis has been made that soluble TNF receptors (sTNFRs) could be involved in protection against CM. Lesslauer et al. [85] have demonstrated that sTNFR competitively inhibits the interaction between TNFα and membrane-bound receptors, and Garcia et al. [86] reported that transgenic mice with high levels of soluble sTNFR1 were protected against experimental CM.

In summary, different experiments showed that TNF receptors are upregulated in CM. This upregulation has been demonstrated in vivo using CM-susceptible mice in comparison with CM-resistant mice, and in vitro, on isolated brain microvascular endothelial cells isolated from these two types of mice. However, conflicting data have been reported about differences of levels of upregulation between these two receptors. TNFR2 was more upregulated in vivo than TNFR1 whereas levels of TNFR regulation did not differ in vitro. Studies performed in CM and other pathological situations suggest that membrane-bound TNF receptors could be involved in the pathogenesis of CM, whereas soluble forms of TNF receptors could be involved in protection against CM by binding

with TNFα and preventing it from binding to membrane bound TNF receptors.

TNFα, NO production and CM

Involvement of nitric oxide (NO) in CM and its relation with TNFα are still unclear. Various studies were performed in severe malaria but not specifically in CM.
Clarke et al. [87, 88] have seriously questioned the obstruction of cerebral blood flow by parasitised erythrocytes and rosettes as the mechanism of CM, because patients recovering from CM usually do not have neurological sequelae, which should be the consequence of cerebral ischaemia severe enough to cause an episode of coma. As an alternative explanation, the authors hypothesised that the high level of TNFα in CM is responsible for induction of NO production in vascular walls. NO would then cross the BBB and cause functional alterations such as inhibition of calcium entry and reduced activity of calcium-dependent NO synthetase, thus reducing NO formation in postsynaptic neurons. NO in the brain is an essential component which controls synaptic plasticity and excitatory neurotransmission. This mechanism, in contrast to vascular obstruction by parasitised red blood cells, would not be followed by neurological sequelae and could explain why increased levels of TNFα tend to correlate with cerebral symptoms in CM.
However, the experiments of Kremsner et al. [89, 90] did not confirm this hypothesis. They investigated the relation between malaria antigen, TNF and reactive nitrogen intermediates (RNIs). Malaria antigen and TNFα induced dose-dependent RNI production in macrophages. RNI production was enhanced when the malaria antigen was coincubated with TNF. Pentoxifylline, an inhibitor of TNFα production, did not significantly influence TNF-induced RNI production. An anti-TNF monoclonal antibody (mAb) did not significantly alter the malaria antigen-induced RNI synthesis by macrophages. In experimental cerebral malaria in mice, inhibitors of nitric oxide synthase, L-*N*-monomethyl arginine and *N* omega-nitro-L-arginine did not exert any significant effect on the development of CM.
Different experiments performed using an in vitro model of primary cultures of human endothelial cells (ECs) and different strains of *P. falciparum* yielded the critical observation that the CD23/NO pathway is functional in endothelial cells. As a matter of fact, (i) *P. falciparum*-infected red blood cells induced expression of CD23 on ECs, (ii) there is increased iNOS expression followed by generation of nitrites upon EC stimulation by anti-CD23 mAb and (iii) addition of L-NMMA inhibited CD23-derived effects in ECs. CD23 and its physiologic ligand, IgE, have been shown to be expressed during multiple infectious diseases, including plasmodia infection. The CD23/NO pathway appears to play a prominent anti-plasmodial role either directly through parasite killing or indirectly by inhibiting cytoadherence of parasited red blood cells, most likely due to a decreased expression of ICAM-1 [91].
In summary, through the hypothesis involving the TNFα/NO interaction looks interesting and could explain the absence of cerebral sequelae in CM, this remains to be proven, and the experiments conducted in macrophages do not seem to go in that direction.

IL-10 : TNFα ratio in CM

IL-10 is a Th2 cytokine which regulates the production of TNFα. Various experiments have shown that IL-10 levels were increased in malaria [92, 93]. Moreover, it mediates protection against experimental CM in mice [94]. Administration of IL-10 to susceptible CBA mice protected them from CM after infection with PbA, and neutralisation of IL-10 in naturally resistant BALB/c mice induced neurological symptoms [95].
Owing to downregulation of TNFα by IL-10, researchers have tried to determine whether the IL-10 : TNFα ratio could be a better marker of the risk of severe malaria and especially of CM, than IL-10 or TNFα considered individually.
In Kenyan children suffering from different severities of malaria, the mean IL-10 : TNFα ratio was significantly lower in children with malarial anaemia (mean ratio, 1.77) than in children with mild malaria or high-density uncomplicated malaria (mean ratio, 4.64) [96]; however, CM was not considered in this study.
In contrast, May et al. [10] compared IL-10 : TNF ratio in Gabonese children with CM, severe anaemia and hypoglycaemia and observed that patients with CM or severe anaemia or hypoglycaemia had always an IL-10 : TNFα ratio of <1, whereas the ratio was >1 in case of hyperparasitaemia.
In a study comparing children from Ghana with severe anaemia, CM or uncomplicated malaria, the ratio of IL-10 : TNFα was significantly lower in severe anaemia but did not differ significantly between patients with CM and uncomplicated malaria [97].
In summary, available data are insufficient to suggest that IL-10 : TNFα ratio could be a better marker of CM risk than IL-10 or TNFα levels taken individually.

TNFα or anti-TNFα therapy as protection against CM

TNFα as protection from CM

Recombinant human TNFα (rhTNFα) is a monomeric nonglycosylated polypeptide of 157 amino acids that

forms a compact homotrimer that is unstable at low concentrations. rhTNFα plays a dual role in experimental CM. Excess levels of rhTNFα have proven to induce cerebral symptoms in mice infected with *P. berghei* [98]; however, such levels are irrelevant to disease states. In contrast, other studies have shown that low levels of rhTNFα protect mice infected with *P. berghei* K173 against CM in mice [99].

The stabilisation of rhTNFα trimers has been performed by Postma et al. [99] by reaction between the protein and the reagent succimidyl-*S*-acetylthioacetate (SATA). Intravenous injections of rhTNFα protected mice from CM, and protection was increased when using the stabilised form of rhTNFα trimers.

After treatment with rhTNFα, plasma levels of soluble TNFR1 were always below the limit of detection. Concentrations of soluble TNFR2 were increased with rhTNFα, depending on the dose, with a return to basal levels 24 h after treatment.

The mechanism by which low levels of TNFα could protect against CM is still unclear. Hypotheses have been suggested, such as an inhibition of parasitaemia, which has been demonstrated in different models [12, 99], although reported data suggest that the level of parasitaemia was not a key factor in the development of CM in an experimental murine model [100]. Another explanation could be the development of tolerance after treatment with low doses, which could be a regulatory mechanism that controls excessive stimulation by this cytokine [99].

The fact that low levels of TNFα protect against CM, whereas high levels induce CM, could partly explain the contradictory results observed in the studies concerning CM. It suggests that in all CM studies, TNFα levels should be carefully monitored.

Anti-TNFα therapy as protection against CM

Whereas high concentrations of rhTNFα have induced cerebral signs in *P. berghei* infected mice [98], monoclonal B-C7 antibody directed to TNFα has failed to improve survival in Gambian children with CM and was even associated with a significant increase in neurological sequelae [101]. The authors suggested that the antibody could have retained TNFα within the circulation and therefore could have prolonged its effects on vascular endothelium.

In a comparative pilot study where a small group of 17 patients with severe falciparum malaria received polyclonal-specific Fab fragment directed against TNFα, and 11 control patients received the corresponding placebo before treatment with artesunate, a faster resolution of clinical manifestations and reduction of fever were observed in the group with anti-TNFα treatment, whereas parasite clearance times were longer [102]. Antibody treatment reduced IFN-γ concentrations but had no obvious effect on levels of other cytokines, although unbound TNFα was undetectable after Fab treatment.

Pentoxifylline is known to inhibit TNFα and could therefore influence prevention or treatment of CM. In combination with artesunate in the treatment of 45 patients with severe falciparum malaria, pentoxifylline at low and high doses failed to improve parasite and fever clearance times and recovery time from coma [103], although high doses of pentoxifylline reduced plasma levels of TNF, IL-6 and TNF receptor in patients with severe malaria [104]. In another study in 51 patients with *P. falciparum* malaria, Hemmer et al. [105] observed that pentoxifylline did not modify the decrease in TNF levels and did not affect the clinical outcomes in a significant way.

In summary, in murine models, injections of low doses of rhTNFα protect from CM, whereas excess TNFα induces neurological symptoms. The mechanism of this dual role remains unknown. In patients infected with *P. falciparum*, anti-TNFα therapy (mAb, polyclonal Fab fragment or pentoxifylline) did not show any efficiency in protection from CM.

Conclusion

The data available in the literature are very controversial according to the authors (table 1). Plasma TNFα levels are not correlated with CM for some authors, whereas they are higher in CM compared with uncomplicated malaria for others. Injections of TNFα may or may not induce cerebral symptoms in infected mice. TNFR2 expression but not TNFR1 expression is increased in CM-susceptible mice, whereas TNFR1 and TNFR2 are both regulated to the same extent on BMVEC from CM-susceptible mice.

One possible explanation of the controversial results in human studies can be related to practical considerations such as different clinical definitions of CM among patients. According to the World Health Organization, CM must be limited to patients unable to localise a painful stimulus with peripheral asexual falciparum parasitaemia and no other causes of encephalopathy. But CM is often difficult to differentiate from cerebral symptoms due to complications related to other organs. The problem is similar in experiments conducted on the murine models where CM is often defined according to the time to death and parasitaemia without considering cerebral symptoms.

Although all these reports are contradictory, TNFα and TNFα receptors seem to be involved in the pathogenesis of CM, but the TNFα/TNFR pathways probably do not work alone and are part of a variety of reactions influencing parasite development, inflammation and cytoadherence.

Further investigations are needed to better clarify the precise role of TNFα and TNFRs in CM before TNFα mod-

Table I. Role of TNFα in the pathogenesis of CM.

	References
Plasma or brain levels of TNFα and CM in human	
Plasma TNFα was not clearly correlated with CM	36
Plasma TNFα levels were higher in CM versus uncomplicated malaria	37–39
Polymorphisms of TNFα and CM in human	
TNF-238 A allele was associated with severe anaemia but not with CM (Gambian children)	46
TNF-308 A allele was associated with CM but not with severe malaria anaemia (Gambian children)	47
TNF-308 A allele was associated with severe anaemia with a trend towards a risk for severe malaria anaemia (Kenyan children)	48
OCT1 binding genotype was associated with a fourfold increase in susceptibility to CM (Africa)	49
TNFP-D was associated with CM in Myanmar	50
Human *P. falciparum* strains inducing CM and TNFα	
There is no evidence that CM is caused by *P. falciparum* strains inducing specifically high levels of TNFα	51
Experimental murine model of CM and TNFα	
High doses of rh-TNFα induced cerebral symptoms in *P. berghei* infected mice	98
Mouse CM was associated with high levels of TNFα, but injections of TNFα did not induce cerebral symptoms	54, 55
In murine experimental CM, levels of TNFα mRNAs paralleled parasite mRNAs in the brain but not in the spleen or liver	56
High levels of TNFα were observed in the brain of mice with CM compared with noninfected mice or infected mice without CM	33
Brain produced TNFα was not directly responsible for the neurological symptoms in CM	68
Lymphotoxin (LTα) and not TNFα would be the major mediator in murine experimental CM	60
TNFα and adhesion molecules in CM	
After TNFα treatment , ICAM-1 and VCAM-1 were upregulated, specifically in brain microvascular endothelial cells coming from CM-sensitive mice	66
Serum TNFα levels were lower and TNFα mRNAs were higher in ICAM-1-deficient mice when compared with wild-type	68
In TNF-alpha/beta-deficient mice, PbA did not induce the upregulation of ICAM-1 expression required for the development of CM.	57
TNFα and TNFR1 and TNFR2 receptors in CM	
TNFR2 expression was increased in CM-susceptible mice when compared with CM-resistant mice, whereas TNFR1 expression was not modified. TNFR2-deficient mice but not TNFR1-deficient mice are protected from PbA-induced CM	83
After TNFα treatment, TNFR1 and TNFR2 were both upregulated at the same level on BMVEC from CM-sensitive mice when compared with CM-resistant mice.	66
Transgenic mice with high levels of soluble sTNFR1 were protected from experimental CM	86
TNFα and NO in CM	
High levels of TNFα in CM would induce NO production in vascular walls which, through retro-control, would reduce NO formation in postsynaptic neurons with cerebral symptoms as a consequence	87, 88
Pentoxifylline (TNFα inhibitor) and anti-TNFα monoclonal antibody did not modify reactive nitrogen intermediates synthesis and NO synthetase inhibitors did not modify occurrence of CM in a murine experimental model.	90
IL10 : TNFα ratio in CM	
IL10 : TNFα ratio was lower in severe anaemia but did not differ significantly between patients with CM or uncomplicated malaria	97
Children with CM consistently had IL10:TNFα ratios of < 1	10
TNFα in the protection from CM	
Low levels of rh-TNFα protected *P. berghei* K173-infected mice from CM	99
Monoclonal antibody anti-TNFα failed to improve survival in Gambian children with CM	101

ulation therapy can be considered in the prevention and/or treatment of CM.

1 Mazier D., Nitcheu J. and Idrissa-Boubou M. (2000) Cerebral malaria and immunogenetics. Parasite Immunol. **22:** 613–623
2 Craig A. and Scherf A. (2001) Molecules on the surface of the *Plasmodium falciparum* infected erythrocyte and their role in malaria pathogenesis and immune evasion. Mol. Biochem. Parasitol. **115:** 129–143
3 Meager A. (1999) Cytokine regulation of cellular adhesion molecule expression in inflammation. Cytokine Growth Factor Rev. **10:** 27–39
4 Nitcheu J., Bonduelle O., Combadiere C., Tefit M., Seilhean D., Mazier D. et al. Perforin-dependent brain-infiltrating cytotoxic $CD8^+$ T Lymphocytes mediate experimental cerebral malaria pathogenesis. J. Immunol. **170:** 2221–2228
5 Brown H., Turner G., Rogerson S., Tembo M., Mwenechanya J., Molyneux M. et al. (1999) Cytokine expression in the brain in human cerebral malaria. J. Infect. Dis. **180:** 1742–1746

6 Bazzoni F. and Beutler B. (1996) The tumor necrosis factor ligand and receptor families. N. Engl. J. Med. **334:** 1717– 1725
7 Munoz-Fernandez M. A. and Fresno M. (1998) The role of tumour necrosis factor, interleukin 6, interferon-gamma and inducible nitric oxide synthase in the development and pathology of the nervous system. Prog. Neurobiol. **56:** 307–340
8 Wallach D. (1996) A decade of accumulated knowledge and emerging answers. The 6th international congress on TNF Rhodes, Greece. May 1996. Eur. Cytokine. Netw. **7:** 713–724
9 Kwiatkowski D., Molyneux M. E., Stephens S., Curtis N., Klein N., Pointaire P. et al. (1993) Anti-TNF therapy inhibits fever in cerebral malaria. Q. J. Med. **86:** 91–98
10 May J., Lell B., Luty A. J., Meyer C. G. and Kremsner P. G. (2000) Plasma interleukin-10:Tumor necrosis factor (TNF)-alpha ratio is associated with TNF promoter variants and predicts malarial complications. J. Infect. Dis. **182:** 1570–1573
11 Tessier P. A., Naccache P. H., Clark-Lewis I., Gladue R. P., Neote K. S. and McColl S. R. (1997) Chemokine networks in vivo: involvement of C-X-C and C-C chemokines in neutrophil extravasation in vivo in response to TNF-alpha. J. Immunol. **159:** 3595–3602
12 Ringwald P., Peyron F., Lepers J. P., Rabarison P., Rakotomalala C., Razanamparany M. et al. (1993) Parasite virulence factors during falciparum malaria: rosetting, cytoadherence and modulation of cytoadherence by cytokines. Infect. Immun. **61:** 5198–5204
13 Gnant M. F., Turner E. M. and Alexander H. R., Jr. (2000) Effects of hyperthermia and tumour necrosis factor on inflammatory cytokine secretion and procoagulant activity in endothelial cells. Cytokine **12:** 339–347
14 Grell M., Zimmermann G., Gottfried E., Chen C. M., Grunwald U., Huang D. C. et al. (1999) Induction of cell death by tumour necrosis factor (TNF) receptor 2, CD40 and CD30: a role for TNF-R1 activation by endogenous membrane-anchored TNF. EMBO J. **18:** 3034–3043
15 Mehler M. F. and Kessler J. A. (1997) Hematolymphopoietic and inflammatory cytokines in neural development. Trends Neurosci. **20:** 357–365
16 Marino M. W., Dunn A., Grail D., Inglese M., Noguchi Y., Richards E. et al. (1997) Characterization of tumor necrosis factor-deficient mice. Proc. Natl. Acad. Sci. USA **94:** 8093–8098
17 Akassoglou K., Probert L., Kontogeorgos G. and Kollias G. (1997) Astrocyte-specific but not neuron-specific transmembrane TNF triggers inflammation and degeneration in the central nervous system of transgenic mice. J. Immunol. **158:** 438–445
18 Lee Y. B., Schrader J. W. and Kim S. U. (2000) p38 map kinase regulates TNF-alpha production in human astrocytes and microglia by multiple mechanisms. Cytokine **12:** 874–880
19 Probert L., Akassoglou K., Pasparakis M., Kontogeorgos G. and Kollias G. (1995) Spontaneous inflammatory demyelinating disease in transgenic mice showing central nervous system-specific expression of tumor necrosis factor alpha. Proc. Natl. Acad. Sci. USA **92:** 11294–11298
20 Beeson J. G. and Brown G. V. (2002) Pathogenesis of *Plasmodium falciparum* malaria: the roles of parasite adhesion and antigenic variation. Cell. Mol. Life Sci. **59:** 258–271
21 Richards A. L. (1997) Tumour necrosis factor and associated cytokines in the host's response to malaria. Int. J. Parasitol. **27:** 1251–1263
22 Nussler A., Drapier J. C., Renia L., Pied S., Miltgen F., Gentilini M. et al. (1991) L-arginine-dependent destruction of intrahepatic malaria parasites in response to tumor necrosis factor and/or interleukin 6 stimulation. Eur. J. Immunol. **21:** 227–230
23 Nussler A., Pied S., Goma J., Renia L., Miltgen F., Grau G. E. et al. (1991) TNF inhibits malaria hepatic stages in vitro via synthesis of IL-6. Int. Immunol. **3:** 317–321
24 Schofield L., Vivas L., Hackett F., Gerold P., Schwarz R. T. and Tachado S. (1993) Neutralizing monoclonal antibodies to glycosylphosphatidylinositol, the dominant TNF-alpha-inducing toxin of Plasmodium falciparum: prospects for the immunotherapy of severe malaria. Ann. Trop. Med. Parasitol. **87:** 617–626
25 Schofield L., Novakovic S., Gerold P., Schwarz R. T., McConville M. J. and Tachado S. D. (1996) Glycosylphosphatidylinositol toxin of *Plasmodium* up-regulates intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and E-selectin expression in vascular endothelial cells and increases leukocyte and parasite cytoadherence via tyrosine kinase-dependent signal transduction. J. Immunol. **156:** 1886–1896
26 Tachado S. D., Gerold P., McConville M. J., Baldwin T., Quilici D., Schwarz R. T. et al. (1996) Glycosylphosphatidylinositol toxin of *Plasmodium* induces nitric oxide synthase expression in macrophages and vascular endothelial cells by a protein tyrosine kinase-dependent and protein kinase C-dependent signaling pathway. J. Immunol. **156:** 1897–1907
27 Schofield L., Hewitt M., Evans K., Siomos M. and Seeberger P. (2002) Synthetic GPI as a candidate antitoxic vaccine in a model of malaria. Nature **418:** 785–789
28 Naotunne T. S., Karunaweera N. D., Del Giudice G., Kularatne M. U., Grau G. E., Carter R. et al. (1991) Cytokines kill malaria parasites during infection crisis: extracellular complementary factors are essential. J. Exp. Med. **173:** 523–529
29 Yoeli M. (1976) Chadwick lecture. Cerebral malaria – the quest for suitable experimental models in parasitic diseases of man. Trans. R. Soc. Trop. Med. Hyg. **70:** 24–35
30 Kwiatkowski D., Cannon J. G., Manogue K. R., Cerami A., Dinarello C. A. and Greenwood B. M. (1989). Tumour necrosis factor production in Falciparum malaria and its association with schizont rupture. Clin. Exp. Immunol. **77:** 361–366
31 Wahlgren M., Carlson J., Helmby H., Hedlund I. and Treutiger C. J. (1992) Molecular mechanisms and biological importance of *Plasmodium falciparum* erythrocyte rosetting. Mem. Inst. Oswaldo Cruz **87:** 323–329
32 Kawai S., Aikawa M., Kano S. and Suzuki M. (1993) A primate model for severe human malaria with cerebral involvement: *Plasmodium coatneyi*-infected *Macaca fuscata*. Am. J. Trop. Med. Hyg. **48:** 630–636
33 Hearn J., Rayment N., Landon D. N., Katz D. R. and de Souza J. B. (2000) Immunopathology of cerebral malaria: morphological evidence of parasite sequestration in murine brain microvasculature. Infect. Immun. **68:** 5364–5376
34 Lou J., Lucas R. and Grau G.E. (2001) Pathogenesis of cerebral malaria: recent experimental data and possible application for humans. Clin. Microbiol. Rev. **14:** 810–820
35 Akanmori B. D., Kurtzhals J. A., Goka B. Q., Adabayeri V., Ofori M. F., Nkrumah F. K. et al. (2000) Distinct patterns of cytokine regulation in discrete clinical forms of *Plasmodium falciparum* malaria. Eur. Cytokine. Netw. **11:** 113–118
36 Shaffer N., Grau G. E., Hedberg K., Davachi F., Lyamba B., Hightower A. W. et al. (1991) Tumor necrosis factor and severe malaria. J. Infect. Dis. **163:** 96–101
37 Grau G. E., Heremans H., Piguet P. F., Pointaire P., Lambert P. H., Billiau A. et al. (1989) Monoclonal antibody against interferon gamma can prevent experimental cerebral malaria and its associated overproduction of tumor necrosis factor. Proc. Natl. Acad. Sci. USA **86:** 5572–5574
38 Kern P., Hemmer C. J., Van Damme J., Gruss H. J. and Dietrich M. (1989) Elevated tumor necrosis factor alpha and interleukin-6 serum levels as markers for complicated *Plasmodium falciparum* malaria. Am. J. Med. **87:** 139–143
39 Kwiatkowski D., Hill A. V., Sambou I., Twumasi P., Castracane J., Manogue K. R. et al. (1990) TNF concentration in fatal cerebral, non-fatal cerebral, and uncomplicated *Plasmodium falciparum* malaria. Lancet **336:** 1201–1204
40 Karunaweera N. D., Grau G. E., Gamage P., Carter R. and Mendis K. N. (1992) Dynamics of fever and serum levels of

tumor necrosis factor are closely associated during clinical paroxysms in *Plasmodium vivax* malaria. Proc. Natl. Acad. Sci. USA **89:** 3200–3203
41 Day N. P., Hien T. T., Schollaardt T., Loc P. P., Chuong L. V., Chau T. T. et al. (1999) The prognostic and pathophysiologic role of pro- and antiinflammatory cytokines in severe malaria. J. Infect. Dis. **180:** 1288–1297
42 Grau G. E., Piguet P. F., Vassalli P. and Lambert P. H. (1989) Tumor-necrosis factor and other cytokines in cerebral malaria: experimental and clinical data. Immunol. Rev. **112:** 49–70
43 Newton C. R. and Krishna S. (1998) Severe falciparum malaria in children: current understanding of pathophysiology and supportive treatment. Pharmacol. Ther. **79:** 1–53
44 Hill A. V., Allsopp C. E., Kwiatkowski D., Anstey N. M., Twumasi P., Rowe P. A. et al. (1991) Common west African HLA antigens are associated with protection from severe malaria. Nature **352:** 595–600
45 Wilson A. G., Symons J. A., McDowell T. L., McDevitt H. O., and Duff G. W. (1997) Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc. Natl. Acad. Sci. USA **94:** 3195–3199
46 McGuire W., Knight J. C., Hill A. V., Allsopp C. E., Greenwood B. M. and Kwiatkowski D. (1999) Severe malarial anemia and cerebral malaria are associated with different tumor necrosis factor promoter alleles. J. Infect. Dis. **179:** 287–290
47 McGuire W., Hill A. V., Allsopp C. E., Greenwood B. M. and Kwiatkowski D. (1994) Variation in the TNF-alpha promoter region associated with susceptibility to cerebral malaria. Nature **371:** 508–510
48 Aidoo M., McElroy P. D., Kolczak M. S., Terlouw D. J., ter Kuile F. O., Nahlen B. et al. (2001) Tumor necrosis factor-alpha promoter variant 2 (TNF2) is associated with pre-term delivery, infant mortality and malaria morbidity in western Kenya: Asembo Bay Cohort Project IX. Genet. Epidemiol. **21:** 201–211
49 Knight J. C., Udalova I., Hill A. V., Greenwood B. M., Peshu N., Marsh K. et al. (1999) A polymorphism that affects OCT-1 binding to the TNF promoter region is associated with severe malaria. Nat. Genet. **22:** 145–150
50 Ubalee R., Suzuki F., Kikuchi M., Tasanor O., Wattanagoon Y., Ruangweerayut R. et al. (2001) Strong association of a tumor necrosis factor-alpha promoter allele with cerebral malaria in Myanmar. Tissue Antigens **58:** 407–410
51 Allan R. J., Beattie P., Bate C., Van Hensbroek M. B., Morris-Jones S., Greenwood B. M. et al. (1995) Strain variation in tumor necrosis factor induction by parasites from children with acute falciparum malaria. Infect. Immun. **63:** 1173–1175
52 Grau G. E., Fajardo L. F., Piguet P. F., Allet B., Lambert P. H. and Vassalli P. (1987) Tumor necrosis factor (cachectin) as an essential mediator in murine cerebral malaria. Science **237:** 1210–1212
53 Grau G. E. and Lou J. (1993) TNF in vascular pathology: the importance of platelet-endothelium interactions. Res. Immunol. **144:** 355–363
54 Clark I. A., Ilschner S., MacMicking J. D. and Cowden W. B. (1990) TNF and *Plasmodium berghei* ANKA-induced cerebral malaria. Immunol. Lett. **25:** 195–198
55 de Kossodo S. and Grau G. E. (1993) Profiles of cytokine production in relation with susceptibility to cerebral malaria. J. Immunol. **151:** 4811–4820
56 Jennings V. M., Actor J. K., Lal A. A. and Hunter R. L. (1997) Cytokine profile suggesting that murine cerebral malaria is an encephalitis. Infect Immun **65:** 4883–4887
57 Rudin W., Eugster H. P., Bordmann G., Bonato J., Muller M., Yamage M. et al. (1997) Resistance to cerebral malaria in tumor necrosis factor-alpha/beta-deficient mice is associated with a reduction of intercellular adhesion molecule-1 up-regulation and T helper type 1 response. Am. J. Pathol. **150:** 257–266
58 Amani V., Vigario A. M., Belnoue E., Marussig M., Fonseca L., Mazier D. et al. (2000) Involvement of IFN-γ receptor-mediated signalling in protection against *Plasmodium berghei* infection and its pathology. Eur. J. Immunol. **30:** 1646–1655
59 Yanez D. M., Manning D. D., Cooley A. J., Weidanz W. P. and van der Heyde H. C. (1996) Participation of lymphocyte subpopulations in the pathogenesis of experimental murine cerebral malaria. J. Immunol. **157:** 1620–1624
60 Engwerda C. R., Mynott T. L., Sawhney S., De Souza J. B., Bickle Q. D. and Kaye P. M. (2002) Locally up-regulated lymphotoxin alpha, not systemic tumor necrosis factor alpha, is the principle mediator of murine cerebral malaria. J. Exp. Med. **195:** 1371–1377
61 Dietrich J. B. (2002) The adhesion molecule ICAM-1 and its regulation in relation with the blood-brain barrier. J. Neuroimmunol. **128:** 58–68
62 Baud V. and Karin M. (2001) Signal transduction by tumor necrosis factor and its relatives. Trends Cell. Biol. **11:** 372–377
63 Brown H., Hien T. T., Day N., Mai N. T., Chuong L. V., Chau T. T. et al. (1999) Evidence of blood-brain barrier dysfunction in human cerebral malaria. Neuropathol. Appl. Neurobiol. **25:** 331–340
64 Prudhomme J. G., Sherman I. W., Land K. M., Moses A. V., Stenglein S. and Nelson J. A. (1996) Studies of *Plasmodium falciparum* cytoadherence using immortalized human brain capillary endothelial cells. Int. J. Parasitol. **26:** 647–655
65 Dobbie M. S., Hurst R. D., Klein N. J. and Surtees R. A. (1999) Upregulation of intercellular adhesion molecule-1 expression on human endothelial cells by tumour necrosis factor-alpha in an in vitro model of the blood-brain barrier. Brain Res. **830:** 330-336
66 Lou J., Gasche Y., Zheng L., Critico B., Monso-Hinard C., Juillard P. et al. (1998) Differential reactivity of brain microvascular endothelial cells to TNF reflects the genetic susceptibility to cerebral malaria. Eur. J. Immunol. **28:** 3989–4000
67 McGuire W., Hill A. V., Greenwood B. M. and Kwiatkowski D. (1996) Circulating ICAM-1 levels in falciparum malaria are high but unrelated to disease severity. Trans. R. Soc. Trop. Med. Hyg. **90:** 274–276
68 Favre N., Da Laperousaz C., Ryffel B., Weiss N. A., Imhof B. A., Rudin W. et al. (1999) Role of ICAM-1 (CD54) in the development of murine cerebral malaria. Microbes Infect. **1:** 961–968
69 Mayhan W. G. (2002) Cellular mechanisms by which tumor necrosis factor-alpha produces disruption of the blood-brain barrier. Brain Res. **927:** 144–152
70 Descamps L., Cecchelli R. and Torpier G. (1997) Effects of tumor necrosis factor on receptor-mediated endocytosis and barrier functions of bovine brain capillary endothelial cell monolayers. J. Neuroimmunol. **74:** 173–184
71 Petito C. K., Adkins B., Tracey K., Roberts B., Torres-Munoz J., McCarthy M. et al. (1999) Chronic systemic administration of tumor necrosis factor alpha and HIV gp120: effects on adult rodent brain and blood-brain barrier. J. Neurovirol. **5:** 314–318
72 Schnell L., Fearn S., Schwab M. E., Perry V. H. and Anthony D. C. (1999) Cytokine-induced acute inflammation in the brain and spinal cord. J. Neuropathol. Exp. Neurol. **58:** 245–254
73 de Vries H.E., Blom-Roosemalen M.C., van Oosten M., de Boer A.G., van Berkel T.J., Breimer D.D. et al. (1996) The influence of cytokines on the integrity of the blood-brain barrier in vitro. J. Neuroimmunol. **64:** 37–43
74 Mark K.S. and Miller D.W. (1999) Increased permeability of primary cultured brain microvessel endothelial cell monolayers following TNF-alpha exposure. Life Sci. **64:** 1941–1953

75 Dickstein J. B., Moldofsky H., Lue F. A. and Hay J. B. (1999) Intracerebroventricular injection of TNF-alpha promotes sleep and is recovered in cervical lymph. Am. J. Physiol. **276:** R1018–1022

76 Tsao N., Hsu H. P. and Lei H. Y. (1999) TNFalpha-induced cyclooxygenase 2 not only increases the vasopermeability of blood-brain barrier but also enhances the neutrophil survival in *Escherichia coli*-induced brain inflammation. Prostaglandins Other Lipid Mediat. **57:** 371–382

77 Saija A., Princi P., Lanza M., Scalese M., Aramnejad E. and De Sarro A. (1995) Systemic cytokine administration can affect blood-brain barrier permeability in the rat. Life Sci. **56:** 775–784

78 Migasena P. and Maegraith B. G. (1968) Factor affecting on the movement of protein across the blood: brain: C.S.F. barriers in *Plasmodium knowlesi* infected *Macaca mulatta*. Med. J. Malaya **22:** 251

79 Pino P., Vouldoukis I., Mahmoudi M., Kolb J. P., Desportes-Livage I., Danis M. et al. (2002) *Plasmodium falciparum*-infected erythrocyte adhesion induces caspase activation and apoptosis in human endothelial cells. J. Infect. Dis., in press

80 Derouich-Guergour D., Brenier-Pinchart M. P., Ambroise-Thomas P. and Pelloux H. (2001) Tumour necrosis factor alpha receptors: role in the physiopathology of protozoan parasite infections. Int. J. Parasitol. **31:** 763–769

81 Lucas R., Lou J. N., Juillard P., Moore M., Bluethmann H. and Grau G. E. (1997) Respective role of TNF receptors in the development of experimental cerebral malaria. J. Neuroimmunol. **72:** 143–148

82 Piguet P.F., Kan C.D. and Vesin C. (2002) Role of the tumor necrosis factor receptor 2 (TNFR2) in cerebral malaria in mice. Lab. Investig. **82:** 1155–1166

83 Lucas R., Lou J., Morel D. R., Ricou B., Suter P. M. and Grau G. E. (1997) TNF receptors in the microvascular pathology of acute respiratory distress syndrome and cerebral malaria. J. Leukoc. Biol. **61:** 551–558

84 Sibson N. R., Blamire A. M., Perry V. H., Gauldie J., Styles P. and Anthony D. C. (2002) TNF-α reduces cerebral blood volume and disrupts tissue homeostasis via an endothelin- and TNFR2-dependent pathway. Brain **125:** 2446–2459

85 Lesslauer W., Tabuchi H., Gentz R., Brockhaus M., Schlaeger E. J., Grau G. et al. (1991) Recombinant soluble tumor necrosis factor receptor proteins protect mice from lipopolysaccharide-induced lethality. Eur. J. Immunol. **21:** 2883–2886

86 Garcia I., Miyazaki Y., Araki K., Araki M., Lucas R., Grau G.E. et al. (1995) Transgenic mice expressing high levels of soluble TNF-R1 fusion protein are protected from lethal septic shock and cerebral malaria, and are highly sensitive to *Listeria monocytogenes* and *Leishmania* major infections. Eur. J. Immunol. **25:** 2401–2407

87 Clark I. A., Rockett K. A. and Cowden W. B. (1991) Role of TNF in cerebral malaria. Lancet **337:** 302–303

88 Clark I. A., Rockett K. A. and Cowden W. B. (1992) Possible central role of nitric oxide in conditions clinically similar to cerebral malaria. Lancet **340:** 894–896

89 Kremsner P. G., Neifer S., Rasenack T. and Bienzle U. (1993) Interference by antimalarial drugs with the in vitro production of reactive nitrogen intermediates by murine macrophages. J. Antimicrob. Chemother. **31:** 385–392

90 Kremsner P. G., Nussler A., Neifer S., Chaves M. F., Bienzle U., Senaldi G. et al. (1993). Malaria antigen and cytokine-induced production of reactive nitrogen intermediates by murine macrophages: no relevance to the development of experimental cerebral malaria. Immunology **78:** 286–290

91 Vouldoukis I., Pino P., Dugas N., Olivier R., Nitcheu J., Traore B. et al. (2002) Nitric oxide production by CD23-bearing human pulmonary endothelial cells down-regulates the expression of ICAM-1 and decreases cyto-adherence of *Plasmodium falciparum* infected erythrocytes. Molecular Medicine, in press

92 Ho M., Sexton M. M., Tongtawe P., Looareesuwan S., Suntharasamai P. and Webster H. K. (1995) Interleukin-10 inhibits, tumor necrosis factor production but not antigen-specific lymphoproliferation in acute *Plasmodium falciparum* malaria. J. Infect. Dis. **172:** 838–844

93 Wenisch C., Parschalk B., Narzt E., Looareesuwan S. and Graninger W. (1995) Elevated serum levels of IL-10 and IFN-gamma in patients with acute *Plasmodium falciparum* malaria. Clin. Immunol. Immunopathol. **74:** 115–117

94 Eckwalanga M., Marussig M., Tavares M. D., Bouanga J. C., Hulier E., Pavlovitch J. H. et al. (1994) Murine AIDS protects mice against experimental cerebral malaria: down- regulation by interleukin 10 of a T-helper type 1 CD4+ cell-mediated pathology. Proc. Natl. Acad. Sci. USA **91:** 8097–8101

95 Kossodo S., Monso C., Juillard P., Velu T., Goldman M. and Grau G. E. (1997) Interleukin-10 modulates susceptibility in experimental cerebral malaria. Immunology **91:** 536–540

96 Othoro C., Lal A. A., Nahlen B., Koech D., Orago A. S. and Udhayakumar V. (1999) A low interleukin-10 tumor necrosis factor-alpha ratio is associated with malaria anemia in children residing in a holoendemic malaria region in western Kenya. J. Infect. Dis. **179:** 279-282

97 Kurtzhals J. A., Adabayeri V., Goka B. Q., Akanmori B. D., Oliver-Commey J. O., Nkrumah F. K. et al. (1998). Low plasma concentrations of interleukin 10 in severe malarial anaemia compared with cerebral and uncomplicated malaria. Lancet **351:** 1768–1772

98 Curfs J. H., Hermsen C. C., Kremsner P., Neifer S., Meuwissen J. H., Van Rooyen N. et al. (1993) Tumour necrosis factor-alpha and macrophages in *Plasmodium berghei*-induced cerebral malaria. Parasitology **107 (Pt 2):** 125–134

99 Postma N. S., Crommelin D. J., Eling W. M. and Zuidema J. (1999) Treatment with liposome-bound recombinant human tumor necrosis factor-alpha suppresses parasitemia and protects against *Plasmodium berghei* k173-induced experimental cerebral malaria in mice. J. Pharmacol. Exp. Ther. **288:** 114–120

100 Curfs J. H., Hermsen C. C., Meuwissen J. H. and Eling W. M. (1992) Immunization against cerebral pathology in *Plasmodium berghei*-infected mice. Parasitology **105 (Pt 1):** 7–14

101 van Hensbroek M. B., Palmer A., Onyiorah E., Schneider G., Jaffar S., Dolan G. et al. (1996) The effect of a monoclonal antibody to tumor necrosis factor on survival from childhood cerebral malaria. J. Infect. Dis. **174:** 1091–1097

102 Looareesuwan S., Sjostrom L., Krudsood S., Wilairatana P., Porter R. S., Hills F. et al. (1999) Polyclonal anti-tumor necrosis factor-alpha Fab used as an ancillary treatment for severe malaria. Am. J. Trop. Med. Hyg. **61:** 26–33

103 Looareesuwan S., Wilairatana P., Vannaphan S., Wanaratana V., Wenisch C., Aikawa M. et al. (1998) Pentoxifylline as an ancillary treatment for severe falciparum malaria in Thailand. Am. J. Trop. Med. Hyg. **58:** 348–353

104 Wenisch C., Looareesuwan S., Wilairatana P., Parschalk B., Vannapann S., Wanaratana V. et al. (1998) Effect of pentoxifylline on cytokine patterns in the therapy of complicated *Plasmodium falciparum* malaria. Am. J. Trop. Med. Hyg. **58:** 343–347

105 Hemmer C. J., Hort G., Chiwakata C. B., Seitz R., Egbring R., Gaus W. et al. (1997) Supportive pentoxifylline in falciparum malaria: no effect on tumor necrosis factor alpha levels or clinical outcome: a prospective, randomized, placebo-controlled study. Am. J. Trop. Med. Hyg. **56:** 397–403