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TITLE:
MMPs and ADAMs in neurological infectious diseases and multiple sclerosis

ABSTRACT:
Metalloproteinases—such as matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinases (ADAMs)—are involved in various diseases of the nervous system but also contribute to nervous system development, synaptic plasticity and neuroregeneration upon injury. MMPs and ADAMs proteolytically cleave many substrates including extracellular matrix components but also signaling molecules and receptors. During neuroinfectious disease with associated neuroinflammation, MMPs and ADAMs regulate blood–brain barrier breakdown, bacterial invasion, neutrophil infiltration and cytokine signaling. Specific and broad-spectrum inhibitors for MMPs and ADAMs have experimentally been shown to decrease neuroinflammation and brain damage in diseases with excessive neuroinflammation as a common denominator, such as pneumococcal meningitis and multiple sclerosis, thereby improving the disease outcome. Timing of metalloproteinase inhibition appears to be critical to effectively target the cascade of pathophysiological processes leading to brain damage without inhibiting the neuroregenerative effects of metalloproteinases. As the critical role of metalloproteinases in neuronal repair mechanisms and regeneration was only lately recognized, the original idea of chronic MMP inhibition needs to be conceptually revised. Recently accumulated research urges for a second chance of metalloproteinase inhibitors, which—when correctly applied and dosed—harbor the potential to improve the outcome of different neuroinflammatory diseases.

Regulation of metalloproteinase activity ::: MMPs and ADAMs and their function in the CNS:
As metalloproteinases have the capacity for extensive tissue destruction, their activity is tightly regulated and controlled [1, 8]. The catalytic activity of MMPs is regulated on four different levels; namely gene expression, compartmentalization, pro-enzyme activation and enzyme inhibition [5]. Pro-MMPs are kept in a catalytically inactive state and can be activated by proteolytic cleavage of the pro-domain or by modification of the pro-domain's cysteine thiol group [5, 9]. Of importance for inflammatory and infectious processes, reactive oxygen species (ROS) have the potential to activate MMPs via oxidation of the pro-domain's thiol group [10–12]. MMP inactivation might be achieved be natural inhibitors, of which the tissue inhibitors of metalloproteinases (TIMPs) are the most prominent ones. By binding to the catalytic site of MMPs, the four TIMPs (TIMP-1, -2, -3 and -4) are able to terminate MMP activity [5, 13]. Many TIMPs target multiple metalloproteinases, but there are also some strict specificities such as TIMP-3, which is the only TIMP to inhibit ADAM17 [14, 15].

Effect of metalloproteinases on BBB integrity:
The contribution of MMPs on blood–brain barrier (BBB) integrity was demonstrated for the first time in the early 1990s by inducing BBB breakdown after intracerebroventricular administration of MMP-2 and MMP-9, an effect that was prevented by simultaneous administration of TIMP-2 [16–18]. Subsequent findings of elevated MMP-9 levels after cerebral insults for the first time established a link between MMPs and inflammation in the CNS [18, 19]. Intracerebral injection of lipopolysaccharide (LPS)—mimicking bacterial brain infections—increased MMP-2, MMP-3 and TNF-α mRNA levels [20] and induced BBB disruption through the action of MMP-9 [21]. Blood–brain barrier opening is associated with a morphological redistribution of tight junction (TJ) and adherens junction (AJ) proteins from the membrane to the cytoplasm [22]. In different neurological pathologies, MMPs are reported to degrade TJ and basal lamina proteins, thereby inducing BBB leakage with subsequent neutrophil infiltration, brain edema and hemorrhage [22, 23].
The role of MMP-2 in BBB opening was intensively studied in cerebral ischemia models, where MMP-2 was found to play an important role in the initial BBB opening [24] with increased activation of MMP-2 being associated with the degradation of the TJ proteins claudin-5 and occludin [17, 25]. Both MMP-2 and MMP-9 (gelatinase A and B) are able to degrade the major components of the basal lamina (type IV collagen, laminin and fibronectin) surrounding the cerebral blood vessels, thus increasing BBB permeability [22]. In vitro and in vivo, MMP-9 is capable of degrade claudin-5, occludin and ZO-1, thereby participating in TJ degradation [25–27].

Experimental data propose that MMP-9—which plays a profound role in delayed BBB breakdown [28]—derives mostly from brain microvascular endothelial cells and infiltrating neutrophils [27, 29–32]. Infiltrating neutrophils contain stores of MMP-9—so-called tertiary granules (or gelatinase granules)—which are rapidly released upon entering the site of injury [1, 33, 34]. MMP-9 levels critically increase during bacterial meningitis and elevated MMP-9 levels are associated with BBB damage and neurological sequelae [32, 35]. In experimental ischemic stroke, increased neutrophil-derived MMP-9 is critically involved in a state of systemic inflammation with sustained degradation of TJ and basal lamina proteins leading to exacerbated brain injury [22, 27]. Apart from these two gelatinases, other MMPs influence BBB integrity. MMP-3 is able to degrade laminin, gelatin, E-cadherin, proteoglycans and fibronectin [36] and has been demonstrated to mediate BBB opening after LPS-induced neuroinflammation, thereby facilitating neutrophil infiltration [37]. Collagenases (MMP-1, -8 and -13) preferentially cleave helical collagens in the ECM [22] and are reported to be activated and/or upregulated in vessels with BBB impairment [38–40]. In bacterial meningitis, cerebrospinal fluid (CSF) levels of MMP-13 and MMP-8 are upregulated [41] and associated with BBB damage [35]. Repeated lumbar punctures in bacterial meningitis patients revealed that MMP-8 levels were regulated independently and did not correlate with CSF granulocyte cell counts, indicating that these MMPs might not only be secreted by infiltrating neutrophils but also from brain-resident cells [35].

Neural vascular barrier function is also regulated by ADAMs. ADAM12 and ADAM17 were shown to control neural vascular barrier function upon hypoxic stimuli by diminishing claudin-5 in brain microvascular endothelial cells, an effect that was prevented by specific inhibition of ADAM12 or ADAM17 [42]. Activated ADAM10 cleaves VE-cadherin and promotes leukocyte migration to inter-endothelial junctions [43, 44].

MMPs in chemokine signaling ::: MMPs in neutrophil migration and cytokine signaling ::: MMPs and ADAMs in progression of neuroinflammation:

To establish an effective immune reaction against invading pathogens, neutrophils must migrate efficiently along chemotactic gradients and extravasate from the blood vessels towards the site of infection. Metalloproteinases have been demonstrated to be involved in these processes not only by degrading ECM and promoting effector cell extravasation but also by regulation of chemotactic gradients [15]. This was first demonstrated with MMP-9 shown to process an amino-terminal fragment of IL-8, thereby increasing its chemoattractant properties to more efficiently recruit neutrophils [46]. On the other hand, MMP-2 was shown to inactivate monocyte chemotactic protein 3 (MCP3, also known as chemokine (C–C motif) ligand 7, CCL7) by removing an amino-terminal tetrapeptide, converting it to an antagonist of its chemokine receptors [47]. Conceptually, these findings showed that MMPs can not only act as effectors but also regulators of inflammatory responses [47]. Subsequently, CCL7 has been also shown to be a specific substrate of MMP-1, -3, -13 and -14 but not MMP-8 and -9 [48]. The closely related chemokines CCL2, CCL8 and CCL13 (MCP-1, -2 and -4) are proteolytically cleaved by MMP-1 and -3 (but not MMP-2 and -14) with the truncated products of CCL8 and CCL13 being potent antagonists of the their respective chemokine receptors [48]. Apart from influencing the activity of C–C motif chemokines, MMPs also contribute to CXC-chemokine function. Stromal cell-derived factor 1 alpha (SDF-1α, also known as CXCL12) is processed and inactivated by MMP-1, -2, -3, -9, -13 and -14 but not by MMP-7 and -8 [49]. The truncated form of CXCL12 after MMP-2 processing demonstrates highly neurotoxic properties [50]. An elegant study with single and double knockout mice for MMP-2 and MMP-9 revealed that these MMPs work synergistically in the initial step of neutrophil recruitment to injury sites by increasing the potency of CXC-chemokine ligand 5 (CXCL5) [51]. Activation of murine LPS-induced CXC chemokine (LIX, similar to the human neutrophil-recruiting chemokine CXCL5 and CXCL8/IL-8) is dependent on MMP-8, with cleaved LIX promoting enhanced chemotaxis [52]. Neutrophil infiltration at LPS-stimulated sites is clearly diminished in mmp8–/– mice [52]. The same study showed that MMP-8 itself is not required for the extravasation and migration of neutrophils, but plays a very crucial role in orchestrating the initial inflammatory response upon LPS stimulation, thereby indicating that chemoattractants rather than collagen are MMP-8's primary substrates [52]. Nevertheless, collagens processed by MMP-8 can also act as chemotactic peptides during neutrophil chemotaxis [53]. Both MMP-8 and -9 are reported to cleave CXCL5 and CXCL6, but no change in biological activity of CXCL6 was demonstrated [54]. MMP-9, in contrast, potently inactivates CXCL5 by multiple cleavages, suggesting a regulatory role of MMP-9 in early activation with subsequent inactivation of CXCL5 [54]. In addition to direct effects of MMPs on chemokines, MMPs might also regulate the

expression of chemokine receptors, thereby providing an additional level by which these proteinases control leukocyte migration [5, 55].

Besides directly processing chemokines and altering their biological function, MMPs can also indirectly influence the availability and activity of chemokines by cleaving accessory macromolecules that bind them [56]. Thereby, MMPs exhibit an additional way of regulating leukocyte migration [5]. MMP-7 is involved in syndecan-1 cleavage, which in turn releases CXCL1 (also known as KC). Thereby, MMP-7 indirectly generates a chemotactic gradient that directs neutrophil migration to sites of injury [56]. Consequently, neutrophil infiltration is massively reduced in mmp7−/− mice at sites of injury because of impaired transepithelial migration [56]. Other MMPs also regulate chemotactic gradients, but their role is less well understood. MMP-2 seems to be critical for establishing a CCL11 (eotaxin) chemotactic gradient during leukocyte recruitment, as allergen-induced asthmatic mmp2−/− mice show leukocyte accumulation in lung parenchyma and decreased CCL11 levels [57]. Notably, this indirect pro-inflammatory effect of MMP-2 is very distinct from the above discussed direct anti-inflammatory function via chemokine cleavage and inactivation [47, 49]. In addition to MMP-2, MMP-9 contributes to the generation of the chemotactic gradient in allergen-induced asthmatic mice, where mmp9−/− mice showed diminished CCL11, CCL7 and CCL17 chemoattractant levels [58]. In summary, these studies demonstrate that MMPs are important regulators of inflammation by controlling chemokine activity and chemotactic gradients, thereby manipulating leukocyte migration.

MMPs in cytokine signaling ::: MMPs in neutrophil migration and cytokine signaling ::: MMPs and ADAMs in progression of neuroinflammation:

Apart from generating chemotactic gradients and recruiting leukocytes to sites of infection, MMPs also have the capacity to directly activate and regulate cytokine signaling. The pro-inflammatory cytokine IL-1β needs proteolytic activation by the IL-1β-converting enzyme (ICE, more recently re-named to caspase-1) [59, 60]. After discovery of caspase-1-independent activation of IL-1β [61], MMP-2, -3 and -9 were described to process human IL-1β precursor into biologically active forms [62]. In addition, MMP-3—to a lesser extent MMP-9, but not collagenases—was found to degrade active IL-1β after longer incubation periods, indicating regulatory roles of MMPs in both IL-1β activation and inactivation [62]. Under physiological conditions, conversion of the membrane-bound pro-TNF-α to its active and soluble form is attributed to TNF-α-converting enzyme (TACE, now known as ADAM17, also see next section) [63, 64]. Even if ADAM17 is the most specific and best convertor of pro-TNF-α, MMP-7—and to lesser but not physiologically relevant extent MMP-1 and -9—harbors the capacity to cleave and activate pro-TNF-α and seems to act as another physiological TNF-α convertor [65]. In contrast to the activation of these pro-inflammatory cytokines, MMPs (MMP-3, -9 and -14) are also able to activate transforming growth factor-β1 (TGF-β1) in vitro [66–68], which restrains mononuclear inflammation [5, 69, 70], thereby again indicating an overall regulatory effect of MMPs in inflammation.

ADAMs in infection and inflammatory responses ::: MMPs and ADAMs in progression of neuroinflammation:

TNF-α-converting enzyme (TACE; ADAM17) was originally thought to be a MMP, as it was effectively inhibited by synthetic metalloproteinase inhibitors [71], but later found to belong the ADAM family and identified as ADAM17 [63, 64]. ADAM17 shows very distinct specificity for shedding membrane-bound pro-TNF-α, thereby releasing biologically active TNF-α [65], with both pro-inflammatory and pro-apoptotic functions [15]. In ADAM17-deficient cells, TNF-α release is reduced by 90%, indicating that ADAM17 is the principal TNF-α converting enzyme [5, 64]. Soluble TNF-α signals via binding of TNF receptor 1 (TNFR1), whereas membrane-bound TNF-α acts preferentially through binding of TNFR2 [72]. The regulation of TNF signaling by ADAM17 is even more complex, since ADAM17 also sheds TNFR1 and TNFR2 [73, 74]. These act as decoy receptors by sequestering soluble TNF-α away from their receptors [15]. ADAM17 deficiency in myeloid cells protected mice from endotoxin-induced septic shock by preventing increased serum levels of TNF-α [75]. As ADAM17-dependent TNF-α conversion is selectively inhibited by TIMP3 [14], Timp3−/− mice show enhanced TNF-α signaling with elevated IL-6 serum levels and increased mortality after LPS-induced sepsis [76]. ADAM17—but also ADAM10—have been described to be involved in IL-6 trans-signaling by shedding IL-6 receptor (IL-6R), which in turn complexes with IL-6 and interacts with gp130-containing membrane receptors that are ubiquitously expressed on many cell types [77, 78].

Most leukocytes express L-selectin on their surface, which is involved in their rolling on inflamed vascular endothelium, followed by firm adhesion and transmigration [79]. L-selectin is rapidly

cleaned near the leukocyte cell surface by ADAM17 [79], during their transmigration [15]. ADAM17-null neutrophils show slower rolling, better adhesion and faster recruitment to site of inflammation [80] with impaired L-selectin shedding being responsible for early neutrophil recruitment [80, 81].

In mucosal barriers, ADAM17 has been shown to be important for EGFR activation, with ADAM17 or EGFR-deficient mice showing similar features [74]. Subsequently, ADAM17 was shown to be the major convertase of the EGFR ligands epiregulin, TGF-α, amphiregulin and heparin-binding EGF-like growth factors, whereas ADAM10 emerged as the major sheddase of EGF and betacellulin [82]. In intestinal inflammation, ADAM17-dependent EGFR ligand shedding is necessary for the production of antimicrobial peptides by epithelial cells and later regeneration [15, 83]. ADAM17 has additionally been associated with shedding of L-selectin from activated T cells, thereby regulating the recruitment of adaptive immune cells [74].

ADAMs are also able to manipulate immune signaling via ectodomain shedding of the Notch receptor, which is required for its activation [84, 85]. Notch activation by ADAM17 was found to regulate atopic barrier function and suppress epithelial cytokine synthesis [86]. In addition, ADAM17 and ADAMTS12 were shown to be involved in neutrophil apoptosis during resolution of acute inflammation [87, 88].

MMPs and microglia ::: MMPs and ADAMs in progression of neuroinflammation:
Microglia are brain-resident immune cells that are involved in important roles of the healthy, infected and injured brain, including post-natal neurodevelopment, neural plasticity and phagocytosis [89, 90]. Upon stimulation, microglia can be polarized into different microglial subsets [91]. The polarization states can be roughly divided into classically activated (M1) microglia that adapt a pro-inflammatory phenotype by secreting TNF-α, IL-1β, IL-6 and IFNγ [92], and alternatively activated (M2) cells, which produce cytokines involved in inflammation termination, restoring homeostasis and promoting tissue repair [91]. MMPs are expressed and produced by microglia at site of infection and inflammation [93, 94]. In macrophages, the two different subsets—M1 and M2—express different MMPs [95]. MMP-9—which depicts pro-inflammatory roles in BBB opening and cytokine activation—is secreted by M2 microglia and involved as a remodeling factor during repair [96, 97]. Kohkha et al. further suggest that MMPs—apart from being differentially expressed in M1 and M2 subsets—might also contribute to phenotype polarization by regulating cytokine and growth factor availability [15]. As microglia polarization towards the M2 phenotype gains interest as therapeutic strategy for different neurological disorders, understanding the critical role of metalloproteinases during this process needs further investigations.

Collectively, these findings demonstrate the crucial role of metalloproteinases in initiation of neuroinflammation—including BBB breakdown, chemokine activation with neutrophil recruitment, and pro-inflammatory cytokine production—but also in inflammation termination and subsequent repair via chemokine and cytokine inactivation and involvement in angiogenesis, neurogenesis and gliosis after damage [98–101]. Therefore, inhibition of metalloproteinase during acute inflammatory processes can lead to beneficial outcomes, whereas inhibition during repair processes might be detrimental [102, 103].

Pathophysiology of brain injury during bacterial meningitis ::: Metalloproteinases in bacterial meningitis ::: MMPs and ADAMs in infectious disease of the CNS:
To establish a CNS infection, the bacterial pathogens have to successfully colonize the host before they gain access to the subarachnoid space or the brain parenchyma. The most common causative agents of bacterial meningitis—Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae type b [106]—colonize the human nasopharynx and are transmitted via the respiratory route [105]. Most cases of bacterial meningitis originate from bacteremia, where the nasopharyngal pathogen gains access to the blood stream and subsequently crosses the BBB or blood–CSF barrier (BCSFB). Other causative pathogens of bacterial meningitis—which cause meningitis mostly in neonates, elderly and immunocompromized patients (group B Streptococcus (GBS), Escherichia coli, Listeria monocytogenes)—colonize the intestinal mucosa from where they access the circulation and invade the brain [105]. Bacterial meningitis might also arise from focal infection in the vicinity of the brain (i.e., otitis media, sinusitis, dental abscess), where local spread rather than bacteremia underlies bacterial brain invasion [105]. Finally, breaches of the anatomical brain barriers (trauma or surgery) may also lead to bacterial meningitis.

Among bacterial meningitis, pneumococcal meningitis (PM) is especially detrimental as it causes high mortality and leads to long-lasting neurofunctional deficits [107, 108]. Neurofunctional sequelae after PM include hearing loss, epilepsy, cerebral palsy, as well as behavioral and cognitive deficits [107–109]. Brain damage during PM is characterized by cortical necrosis and apoptosis of dentate gyrus granular cell progenitors in the hippocampus, being in part responsible for behavioral and cognitive deficits [110–114]. In experimental models, cortical necrosis is found as early as 18 h after infection and might arise as a consequence of focal and global ischemia [107, 115]. Neural cell death is caused by multiple factors including bacterial toxins and an excessive inflammatory reaction from the host [105, 116–118]. Inflammatory mediators (cytokines, chemokines, reactive oxygen and nitrogen species, MMPs) released from recruited neutrophils, endothelial cells, leptomeningeal macrophages and brain-resident microglia and astrocytes contribute to pathogen eradication but also act as neurotoxins and induce neuronal damage [107, 116, 119, 120]. Moreover, PM induces damage to hair cells and spiral ganglion neurons in the inner ear [121–123] provoking sensorineural hearing impairments in up to 30% of survivors [108, 124, 125]. In an infant rat PM model, increased CSF levels of TNF-α during acute infection were positively correlated with increased hearing loss in surviving animals [122].

The pathogenesis and involvement of inflammatory processes in inducing brain damage during meningitis have been intensively studied in different animal models. Upon bacterial invasion into the CNS, a wide range of cytokine is produced and secreted into the CSF [126]. In experimental pneumococcal meningitis, increased CSF levels of TNF-α and MMP-9 are already detected at 4 h after infection and peaked at 12 h after infection [127]. Since levels of TNF-α, IL-1β, IL-6 and IL-8 in the CSF increase before evidence of neutrophil recruitment, brain-resident cells are considered to contribute significantly to cytokine production [126]. As a result of bacterial proliferation, an excessive inflammatory reaction takes place in the CSF with BBB breakdown causing brain edema via leakage of plasma into the CNS, increased intracranial pressure, hydrocephalus and cerebral ischemia [116]. In patients with bacterial meningitis, cerebrovascular complications are frequently observed [124] with vasculitis being the cause for cerebral infarction, hemorrhages and subsequent cortical damage during meningitis [18, 116, 124].

Metalloproteinases in the pathogenesis of bacterial meningitis ::: Metalloproteinases in bacterial meningitis ::: MMPs and ADAMs in infectious disease of the CNS:
Data from clinical and experimental studies have shed light onto the crucial role of metalloproteinases during pathogenesis of bacterial meningitis and other neuro-infectious diseases. Metalloproteinases—expressed or activated in response to the invading pathogen—contribute to acute neuroinflammatory reaction and damage upon bacterial invasion but might also be involved later during the disease in the resolution of inflammation and repair mechanisms [41, 127–129]. During bacterial meningitis, the expression and activation of metalloproteinases and their inhibitors are altered compared to physiologic conditions. During experimental PM in infant rats, MMP-8 and MMP-9 are transcriptionally upregulated in CSF cells, whereas the expression of MMP-2 and MMP-7 remains unaffected. In brain tissue, expression of MMP-3, -8, -9, -12, -13 and -14 was significantly upregulated (100- to 1000-fold), while expression levels of MMP-2 and MMP-7 remained unchanged [127]. The upregulation of MMP-8 (mostly released from infiltrating neutrophils) is a specific hallmark of bacterial meningitis, which is not found in other neuroinflammatory disease [130]. MMP-8 induces increased barrier permeability via proteolytic cleavage of the tight junction protein occludin in meningococcal neuroinfection [131]. Other experimental data of meningitis caused by heat-inactivated N. meningitidis showed a significant upregulation of MMP-9 mRNA expression with stable MMP-2 and MMP-7 expression [41]. On the protein level, MMP-9 in the CSF correlated with TNF-α levels, with a concentration peak for both at 12 h after intracisternal infection with S. pneumoniae [127]. MMP-9 in the CSF was detected as early as 15 min after intracisternal infection, indicating its early release from brain-resident cells in this experimental model. Further recruitment and infiltration of neutrophils contribute to the peak MMP-9 levels at 12 h after infection [127]. Gelatinase activity (MMP-2 and/or MMP-9) has been associated with the occurrence of cortical necrotic lesions in experimental PM [23, 132]. During the initiation of neuroinflammation, ADAM17 plays a crucial role in releasing TNF-α, which in turn acts as a stimulus to induce MMP upregulation via a positive feedback loop [127, 133]. The fact that MMP-7—being the second most potent TNF-α activator apart from ADAM17 [65]—remains unchanged in bacterial meningitis [127] emphasizes the importance of ADAM17 during this early neuroinflammatory process. In addition to increased TNF-α and MMP-9 levels, TIMP-1 expression is also increased in the CSF of infant rats with PM, however, with a short delay [23]. TIMPs are suggested to regulate protein degradation and cytokine shedding during PM, thereby controlling

metalloproteinase-induced neuroinflammation. In experimental PM, the upregulation of MMP-9, however, exceeds the compensatory effect of TIMP-1 during the acute phase. This imbalance between MMPs and TIMPs during bacterial meningitis has, therefore, been implicated as a key event during pathophysiology of the disease [23]. In an experimental model of cerebral ischemia, MMP-9-deficient mice showed BBB preservation and reduced levels of tight junction protein degradation with subsequent better outcome [134]. MMP-9 deficiency during experimental PM, however, was shown to be associated with impaired bacterial clearance from blood and spleen, without showing an impact on clinical course of the disease, leukocyte infiltration of the subarachnoid space or bacterial titers in the brain [135]. Non-infectious experimental models of brain damage have shown a direct effect of MMP-9 on laminin degradation and associated hippocampal apoptosis [136, 137].

Matrix metalloproteinase expression profiles in patients with bacterial meningitis are consistent with the data generated in experimental settings. In patients with bacterial meningitis, CSF levels of MMP-1, -3, -7, -8, -9 and -10 have been found to be elevated, whereas CSF levels of MMP-2 remain unaffected [35, 138–143]. Similar to animal models, TIMP-1 levels have also been found to be upregulated, whereas TIMP-4 was significantly downregulated compared to control patients [142]. One week after infection, TIMP-1 continues to be highly expressed, whereas MMP-9 is reduced compared to the acute phase of infection [144]. In pediatric PM, MMP-9 levels were found to correlate with CSF cell counts, with high CSF MMP-9 levels being a risk factor for fatal outcome or development of neurologic sequelae [35, 141, 144]. The causative pathogen determines the inflammatory response in patients with bacterial meningitis. Pneumococcal meningitis is associated with significantly increased mortality and elevated levels of IFN-γ, MCP-1 and MMP-9 compared to meningitis caused by N. meningitidis or H. influenzae [145]. Tuberculous meningitis (TBM), which arises rarely as an extrapulmonary form of tuberculosis, is associated with neurofunctional complications [146]. In patients with TBM, levels of MMP-2 and MMP-9 were found to be elevated, and high levels of MMP-9 were associated with late neurofunctional deficits [147, 148]. Adjuvant dexamethasone during TBM was shown to reduce MMP-9 levels early in treatment and might represent one way by which dexamethasone reduces mortality in TBM [149, 150]. Despite the beneficial effect of dexamethasone in terms of mortality, it could not successfully prevent severe disabilities [150].

MMPs and ADAMs in viral neuro-infectious diseases ::: MMPs and ADAMs in infectious disease of the CNS:

The involvement of metalloproteinase in BBB integrity during viral neuroinfections has been intensively reviewed elsewhere [151]. Clinical data from patients with viral meningitis report elevated CSF levels of MMP-9 and TIMP-1 compared to control patients, with MMP-9 levels correlating to neutrophil cell number in CSF [152]. Involvement of metalloproteinases during viral neuroinfection has mostly been analyzed in experimental studies, though.

Japanese encephalitis virus (JEV) is an arthropod-borne virus and a major cause of acute encephalopathy in children [153]. In vitro, MMP-9 expression after JEV infection is mediated via NF-κB activation and the generation of ROS [153]. The expression of MMP-2, -7 and -9, as well as TIMP-1 and -3 is upregulated in mice infected with JEV and their overexpression is associated with disease severity [154].

In experimental herpes-simplex virus encephalitis (HSE), MMP-9 is upregulated and insufficiently counterbalanced by TIMP-1 resulting in a loss of collagen type IV, indicating MMP-9's role during the pathogenesis of HSE [155].

In vitro, West Nile virus (WNV)-infected human endothelial cells show significant upregulation of multiple MMPs with subsequent loss of tight junction proteins, an effect successfully prevented by the MMP inhibitor GM6001 [156]. MMP-9 levels are also found to be elevated in WNV-infected mice as well as in patients, suggesting that MMP-9 plays a role in mediating WNV entry into the CNS [157].

During HIV infection, a CCL2-dependent mechanism was postulated to be involved in infiltration of HIV-infected leukocytes into the CNS causing neuropathology. In vitro, this mechanism seems to rely on MMP-2 and -9 upregulation with an associated reduction of tight junction proteins [158]. In experimental HIV infection, the envelope protein gp120 increased MMP-2 and -9 expression with subsequent laminin and claudin-5 reduction and causing increased BBB permeability [159]. In patients with HIV-associated dementia, CSF levels of MMP-2, -7 and 9 are increased and might reflect a link to symptomatic neurological disease [160, 161]. Increased MMP activity with its detrimental effects on BBB integrity might contribute to transendothelial migration of HIV-infected cells into the CNS and development of HIV-associated neurologic damage [161].

Mouse adenovirus type-1 (MAV-1) infection increases levels of MMP-2 and -9 in brains [162]. Additional ex vivo data revealed that MMP-2 and -9 are produced by astrocytes and microglia in response to mouse encephalitic adenovirus-1, which might contribute to BBB disruption and encephalitis [163]. MAV-1 may also induce BBB breakdown by reducing surface expression of TJ proteins occludin and claudin-5 [164]. Murine coronavirus-induced viral encephalitis induces expression of MMP-3 and -12 plus TIMP-1, with MMP-3 expression exclusively localized in astrocytes, whereas TIMP-1 originates from infiltrating cells [165]. Increased MMP and TIMP levels are also associated with increased viral replication during neurotropic mouse hepatitis infection [166].

Neuroinflammation and BBB breakdown ::: MMP and ADAM inhibitors as potential treatment options in bacterial meningitis ::: MMPs and ADAMs in infectious disease of the CNS:
Matrix metalloproteinases and ADAMs function as ECM degrading enzymes and sheddases, thereby controlling BBB breakdown and production of inflammatory cytokines [15]. In experimental bacterial meningitis, MMP and ADAM inhibitors significantly reduce CSF levels of MMPs and pro-inflammatory cytokines (TNF-α, IL-6, IL-1β, IL-10) [128, 132, 167–169]. Notably, CSF levels of TNF-α are also significantly reduced by MMP inhibitors (MMPIs) without specificity for ADAM17 (i.e., Trocade) [167], indicating that MMPIs are able to indirectly reduce pro-inflammatory cytokine production apart from direct ADAM17 inhibition. MMPIs partially preserved BBB integrity by preventing ECM protein degradation [132, 167]. In a PM mouse model, TNF484 reduced neuroinflammation, thereby improving survival in animals without antibiotic therapy [170]. Notably, there are remarkable differences in different mouse and rat strains in terms of BBB breakdown prevention by metalloproteinase inhibitors after LPS-induced neuroinflammation [171].

Cortical necrosis ::: MMP and ADAM inhibitors as potential treatment options in bacterial meningitis ::: MMPs and ADAMs in infectious disease of the CNS:
As a consequence of bacterial meningitis and its associated inflammatory reaction, cerebrovascular complications are frequently observed in patients [124]. Focal ischemia caused by vasculitis results in cortical necrosis during meningitis [116, 124]. Breakdown of the BBB during the excessive neuroinflammatory reaction is a crucial step in the development of cerebral ischemia [116]. Since metalloproteinases are central regulators of BBB breakdown during bacterial meningitis (discussed above), their inhibition during the acute disease is a valuable therapeutic strategy to reduce cortical necrosis. Pre-treatment or treatment early in the course of disease with MMPIs is associated with reduced cortical necrosis in pneumococcal meningitis [128, 132, 167, 168, 172, 173] and with lower rates of intracerebral hemorrhage in meningococcal meningitis [169]. This neuroprotective effect is, however, reduced when the application of the inhibitors is delayed until the time of antibiotic therapy at the first appearance of disease symptoms [127, 174]. The early inhibition of MMPs during bacterial meningitis might be responsible for an overall reduction of the neuroinflammatory reaction with reduced BBB breakdown and cytokine production, thereby limiting the pathophysiological consequences of the disease [116].

Hippocampal apoptosis ::: MMP and ADAM inhibitors as potential treatment options in bacterial meningitis ::: MMPs and ADAMs in infectious disease of the CNS:
Apoptosis of immature neurons in the dentate gyrus of the hippocampus during bacterial meningitis is caused by bacterial toxins, but also by an excessive inflammatory reaction from the host [105, 116–118]. Several metalloproteinase inhibitors that inhibit ADAM17 (i.e., BB1001, Ro 32-7315) were found to prevent hippocampal apoptosis [127, 167]. However, other ADAM17 inhibitors were unable to prevent neural cell death in the hippocampus [132]. On the other hand, Trocade—without having a specific ADAM17-inhibitory profile—successfully prevented hippocampal apoptosis [167, 174]. As MMP-9 is directly involved in hippocampal cell death via the degradation of laminin [136, 137], inhibition of MMP-9—rather than ADAM17—might explain this neuroprotective effect of Trocade during bacterial meningitis. The protective effect on hippocampal neurons is lower—but still present—when BB1001 or Trocade therapy is initiated together with antibiotics at time of symptom onset [127, 167, 174].

Neurofunctional outcome ::: MMP and ADAM inhibitors as potential treatment options in bacterial meningitis ::: MMPs and ADAMs in infectious disease of the CNS:
Apoptosis of dentate gyrus granular cell progenitors found in the hippocampus of humans [114] and animal models with bacterial meningitis [175, 176] correlates with behavioural and learning

deficits [110–113], with increased hippocampal apoptosis being associated with impaired learning and memory performance in rat PM [111, 113]. Protection from hippocampal apoptosis by BB1001 improved learning performance in rats with PM [127]. Similarly, in PM-infected rats treated with Trocade plus daptomycin, the observed reduction in hippocampal apoptosis was associated with a significant improvement of learning and memory function [174]. GM6001 therapy significantly protected hippocampal neurons and prevented PM-induced learning and memory impairments in neonatal rats [177]. Notably, all three therapies also significantly protected from cortical necrosis [127, 174, 177], which might also positively influence learning and memory performance after PM. Correspondingly, in adult rats, MMP-2 and -9 inhibition further prevented cognitive impairment after PM possibly due to the observed successful preservation of BBB integrity during acute infection [178].

Bacterial meningitis-induced hearing loss ::: MMP and ADAM inhibitors as potential treatment options in bacterial meningitis ::: MMPs and ADAMs in infectious disease of the CNS: Bacterial meningitis induces sensorineural hearing loss by damaging hair cells and spiral ganglion neurons in the inner ear [122, 123]. In PM, CSF levels of TNF-α during acute infection are positively correlated with increased hearing threshold after infection [122]. Treatment with doxycycline or Trocade plus daptomycin significantly reduces CSF levels of TNF-α and other pro-inflammatory cytokines and significantly improves hearing thresholds after PM [173, 174]. Doxycycline—with its broad spectrum MMP inhibition profile—preserves spiral ganglion neurons from PM-induced cell death [173]. The overall reduction of neuroinflammation in both therapies might be attributable to a reduced concentration of ototoxic compounds in the cochlear duct, thereby preventing ototoxic damage in spiral ganglion neurons and hair cells of the cochlea [174].

Detrimental effects after metalloproteinase inhibition ::: MMP and ADAM inhibitors as potential treatment options in bacterial meningitis ::: MMPs and ADAMs in infectious disease of the CNS: Two metalloproteinase inhibitors—Ro 32-7315 and high-dose TNF484, both with high specificity for ADAM17—were reported to increase mortality during acute bacterial meningitis when administrated early after infection [132, 167]. In an experimental model of PM with TNF-α-deficient mice, mortality was significantly increased despite treatment with antibiotics, indicating that TNF-α may play an essential role during infection and inflammation, being necessary for host defense in the early stages of infection [179]. In PM, TNF-α inhibition by very potent ADAM17 inhibitors might, therefore, be rather detrimental than beneficial despite being neuroprotective in survivors, especially when applied at a time when TNF-α is a major contributor of the initial host response.

MMP inhibitors as treatment options for MS ::: Matrix metalloproteinases in multiple sclerosis: Based on the evidence concerning the role of MMPs in BBB opening and lesion formation, MMP inhibitors (MMPIs) were thought to be an ideal class of compounds for MS therapy in the late 1990s. The development of specific MMPIs for oncology was followed with great interest by the neurology field. In vitro models of metastasis showed dramatic reduction of dissemination and growth of tumor cells, which were thought to be transferable to suppression of BBB transmigration of blood-derived leukocytes in MS. This was bolstered by the observation that current therapies such as IFNβ, or later natalizumab [207] downregulate MMPs as part of their mode of action. The hypothesis that the therapeutic effect of IFNβ may be mediated via its suppressive effect on MMP-9 expression in T cells was further corroborated by a reduction of their migration across an artificial BBB [208, 209].
A number of hydroxamic acid inhibitor type compounds, which inactivate the enzymatic activity of MMPs by binding to the catalytic zinc of MMPs, have led to disease severity attenuation in EAE [210–212]. Similarly, such compounds reduced the capacity of T cells to migrate across an in vitro BBB model [213]. However, for several reasons clinical studies have never started. In oncology, the initially promising preclinical results with MMPIs were not confirmed in clinical trials. The same was the case in rheumatoid arthritis. Additionally, depending on their enzymatic target profile, adverse events and specific signs of toxicity were recognized during chronic dosing, which precluded the intended long-term use as maintenance therapy in MS (for review see [214]). Evidence for the role of MMPs in tissue repair in subacute and stable phases of the disease suggests that long-term MMP inhibition could have detrimental effects. The intent to overcome this by targeting only 'bad' MMPs via hyper-selective substrate profiles fell short on the basis that tissue destruction and repair can be mediated by the very same MMPs (e.g., MMP-7 and -9) [204], depending on the acuity of disease at a given time point.

There is, however, one example of translation of the knowledge on the role of MMPs in MS. Minocycline, a tetracycline antibiotic that exerts broad-spectrum inhibition of MMPs, demonstrated in two phase 2 studies in MS efficacy in reducing gadolinium-enhancing lesions [215, 216], which encouraged the study group to execute a phase 3 clinical trial [217] with 142 patients with a first MS attack. Clinical and MRI findings suggested that minocycline in comparison to placebo reduced the risk for patients after a first attack to reach the criteria for formal MS diagnosis within 6 months by 28% (p = 0.006). The site of primary action of minocycline is likely in the 'periphery', i.e., the immune system outside the CNS, while it is not known which MMP's inhibition is responsible for the clinical effect. Another potential mode of action apart from direct inhibition of enzymatic activity of several MMPs is interference with their activation. EMMPRIN (extracellular matrix metalloproteinase inducer, CD147) is a membrane glycoprotein of the immunoglobulin superfamily whose levels are reduced in T cells by minocycline [218]. Thus, minocycline treatment likely reduces the activity and secondarily the release and production of MMPs resulting in diminished leukocyte extravasation of leukocytes into the CNS. The Canadian study group that has run all minocycline trials in MS so far is preparing currently for another trial (Yong VW, personal communication).

Severity and frequency of relapses are important determinants for the long-term outcome of MS. Steroids are the current standard for intervention therapy to attenuate acute exacerbations in MS; however, their effect on the disability development is very limited. More powerful compounds for this situation represent a high unmet medical need. The effect of hydroxamic acid type MMPIs in EAE is strong pre-clinical evidence for their use in acute exacerbations in MS. Broad-spectrum MMPIs are the obvious drug candidates here, given the array of MMPs upregulated in this condition, as they have been shown to be free of side effects for a dosing period of several days [219]. Despite Health Authorities have defined the development path for the development of compounds for relapse therapy [220], the pharmaceutical industry has so far not engaged in such development activities. However, this may be an opportunity for a 'second life' for this class of candidate drugs.

Conclusion:
Metalloproteinases are mediators of neuroinflammatory processes upon neuroinfections and in MS. During these neuroinflammatory diseases, metalloproteinases regulate BBB breakdown, neutrophil infiltration and cytokine signaling. Metalloproteinase inhibitors have experimentally been shown to decrease neuroinflammation and brain damage in diseases with excessive neuroinflammation as a common denominator, thereby improving the outcome of such diseases. Nevertheless, timing of metalloproteinase inhibition appears to be critical to effectively downmodulate the cascade of pathophysiological processes leading to brain damage during PM. On the other hand, metalloproteinases are also mediators of neuroregeneration and synaptogenesis, processes that are therapeutically not intended to be inhibited. As this critical role of metalloproteinases in neuronal repair mechanisms and regeneration was only lately recognized, the original idea of chronic MMP inhibition needs to be conceptually revised. Recently accumulated research urges for a second chance of MMPIs, which—when correctly applied and dosed—harbor the potential to improve many different neuroinflammatory diseases.