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Production and Roles of Glial Tissue Inhibitor of Metalloproteinases-1 in Human Immunodeficiency Virus-1-Associated Dementia Neuroinflammation: A Review

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Abstract: Problem statement: Tissue Inhibitor of Metalloproteinases-1 (TIMP-1) and its cognate targets, the Matrix Metalloproteinases (MMPs), were differentially expressed in human brain samples with or without HIV-1 infection or HIV-1 Encephalitis (HIVE). **Approach:** A through literature review demonstrated that cell culture models of Central Nervous System (CNS) cell types had been used to illustrate the intricate temporal patterns of TIMP-1/MMP expression, regulated by a variety of inflammatory cytokines. **Results:** As MMPs and TIMP-1 can significantly altered the extracellular environment and cell signaling, the differential regulation of TIMP-1/MMP expression in neuroinflammation can impact neuronal function and survival in disease conditions. TIMP-1 pro-survival effects had been demonstrated in a variety of cell types including CNS neurons, protecting cells from a wide range of stress and insults. TIMP-1, also known to interact with non-MMP targets, altered cell behavior. In this review, we discussed the possibility that the upregulation of TIMP-1 by glia in acute neuroinflammation may be a neuroprotective response. **Conclusion:** It will be important to delineate the effects of TIMP-1 on neurons and identify receptors and downstream signaling pathways, in order to evaluate TIMP-1 as a therapeutic strategy for neuroinflammatory and neurodegenerative diseases.

Key words: Astrocyte, interleukin-1-beta, matrix metalloproteinases, microglia, transforming growth factor-beta

INTRODUCTION

***In vivo* expression of TIMP-1 and MMPs in HAD/HIVE disease:** In advanced Human Immunodeficiency Virus type 1 (HIV-1) infection, virus and activated immune cells perturb the Central Nervous System (CNS), leading to HIV-1-Associated Dementia (HAD, lately termed HAND, HIV-1-associated neurocognitive disorders), with devastating symptoms such as cognitive changes and motor dysfunction. Neuropathology examination of patient samples usually reveal nodules of infiltrating, activated Mononuclear Phagocytes (MP) and reactive gliosis in their vicinity[1], a condition termed HIV-1 Encephalitis (HIVE). Analysis of patient brain and Cerebral Spinal Fluid (CSF) samples revealed a high upregulation of Matrix Metalloproteinases (MMP)-9 and MMP-2 mRNA in HIVE samples than in HIV-1 seropositive patients without HAD (designated as HIV+) or healthy controls[2]. Further immunohistochemical studies comparing these three categories demonstrated that, in patients with severe HIVE, activated MP (positive for CD68 and HLA-DR) infected with HIV-1 (positive for HIV-1p24) in microglial nodules were the main source of MMP-2[2]. MMP-9 was detected on most activated MP, even those that were negative for HIV-1p24 in HIVE samples and HIV+ samples[2]. MMP-2 protein was significantly upregulated in both HIVE and HIV+ brain samples compared to seronegative controls without neurological disease[3].

Expression of MMPs and TIMP-1 is known to be regulated by inflammatory cytokines[4], which are in turn differentially expressed in neuroinflammation. Compared to healthy controls, both HIV+ and HIVE brain samples exhibited significantly higher mRNA levels for IL-1beta[3] and higher protein levels of TGF-

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beta2, while TGF-beta2 mRNA was significantly increased only in HIVE samples[5]. TIMP-1 protein levels, however, were significantly lower in both HIVE and HIV+ patients as compared to healthy controls[3]. Of possible interest, TIMP-1 protein levels were significantly lower in the white matter than in basal ganglia or cerebellum of a HIVE patient[3]. It was sufficiently obvious that inflammatory cytokines were differentially expressed in HAD/HIVE as communication signals between immune cells and responding cell types. What, then, were the roles of the differentially expressed MMPs and TIMP-1? Were they beneficial to neuronal function, or detrimental in the degrading extracellular environment? The general functions of TIMP-1 and MMPs are briefly reviewed below to suggest their possible roles in HAD/HIVE.

General review of TIMPs and MMPs: Extracellular Matrix (ECM) homeostasis and remodeling are accomplished through activities of Matrix Metalloproteinases (MMPs), a large family of zinc-dependent proteases. The homeostasis of ECM is important for normal function of many organs and remodeling by MMPs is essential in diverse physiological processes including angiogenesis and wound healing[7]. MMPs are classified according to the type of substrates: MMP-2 and -9 are the prominent gelatinases, MMP-1 is the most well-studied of the collagenases and other types of MMPs include stromelysins (e.g., MMP-3), matrilysins (e.g., MMP-7) and Membrane-Type (MT)-MMPs which are attached to the outer surface of cell membranes rather than soluble in the extracellular milieu[5]. MMPs are also responsible for processing cell-surface signaling receptors and their ligands, such as the apoptosis-inducing Fas Ligand (FasL)[7]. Furthermore, aberrant MMP expression or activity has been observed in many pathological conditions, including tumor metastasis[8]. It follows that proper regulation of MMP activity is crucial for an extracellular environment permissive to normal cell functioning.

In addition to controls in gene expression and protein secretion, MMP activity is further regulated by activation in the extracellular environment from proenzymes to active MMPs and sequestration and inhibition by their endogenous inhibitors, the four members of tissue inhibitor of metalloproteinases[6,9]. These members, termed TIMP-1 through -4, are small extracellular proteins which reversibly bind to the active site of MMPs and inhibit their interaction with substrates[9]. Each TIMP can inhibit a wide spectrum of MMPs. TIMPs may also regulate MMPs by mechanisms other than direct inhibition. TIMP-2, for example, with MT1-MMP can activate proMMP-2 to its active form[9,10]. As MMPs are involved in many extracellular functions; TIMPs, as their inhibitors, have been found to affect many cellular functions that are dependent on the ECM or other extracellular signals. TIMP-1 is of particular interest in disease processes because it is expressed at a non-detectable or very low level in most tissues in homeostasis, but is highly inducible by various stimuli, especially inflammation. TIMP-1 regulates, among many other pathways, the JAK, PI3 kinase, Bcl-2, FAK and MAP kinase pathways in a variety of cell types, leading to cell survival[11-14] and differentiation in the case of B cells[15]. Intricate cell-signaling pathways aside, it is just as likely that TIMP-1 can protect neurons by inhibiting the overly-high activity levels of MMPs in neuroinflammation, thereby maintaining the integrity of the Blood-Brain Barrier (BBB) and decreasing trafficking immune cells, the cytokines of which may be detrimental to neurons.

Glial expression of TIMP-1 and MMP in HAD conditions: Primary human microglia in culture exhibited detectable expression of MMP-1 and -3 and higher expression levels of MMP-2 and -9[2]. CD40 ligand (CD40L), an inflammatory signal, increased all of these MMPs in cultured microglia. Moreover, monocytes isolated from healthy donors exhibited a high increase in MMP-2 and -9 expression when cells differentiated into Monocyte-Derived Macrophages (MDM) in culture. While these results may be applicable to all neuroinflammatory conditions, further studies revealed that HIV-1 infection impacted MP production of MMPs. Infection with CNS isolates HIV-1_{DJV}, HIV-1_{JR-FL}, or Cerebrospinal Fluid (CSF) HIV-1 isolates HIV-1_{SF162} and HIV-1_{MSCSF} all downregulated MDM expression of MMP-9[2]. Among the HIV-1 strains tested, only the CNS isolate HIV-1_{YU-2} had no effect on MDM expression of MMP-9. More importantly, inflammatory stimuli such as CD40L still upregulated MMP-9 expression in MDM infected with the tested HIV-1 strains, except in MDM infected with HIV-1_{YU-2}. Thus, MMPs from MP could have important roles in the neuroinflammatory disease of HAD. Meanwhile, stimulation with CD40L and differentiation also increased MDM secretion of TIMP-1, thus TIMP-1 expression from MP was concurrently increased with MMPs[2].

Although MP initiate the inflammatory cascade in HAD neuroinflammation, contribution into the MMP/TIMP axis by the numerically and spatially prevalent astroglia should not be overlooked. Astrocyte expression of MMP-2 was increased after 6 days in

culture without any cytokine stimulation[3] and MMP-1 after 14 days[16]. When stimulated with IL-1beta, astrocyte expression of MMP-2 peaked between 6-20 days[3] and MMP-1 level rose significantly after 20 days[16]. MMP-7 production by astrocytes, on the other hand, was drastically increased by IL-1beta stimulation after 24 h, but decreased over time to no significant difference to unstimulated cells by day 14[16]. As for within the first 24 h of stimulation, IL-1beta did not increase astrocyte secretion of MMP-2 unless simultaneously stimulated with TGF-beta1 or TGF-beta2, while the two TGF-beta isoforms significantly downregulated the IL-1beta-stimulated increase of proMMP-1[5]. Moreover, IL-1beta stimulation significantly upregulated astrocyte secretion of TGF-beta2 after 7 days and TGF-beta1 after 14 days[5]. These studies demonstrated the intricate network of inflammatory cytokine signaling and feedback to astrocytes and the inherent differences in expression patterns of different MMPs in astrocytes. In general, astrocyte production of MMPs is upregulated by IL-1beta stimulation and increased over time.

With astrocytes composing the majority of cells in the CNS, such drastic changes in their MMP expression can be balanced only by their expression of the TIMPs. Primary human astrocytes significantly upregulated TIMP-1 expression in acute stimulation with IL-1beta within 24 h[3,16]. Furthermore, astrocyte production of TIMP-1 was also modulated by the presence of HIV-1 virions and products of HIV-1-infected MP[3]. Astrocytes treated with supernatant from control MP and IL-1beta-stimulated MP supernatant exhibited comparable TIMP-1 production. However, there was a significant increase in TIMP-1 production in astrocytes treated with HIV-1-infected MP supernatant and further increase in astrocytes treated with supernatant from HIV-1-infected, IL-1beta-stimulated MP. Astrocyte TIMP-1 was also differentially regulated in the presence of HIV-1 viral particles. While treatment with HIV-1_{ADA} particles did not alter astrocyte secretion of TIMP-1, presence of HIV-1_{SF162} caused a slight increase and production in astrocytes treated with HIV-1_{JR-FL} or HIV-1_{DJV} was significantly increased[3]. Taken together, these studies revealed that TIMP-1 expression was differentially regulated in astrocytes by inflammatory stimuli and HAD conditions.

In summary, many of the MMPs under study were upregulated by inflammatory cytokines over a course of days, while TIMP-1 was upregulated by an acute stimulation of approximately 24 h. Under IL-1beta stimulation, a robust increase of TIMP-1 mRNA and protein level observed in the first 24 h was virtually obliterated by day 7 of stimulation[4,15]. Since astrocytes *in vivo* in HIVE and HIV+ patients had chronic exposure to IL-1beta as the disease progressed, these *in vitro* results explained the consistent drop in TIMP-1 mRNA and protein levels in the CNS of these patients compared to healthy controls.

Further studies into the control mechanism of this downregulation of TIMP-1 in chronic neuroinflammation were also conducted in primary human astrocytes. In acute stimulation, IL-1beta considerably upregulated TIMP-1 mRNA and protein secretion in astrocytes, but both TGF-beta1 and TGF-beta2 could knock down this increase, although not to baseline level in acute stimulation[4]. Studies using reporter constructs of the TIMP-1 promoter demonstrated that following IL-1beta stimulation, TGF-beta1 and TGF-beta2 could decrease the promoter activity and/or mRNA stability of TIMP-1[15]. Thus, in the context of HAD neuroinflammation, IL-1beta upregulates astrocyte TIMP-1 expression only in a very short, initial time frame, while also inducing expression of the two forms of TGF-beta. TGF-beta1 and -beta2 then begin to downregulate TIMP-1 expression. Meanwhile, expressions of MMP-2 and MMP-9 are increased. In summary, over the time course of neuroinflammation, the MMP/TIMP balance is considerably shifted in favor of the MMPs.

Neuroprotective capabilities of TIMP-1 against a variety of insults: The differential regulation of MMPs and TIMP-1 by glia in neuroinflammation serves multiple purposes. First of all, the changes in the MMP/TIMP axis alter the homeostatic state of the ECM. Increased MMP expression can facilitate MP infiltration into the CNS in HAD and this can be countered by TIMP-1. A parallel was demonstrated in an experimental autoimmune encephalomyelitis (EAE) mouse model for multiple sclerosis, another important and well-studied neuroinflammatory degenerative disease with immune cell infiltration into the CNS. Compared to “wild-type” EAE mice, TIMP-1 knockout mice had more severe pathology after EAE induction, evaluated in terms of EAE disease score, numbers and activation states of infiltrating T cells and MP and demyelination[17]. Logically, in both HAD and EAE, TIMP-1 can protect the basement membrane on the vasculature, thereby decreasing the number of infiltrating and activated immune cells that can exacerbate inflammation, which is detrimental to neurons.

MMP/TIMP effect on neurons, however, may be far beyond the maintenance of the ECM. In a study with kainate-induced seizure, a mouse model for epilepsy and acute excitotoxicity, induction or addition of the gelatinase MMP-9 was correlated with neuronal

death after kainate injection[13]. This was not entirely due to MMP activity on the ECM, since in the same study, MMP-2, another major gelatinase, had no effect on neuron death after kainate[18]. A subsequent study observed that TIMP-1 expression was induced as an immediate-early gene after kainate treatment[19]. This, however, was again beyond inhibiting the neurotoxic MMP-9, since, paradoxically, wild-type mice able to express TIMP-1 had more neuron death in specific regions of the hippocampus than in TIMP-1 knockout mice after kainate-induced seizure[19]. Moreover, expression of MMP-2, MMP-9 and gelatinolytic activity were elevated after kainate in wild-type mice, but not in TIMP-1 knockout mice, suggesting that TIMP-1 may help activate the gelatinases, or feed-back upregulating expression of these gelatinases[19]. Behaviorally, TIMP-1 knockout mice had normal susceptibility to kainate-induced seizure, but exhibited deficits in learning and memory[19]. While this may not have a bearing on the survival of neurons *per se* in CNS pathology, the presence and activity of TIMP-1 may improve or preserve cognitive performance of patients with neurodegenerative diseases.

An intricate study revealed that TIMP-1 neuroprotection may have nothing to do with inhibition of MMP activity, but is specific to an intact TIMP-1 protein and specific to the type of insult to neurons[20]. In this study of acute glutamate excitotoxicity, neurons transfected with TIMP-1 in an adenoviral vector were protected from glutamate toxicity. Neurons with control transfection of beta-galactosidase were not protected, showing that the effect was not due to the transfection procedure. More importantly, neurons transfected with TIMP-3, or treated with broad-spectrum MMP inhibitors BB94 or MMP inhibitor (MMPI)-1 were not protected from glutamate toxicity, demonstrating that neuroprotection from glutamate excitotoxicity in this model was TIMP-1-specific and inhibition of MMPs was not sufficient for neuroprotection. To further elucidate the neuroprotective mechanism, Tan *et al*.[20] used N-TIMP-1-T2G, a non-MMP-interacting mutant of TIMP-1, with the threonine at the second amino acid position mutated to glycine, expressed as the N-terminal domain of TIMP-1[21,22]. Neither N-TIMP-1-T2G added to the culture nor transfection with a non-secretable form of TIMP-1 provided neuroprotection against glutamate toxicity, demonstrating that TIMP-1 neuroprotection was mediated by an intact TIMP-1 protein in an extracellular position[20]. Calcium imaging studies revealed that the calcium influx into neurons after glutamate treatment was significantly decreased in neurons treated with TIMP-1, implicating TIMP-1 in the modulation of calcium-permeable glutamate receptors[20]. Of interest, TIMP-1 did not protect neurons from chemical ischemia induced by potassium cyanide, indicating that TIMP-1 neuroprotection was specific to the type of insults on neurons[20].

TIMP-1-mediated neuroprotection was thus seen in diverse *in vivo* and *in vitro* models, including the EAE mouse model and TIMP-1 knockout mouse model. While the mechanism of neuronal injury in the EAE model was inflammatory, glutamate excitoxicity *in vitro* and injuries in kainate-seizure mouse model were dependent on neuronal activity. Based on this wide variety of injury mechanisms in these different models, TIMP-1 was implicated in different neuroprotection pathways. TIMP-1 was linked to preservation of the ECM through inhibition of MMPs[17], non-inhibiting regulation of MMPs[19] and the completely non-classical modulation of ionotropic glutamate receptors[20]. The studies with potassium cyanide[20] and TIMP-1 knockout[19] also indicate that TIMP-1-mediated neuroprotection may not be evident in all types of neuronal injuries. Thus, TIMP-1 has differential effects on neurons depending on the mechanism of the specific insult and tissue context.

A novel TIMP-1 receptor: CD63: As discussed earlier, three independent studies indicate TIMP-1 neuroprotection to be mediated through mechanisms dependent on either MMPs or neuron-specific ionotropic glutamate receptors. Many other studies have demonstrated MMP-independent TIMP-1 pro-survival effects, however, in cell types that do not express ionotropic glutamate receptors, such as Burkitt's lymphoma B cell lines, mouse bone marrow stromal cell line MBA-1 and human breast epithelial cell line MCF10A[11,13,14]. Such results strongly indicate that TIMP-1 may signal through a variety of "receptors" in different cell types. Conversely, some "TIMP-1 receptors" other than MMPs or ionotropic glutamate receptors may also be active in TIMP-1-mediated neuroprotection in other contexts such as HIVE neuroinflammatory environment.

In search for a novel TIMP-1 receptor, CD63, a tetraspanin (transmembrane-4 superfamily; TM4SF) was identified by yeast two-hybrid screening as interacting with the C-terminal, non-MMP-inhibiting domain of TIMP-1[23]. Further experiments revealed cell-surface co-localization of TIMP-1, CD63 and beta1 integrins[23]. The tetraspanins are known for promiscuous protein-protein associations on the cell membrane, forming functional microdomains with other tetraspanins and integrins[24,25]. Similarly, the beta1 subunit of integrins is known to associate with a variety of integrin alpha subunits, forming integrin dimers with diverse

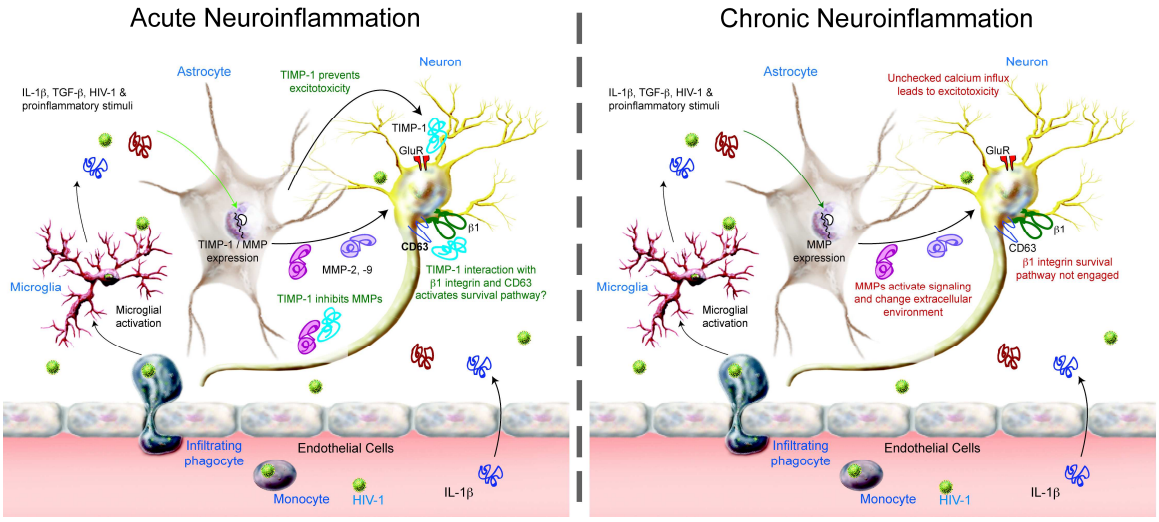

Fig. 1: TIMP-1 neuroprotective mechanisms are disengaged in chronic inflammation

functions including modulation of cell growth and survival[26]. In accordance with the wide variety of cell signaling associated with CD63 and beta1 integrins, experiments found CD63 necessary for TIMP-1 mediated ERK activation and inhibition of apoptosis in MCF10A cells[23]. Beyond regulation of cell survival, overexpression of TIMP-1 in these cells also caused loss of cell polarization behavior necessary for formation of normal acini structure, a phenotype restored by downregulation of CD63[23]. This study demonstrated not only that TIMP-1 could promote cell survival through integrins and a tetraspanin instead of MMPs, but also opened up a wide field of research for intracellular pathways signaling TIMP-1 non-classical effects, as all pathways associated with CD63 and beta1 integrin could potentially be modulated by TIMP-1.

CONCLUSION

In this review, we discussed the differential regulation of MMP/TIMP-1 expression in disease and TIMP-1-mediated neuroprotection. TIMP-1 pro-survival and differentiation-inducing effects have been demonstrated in a wide variety of cell types and disease models. Several divergent protein families including MMPs, tetraspanins and integrins have been indicated as functional "TIMP-1 receptors". Both classical and non-classical pathways mediating TIMP-1 neuroprotection have been shown in different neural injury models. A complex alteration in the MMP/TIMP axis has also been demonstrated in neuroinflammatory diseases including HAD. Much remains to be elucidated, however, as to the precise and divergent roles that TIMP-1 plays in HAD neurodegeneration. Here we include a schematic summarizing TIMP-1 regulation and activities in HAD and neuroprotective mechanisms, including CD63 as a potential TIMP-1 receptor in neurons (Fig. 1).

Based on results discussed above, TIMP-1 in HAD can potentially decrease infiltration of HIV-1-infected and activated immune cells, reduce neuroinflammation, confer neuroprotection and improve cognitive function of patients. A marked downregulation of TIMP-1 in chronic neuroinflammation may contribute to the exacerbated neurodegeneration in HAD. Thus, studies into TIMP-1 neuroprotective mechanisms as may be found in HAD conditions are important for both basic science advancement and development of novel therapeutic intervention strategies.

In HIV-1-infection, activated/infected phagocyte crosses the blood-brain barrier and enters the CNS. Cytokine communication and HIV particles from the MP activate microglia, which contribute to the inflammatory cascade and activate astroglia. Activated astrocytes alter their expression patterns of TIMP-1, MMP-2 and -9. TIMP-1 can moderate MMP activity level. TIMP-1 can also prevent excitotoxicity through ionotropic glutamate receptors[20]. Further, TIMP-1 can potentially interact with neuronal beta1 integrins and CD63 and activate survival pathways[23]. Overall, upregulation of TIMP-1 may be beneficial to the HIV-1-infected CNS. As chronic inflammatory stimulation downregulates astrocyte expression of TIMP-1, TIMP-1 neuroprotection is lost in HAD/HIVE patients.

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