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Macrophages/microglial cells in patients with cerebral malaria

Abstract

Cerebral malaria is a life threatening sequel of Plasmodium falciparum infection and contributes significantly to malaria mortality, especially among children. Accumulation of macrophages and proliferation of microglial cells play key roles in cerebral malaria and are thought to contribute to the pathophysiological alterations observed in these patients, which include enhanced adherence of infected erythrocytes to the cerebral vasculature by expression and secretion of proinflammatory molecules, disruption of the blood-brain barrier, recruitment of other inflammatory cells to the lesion site. In this review, recent advances in

the understanding of the involvement of macrophages/microglial cells in the development of cerebral malaria are summarized.

Full text

INTRODUCTION

Plasmodium falciparum can cause the most severe and life threatening form of malaria in man. While the majority of infected individuals suffer a mild febrile illness, some patients develop severe disease. Frequently observed clinical sequelae of severe malaria include severe anemia, hypoglycemia, hyperlactatemia, renal failure, respiratory distress and cerebral malaria (CM). The latter is considered one of the most serious complications, with a high mortality rate at around 1 million deaths per year. The clinical course of CM is characterized by a loss of consciousness. The detailed pathophysiology of CM remains far from completely resolved. However, inflammatory cytokines have been suspected of contributing to CM by modulating key parameters of brain parenchyma homeostasis, such as endothelial cell integrity, activation of astrocytes and immune response. Pathological hallmarks are cerebral venules packed with parasitized erythrocytes, microhemorrhages, local and global hypoxia-ischemia and glial proliferation. The final stage of this pathological process is the formation of lesions, termed Dürck´s granulomas, characterized by aggregates of astrocytes and microglial cells [1]. Although formal proof is lacking, these alterations are thought to constitute the pathophysiological counterpart of the neurological impairments observed in survivors and could be the basis of fatal brain edema.

Adhesion of parasitized erythrocytes to brain endothelial cells, inflammatory burst, disruption of the blood-brain barrier, immigration of blood-borne inflammatory cells, microvessel hemorrhage, formation of Dürck´s granulomas and finally, glial scarring, are complex mechanisms involved in the development of clinically apparent cerebral malaria. Although a wide range of cell types has been identified, macrophages/microglial cells are key determinators of these processes.

Direct effects of *Plasmodium falciparum* are well defined, and are thought to contribute to the activation of endothelial cells that results in increased leukocyte and parasited erythrocyte adherence to brain endothelial cells [2].

However, modulation of the activation of endothelial cells and macrophages, and disruption of endothelial intercellular junctions in vessels containing sequestered parasitized erythrocytes observed in patients with cerebral malaria, is thought to be mediated by cytokines [3, 4]. Interestingly, tumor necrosis factor alpha (TNF-alpha) a cytokine with a wide range of biological activities, is produced mainly by macrophages [5]. Secreted TNF-alpha is considered a key player in lesion formation in brains of patients with cerebral malaria. It induces a cascade of pathophysiological alterations in adjacent endothelial cells that leads to the disruption of the blood-brain barrier by induction of ICAM-1 [6, 7]. Other mechanisms are thought to contribute to this process: the ring-infected erythrocyte surface antigen is another inducer of TNF-alpha [8]. Although the pathophysiological importance of TNF-alpha is undoubted, detrimental as well as beneficial overall effects have been ascribed to TNF production during malaria [9, 10]. One reason for this phenomenon might be the fact that the signal transduction leading to TNF-alpha secretion is mediated partly through protein kinase C and calmodulin-dependent protein kinase activation and it was suggested from these data that a wide range of cytokines is capable of modulating the course of cerebral malaria *via* the modulation of TNF-alpha production [11]. In clinical trials, TNF-alpha reduction has nevertheless become an important hallmark alteration in the pharmacological modulation of cerebral malaria [12, 13].

Evidence is accumulating that accessory TNF-alpha alpha interdependent mechanisms are capable of mediating some of the pathophysiological phenomena observed in cerebral malaria. One prominent candidate is the nitric oxide synthase 2 (i-NOS, NOS2). NOS2 expression was found in endothelial cells, neurones, astrocytes and microglial cells in cases of CM. There was also strong staining in macrophages surrounding ring haemorrhages. NOS2 staining was decreased in recovering malaria cases compared to acute CM, and was low in controls. Quantification showed a significant association between the intensity and number of iNOS positive vessels with the severity of malaria as assessed by histopathological changes, although the total number of cells stained was not increased compared to recovering CM cases [14]. Overall induction of NOS2 expression during *Plasmodium falciparum* malaria reflects disease severity. High plasma levels of nitrogen oxides are associated with severe disease and correlate with rapid parasitological and clinical cure in *Plasmodium falciparum* malaria [15]. Moreover, peroxynitrite, which is formed after chemical reaction of NO with superoxide, appears to be the principal effector molecule for

macrophage-mediated cytotoxicity toward *Plasmodium falciparum*. Interferon-gamma is thought to be a major regulatory cytokine for this process [16]. As nitric oxide (NO) may activate a number of secondary neuropathological mechanisms in the brain, including modulators of synaptic function, induction of NOS2 expression in cerebral malaria may contribute to coma, seizures and death, but it also may have beneficial effects. For example, cytokine stimulation of human macrophages resulted in increased NO formation and decreased survival of plasmodia within cocultured human erythrocytes. Here, addition of desferrioxamine before cytokine treatment increased both NO formation and parasite killing, but had no effect in the presence of an inhibitor of NO formation [16]. Not only do macrophages contribute to cerebral malaria, but also brain histiocytes called microglia. Microglial cells are strategically placed, cellular sensors responding to a variety of stressors of the central nervous system. Central nervous system microglia are a source of defense cells in the CNS capable of carrying out certain immune functions autonomously. During *Plasmodium falciparum* infection, they become activated, and contribute to lesion formation in brains of patients with cerebral malaria by modulating the interdependent mechanisms of the disease: blood flow impairment by parasitized red blood cells, secretion of proinflammatory cytokines, release of free radicals and immunological dysfunction. Microglial cells express a broad spectrum of cytokines that are well known modulators of blood-brain barrier breakdown, endothelial activation and inflammation, and their involvement in degenerative, inflammatory and neoplastic brain disease is undoubted. In this review, current data on the role and function of both macrophages and microglial cells in cerebral malaria, with respect to the pathophysiological mechanisms observed in these patients, are summarized.

Specimens of brain material were collected from adult Europeans who had travelled to Africa, acquired *Plasmodium falciparum* malaria there, and died with cerebral malaria in primary care hospitals after returning to Germany. To ensure the clinical diagnosis of cerebral malaria and to exclude other infectious diseases of the brain, CT scans and lumbar punctures were conducted. *Plasmodium falciparum* was detected in peripheral venous blood smears of all patients. No differences were observed between patients in the stage of the 48 hours red cell stage cycle. All patients were in the late trophozoite and schizont stage. Clinical condition was monitored using the Glasgow coma scale. In all tissue specimens examined, prominent intravascular sequestration, with parasitized erythrocytes can readily be observed. Samples were taken from

affected regions of each brain characterized either by granuloma formation or intravascular sequestration (Table).

TRANSFORMING GROWTH FACTORS (TGF)-beta1, -2 AND -3

Transforming growth factors (TGF)-beta1, -2 and -3 are thought to contribute to some phenomena observed in patients with malaria. As a consequence, a reduction of peripheral TGF-beta concentrations during systemic and cerebral malaria has been observed and may be associated with TGF-beta tissue accumulation [17, 18]. Only limited information is available about TGF-beta function in cerebral malaria. However, there is convincing evidence that TGF-betas are candidate cytokines involved in the formation of pathophysiological hallmark alterations in CM patients. Predominantly, TGF-betas modulate functional properties of cells that participate in the immune response. In the rat brain, TGF-betas contribute to global inflammation in experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis [19], astrocytic scar formation in rat cerebral cortex after a penetrating brain injury [20] and endothelial cell activation *in vitro* [21]. By using RT-PCR on *postmortem* human tissues from patients with CM, the induction of the proinflammatory cytokines TNF-alpha and IL-1beta was recently revealed. TGF-beta was expressed in normal brain, in cerebral malaria, and in meningitis and encephalitis. IL-1beta was absent from normal brain, but was detected in CM and other cerebral infections. TNF-alpha mRNA was expressed only in CM, although TNF-alpha protein was also seen in meningitis [4].

In an immunohistochemical study however, we conversely observed prominent TGF-beta1 immunoreactivity in astrocytes that form the blood-brain barrier around cerebral capillaries, characterized by deposition of malaria pigment and sequestration (Figure 1a) [22]. Double labeling experiments revealed the coimmunoreactivity of S-100beta (astrocytes), but not of HLA-DR, -DP, -DQ or CD68 (macrophages/microglial cells) in TGF-beta1 immunoreactive cells. Statistical analysis using the two-tailed Mann-Whitney rank sum test revealed significantly ($p = 0.0003$) more TGF-beta1 immunoreactive astrocytes than in neuropathologically unaltered brains. Gross effects of TGF-beta1 in the brain are aimed at the reconstruction of neural tissues following their disruption during inflammation and degeneration, and to maintain neuronal viability [23]. More specifically, TGF-beta1 induces apoptosis of microglial cells [24], controls CNS inflammation by regulating cytokine - induced vascular cellular adhesion

molecule-1 expression [25], and promotes angiogenesis by eliciting vascular endothelial growth factor expression [26]. Therefore, these findings provide further evidence for the involvement of TGF-beta1 in the process of the reorganization of brain parenchyma in patients who suffered from CM.

TGF-beta2 was found in macrophages/microglial cells that accumulated in Dürck's granulomas and in glioses of ring hemorrhages (Figure 1b). TGF-beta2 immunoreactive cells were characterized by the coimmunoreactivity of CD68 and HLA-DR, -DP, -DQ, but not of S100b. Perivascular TGF-beta2 immunoreactive cells were only double labeled with CD68. No colocalization of HLA-DR, -DP, -DQ was observed in these cells. Statistical analysis revealed significantly ($p = 0.0081$) more TGF-beta2 immunoreactive macrophages/microglial cells than in neuropathologically unaltered brains. Accumulation of TGF-beta2 immunoreactive cells was occasionally found in areas of perivascular cellular infiltration. In addition, few
TGF-beta1 and -beta2 immunoreactive neurons and macrophages/microglial cells were scattered in the brain parenchymas examined. The accumulation of TGF-beta2 immunoreactive macrophages/microglia cells in Dürck´s granulomas and in glioses of ring hemorrhages elicits new functional insights into the detailed pathophysiology of these lesions. TGF-beta2 is thought to interfere with key funtions of the immune system. In the brain, TGF-beta2 mediates immunosuppression, inhibits leukocyte transmigration across the blood-brain barrier in CNS inflammation and promotes intracerebral macrophage proliferation [27]. Accumulation of TGF-beta2 immunoreactive macrophages/microglial cells in granulomas and glioses of ring hemorrhages therefore indicates complex interactions with the immunological dysfunction observed in these patients.

TGF-beta3 immunoreactivity was prominently expressed in endothelial cells and smooth muscle cells of the CM brain vasculature, characterized by deposition of malarial pigment and sequestration (Figure 1c). TGF-beta3 immunoreactivity in endothelial cells was confirmed by colocalization of vWF (von-Willebrand-factor). TGF-beta3 immunoreactive cells of the vascular wall were double labeled for smooth muscle actin. No colocalization of the T cell marker CD3 and the B cell marker CD20 was observed in either TGF-beta1, -2 and -3 immunoreactive cells. Statistical analysis revealed significantly ($p = 0.0022$) more TGF-beta3 immunoreactive endothelial cells than in neuropathologically unaltered brains.

TGF-beta3 expression in endothelial and smooth muscle cells is considered to be an important modulator of cellular activation and transformation, and reorganization of complex vascular structures has been suggested to involve, at least in part, TGF-beta3. Only limited data are available concerning the involvement of TGF-beta3 in brain pathologies. However, expression of TGF-beta3 was identified in pathologically altered smooth muscle cells of atherosclerotic vessels and in fibrotic vasculature of hepatic human allografts, and has therefore been associated with thrombosis, atherosclerosis and endothelial cell activation [28]. Immunolocalization of TGF-beta3 in the brain vasculature, characterized by deposition of malarial pigment and sequestration in patients who died with CM, therefore suggests its involvement in sequestration and thrombosis.

CYCLOOXYGENASE-1 AND CYCLOOXYGENASE-2

Altered prostaglandin concentrations were revealed by clinical trials in peripheral blood of malaria patients [29]. Prostaglandin synthesis is controlled by cyclooxygenases (COX, prostaglandin endoperoxide synthase, PGG/H synthase), and COX expression has been attributed a key role in immunomodulation, hemostasis and inflammation in a wide variety of pathologically altered brain tissues. An indication for the involvement of prostaglandins (PG) in the control of parasitemia is suggested by prolonged parasite clearance time following administration of the PG synthesis inhibitor paracetamol [30]. Administration of glucocorticoid inhibitors of PG synthesis has been reported to be detrimental particularly in CM [31].

The rate limiting production of PG metabolites is controlled by cyclooxygenases. Two cyclooxygenase (COX) isoforms have been described [32] that catalyze identical PG reaction products [33]. PGs are mediators of inflammation, brain-blood flow, cellular aggregation and fever. While COX-1 is constitutively expressed in a range of tissues, COX-2 is cytokine-inducible following proinflammatory stimuli. Expression of COX-2 is associated with inflammation in Alzheimer´s disease [34] and early induction of COX-2 has been associated with tissue damage and remodeling of the surviving neural networks [35]. In an animal model of focal brain ischemia, COX-2 is induced in cortical neurons that are at a high risk of dying [36]. PGs of the E series inhibit the proliferation of T cells [37]. However, evidence is accumulating that cyclooxygenases exert more complex functions in angiogenesis, apoptosis and cell adhesion [38, 39].

In neuropathologically unaltered controls, only single COX-1 immunoreactive microglial cells were scattered throughout all examined brain areas (Figure 2a). In patients who died of cerebral malaria, accumulation of COX-1 immunoreactive macrophages/microglial cells was observed in Dürck´s granulomas (Figure 2b). The number of COX-1 immunoreactive macrophages/microglial cells was significantly ($p = 0.0006$) higher in Dürck´s granulomas than in the brain parenchyma of control brains. Occasionally, COX-1 immunoreactive macrophages/microglial cells were localized in perivascular spaces of capillaries clotted with parasitized erythrocytes. Double labeling revealed the coimmunoreactivity of the macrophage marker CD68 in < 90% of all COX-1 immunoreactive cells (Figures 2c, d). However, cells coimmunoreactive to CD68 and COX-1 were predominantly located in the center of Dürck´s granulomas. Approximately 10% of COX-1 immunoreactive cells coexpressed the MHC class II antigens HLA-DR, -DP, -DQ (Figures 2e, f). These cells were mainly located at the outer boundaries of Dürck´s granulomas. Further, few endothelial cells and single neurons expressed COX-1. No accumulation of COX-1 immunoreactive macrophages/microglial cells was observed in hemorrhages. No colocalization of the glial markers GFAP, S-100beta, the T cell marker CD43 and the B cell marker CD20 was observed in COX-1 immunoreactive cells.

COX-2 immunoreactivity was observed in single or grouped neurons (Figure 2g). COX-2 immunoreactivity in patients who died of CM was most prominently detected in endothelial cells (Figure 2h) and in a subset of astrocytes (Figure 2i). The number of COX-2 immunoreactive endothelial cells was significantly ($p = 0.0006$) higher than in the brain parenchyma of control patients. The number of COX-2 immunoreactive astrocyte subpopulations was significantly ($p = 0.0012$) higher in patients with CM than in the brain parenchyma of control patients. Further, COX-2 was expressed in single macrophages and in neurons. Double labeling experiments confirmed COX-2 immunoreactivity predominantly in cells with endothelial vWF (von-Willebrand factor) antigen immunoreactivity, the macrophage antigen CD68 (Figure 2k) or the astrocytic antigens S-100beta and GFAP (Figure 2l) [40].

Eicosanoids have been suspected of playing pivotal roles in the development of pathophysiological alterations in CM before. Clinical trials revealed beneficial effects of glucocorticoids in post-CM encephalopathy [41], but aggravation of the clinical course of CM after administration of glucocorticoids [31] resulted in their application in CM patients being abandoned. *In vitro* results suggest COX-1

expression in a housekeeping function and COX-2 expression in response to pathogenic stimuli. More specifically, COX-1 expression in macrophages/microglial cells was found to be constitutive and unrelated to pyogenic stimuli in a rat model of proinflammatory lipopolysaccharide challenge [42]. However, numerical increases in COX-1 immunoreactive macrophages/microglial cells in CM provides evidence for the involvement of COX-1 in the local production of prostaglandins because COX-1 as well as COX-2 catalyze the eicosanoid reaction products PGG_2 and PGH_2 [33]. In this context, accumulation of COX-1 macrophages/microglial cells in Dürck´s granulomas, but not in hemorrhages, might be indicative of the involvement of COX-1 expression only during a distinct temporal window during the formation of granulomas following hemorrhage.

COX-2 overexpression in the brain has been associated with inflammation disorders [43], experimental trauma [36], ischemia [35] and Alzheimer´s disease [34]. Distinct cell-type-specific COX-2 functions were identified. Animal models suggested that in brain blood vessels, induction of COX-2 expression is thought to be a primary source of fever [44]. However, recent reports have revealed the involvement of COX-2 in angiogenesis, apoptosis and cell adhesion [38, 39]. COX-2 expression in astrocytes has been associated with astrogliosis before [45]. Taken together, these data suggest that COX-2 may be associated with glial scarring, a final stage alteration in cerebral malaria brains.

HEME OXYGENASE-1

During human cerebral malaria, oxidative stress is considered a crucial mechanism generating damage to the vascular endothelial lining. Adaptive cellular antioxidative mechanisms might limit local tissue destruction. In addition, the typical petechial bleedings results in the liberation of hemoglobin and heme, a powerful oxidant. Heme oxygenases degrade heme to CO, Fe and biliverdin, which is then further reduced to bilirubin. Biliverdin and bilirubin are both powerful antioxidants [46]. Heme oxygenases protect cells from the deleterious, immunostimulatory and oxidative effects of free heme both *in vitro* and in animal models [47, 48] and we have therefore analyzed the localization of heme oxygenase 1 (HO-1 (heat-shock protein hsp-32) in human cerebral malaria [49].

HO-1 was selectively expressed in areas of bleedings. Massive immunoreactivity of HO-1 was seen in Dürck's granulomas, and almost all HO-1$^+$ cells coexpressed the macrophage marker CD68 (Figures 3a, c, d). In general, astrocytes were negative for HO-1: GFAP and HO-1 were rarely colocalized in the same cytoplasm (Figure 3b). Ramified microglial cells in the vicinity of the bleedings expressed HO-1 as well. The majority of ramified cells within the non-affected parenchyma was HO-1 negative. They were, however, activated, as shown by myeloid-related protein-8 immunoreactivity (Figure 3c). Endothelia were mostly negative for HO-1 (Figure 3b, c, d) and only rarely positive endothelial cells, unrelated to histological manifestation of malaria lesions, were encountered. HO-1 was not found in glial cells of control brains from patients who died of acute heart attack.

In animal models of brain disease, expression of HO-1 is induced by a variety of stressors, such as ischemia [50], trauma [51], neurotoxins [52]. Interestingly, HO-1 expression was also observed in human senescent and morbus Alzheimer degenerative brain disease [53]. In animal and *in vitro* models, HO-1 is induced in glial and some neuronal cells [54] and expression in glia throughout the brain can be induced by subarachnoid hemoglobin [55]. Further more, interleukin-1, TNF-alpha, reactive oxygen species and nitric oxide, which are considered mediators involved in the development of cerebral malaria, with a major role in initiation and propagation of local pathological processes, have all been demonstrated to induce HO-1 expression [56]. Animal models revealed that overexpression of HO-1 is cyto- [57] and neuroprotective [58]. Transfection of the human HO-1 gene in endothelial cells attenuates the damaging effect of free heme *in vitro* [59]. HO-1 is considered an endogenous factor responsible for the resolution of local inflammation, and increased HO-1 activity has been implicated in tissue protection against inflammation-associated oxidative stress. Further, CO generated by HO-1 is involved in the regulatory cascades of nitric oxide production and has effects on neural function. The notion that HO-1 plays an important anti-inflammatory role [60] is supported by the effects of HO-1-generated CO on NO production and vice versa [61], and it has been suggested that induction of HO-1 may represent a decisive defensive response to ameliorate cytotoxicity caused by NO and/or secondary oxidative stress [62]. The role of CO in the neurological signs of CM and in coma has not been studied so far, but appears worthy of further investigation. Induction of human HO-1 expression represents an important adaptive mechanism for moderating the severity of cell damage, and heme oxygenases are therefore considered novel targets for the modulation of the local inflammatory response.

MYELOID-RELATED PROTEINS MRP8 (S100A8) AND MRP14 (S100A9)

MRP8 and MRP14 are intracellular Ca^{2+}-binding proteins associated with a number of inflammatory disorders. Both proteins have been described as the L1 complex or the cystic fibrosis antigen. MRP gene expression during inflammation has been observed predominantly on myelomonocytic cells and thus MRP- immunostaining has been proposed as a tool for staging the inflammatory response. MRP8 and MRP14 are predominantly expressed by myelomonocytic cells and are considered to be macrophage activation antigens [63]. MRP8 and MRP14 complexes have a variety of functions. They are antimicrobial [64], and the heterodimers specifically bind arachidonic acid [65]. MRP8 and MRP14 expression is associated with inflammatory processes and correlates with inflammatory changes in vascular adhesion molecule activation [66].

Macrophages/microglial cells showed typical distribution in non-lesioned areas of the grey and white matter in brains of patients with CM [67] (Figures 4a, b). Within the petechial bleedings, microglial cells expressed CD68, which is considered an indicator of activation and phagocytic activity (Figure 4c). CD68 was also expressed on grey matter microglia (Figure 4d). Petechial hemorrhages were surrounded by a rim of amoeboid microglial cells. Rounded macrophages had invaded the hemorrhages. Such changes were also found in areas which were microscopically free of sequestrated erythrocytes. In addition, some areas with infected erythrocytes within the blood vessel lacked petechial bleedings. But even in these areas, microglial cells adopted an amoeboid form typical of activated microglia. Within the grey matter, the majority of neurons appeared shrunken and *in situ* nick translation demonstrated DNA damage related to apoptosis/necrosis. Expression of differentiation and activation antigens of human microglial cells was analyzed with a set of monoclonal antibodies. As described recently [68], IL-16 (LCF) is expressed by microglial cells in CM brains (Figure 4e). In the normal adult brain, expression of IL-16 appears to be constitutive. IL-16 is a natural ligand for CD4. As microglial cells express low levels of CD4, IL-16 might be an autocrine factor but, although it is considered a proinflammatory cytokine, its role in brain pathology is unknown so far. However, IL-16 immunoreactivity can be used to immunotype human microglial cells [68]. MRP8 and MRP14 proteins were detected by monoclonal antibodies 8-5C2, detecting a distinct epitope of MRP 8 and S36.48, binding specifically to MRP14. MRP8 and -14 markers of early microglial cell activation, were expressed by

microglial cells of the whole brain including the grey matter (Figure 4f). In addition and in support of this observation, microglial cells often adopted an amoeboid morphology typical migrating microglia.

In comparison, MRP expression has been observed in multiple sclerosis lesions, in Alzheimer plaques, in ischemia and neurotrauma. Expression of MRP-proteins is a marker of microglial cell activation [69]. We observed immunoreactivity of both MRP-8 and MRP-14 by microglial cells in human CM. MRPs are expressed in response to inflammatory stimuli but are even more rapidly expressed after onset of anoxia [70]. Thus, in CM MRP immunoreactivity is probably not attributable to a single type of stimulus. Inducing factors might be immunoactive proteins of the parasite or immunoregulatory cytokines. In addition, immunoreactivity of MRP8 and MRP14 could be attributed to microcirculatory sludging. The widespread activation of microglia, notably in the grey matter and in areas rather remote of lesions, or adherent infected erythrocytes is reminescent of observations in human ischemia [70] and suggest that malaria-induced failure of the microcirculation and resulting damage due to hypoxia is a major feature of CM. In addition, MRP8/14 are signalling molecules that might well contribute to microvascular changes [66] observed in CM and to the massive apoptosis seen in brain tissue and in circulating leukocytes [71].

UROKINASE-TYPE PLASMINOGEN ACTIVATOR RECEPTOR (UPAR, CD87)

Recent data indicate that focal immune response contributes to CM progression by modulation of endothelial cell integrity, inflammatory mediator release and (auto-)immune reactions [1]. More specifically, disruption of the blood-brain barrier and inflammatory cell recruitment are known to be facilitated by proteolytic degradation of extracellular matrix barriers in degenerative cerebral diseases. Interestingly, a previous report showed activation of the fibrinolytic cascade in patients with malaria [72]. Among others, the serine protease urokinase type plasminogen activator (uPA), together with its receptor uPAR (CD87), generates a specific serine protease-mediated plasminogen activation pathway. Plasminogen is the precursor of plasmin which in turn degrades various glycoproteins in the basement membrane and extracellular matrix. Besides its function in focussing uPA activity to the cell surface, uPAR promotes cell adhesion and spreading *via* a binding site for vitronectin [73]. Detailed investigations on the uPA/uPAR system exist for various malignant tumors [74-76], and the first studies have been published on the role of the

plasminogen activator system in ischemic and traumatic brain injury [77]. Information concerning uPAR expression in cerebral infectious diseases is rare. However, protease systems and their inhibitors have been considered to be important in plasmodial infections, in the context of the erythrocytic life cycle of parasites [78].

In brains of CM patients, CD87 reactivity was spatially limited to the pathologically altered areas with parasitized erythrocytes sequestered in the microvasculature, petechial bleedings and Dürck´s granulomas [79]. uPAR immunoreactivity was detected in 3 different cell types: microglia/macrophages, astrocytes and endothelial cells. Distinct immunoreactivity of uPAR was observed in ramified microglial cells accumulating in the microglial nodules (Dürck´s granuloma) (Figure 5a). Moreover, uPAR immunoreactivity was found in ramified microglia/macrophages in tissue adjacent to the petechial bleedings (Figure 5b). In the center of petechial bleedings, rounded, uPAR positive mononuclear cells predominated. Coimmunoreactivity with CD68 (Figure 5a, insert) and MRP8 confirmed their microglial/macrophage identity. In the vicinity of Dürck´s granuloma and petechial hemorrhages, uPAR immunoreactivity was seen on the cytoplasmatic processes of reactive astrocytes. Double labeling experiments revealed coimmunoreactivity with the glial marker, glial fibrillary acidic protein (Figure 5c), but not with the macrophage/microglial activation markers, CD68 and MRP8. Quiescent astrocytes remote from the lesion site, in contrast, showed no uPAR immunoreactivity on their cytoplasmatic processes (not shown). Moreover, uPAR was prominently expressed in endothelial cells of brain vessels characterized by deposition of malaria pigment and sequestration (Figure 5d). This striking upregulation of uPAR was confined to histologically altered regions and was not seen in remote areas.

uPAR expression on cell surfaces is known to be an indicator for activation and /or stimulation of cells [80]. Enhanced uPAR expression on macrophages/microglial cells was shown to be confined to injured areas and thus indicates a locally limited process. Microglial activation in CM, as shown by MRP8 expression on microglial cells in human CM, however, was shown to be more general [67]. This observation supports the idea, that uPAR is selectively upregulated in cells which are primed for migration and does not generally correlate with microglial activation markers such as MRP8. A functional heterogeneity has already been shown in peripheral blood monocytes, where uPAR expression did not always correlate with MHC class II expression or other

monocyte activation markers [81]. uPAR expression in macrophages/microglial cells in CM may thus play a relevant role in microglia/macrophage recruitment and migration into diseased brain tissue, where these cells probably contribute to local release of inflammatory mediators and are involved in reorganization processes such as clearance of infected and uninfected erythrocytes from hemorrhages [1].

Cerebral endothelial cells are key interfaces between the blood compartment and the brain parenchyma in CM. Focal areas of endothelial cell degeneration, hyperplasia and necrosis can be seen in CM, frequently in areas where hemorrhages and granulomas are found. The striking lesion-associated uPAR expression suggests that the uPA/uPAR system is also involved in this process. Similarly, in cerebral ischemia and traumatic injury, uPAR expression in endothelial cells was shown to be concomitant with small hemorrhages or edematous extravasates [77]. Furthermore, we speculate that uPAR on activated endothelial cells may serve as an additional adhesion molecule for parasitized erythrocytes as parasitized erythrocytes are located adjacent to uPAR immunoreactive endothelial cells.

uPAR expression in adult, non-neoplastic astrocytes is an interesting finding which has yet been described on protoplasmatic astrocytes in cerebral ischemia and traumatic lesions [77]. However, neither under those conditions nor in toxoplasmic encephalitis and bacterial meningitis, as a prominent uPAR expression on the astrocytic processes been shown as found in CM. It is tempting to speculate, that the uPAR expression on astrocytes is the result of enhanced inflammatory mediators such as various cytokines. TNF-alpha alpha for example is known to play an important role in cerebral malaria, and enhances uPAR expression in macrophage-like U937 cells, colon carcinoma cell lines, and endothelial cells [82, 83]. The importance of uPAR expression on adult non-neoplastic astrocytes in CM remains unclear. Through the enhanced production of plasmin, they may contribute to the activation of other inflammatory mediators such as TGF-beta and thus play a role in the complicated cascade of local immunity in the brain. Another possible explanation is the involvement of astrocytes in the degradation of plasma proteins, which occurs in the brain parenchyma of CM patients due to leakage across an impaired blood-brain barrier [84]. In this context, an apparent uptake of fibrinogen by perivascular astrocyte foot processes can be observed in CM.

CONCLUSION

Cerebral malaria is caused by *Plasmodium falciparum* and is considered to be a major contributor to mortality and morbidity in malaria, predominantly in non-immune individuals and children [1].

Neuropathological alterations include sequestration of parasitized red blood cells in cerebral microvasculature, petechial hemorrhages in the brain parenchyma, ring hemorrhages and microglial-astroglial nodules, so-called Dürck´s granulomas. CM is principally a hematogenous infection. Thus, cerebral endothelial cells are key interfaces between the blood compartment and the brain parenchyma in CM. Focal areas of endothelial cell degeneration, hyperplasia and necrosis can be seen in CM, frequently in areas where hemorrhages and granulomas are found. Several different mechanisms have been proposed to account for damage to endothelial cell integrity.

Macrophages/microglial cells are strategically placed cellular sensors responding to a variety of stressors to the central nervous system. CNS microglia are a source of defense cells in the CNS capable of carrying out certain immune functions autonomously. Resting microglia are scattered uniformly throughout the CNS, forming a network of potential immunoeffector cells, which can be activated by stimuli ranging from peripheral nerve injury resulting from viral infections to direct, mechanical brain trauma. Activated microglia are proliferating cells that demonstrate changes in their immunophenotype but which have not undergone transformation into brain macrophages. Such a transformation can be stimulated by neuronal death but not by sublethal neuronal injury. Microglia may function as antigen-presenting cells and may thus represent the effector cell responsible for the recruitment of lymphocytes to the brain, resulting in an inflammatory reaction [85]. However, immigration of blood-borne macrophages is an additional mechanism of the host with which combat to cerebral malaria. Both, macrophages and microglial cells produce a series of cytokines and other signalling molecules, and their involvement in the regulation of local tissue responses has been shown in a wide range of diseases including inflammation, trauma and ischemia [86, 87].

In brains of patients with cerebral malaria, microglial cells, and in addition macrophages, secrete a series of cytokines and other signalling molecules and are thus involved in the regulation of local tissue responses in inflammation,

blood-brain barrier breakdown and lesion formation. Morphological changes include retraction of ramified processes, soma enlargement, an increasingly amoeboid appearance, and vacuolation.

The identification of factors that are involved in downstream processes associated with brain inflammation, blood-brain barrier distortion, endothelial and microglial activation therefore raises the hope of identifying important regulatory mechanisms that may lead to future treatment for patients with cerebral malaria.

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Figures

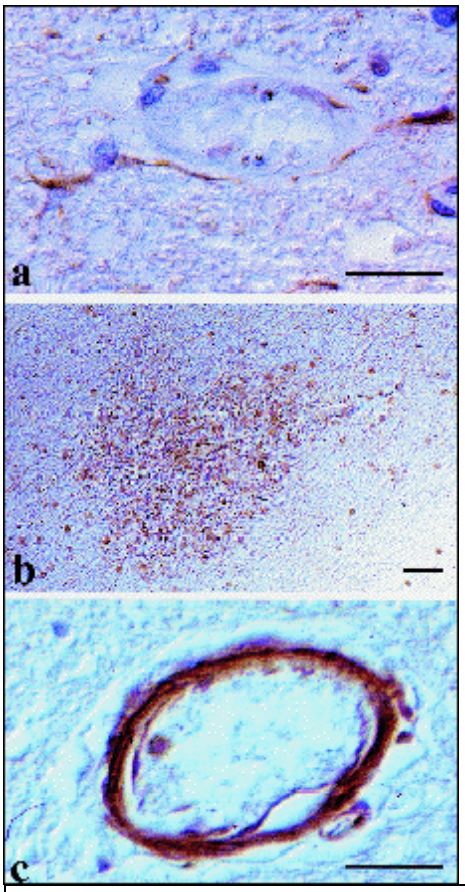


Figure 1

In brains of patients who died with cerebral malaria, TGF-beta**1 immunoreactivity (brown color) was found in astrocytes that form the blood-brain barrier around cerebral capillaries characterized by deposition of malarial pigment and sequestration** (a).

TGF-beta2 (brown color) immunoreactivity was found in macrophages/microglial cells in Dürck´s granulomas and in glioses of ring hemorrhages **(b)**. TGF-beta3 immunoreactivity (brown color) was found in endothelial and smooth muscle cells in capillaries with deposition of malarial pigment and sequestration **©**. All slices were counterstained with hematoxylin-eosin. Bars = 25 mum.

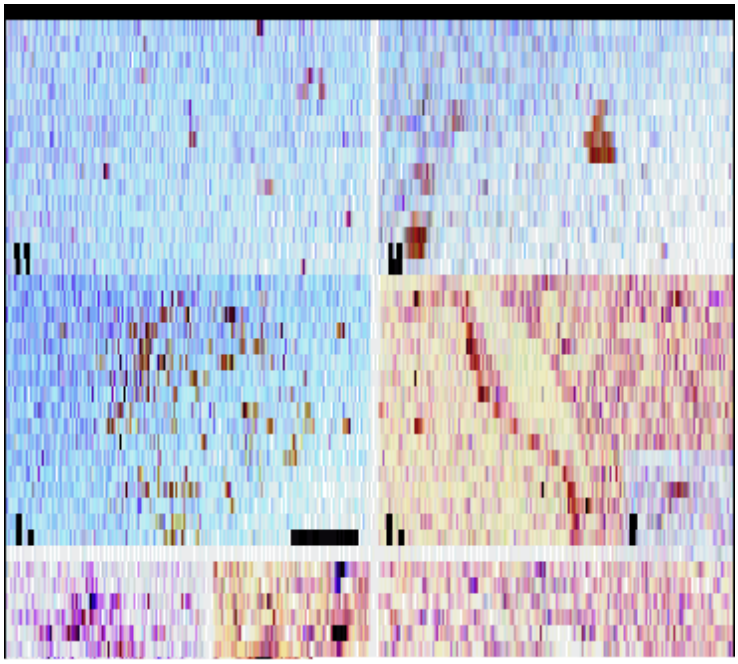


Figure 2

In patients without neuropathological alterations, few COX-1$^+$ macrophages/microglial cells (a) **and singular COX-2$^+$ neurons** (g) **were observed.**

In patients with cerebral malaria, accumulation of COX-1$^+$ macrophages/microglial cells was observed predominantly in Dürck's granulomas **(b)**, and of COX-2$^+$ endothelial cells in vessels clotted with parasitized erythrocytes **(h)** and in singular astrocytes **(i)**. Double labeling experiments revealed the coimmunoreactivity of COX-1 (brown color) in CD68$^+$ (blue color) **(c, d)** and in HLA-DR, -DP, -DQ$^+$ cells (blue color, **e, f**). COX-2$^+$ cells colocalized within cells that were immunoreactive with

the macrophage antigen CD68 (Figure 2k) or the astrocytic antigens S-100b and GFAP (Figure 2l). Bars = 25 mum.

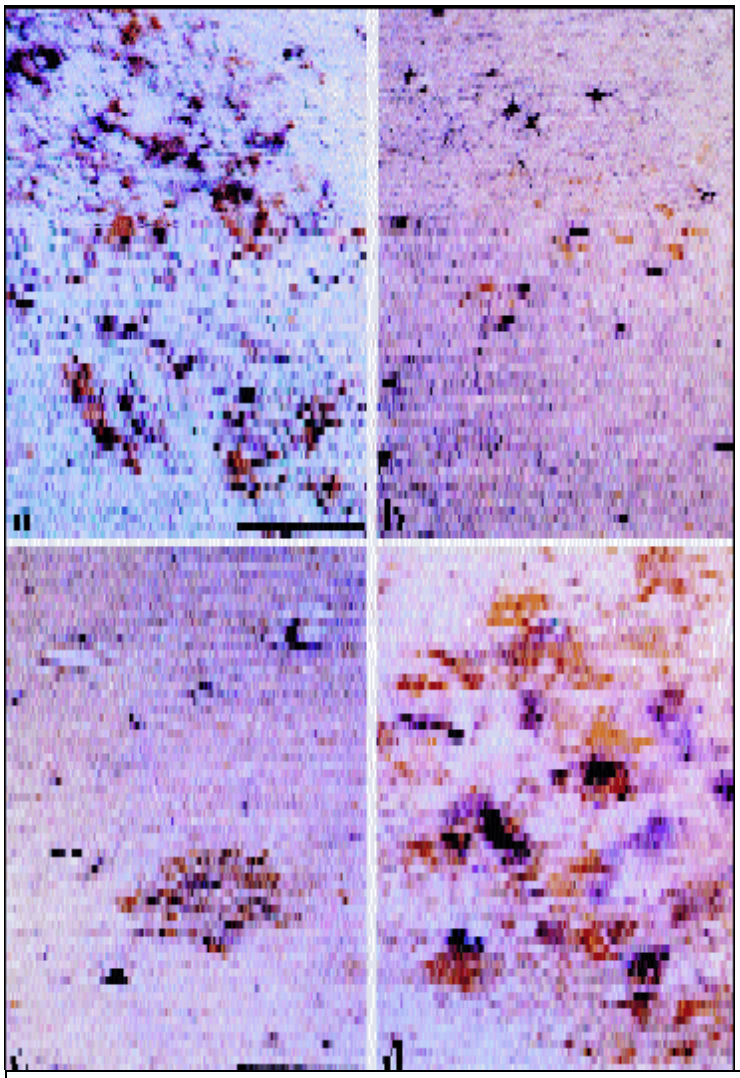


Figure 3

In Dürck's granulomas, HO-1 positive cells (brown) coexpress the macrophage marker CD68 (a, blue). Endothelia are negative for HO-1. Labeling of GFAP (blue) and HO-1 (brown) reveals that HO-1 is rarely expressed by astrocytes **(b)**. HO-1 (brown) is expressed by activated macrophages as shown by double labeling with MRP8 (blue), which is a well defined activation marker of macrophages. Ramified cells in the vicinity of

Dürck's granulomas express both antigens (**c**, arrow). In areas remote from the granulomas, ramified cells express only MRP8. Endothelia are negative for HO-1. This higher magnification shows coimmunoreactivity of MRP8 (blue) and HO-1 (brown) by monocytic cells in Dürck's granuloma **(d)**. Bars = 25 mm.

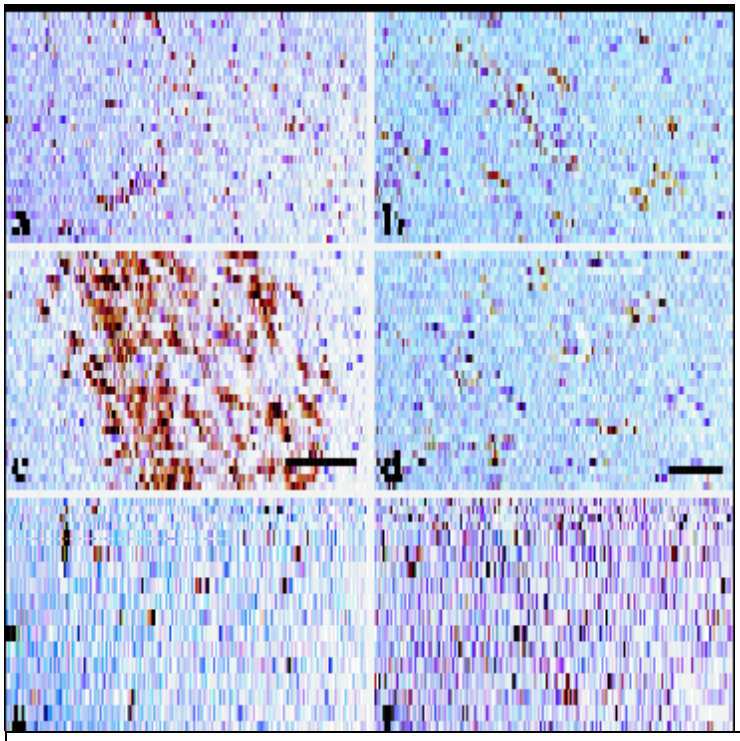


Figure 4

MHC class II antigens are expressed on both ramified and amoeboid microglial cells in the whole brain.

They are found in both the grey **(a)** and the white matter **(b)**. CD-68, expressed by phagocytosing microglial cells, is only found on few microglial cells within the white matter. In contrast, this antigen is highly expressed on macrophages of the perivascular bleedings **©**. Surprisingly, this antigen is also expressed in grey matter of diseased brains **(d)**. IL-16 is expressed by microglial cells within the white matter. These cells have a similar

position to those which express MHC class II antigens **(e)**. Interestingly, MRP8 is expressed on microglial cells within the grey matter **(f)**, which reflects an early stage of microglial activation. Bars = 25 mum.

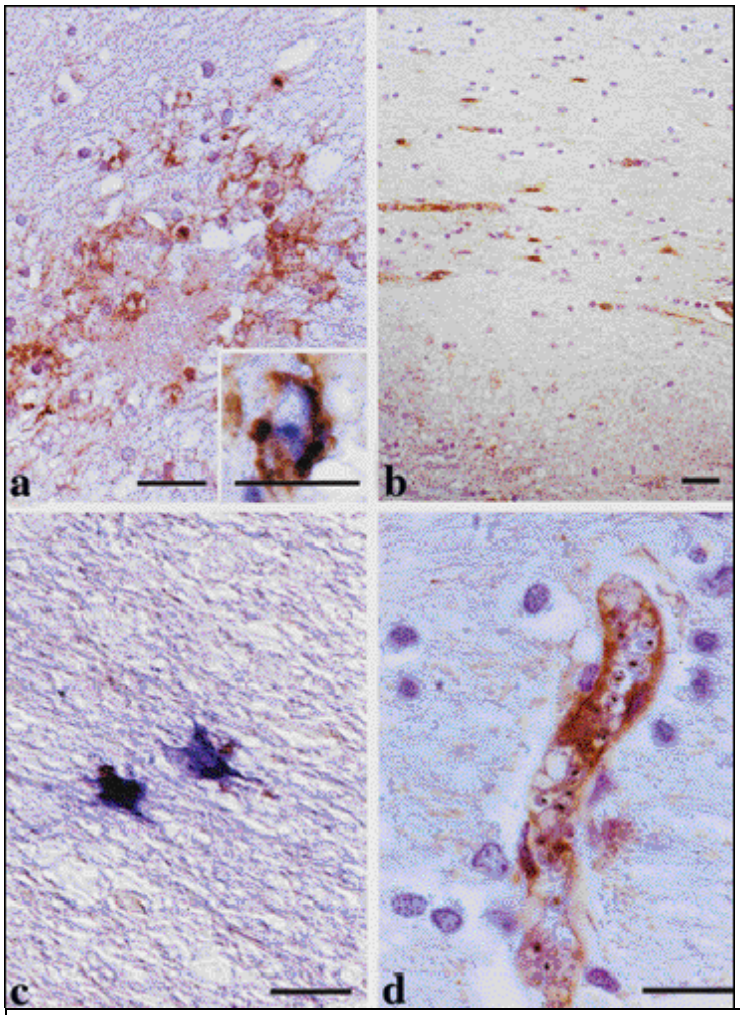


Figure 5

In brains of patients who died with cerebral malaria, uPAR-immunoreactivity (brown color) is predominantly found in Dürck's granuloma (a). Insert: uPAR positive cells (brown color) in the center of Dürck's granuloma coexpress the macrophage/microglia marker CD68 (blue color).
Accumulation of uPAR-positive macrophages/microglia in the vicinity of petechial hemorrhages **(b)**. Astrocytes in

lesioned areas show coimmunoreactivity of the glial fibrillary acid protein GFAP (blue color) and uPAR on their cytoplasmic processes (brown color) **©**. Endothelial cells of capillaries (arrow) characterized by deposition of malaria pigment and sequestration demonstrate high uPAR-immunoreactivity (brown color) **(d)**. Bars = 25 mum.

Table
Histological characterization of patients with cerebral malaria

Patient – ID	Diagnosis/Histology
1	Cerebral malaria parasitized erythrocytes in cerebral microvasculature, small Dürck's granulomas adjacent to vessels, petechial hemorrhages
2	Cerebral malaria small Dürck's granulomas adjacent to the vasculature, no hemorrhages, parasitized erythrocytes within microvasculature
3	Cerebral malaria parasitized erythrocytes in cerebral microvasculature, several ring hemorrhages
4	Cerebral malaria parasitized erythrocytes, sludge phenomenon, petechial and ring hemorrhages, Dürck's granuloma
5	Cerebral malaria brain edema, parasitized erythrocytes in the microvasculature, no Dürck's granuloma, no ring hemorrhages
6	Cerebral malaria parasitized erythrocytes in the vasculature, no hemorrhages, no Dürck's granulomas
7	Cerebral malaria parasitized erythrocytes in the vasculature, no hemorrhages, no Dürck's granuloma
8	Cerebral malaria parasitized erythrocytes in the vasculature, no hemorrhages, no Dürck's granuloma

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Interferon-alpha suppresses leptin levels: studies in interferon-alpha treated patients with hepatitis C virus infection and murine adipocytes

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interferon-alpha, leptin, chronic hepatitis C virus infection, adipocytes.

The CD14-260 C => T promoter polymorphism co-segregates with the tumor necrosis factor-alpha (TNF-alpha)-308 G => A polymorphism and is associated with the interleukin-1beta (IL-1beta) synthesis capacity of human leukocytes

M. Heesen, B. Bloemeke, B. Bachmann-Mennenga, D. Kunz
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Extracellular matrix proteins influence phenotype and cytokine expression in human breast cancer cell lines

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breast cancer, cell line, extracellular matrix (ECM), tumor cell phenotype, malignant potential, cytokine expression pattern.

Expression and regulation of the macrophage inflammatory protein-1alpha gene by nicotine in rat alveolar macrophages

I.-W. Chong, S.-R. Lin, J.-J. Hwang, M.-S. Huang, T.-H. Wang, J.-Y. Hung, J. D. Paulauskis

nicotine, macrophage inflammatory protein-1alpha (MIP-1alpha), rat alveolar macrophage (RAM).

Recombinant human interleukin (IL)-1beta-mediated regulation of hypoxia-inducible factor-1alpha (HIF-1alpha) stabilization, nuclear translocation and activation requires an antioxidant/reactive oxygen species (ROS)-sensitive mechanism

J. J. Haddad

antioxidant, airway epithelium, cytokine, HIF-1alpha, IL-1beta, mitochondria, oxygen sensing, reactive oxygen species.

Thioredoxin specifically cross-desensitizes monocytes to MCP-1

S. Pagliei, P. Ghezzi, C. Bizzarri, V. Sabbatini, G. Frascaroli, S. Sozzani, G. Caselli, R. Bertini

thioredoxin, monocytes, MCP-1.

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