Astrocyte–leucocyte interactions and the mechanisms regulating matrix degradation in CNS tuberculosis

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Abstract
The CNS (central nervous system) has a unique pattern of immune response to infection. TB (tuberculosis) of the CNS is devastating with widespread tissue destruction. In TB, astrocyte–leucocyte interactions are key in regulating MMP (matrix metalloproteinase) activity and are regulated by complex signalling pathways. A synergistic interaction between interferon γ and monocyte-derived mediators drives high-level astrocyte MMP-9 secretion; and other networking effects are inhibited by steroids. Better understanding of regulatory mechanisms may identify potential switch points that could be future therapeutic targets.

MMPs (matrix metalloproteinases) and the CNS (central nervous system)
MMPs are proteases whose diverse roles include degradation of extracellular matrix, activation of cytokines and chemokines and facilitation of leucocyte recruitment to sites of infection and inflammation. In excess, MMPs may cause direct tissue destruction. MMP activity is controlled by transcriptional regulation, compartmentalization, secretion of pro-forms (zymogens), and by four specific tissue inhibitors of MMPs [TIMPs (tissue inhibitors of metalloproteinases)] [1,2]. Specific MMPs and TIMPs have varying tissue- and stimulus-dependent expression as well as distinct substrates.

The CNS is often considered an 'immunologically privileged' site but is nevertheless affected by inflammatory diseases such as multiple sclerosis and autoimmunity. The CNS is better viewed as an immunologically restricted site divided from the systemic immune system by the BBB (blood–brain barrier). MMPs are secreted by many cells within the CNS as well as infiltrating leucocytes. Numerous MMPs have been detected in the CNS in diverse diseases. For example, in active MS lesions, MMP-9 may be most abundant. CSF (cerebrospinal fluid) and serum levels of active MMP-9 have been suggested as potential biomarkers for monitoring MS disease activity, particularly since IFN-γ (interferon γ) therapy may lower CSF MMP-9 concentrations [3]. However, there is a growing body of literature indicating that other MMPs may have key roles within the CNS. For example, MMP-3 (stromelysin-1) is released from apoptotic neuronal cells and this results in the activation of microglia, the resident CNS macrophages.

MMP secretion is likely to be key in contributing to breakdown of the BBB which is rich in type IV collagen as well as fibronectin and laminin. In the context of infection, loss of integrity of the BBB will facilitate leucocyte infiltration, which in turn leads to further MMP secretion either directly or following stimulation of resident CNS cells activated by pro-inflammatory cytokines. Diapedesis of monocytes cells through the brain capillary endothelium is an important first step in this process and appears to involve the disruption of intercellular tight junctions by gelatinase (MMP-2 and -9)-mediated occludin degradation along an MIP-1α (macrophage inflammatory protein-1α) gradient [4]. Astrocytes have many long cellular processes forming the glial (limiting) membrane, which is an integral part of the BBB, and they may secrete MMPs, causing direct local damage.

MMPs in infection
Successful eradication of an invading pathogen requires a co-ordinated immune response generally involving recruitment of neutrophils, T-cells and macrophages followed by resolution of inflammation once the source has been eliminated. Studies of Staphylococcus aureus septic arthritis in MMP-7 knockout mice reveal decreased joint destruction despite increased numbers of pathogens in knockout animals [5]. There are similar findings in a murine model of Streptococcus pneumoniae meningitis in MMP-9 knockout mice in which the knockout was less able to clear the pathogen [6]. Such results suggest a role for MMPs both in tissue destruction and in host defence; cell migration is key in both processes. The role of MMPs in defence to pathogens is complex since these enzymes may activate pro-α-defensins, microbicidal peptides important in the early phase of the innate immune responses [7].

Even classical paradigms such as the one in which Th2 cytokines down-regulate MMP secretion are subject to
exceptions and IL-5 (interleukin 5) may up-regulate MMP-9 secretion, facilitating eosinophil migration through basement membranes [8].

A broad range of CNS infections may induce MMP secretion but evidence for their pathological role is strongest in bacterial meningitis, HIV-associated dementia and TB (tuberculosis). MMP-3, MMP-7, MMP-8 and MMP-9 mRNA levels were raised in neural tissue and MMP-9 in CSF in a rat model of pneumococcal meningitis, and MMP inhibition led to significant abrogation of neuronal injury [9]. Raised MMP-9 CSF concentrations are also found clinically, although the source of this may be infiltrating leukocyte [10]. A further example of the interaction with CNS infiltrating leukocytes is found in HIV where infected macrophages secrete MMP-2 activated by MMP-14 on neurons, which results in cleavage of the astrocyte-derived chemokine SDF-1 (stromal-cell-derived factor 1) and subsequent neuronal death [11].

CNS TB is almost uniformly fatal if untreated and even patients receiving appropriate therapy are often left with significant neurological sequelae as a result of tissue destruction. Our group has proposed that in TB, a matrix degrading phenotype develops in which MMP activity is relatively unopposed by TIMPs. For example, there was strong immunohistochemical MMP-9 but not TIMP-1 staining in granuloma-associated monocytic cells as well as adjacent to caseous necrosis in TB-infected patient lymph node biopsies [12]. The activation of MMP-1 in response to TB but not to the vaccine strain BCG (Bacille Calmette-Guérin) may be one reason why BCG seldom is pathogenic and it is TB that is associated with CNS disease [13]. CNS TB provides a useful paradigm in which to study networking effects regulating MMP activity in a CNS infection characterized by widespread tissue necrosis.

Neuroglial–leucocyte interactions in CNS TB

Clinical experimental studies of CNSTB have mainly concentrated on TBM (TB meningitis). Elevated CSF MMP-9 concentrations were associated with signs of local but not systemic tissue damage and patient death [14]. Initial high concentrations of MMP-9 fall very slowly during TBM treatment, suggesting that there may be ongoing BBB permeability in the absence of infection [15]. Dissecting the mechanisms behind such observations requires studies of the interactions between cells in the CNS, since the inflammatory response in CNS TB is largely due to networking effects as there are relatively small numbers of organisms present in the brain. A number of cell types may be involved in such networks but the most interesting initial data have arisen from the study of astrocyte–leucocyte interactions (see Figure 1).

TB may infect or stimulate any cell within the CNS. However, direct infection of astrocytes with the pathogen does not result in MMP gene expression or secretion. Furthermore, conditioned medium from astrocytes stimulated with Mycobacterium tuberculosis or CoMTB (conditioned medium from M. tuberculosis-infected monocytes) did not drive MMP activity from monocytes or from other astrocytes. In contrast, CoMTB was a major stimulus to MMP gene expression and secretion. Real-time RT (reverse transcriptase)–PCR analysis of all known MMPs and TIMPs showed that gene expression of MMP-1, -2, -3, -7 and -9 was increased in human astrocytic cells (U373-MG) stimulated by CoMTB [16]. In contrast, TIMP gene expression was only minimally altered by CoMTB. Although CoMTB up-regulated MMP-9 secretion, it was not possible to detect MMP-1, -3 and -7 secretion, which may mean that a second factor is required. Although both TNF (tumour necrosis factor) and IL-1 were required for the network-dependent activation of astrocytes, at concentrations present in CoMTB neither was able to stimulate MMP-9 secretion alone or in combination, indicating that these pro-inflammatory mediators are not sufficient to drive the leucocyte–astrocyte interaction. The mechanisms regulating astrocyte MMP-9 secretion are complex. All three MAPKs (mitogen-activated protein kinases) were involved in regulating MMP-9 secretion [17] and there was activation of AP-1 (activator protein 1) and NF-κB (nuclear factor κB). In particular, the results indicate that homodimers or monomers of p65 NF-κB are involved in control of MMP-9 gene transcription.

IFN-γ is an important cytokine in host defence to TB that usually down-regulates MMP secretion. Unexpectedly, IFN-γ synergized with CoMTB to drive astrocyte MMP-9 secretion to very high concentrations [18]. This was in part due to an interaction with IL-1β. However, although IL-1β up-regulated MAPK paths, the synergistic interaction did not involve this path but was at least in part mediated by a JAK-2 (Janus kinase 2) and STAT-3 (signal transducer and activator of transcription 3)-dependent mechanism. Interestingly, the steroid dexamethasone inhibited both the CoMTB network-dependent astrocyte MMP-9 secretion and the IFN-γ-dependent synergy. This provides a potential mechanism to

Figure 1 | Astrocyte–leucocyte interactions in TB

Networks between astrocytes, leucocytes and microglial cells in the CNS amplify the effects of infection by Mycobacterium tuberculosis.
explain the proven beneficial effect of steroid therapy in CNS TB, the mechanism for which is so far unexplained [19]. This is the subject of active ongoing investigations.

Conclusions
There is no doubt that MMPs have important physiological and pathological roles in the response to CNS infection. The mechanisms underlying this are complex and a key role is played by networking interactions involving different CNS cell types. Although this review has focused on the central role of astrocytes interacting with leucocytes infiltrating across the BBB in TB, it is very likely that other resident CNS cells such as microglia have important roles in such networks, and preliminary results confirm this. Interfering with pro-inflammatory networks may offer an opportunity to modulate MMP-dependent pathology without blocking direct MMP responses to pathogens which are likely to be necessary for a successful host CNS immune response.

References

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