Journal of NeuroVirology, 13: 2–10, 2007

ISSN: 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280701258084

informa
healthcare

Review

Glial activation and matrix metalloproteinase release in cerebral malaria

A Szklarczyk,[1] M Stins,[2] EA Milward,[4] H Ryu,[1] C Fitzsimmons,[4] D Sullivan,[3] and K Conant [1]

Departments of [1]Neurology, [2]Pediatrics and [3]Molecular Microbiology and Immunology, Johns Hopkins University, Baltimore, Maryland, USA; [4]The University of Newcastle, Callaghan, NSW Australia

Although neurological symptoms associated with cerebral malaria (CM) are largely reversible, recent studies suggest that lasting neurological sequelae can occur. This may be especially true for children, in whom persistent deficits include problems with memory and attention. Because the malaria parasite is not thought to enter the brain parenchyma, lasting deficits are likely related to factors including the host response to disease. Studies with a rodent model, and with human postmortem tissue, suggest that glial activation occurs with CM. In this review, the authors will highlight studies focused on such activation in CM. Likely causes will be discussed, which include ischemia and activation of blood brain barrier endothelial cells. The potential consequences of glial activation will also be discussed, highlighting the possibility that glial-derived proteinases contribute to structural damage of the central nervous system (CNS). Of note, for the purposes of this focused review, glial activation will refer to the activation of astrocytes and microglial cells; discussion of oligodendroglial cells will not be included. In addition, although events thought to be critical to the pathogenesis of CM and glial activation will be covered, a comprehensive review of cerebral malaria will not be presented. Excellent reviews are already available, including Coltel *et al* (2004; *Curr Neurovasc Res 1:* 91–110), Medana and Turner (2006; *Int J Parasitol* 36: 555–568), and Hunt *et al* (2006; *Int J Parasitol* 36: 569–582). *Journal of NeuroVirology* (2007) **13,** 2–10.

Background—cerebral malaria (CM) is a major cause of morbidity and mortality

The *2005 World Malaria Report* released by the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) indicates that there are an estimated 350 to 500 million malaria cases, of which 270 to 400 million are falciparum malaria (the strain associated with CM). According to this report, about 70% of the burden of falciparum malaria is estimated to be in Africa and about 20% in Southeast Asia. Malarial pathogens are not, however, limited to Africa and Southeast Asia. Endemic areas include India, the Caribbean, and Central and South America.

Address correspondence to Katherine Conant, Department of Neurology, Johns Hopkins University, Meyer 6-109, 600 N. Wolfe Street, Baltimore, Maryland 21287, USA. E-mail: kconant@jhmi.edu

Received 8 November 2006; revised 14 January 2007; accepted 31 January 2007.

As a single infectious disease, malaria is close to tuberculosis and acquired immunodeficiency syndrome (AIDS) in terms of the number of lives it claims (Lou *et al*, 2001). Cerebral malaria (CM) is among the most severe complications of infection, and a major cause of death (Lou *et al*, 2001). In some series, CM accounts for up to 80% of malaria fatalities (Lou *et al*, 2001). Though malaria can strike people at varied ages, it is predominantly children, and those with poor access to health care, who die (Lou *et al*, 2001).

Neurological symptoms in CM include impaired consciousness, coma, delirium, seizures, and intracranial hypertension (MacPherson *et al*, 1985). Strict research definitions of CM include coma lasting for more than 30 min, confirmed *Plasmodium falciparum* infection, and exclusion of other causes of encephalopathy. Neuroimaging studies may show diffuse cerebral edema, as well as thalamic and cerebellar white matter hypoattenuation. Such studies may, however, underestimate pathology. For

example, diffuse petechial hemorrhages seen at autopsy may be missed by computerized tomography (Patankar *et al*, 2002).

Although nonfatal CM may be associated with what appears to be a dramatic recovery in that those who do not die may wake from coma, it is becoming increasingly recognized that the disease may cause serious long-term neurological disability (MacPherson *et al*, 1985). CM often results in permanent neurological sequelae, including seizures. In addition, children who survive CM may be left with acquired language disorders, motor deficits, and problems with memory and attention (Carter *et al*, 2006; Idro *et al*, 2006). A recent review suggests that *P. falciparum* may affect the brain globally rather than in a localized fashion (Kihara *et al*, 2006).

Sequestration of *Plasmodium falciparum*-infected red blood cells (Pf-IRBCs) in postcapillary brain endothelium is a hallmark of CM pathogenesis

Central to CM pathogenesis is the sequestration of *P. falciparum*-infected red blood cells, containing mature forms of the parasite, within brain microvessels. Studies show there is a strong correlation between adhesiveness of *Plasmodium*-infected red blood cells (RBCs) to endothelium and the risk of CM development (Urban and Roberts, 2002).

Histopathologic studies of CM have shown that the tightly packed and adherent *P. falciparum*-infected red blood cells (Pf-IRBCs) associate with the blood-brain barrier (BBB) endothelium via Pf-IRBC surface knob-like protrusions (Pongponratn *et al*, 1985). The knobs of 200 kDa, or larger, contain parasite-derived variant surface antigens that comprise a family of erythrocyte membrane proteins (PfEMP1), which can act as ligands for Pf-IRBC attachment to host cells (Baruch *et al*, 1995).

The attachment of Pf-IRBCs to endothelium is mediated by specific host-encoded receptor molecules such as intercellular adhesion molecule (ICAM)-1, which may be especially important in the brain. Peripheral parasites isolated from CM patients bind to ICAM-1, and postmortem brain samples of CM patients indicate that IRBC sequestration correlates with increased endothelial ICAM-1 expression (Newbold *et al*, 1997; Porta *et al*, 1993; Silamut *et al*, 1999; Turner *et al*, 1994). Other adhesion molecules may also play a role, including the $\alpha_v\beta_3$ integrin, vascular cell adhesion molecule (VCAM)-1, and thrombospondin, which colocalize with sequestered Pf-IRBCs in postmortem brain samples of CM patients (Newbold *et al*, 1997; Porta *et al*, 1993; Turner *et al*, 1994). Chondroitin sulfate A and CD36 can also interact with Pf-IRBCs (Ockenhouse *et al*, 1991; Sherman *et al*, 2003).

Adhesive interactions may cause glial activation by varied mechanisms including hypoxia, endothelial cell activation, and changes in blood-brain barrier permeability

Adhesion of malarial infected RBCs to endothelia may be associated with vascular occlusion and localized hypoxia (Hunt *et al*, 2006). Hypoxia is damaging to cells and can stimulate aberrant protein expression. Hypoxia has been linked to increased stability of the transcription factor subunit hypoxia-inducible factor-1α (HIF-1α) (Ke and Costa, 2006). Hypoxia-inducible transcription factors may up-regulate the expression of proteins involved in angiogenesis, which include the urokinase plasminogen activator receptor and the matrix metalloproteinases (Dachs and Tozer, 2000; Graham *et al*, 1998). These proteins are important in a range of activation-related responses, as detailed below.

Adhesion of IRBCs to BBB endothelium can also lead to further endothelial cell activation, with a consequent increase in their release of soluble molecules such as cytokines and prostaglandins that can in turn activate cells of the brain parenchyma. Matrix metalloproteinase (MMP) release by endothelial cells may also be stimulated by adhesive interactions and may contribute to changes in BBB permeability. Moreover, altered BBB permeability, with ingress of plasma components, may contribute in some part to microglial activation (Medana *et al*, 2000). In addition, changes in BBB integrity may allow products of malarial infected RBCs, such as hemozoin, to come into contact with cells of the BBB and brain parenchyma. This is significant in that products such as haemozoin can activate select cells and increase their release of MMPs via interactions with toll like receptors (Coban *et al*, 2005).

Hypoxia and endothelial cell activation are not, however, the only mechanisms by which glial activation may occur. Elegant studies have shown that increases in levels of circulating cytokines can activate parenchymal cells, especially in areas without a fully formed BBB (Thibeault *et al*, 2001). This sort of mechanism may be important in CM cases associated with activation of leukocytes and changes in levels of circulating cytokines.

One group that has examined the potential mechanisms underlying glial activation in CM has investigated the potential contribution of changes in BBB permeability, as well as that of the immune response to the malaria parasite (Medana *et al*, 2000). This group relied on a murine model of malaria, experimental cerebral malaria. Murine and human CM share critical features in terms of behavior, histopathology, and immunological manifestations (Hunt *et al*, 2006). Although there may be differences in terms of the predominant cell type that is sequestered in brain microvasculature, with the murine model having relatively higher leukocyte

sequestration and the human having relatively higher IRBC sequestration, significant leukocyte sequestration is seen in pediatric patients and both human and murine malaria share disease sequelae such as central nervous system (CNS) inflammation (Hearn *et al*, 2000).

In one set of experiments, Medana *et al* showed that BBB function was compromised in noninfected animals that received an intracarotid injection of arabinose. Such compromise was followed by an increase in BBB permeability, thickening of microglial processes, and redistribution of microglia to the vasculature, but not with other changes in microglial morphology that are typically seen in the murine model of disease. In contrast, dexamethasone treatment of infected mice, initiated 4 days post inoculation and not affecting parasite growth, could prevent cerebral complications and morphological changes in microglia (Medana *et al*, 2000). The authors concluded that a dexamethasone sensitive event(s), possibly associated with the immune response and occurring within the first few days of malaria infection, could prevent development of reactive microglia and cerebral complications.

Evidence for activation of glial cells in CM

Early disease histopathology findings in CM involve cerebral venules packed with parasitized erythrocytes, as well as microhemorrhages and ischemic foci. Later, lesions known as Durck's granulomas may become apparent. Durck's granulomas are characterized by aggregates of reactive astrocytes and microglial cells in the vicinity of cerebral capillaries (Turner, 1997). Reactive astrocytes proximal to Durck's granulomas show cell surface expression of urokinase plasminogen activator receptor whereas quiescent astrocytes do not (Fauser *et al*, 2000). Microglial cell expression of macrophage-related proteins (MRP), a correlate of activation, is also observed as is increased expression of cyclooxygenase-1 (Bruck *et al*, 1995; Deininger *et al*, 2002). In addition, nitric oxide synthase (NOS)-2 expression in astrocytes, microglia, and macrophages is increased (Deininger *et al*, 2002).

Of interest, endothelial cell activation has been noted in a study of brains from Ghanaian children who died with conditions including CM, severe malarial anemia (SMA), or non–central nervous system infection (NCNSI). Sequestered leukocytes were present in most of the sections from the CM cases but none of the sections from the SMA cases. Elevated vascular expression of ICAM-1, VCAM-1, and E-selectin was noted and showed significant co-localization with areas of erythrocyte sequestration (Armah *et al*, 2005). In this study, CM cases also showed increased staining for interleukin (IL)-1β and tumor necrosis factor (TNF)-α in the brain parenchyma, especially in cerebellar sections.

The murine model also supports the idea that glial activation occurs with CM. For example, in one recent study, significant inflammation was observed with an increase in F4/80+ microglia/macrophages and glial fibrillary acidic protein positive astrocytes (Wiese *et al*, 2006). Increased immunoreactivity for 8-oxoguanine (marker of oxidative stress) was also noted. In another murine study, activated caspase 3-positive apoptotic astrocytes were noted (Potter *et al*, 2006). Of note, although there may be differences in terms of the predominant cell type that is sequestered in brain microvasculature, with the murine model having relatively higher leukocyte sequestration and the human having substantial IRBC sequestration, it is becoming increasingly apparent that murine and human CM share critical features in terms of behavior, histopathology, and immunological manifestations (Hunt *et al*, 2006).

Shown in Figure 1 are results demonstrating increased immunostaining for glial fibrillary acidic protein in *P. berghei* ANKA-infected, as compared to uninfected, mice.

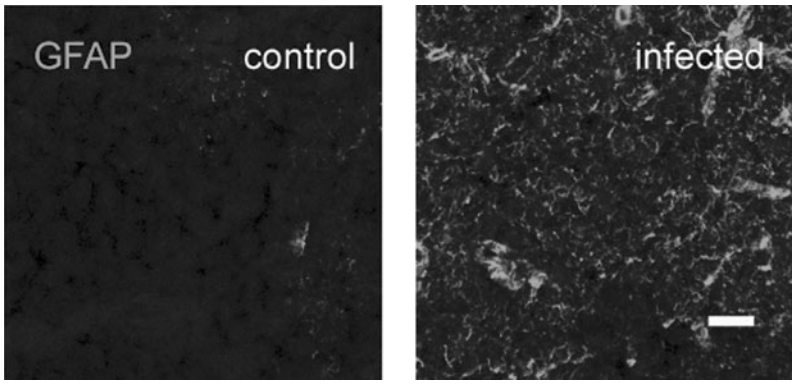

Figure 1 Glial activation in the murine model of CM, experimental cerebral malaria (ECM). Shown are results from immunohistochemical analysis of control and ECM brain tissue. These studies relied on C57Bl/6 mice, which, as compared to other strains of mice, consistently develop ECM (Hunt *et al*, 2006). Mice were given a saline injection or infected with *P. berghei* ANKA. Animals were euthanized 9 days later and brains harvested for preparation of fresh frozen sections. Immunohistochemistry of the cerebral cortex (layers 1 to 6) was subsequently performed as described (Szklarczyk *et al*, using a commercial antibody to glial fibrillary acidic protein (Zymed).

The products of activated glial cells may contribute to neurological sequelae—focus on matrix metalloproteinases

As alluded to above, both hypoxia and the products of activated endothelial cells can lead to activation of brain parenchymal cells. Among the soluble factors released by activated glia are potential neurotoxins such as platelet-activating factor, nitric oxide, proinflammatory cytokines, and quinolinic acid. Activated glia also release matrix metalloproteinases (MMPs). We (see data to follow) and others have noted that MMPs may be elevated with CM (Brown *et al*, 2000). This may be significant in that, as will be discussed in the section to follow, MMPs can target matrix proteins that support BBB integrity and neuronal survival.

The matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases that can cleave extracellular matrix (ECM) proteins as well as secreted cytokines and cell surface receptors (Blobel, 2000; Lochter *et al*, 1998; McCawley and Matrisian, 2001; Sternlicht and Werb, 2001; Yong *et al*, 1998). Members of the MMP family are differentially expressed by all major resident cells of the brain, including neurons, astrocytes, and microglia. For example, MMP-9 is localized to both neurons and glia in mature brain, whereas MMP-2 is expressed almost exclusively by astrocytes. A particularly important source of MMPs in the context of neuroinflammation is the macrophage, a cell type known to transverse the blood-brain barrier.

When produced in a physiological manner during development or repair, the expression of select MMPs may benefit the host. For example, MMPs may increase the bioavailability of neurotrophic factors including brain-derived nerve factor (BDNF) and insulin-like growth factor (IGF)-1 (Fowlkes *et al*, 2004; Lee *et al*, 2001), and they may play a role in postinjury synaptogenesis (Reeves *et al*, 2003; Szklarczyk *et al*, 2002).

In the setting of disease with excess proteinase release, however, MMPs may have overall effects that are deleterious to the host. Several studies have shown that MMPs can be neurotoxic, and a variety of mechanisms have been implicated (Gu *et al*, 2002; Vos *et al*, 2000; Zhang *et al*, 2003). MMPs cleave integrin-binding matrix proteins and may thus stimulate cell death through detachment. MMPs also bind to integrins and may thereby stimulate changes in intracellular signaling cascades (Alimenti *et al*, 2004; Chung *et al*, 2004; Conant *et al*, 2004; Hong *et al*, 2003; Zigrino *et al*, 2002). In addition, MMP-2 can cleave the chemokine stromal-derived factor-1α to generate a neurotoxic protein fragment (Zhang *et al*, 2003). Moreover, at least one MMP can activate proteinase-activated receptor-1 (Boire *et al*, 2005). Activation of this receptor by thrombin has been linked to the death of motor neurons (Turgeon *et al*, 1998).

MMPs may also influence synaptic structure or function (Table 1). MMPs can cleave adhesion molecules that may stabilize the synapse, including syndecans, cadherins, and signal regulatory protein (SIRP)-1α (Brule *et al*, 2006; Monea *et al*, 2006; Ohnishi *et al*, 2004). Consistent with this possibility, an membrane-type (MT)-MMP has recently

Table 1 Select brain parenchymal proteins that are critical to CNS function and are candidate substrates for MMPs or MT-MMPs (membrane-type MMPs)

Protease	*Substrate*	*CNS localization*	*Function of the substrate*	*Reference*
MMP-7	Connexin 43	Astrocytes	Component of astrocytic gap junctions	(Lindsey *et al*, 2006)
MMP-7	VE-cadherin	Endothelium	Adhesion and signaling	(Ichikawa *et al*, 2006)
MMP-7	E-cadherin	Endothelium	Adhesion and signaling	(Davies *et al*, 2001)
MMP-3		neurons, synapses, astrocytes		(Noe *et al*, 2001)
MT1-MMP				(Covington *et al*, 2006)
MT5-MMP				(Monea *et al*, 2006)
MMP-9	ZO-1	Endothelium neurons	Epithelial barrier permeability Component of the electrical synapse	(Asahi *et al*, 2001)
MMP-7	Fas ligand	Neurons	Cell death and signaling	(Ethell *et al*, 2002) (Vargo-Gogola *et al*, 2002)
MMP-7	IGF-BP	Neurons	Bioavailability of the trophic factor IGF	(Hemers *et al*, 2005)
MMP-3				(Nakamura *et al*, 2005)
MMP-9				(Coppock *et al*, 2004)
MMP-7	Osteopontin	Astrocytes microglia	Neuronal survival mediator	(Agnihotri *et al*, 2001)
MMP-3				
MMP-7	Integrin	Neurons, astrocytes	Adhesion and signaling	(Ratnikov *et al*, 2002)
MT1-MMP				(von Bredow *et al*, 1997)
MMP-3	Extracellular α-synuclein	Neurons, astrocytes	Unknown	(Sung *et al*, 2005)
MMP-3	Agrin	Synapses	Receptor clustering	(Sole *et al*, 2004) (VanSaun and Werle, 2000)
Putative MMP	SHPS	Synapses	Adhesion and signaling	(Ohnishi *et al*, 2004)
Plasmin MMP-7	Pro-BDNF, pro-NGF	Periglial, perineuronal	Neuronal survival	(Lee *et al*, 2001)

been shown to process synaptic cadherin (Monea *et al*, 2006). In addition, MMP-7 has been shown to influence the morphology of dendritic spines (Bilousova *et al*, 2006), and MMP-9 has been linked to altered long-term potentiation (Meighan *et al*, 2006; Nagy *et al*, 2006). This effect may involve MMP-9-mediated generation of a matrix fragment that influences the phosphorylation of select NMDA receptor (NMDAR) subunits (Nagy *et al*, 2006).

Evidence that MMP levels are increased with CM

Analysis of postmortem CM brains reveals that MMP-dependent proteolysis may be enhanced in the brain vasculature. For example, it has been shown that MMP-1 protein accumulates in macrophages/microglial cells in Durck's granulomas (Deininger *et al*, 2003). Similarly, Durck's granulomas are immunopositive for endostatin, which may be generated by MMPs. Granulomas are also positive for urokinase plasminogen activator receptor (uPAR), which can activate MMPs (Deininger *et al*, 2002, 2003). Of interest, animal studies have shown that urokinase- and urokinase receptor-deficient mice have delayed mortality and attenuated thrombocytopenia associated with severe malaria (Piguet *et al*, 2000).

Human studies have also shown that circulating levels of MMP-9 may be increased with CM (Brown *et al*, 2000), although increases in cerebrospinal fluid (CSF) levels in particular were not apparent, possibly because of the enzyme's tight association with the extracellular matrix. Although no studies of MMPs in human postmortem brain tissue have so far been reported, it has been shown that MMP-2 and MMP-9 levels are heavily up-regulated in C57BL/6 mice brain infected with *P. berghei* ANKA (Van den Steen *et al*, 2006). Elevated MMP-9 levels were selective for the CNS, where they were found in association with the vasculature and parenchyma. Elevated levels of MMP-9 were not observed in other organs. CM associated survival in MMP-9-null mice did not differ, however, from that in the control group, suggesting that the enzyme does not significantly contribute to lethality. Nonetheless, the application of a synthetic broad-spectrum inhibitor of MMPs did result in enhanced survival of animals. Gene expression of other MMPs including MMP-3 was enhanced at the message level in infected brains. It is therefore possible that an increase in the activity of a variety of MMPs is required for lethality, or that an MMP other than MMP-9 may contribute in a significant manner (Van den Steen *et al*, 2006).

As shown in Figure 2, we observe increased levels of MMP-7 in brain tissues from *P. berghei*-infected mice. Increased release of this proteinase proximal to

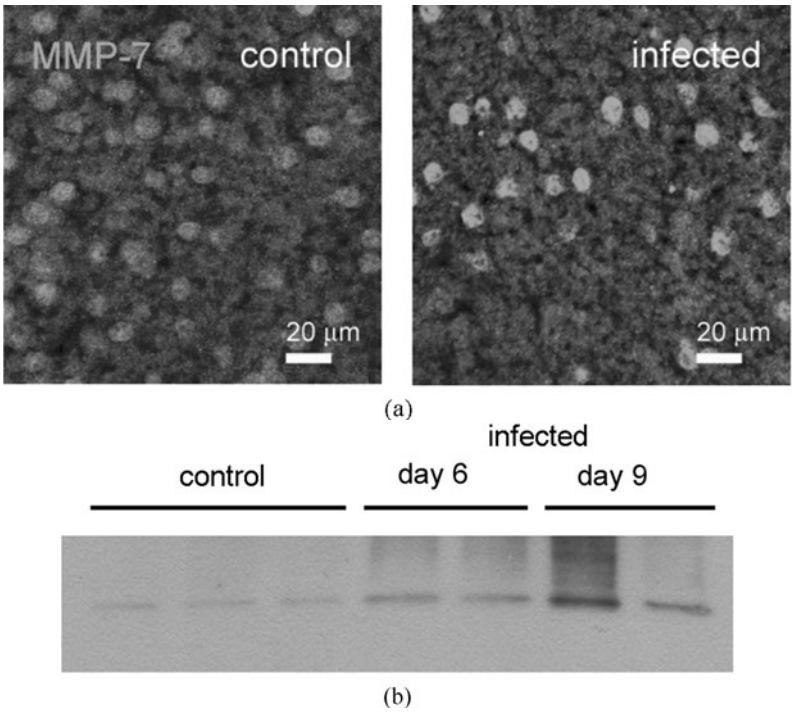

Figure 2 MMP-7 protein expression in cerebral neocortex of control and *P. bergheri* ANKA infected mice. **Upper panel**: High-magnification image of cerebral neocortex demonstrating immunofluorescent detection of MMP-7 protein. Note that in noninfected animals (control), MMP-7 protein localizes to cortical neurons. In the infected animal (day 9 post infection), MMP-7 was up-regulated *(right)*. The scale bar is 20 μm. **Lower panel**: Detection of MMP-7 protein in cortical extracts from control and infected mice. MMP-7 protein was first immunoprecipitated from cortical extracts by means of a selective antibody (Calbiochem IM71), and immunoprecipitates were then analyzed by Western blot. Lanes show replicate results from three control, two day 6, and two day 9 animals.

the neuronal synapse may in turn influence synaptic structure and function.

Potential MMP targets that are altered in CM

Consistent with potential roles in disease pathogenesis, evidence from *in vitro* and animal studies shows that MMPs can target CNS structures and substrates that are damaged in CM, including BBB and myelin components. As previously mentioned, MMPs are well known to target proteins of the BBB basement membrane (Yong *et al*, 1998). Moreover, MMP-9 and -12 can degrade myelin basic protein (Chandler *et al*, 1996; Proost *et al*, 1993).

Evidence for BBB damage in CM is supported by both human and animal studies. For example, disruption of endothelial cell tight junctions and reduced staining for the junctional protein zonae occludens-1 has been observed in Malawian children and Vietnamese adults (Brown *et al*, 1999, 2001). In addition, loss of BBB basement membrane fragments has been observed in the murine model (Polder *et al*, 1992). MMPs released by activated endothelium or glial endfeet may be uniquely positioned to act on the BBB. Sequestered leukocytes are another likely source of BBB-degrading MMPs, especially if activated by events such as adhesion to infected erythrocytes.

Evidence for damage to myelin in CM also comes from both human and animal studies (Ma *et al*, 1997; Medana *et al*, 2002). Axonal damage, another potential consequence of MMP activity, has also been described in CM (Medana *et al*, 2002). MMPs can also act on soluble molecules that have been linked to CM. For example, endostatin and proinflammatory cytokines including TNF-α and IL-1β may be generated through MMP activity (Chandler *et al*, 1996; Schonbeck *et al*, 1998). For example, IL-1β can be activated by MMP-9 (Schonbeck *et al*, 1998).

As previously discussed, MMPs can also be neurotoxic and a recent report has demonstrated that neuronal apoptosis can occur with murine malaria (Wiese *et al*, 2006).

In summary, various MMP targets are altered with CM and given the range of CNS molecules processed by MMPs, alterations in other targets will probably be discovered. However, because a number of the described targets are influenced by molecules other than MMPs, additional studies will be necessary to determine to what extent MMPs play a role in observed changes.

Conclusions and future directions

In conclusion, we have discussed events that may lead to activation of glia with CM. Such events are likely stimulated to some degree by adhesive interactions between infected red blood cells and blood-brain barrier endothelium, and are likely to include localized hypoxia and endothelial cell activation with increased release of proinflammatory molecules that act on glia. We have also discussed proteinase release as a potential consequence of neuronal and glial activation. Given that MMPs act on critical proteins of the brain parenchyma, it is tempting to speculate that these enzymes may contribute to tissue damage in this disease. Future studies may thus be warranted to determine whether safe and inexpensive inhibitors of MMP expression and activity, such as minocycline, should be considered for the treatment of CM.

References

Agnihotri R, Crawford HC, Haro H, Matrisian LM, Havrda MC, Liaw L (2001). Osteopontin, a novel substrate for matrix metalloproteinase-3 (stromelysin-1) and matrix metalloproteinase-7 (matrilysin). *J Biol Chem* **276:** 28261–28267.

Alimenti E, Tafuri S, Scibelli A, D'Angelo D, Manna L, Pavone LM, Belisario MA, Staiano N (2004). Pro-apoptotic signaling pathway activated by echistatin in GD25 cells. *Biochim Biophys Acta* **1693:** 73–80.

Armah H, Dodoo AK, Wiredu EK, Stiles JK, Adjei AA, Gyasi RK, Tettey Y (2005). High-level cerebellar expression of cytokines and adhesion molecules in fatal, paediatric, cerebral malaria. *Ann Trop Med Parasitol* **99:** 629–647.

Asahi M, Wang X, Mori T, Sumii T, Jung JC, Moskowitz MA, Fini ME, Lo EH (2001). Effects of matrix metalloproteinase-9 gene knock-out on the proteolysis of blood-brain barrier and white matter components after cerebral ischemia. *J Neurosci* **21:** 7724–7732.

Baruch DI, Pasloske BL, Singh HB, Bi X, Ma XC, Feldman M, Taraschi TF, Howard RJ (1995). Cloning the P. falciparum gene encoding PfEMP1, a malarial variant antigen and adherence receptor on the surface of parasitized human erythrocytes. *Cell* **82:** 77–87.

Bilousova TV, Rusakov DA, Ethell DW, Ethell IM (2006). Matrix metalloproteinase-7 disrupts dendritic spines in hippocampal neurons through NMDA receptor activation. *J Neurochem* **97:** 44–56.

Blobel CP (2000). Remarkable roles of proteolysis on and beyond the cell surface. *Curr Opin Cell Biol* **12:** 606–612.

Boire A, Covic L, Agarwal A, Jacques S, Sherifi S, Kuliopulos A (2005). PAR1 is a matrix metalloprotease-1 receptor that promotes invasion and tumorigenesis of breast cancer cells. *Cell* **120:** 303–313.

Brown H, Hien TT, Day N, Mai NTH, Chuong LV, Chau TTH, Loc PP, Phu NH, Bethell D, Farrar J, Gatter K, White N, Turner G (1999). Evidence of blood–brain barrier dysfunction in human cerebral malaria. *Neuropathol Appl Neurobiol* **25:** 331–340.

Brown H, Rogerson S, Taylor T, Tembo M, Mwenechanya J, Molyneux M, Turner G (2001). Blood-brain barrier function in cerebral malaria in Malawian children. *Am J Trop Med Hyg* **64:** 207–213.

Brown HC, Chau TT, Mai NT, Day NP, Sinh DX, White NJ, Hien TT, Farrar J, Turner GD (2000). Blood-brain barrier function in cerebral malaria and CNS infections in Vietnam. *Neurology* **55:** 104–111.

Bruck W, Porada P, Poser S, Rieckmann P, Hanefeld F, Kretzschmar HA, Lassmann H (1995). Monocyte/macrophage differentiation in early multiple sclerosis lesions. *Ann Neurol* **38:** 788–796.

Brule S, Charnaux N, Sutton A, Ledoux D, Chaigneau T, Saffar L, Gattegno L (2006). The shedding of syndecan-4 and syndecan-1 from HeLa cells and human primary macrophages is accelerated by SDF-1/CXCL12 and mediated by the matrix metalloproteinase-9. *Glycobiology* **16:** 488–501.

Carter JA, Lees JA, Gona JK, Murira G, Rimba K, Neville BG, Newton CR (2006). Severe falciparum malaria and acquired childhood language disorder. *Dev Med Child Neurol* **48:** 51–57.

Chandler S, Cossins J, Lury J, Wells G (1996). Macrophage metalloelastase degrades matrix and myelin proteins and processes a tumour necrosis factor-alpha fusion protein. *Biochem Biophys Res Commun* **228:** 421–429.

Chung CH, Wu WB, Huang TF (2004). Aggretin, a snake venom-derived endothelial integrin alpha 2 beta 1 agonist, induces angiogenesis via expression of vascular endothelial growth factor. *Blood* **103:** 2105–2113.

Coban C, Ishii KJ, Kawai T, Hemmi H, Sato S, Uematsu S, Yamamoto M, Takeuchi O, Itagaki S, Kumar N, Horii T, Akira S (2005). Toll-like receptor 9 mediates innate immune activation by the malaria pigment hemozoin. *J Exp Med* **201:** 19–25.

Coltel NV, Combes V, Hunt NH, Grau GE (2004). Cerebral malaria—a neurovascular pathology with many riddles still to be solved. *Curr Neurovasc Res* **1:** 91–110.

Conant K, St Hillaire C, Nagase H, Visse R, Gary D, Haughey N, Anderson C, Turchan J, Nath A (2004). Matrix metalloproteinase 1 interacts with neuronal integrins and stimulates dephosphorylation of Akt. *J Biol Chem* **279:** 8056–8062.

Coppock HA, White A, Aplin JD, Westwood M (2004). Matrix metalloprotease-3 and -9 proteolyze insulin-like growth factor-binding protein-1. *Biol Reprod* **71:** 438–443.

Covington MD, Burghardt RC, Parrish AR (2006). Ischemia-induced cleavage of cadherins in NRK cells requires MT1-MMP (MMP-14). *Am J Physiol Renal Physiol* **290:** F43–F51.

Dachs GU, Tozer GM (2000). Hypoxia modulated gene expression: angiogenesis, metastasis and therapeutic exploitation. *Eur J Cancer* **36:** 1649–1660.

Davies G, Jiang WG, Mason MD (2001). Matrilysin mediates extracellular cleavage of E-cadherin from prostate cancer cells: a key mechanism in hepatocyte growth factor/scatter factor-induced cell-cell dissociation and in vitro invasion. *Clin Cancer Res* **7:** 3289–3297.

Deininger MH, Fimmen B, Kremsner PG, Meyermann R, Schluesener HJ (2002). Accumulation of endostatin/collagenXVIII in brains of patients who died with cerebral malaria. *J Neuroimmunol* **131:** 216–221.

Deininger MH, Kremsner PG, Meyermann R, Schluesener H (2002). Macrophages/microglial cells in patients with cerebral malaria. *Eur Cytokine Netw* **13:** 173–185.

Deininger MH, Winkler S, Kremsner PG, Meyermann R, Schluesener HJ (2003). Angiogenic proteins in brains of patients who died with cerebral malaria. *J Neuroimmunol* **142:** 101–111.

Ethell DW, Kinloch R, Green DR (2002). Metalloproteinase shedding of Fas ligand regulates beta-amyloid neurotoxicity. *Curr Biol* **12:** 1595–600.

Fauser S, Deininger MH, Kremsner PG, Magdolen V, Luther T, Meyermann R, Schluesener HJ (2000). Lesion associated expression of urokinase-type plasminogen activator receptor (uPAR, CD87) in human cerebral malaria. *J Neuroimmunol* **111:** 234–240.

Fowlkes JL, Serra DM, Bunn RC, Thrailkill KM, Enghild JJ, Nagase H (2004). Regulation of insulin-like growth factor (IGF)-I action by matrix metalloproteinase-3 involves selective disruption of IGF-I/IGF-binding protein-3 complexes. *Endocrinology* **145:** 620–626.

Graham CH, Fitzpatrick TE, McCrae KR (1998). Hypoxia stimulates urokinase receptor expression through a heme protein-dependent pathway. *Blood* **91:** 3300–3307.

Gu Z, Kaul M, Yan B, Kridel SJ, Cui J, Strongin A, Smith JW, Liddington RC, Lipton SA (2002). *S*-nitrosylation of matrix metalloproteinases: signaling pathway to neuronal cell death. *Science* **297:** 1186–1190.

Hearn J, Rayment D, Landon DN, Katz DR, de Souza. JB (2000). Immunopathology of cerebral malaria: morphological evidence of parasite sequestration in murine brain microvasculature. *Infect Immun* **68:** 5364–5376.

Hemers E, Duval C, McCaig C, Handley M, Dockray GJ, Varro A (2005). Insulin-like growth factor binding protein-5 is a target of matrix metalloproteinase-7: implications for epithelial-mesenchymal signaling. *Cancer Res* **65:** 7363–7369.

Hong SY, Lee H, You WK, Chung KH, Kim DS, Song K (2003). The snake venom disintegrin salmosin induces apoptosis by disassembly of focal adhesions in bovine capillary endothelial cells. *Biochem Biophys Res Commun* **302:** 502–508.

Hunt NH, Golenser J, Chan-Ling T, Parekh S, Rae C, Potter S, Medana IM, Miu J, Ball HJ (2006). Immunopathogenesis of cerebral malaria. *Int J Parasitol* **36:** 569–582.

Ichikawa Y, Ishikawa T, Momiyama N, Kamiyama M, Sakurada H, Matsuyama R, Hasegawa S, Chishima T, Hamaguchi Y, Fujii S, Saito S, Kubota K, Hasegawa S, Ike H, Oki S, Shimada H (2006). Matrilysin (MMP-7) degrades VE-cadherin and accelerates accumulation of beta-catenin in the nucleus of human umbilical vein endothelial cells. *Oncol Rep* **15:** 311–315.

Idro R, Carter JA, Fegan G, Neville BG, Newton CR (2006). Risk factors for persisting neurological and cognitive impairments following cerebral malaria. *Arch Dis Child* **91:** 142–148.

Ke Q, Costa M (2006). Hypoxia-inducible factor-1 (HIF-1). *Mol Pharmacol* **70:** 1469–1480.

Kihara M, Carter JA, Newton CR (2006). The effect of *Plasmodium falciparum* on cognition: a systematic review. *Trop Med Int Health* **11:** 386–397.

Lee R, Kermani P, Teng KK, Hempstead BL (2001). Regulation of cell survival by secreted proneurotrophins. *Science* **294:** 1945–1948.

Lindsey ML, Escobar GP, Mukherjee R, Goshorn DK, Sheats NJ, Bruce JA, Mains IM, Hendrick JK, Hewett KW, Gourdie RG, Matrisian LM, Spinale FG (2006). Matrix metalloproteinase-7 affects connexin-43 levels, electrical conduction, and survival after myocardial infarction. *Circulation* **113:** 2919–2928.

Lochter A, Sternlicht MD, Werb Z, Bissell MJ (1998). The significance of matrix metalloproteinases during early stages of tumor progression. *Ann N Y Acad Sci* **857:** 180–193.

Lou J, Lucas R, Grau GE (2001). Pathogenesis of cerebral malaria: recent experimental data and possible applications for humans. *Clin Microbiol Rev* **14:** 810–820, table of contents.

Ma N, Madigan MC, Chan-Ling T, Hunt NH (1997). Compromised blood-nerve barrier, astrogliosis, and myelin disruption in optic nerves during fatal murine cerebral malaria. *Glia* **19:** 135–151.

MacPherson GG, Warrell MJ, White NJ, Looareesuwan S, Warrell DA (1985). Human cerebral malaria. A quantitative ultrastructural analysis of parasitized erythrocyte sequestration. *Am J Pathol* **119:** 385–401.

McCawley LJ, Matrisian LM (2001). Matrix metalloproteinases: they're not just for matrix anymore! *Curr Opin Cell Biol* **13:** 534–540.

Medana IM, Chan-Ling T, Hunt NH (2000). Reactive changes of retinal microglia during fatal murine cerebral malaria: effects of dexamethasone and experimental permeabilization of the blood-brain barrier. *Am J Pathol* **156:** 1055–1065.

Medana IM, Day NP, Hien TT, Mai NT, Bethell D, Phu NH, Farrar J, Esiri MM, White NJ, Turner GD (2002). Axonal injury in cerebral malaria. *Am J Pathol* **160:** 655–666.

Medana IM, Turner GD (2006). Human cerebral malaria and the blood-brain barrier. *Int J Parasitol* **36:** 555–568.

Meighan SE, Meighan PC, Choudhury P, Davis CJ, Olson ML, Zornes PA, Wright JW, Harding JW (2006). Effects of extracellular matrix-degrading proteases matrix metalloproteinases 3 and 9 on spatial learning and synaptic plasticity. *J Neurochem* **96:** 1227–1241.

Monea S, Jordan BA, Srivastava S, DeSouza S, Ziff EB (2006). Membrane localization of membrane type 5 matrix metalloproteinase by AMPA receptor binding protein and cleavage of cadherins. *J Neurosci* **26:** 2300–2312.

Nagy V, Bozdagi O, Matynia A, Balcerzyk M, Okulski P, Dzwonek J, Costa RM, Silva AJ, Kaczmarek L, Huntley GW (2006). Matrix metalloproteinase-9 is required for hippocampal late-phase long-term potentiation and memory. *J Neurosci* **26:** 1923–1934.

Nakamura M, Miyamoto S, Maeda H, Ishii G, Hasebe T, Chiba T, Asaka M, Ochiai A (2005). Matrix metalloproteinase-7 degrades all insulin-like growth factor binding proteins and facilitates insulin-like growth factor bioavailability. *Biochem Biophys Res Commun* **333:** 1011–1016.

Newbold C, Warn P, Black G, Berendt A, Craig A, Snow B, Msobo M, Peshu N, Marsh K (1997). Receptor-specific adhesion and clinical disease in *Plasmodium falciparum. Am J Trop Med Hyg* **57:** 389–398.

Noe V, Fingleton B, Jacobs K, Crawford HC, Vermeulen S, Steelant W, Bruyneel E, Matrisian LM, Mareel M (2001). Release of an invasion promoter E-cadherin fragment by matrilysin and stromelysin-1. *J Cell Sci* **114:** 111–118.

Ockenhouse CF, Ho M, Tandon NN, Van Seventer GA, Shaw S, White NJ, Jamieson GA, Chulay JD, Webster HK (1991). Molecular basis of sequestration in severe and uncomplicated *Plasmodium falciparum* malaria: differential adhesion of infected erythrocytes to CD36 and ICAM-1. *J Infect Dis* **164:** 163–169.

Ohnishi H, Kobayashi H, Okazawa H, Ohe Y, Tomizawa K, Sato R, Matozaki T (2004). Ectodomain shedding of SHPS-1 and its role in regulation of cell migration. *J Biol Chem* **279:** 27878–27887.

Patankar TF, Karnad DR, Shetty PG, Desai AP, Prasad SR (2002). Adult cerebral malaria: prognostic importance of imaging findings and correlation withpostmortem findings. *Radiology* **224:** 811–816.

Piguet PF, Da Laperrousaz C, Vesin C, Tacchini-Cottier F, Senaldi G, Grau GE (2000). Delayed mortality and attenuated thrombocytopenia associated with severe malaria in urokinase- and urokinase receptor-deficient mice. *Infect Immun* **68:** 3822–3829.

Polder TW, Eling WM, Curfs JH, Jerusalem CR, Wijers-Rouw M (1992). Ultrastructural changes in the blood-brain barrier of mice infected with *Plasmodium berghei*. *Acta Leiden* **60:** 31–46.

Pongponratn E, Riganti M, Harinasuta T, Bunnag D (1985). Electron microscopy of the human brain in cerebral malaria. *Southeast Asian J Trop Med Public Health* **16:** 219–227.

Porta J, Carota A, Pizzolato GP, Wildi E, Widmer MC, Margairaz C, Grau GE (1993). Immunopathological changes in human cerebral malaria. *Clin Neuropathol* **12:** 142–146.

Potter SM, Chan-Ling T, Rosinova E, Ball HJ, Mitchell AJ, Hunt NH (2006). A role for Fas-Fas ligand interactions during the late-stage neuropathological processes of experimental cerebral malaria. *J Neuroimmunol* **173:** 96–107.

Proost P, Van Damme J, Opdenakker G (1993). Leukocyte gelatinase B cleavage releases encephalitogens from human myelin basic protein. *Biochem Biophys Res Commun* **192:** 1175–1181.

Ratnikov BI, Rozanov DV, Postnova TI, Baciu PG, Zhang H, DiScipio RG, Chestukhina GG, Smith JW, Deryugina EI, Strongin AY (2002). An alternative processing of integrin alpha(v) subunit in tumor cells by membrane type-1 matrix metalloproteinase. *J Biol Chem* **277:** 7377–7385.

Reeves TM, Prins ML, Zhu J, Povlishock JT, Phillips LL (2003). Matrix metalloproteinase inhibition alters functional and structural correlates of deafferentation-induced sprouting in the dentate gyrus. *J Neurosci* **23:** 10182–10189.

Schonbeck U, Mach F, Libby P (1998). Generation of biologically active IL-1 beta by matrix metalloproteinases: a novel caspase-1-independent pathway of IL-1 beta processing. *J Immunol* **161:** 3340–3346.

Sherman IW, Eda S, Winograd E (2003). Cytoadherence and sequestration in *Plasmodium falciparum*: defining the ties that bind. *Microbes Infect* **10:** 897–909.

Silamut K, Phu NH, Whitty C, Turner GD, Louwrier K, Mai NT, Simpson JA, Hien TT, White NJ (1999). A quantitative analysis of the microvascular sequestration of malaria parasites in the human brain. *Am J Pathol* **155:** 395–410.

Sole S, Petegnief V, Gorina R, Chamorro A, Planas AM (2004). Activation of matrix metalloproteinase-3 and agrin cleavage in cerebral ischemia/reperfusion. *J Neuropathol Exp Neurol* **63:** 338–349.

Sternlicht MD, Werb Z (2001). How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol* **17:** 463–516.

Sung JY, Park SM, Lee CH, Um JW, Lee HJ, Kim J, Oh YJ, Lee ST, Paik SR, Chung KC (2005). Proteolytic cleavage

of extracellular secreted alpha-synuclein via matrix metalloproteinases. *J Biol Chem* **280:** 25216–25224.

Szklarczyk A, Lapinska J, Rylski M, McKay RD, Kaczmarek L (2002). Matrix metalloproteinase-9 undergoes expression and activation during dendritic remodeling in adult hippocampus. *J Neurosci* **22:** 920–930.

Thibeault I, Laflamme N, Rivest S (2001). Regulation of the gene encoding the monocyte chemoattractant protein 1 (MCP-1) in the mouse and rat brain in response to circulating LPS and proinflammatory cytokines. *J Comp Neurol* **434:** 461–477.

Turgeon VL, Lloyd ED, Wang S, Festoff BW, Houenou LJ (1998). Thrombin perturbs neurite outgrowth and induces apoptotic cell death in enriched chick spinal motoneuron cultures through caspase activation. *J Neurosci* **18:** 6882–6891.

Turner G (1997). Cerebral malaria. *Brain Pathol* **7:** 569–582.

Turner GD, Morrison H, Jones M, Davis TM, Looareesuwan S, Buley ID, Gatter KC, Newbold CI, Pukritayakamee S, Nagachinta B, *et al* (1994). An immunohistochemical study of the pathology of fatal malaria. Evidence for widespread endothelial activation and a potential role for intercellular adhesion molecule-1 in cerebral sequestration. *Am J Pathol* **145:** 1057–1069.

Urban BC, Roberts DJ (2002). Malaria, monocytes, macrophages and myeloid dendritic cells: sticking of infected erythrocytes switches off host cells. *Curr Opin Immunol* **14:** 458–465.

Van den Steen PE, Van Aelst I, Starckx S, Maskos K, Opdenakker G, Pagenstecher A (2006). Matrix metalloproteinases, tissue inhibitors of MMPs and TACE in experimental cerebral malaria. *Lab Invest* **86:** 873–888.

VanSaun M, Werle MJ (2000). Matrix metalloproteinase-3 removes agrin from synaptic basal lamina. *J Neurobiol* **43:** 140–149.

Vargo-Gogola T, Crawford HC, Fingleton B, Matrisian LM (2002). Identification of novel matrix metalloproteinase-7 (matrilysin) cleavage sites in murine and human Fas ligand. *Arch Biochem Biophys* **408:** 155–161.

von Bredow DC, Nagle RB, Bowden GT, Cress AE (1997). Cleavage of beta 4 integrin by matrilysin. *Exp Cell Res* **236:** 341–345.

Vos CM, Sjulson L, Nath A, McArthur JC, Pardo CA, Rothstein J, Conant K (2000). Cytotoxicity by matrix metalloprotease-1 in organotypic spinal cord and dissociated neuronal cultures. *Exp Neurol* **163:** 324–330.

Wiese L, Kurtzhals JA, Penkowa M (2006). Neuronal apoptosis, metallothionein expression and proinflammatory responses during cerebral malaria in mice. *Exp Neurol* **200:** 216–226.

Yong VW, Krekoski CA, Forsyth PA, Bell R, Edwards DR (1998). Matrix metalloproteinases and diseases of the CNS. *Trends Neurosci* **21:** 75–80.

Zhang KMG, Silva C, Butler GS, Johnston JB, Holden J, Clark-Lewis I, Overall CM, and Power C (2003). HIV-induced metalloproteinase cleavage of the chemokine SDF-1alpha causes neurodegeneration. *Nat Neurosci* **6:** 1064–1011.

Zigrino P, Kamiguti AS, Eble J, Drescher C, Nischt R, Fox JW, Mauch C (2002). The reprolysin jararhagin, a snake venom metalloproteinase, functions as a fibrillar collagen agonist involved in fibroblast cell adhesion and signaling. *J Biol Chem* **277:** 40528–40535.