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REVIEW

The Significance of Matrix Metalloproteinases in the Immunopathogenesis and Treatment of Multiple Sclerosis

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أهمية الأنزيمات المعدنية المحللة للبروتين في تطور مرض التصلب العصبي المتعدد وعلاجه

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abstract: Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS). The major pathological outcomes of the disease are the loss of blood-brain barrier (BBB) integrity and the development of reactive astrogliosis and MS plaque. For the disease to occur, the non-resident cells must enter into the immune-privileged CNS through a breach in the relatively impermeable BBB. It has been demonstrated that matrix metalloproteinases (MMPs) play an important role in the immunopathogenesis of MS, in part through the disruption of the BBB and the recruitment of inflammatory cells into the CNS. Moreover, MMPs can also enhance the cleavage of myelin basic protein (MBP) and the demyelination process. Regarding the growing data on the roles of MMPs and their tissue inhibitors (TIMPs) in the pathogenesis of MS, this review discusses the role of different types of MMPs, including MMP-2, -3, -7, -9, -12 and -25, in the immunopathogenesis and treatment of MS.

Keywords: Multiple Sclerosis; Blood-Brain Barrier; Matrix Metalloproteinases; Inflammation; Central Nervous System.

الملخص: يعد مرض التصلب العصبي المتعدد (MS) أحد أمراض المناعة الذاتية في الجهاز العصبي المركزي (CNS). ونتيجه للإصابه به يحدث خلل في سلامة حاجز الدم في الدماغ (BBB) إلى جانب تطور في رد فعل الدباق النجمي و لويحات التصلب العصبي المتعدد. يحدث هذا المرض عندما تدخل الخلايا غير المقيمه الى الجهاز العصبي المركزي المحمي مناعيا من خلال ثغرة في حاجز الدم في الدماغ. وقد تبيّن أن الأنزيمات المعدنية المحللة للبروتين (MPPs) تلعب دورا مهما في تطور مرض التصلب العصبي المتعدد، من خلال تعطيل حاجز الدم وتوجيه الخلايا الالتهابية الى الجهاز العصبي المركزي . يضاف الى ذلك أن هذه الأنزيمات يمكن أن تقوم بتعزيز الانقسام في بروتين الاساس للمايلين ونزع غطائه. أما فيما يتعلق بالمعلومات المتنامية حول دور الأنزيمات المعدنية المحللة للبروتين و مثبطاتها في الانسجة في التسبب في مرض التصلب العصبي المتعدد، فانناناقش هنا دورالأنواع المختلفة من الأنزيمات المعدنية المحللة للبروتين ، بما في ذلك MMP-2، -3، -7، -9، -25، في تطور مرض التصلب العصبي المتعدد وعلاجه.

مفتاح الكلمات: مرض التصلب العصبي المتعدد؛ حاجز الدم في الدماغ؛ الأنزيمات المعدنية المحللة للبروتين؛ التهاب؛ الجهاز العصبي المركزي.

Multiple sclerosis (MS) is the most common prototypic inflammatory disorder, characterised by inflammation, oligodendrocyte depletion, reactive astrogliosis and demyelination in the brain, optic nerve and spinal cord.[1] MS plaque is the major pathological hallmark of MS. It is a unique feature of central nervous system (CNS) demyelination, which is characterised by oligodendrocyte destruction along with the loss of myelin, axonal damage and loss, and glial scar formation.[2]

MS usually occurs in young adults and is more common in women than men, with about 300,000 patients suffering from MS in North America alone. Literature reviews robustly imply an increased prevalence of MS in recent times.[3]

MS patients show a variety of clinical symptoms, including visual difficulties, muscle weakness, sensory damage and difficulties with speech and coordination. Briefly, there are four types of MS, with each having a mild, moderate or severe course.[4] Most patients (~85%) initially experience relapsing-

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remitting MS (RRMS); the majority is predisposed to the establishment of secondary-progressive MS (SPMS) which is characterised by an initial RRMS disease course followed by a progression with or without occasional relapses, minor remissions, and plateaus. Nearly 10% of patients display primary-progressive MS (PPMS), which indicates the disease progression from onset with occasional plateaus and temporary minor improvements. Finally, the least common form of the disease is progressive-relapsing MS (PRMS), which occurs in ~5% of MS patients and is characterised by the progressive disease from onset, with clear acute relapses, with or without full recovery.[5,6]

Although the precise cause of MS is unknown, the aetiology of MS seems to be linked to a variety of genetic and environmental factors. Current opinion suggests that the activation of autoreactive, adaptive and innate immune responses which target neural antigens leads to MS.[2] The self-reactive T cells are the main factors in the immunopathogenesis of MS. There is consistent evidence showing the central role of T helper 17 cell (Th17)-producing interleukin 17 and interferon γ (IFN-γ)-secreting type 1 T helper (Th1) cells in the disease's development, in part through the secretion of inflammatory mediators and the activation of microglial cells.[7]

Although the microglia is involved in the phagocytosis of myelin debris and apoptotic cells during demyelination, the activation of the microglia and the release of inflammatory mediators has been suggested as a possible mechanism by which innate immunity enhances the demyelination process in MS.[2,8] Therefore, microglial cells, which constitute about 10% of the CNS, are one of the first cells that cause neuronal damage and play an important role in neuroinflammatory processes.[8]

In MS, neural factors generated by acute stress, including myelin basic protein (MBP), substance P and corticotropin-releasing hormones, can activate brain mast cells to release inflammatory mediators and stimulate the autoreactive T cells.[9]

Astrocytes are involved in nearly all immunopathological processes in the brain. It has been reported that astrocytes could release factors which regulate the oligodendrocyte progenitor differentiation and myelin formation.[1,10] Oligodendrocytes enhance the inflammatory cytokine production in T cells as well as chemokines that may recruit additional peripheral inflammatory leukocytes.[10] The localisation of astrocytes in MS is similar to mast cells in the perivascular area.[11]

Two types of phagocytes (including the microglia and inflammatory macrophages) in MS and its animal model, experimental autoimmune encephalomyelitis (EAE), are derived from proliferating resident precursors and the recruitment of blood-borne progenitors, respectively.[12] These phagocytes and astrocytes can recognise pathogen-associated molecular patterns (PAMPs) via Toll-like receptors (TLRs) and generate pro-inflammatory signals that trigger adaptive immune responses. Moreover, these cells enhance the autoimmune responses through the secretion of effector molecules such as nitric oxide (NO), matrix metalloproteinases (MMPs) and calpain-1 that may also play a role in the initiation of myelin and axon damage.[13,14] The recruited inflammatory cells are mainly composed of cluster of differentiation 4 ($CD4^+$) T cells, B cells, monocyte/macrophages, neutrophils and dendritic cells.[15] However, these non-resident cells have to penetrate the blood-brain barrier (BBB) to reach the immunologically-privileged CNS.[16] The BBB is a dynamic interface that separates the brain from the circulatory system and protects the CNS from potentially harmful chemicals. Therefore, the BBB restricts the exchange of humoral factors and cells between the blood and brain, thus playing a crucial role in maintaining cerebral homeostasis. Disruption of the BBB is considered an initial key step of the disease process in MS.[17]

The breakdown of the BBB usually lasts for about a month and then resolves, leaving a site of damage that can be investigated by conventional magnetic resonance imaging (MRI).[18] In MS, the BBB may be impaired by MMPs attacking the basal *lamina* macromolecules before the formation of demyelinating *foci* or T-cell infiltration around the small vessels; however, once the BBB is disrupted, the massive infiltration of T cells, the augmented expression of adhesion molecules on the endothelial cell surface and the leakage of the inflammatory cytokines and antibodies aggravate the MS lesions.[17] In the brains of MS patients, mast cells are placed on the perivascular area and secrete numerous vasoactive molecules and pro-inflammatory mediators that can contribute to the BBB disruption.[9]

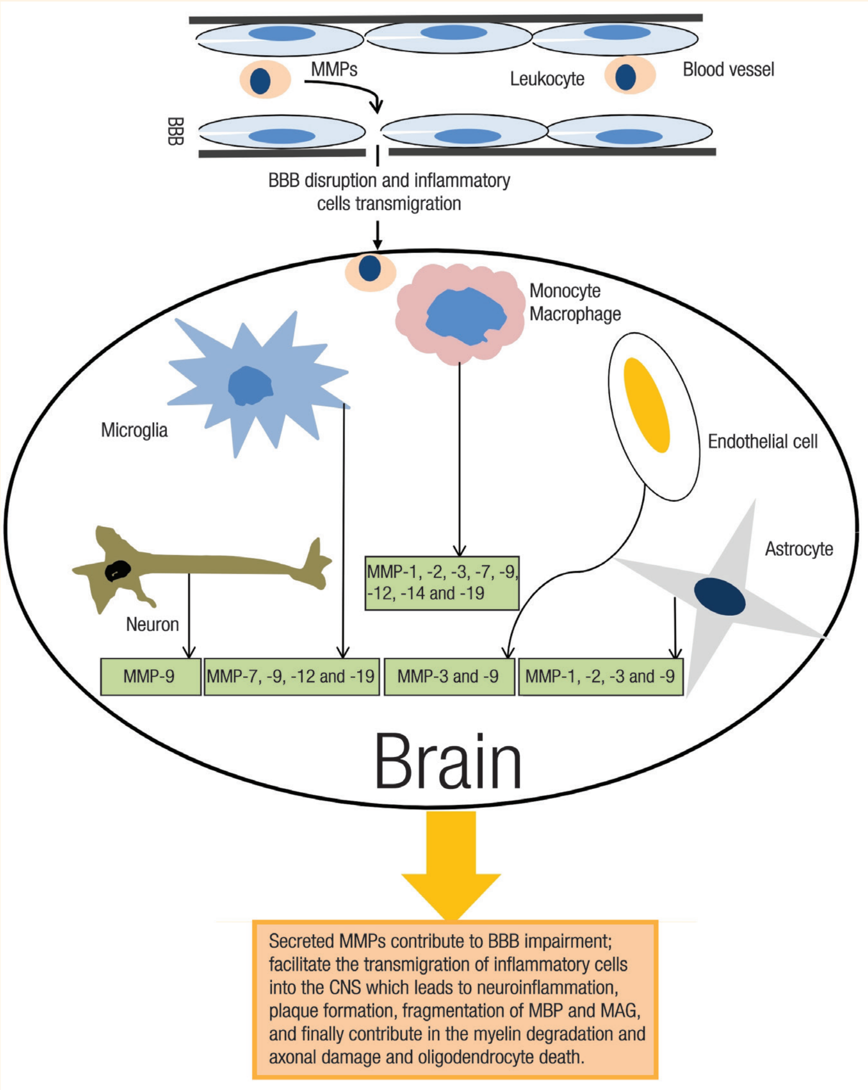

Figure 1: In multiple sclerosis, matrix metalloproteinases (MMPs) are expressed in the central nervous system (CNS) by various cell types, including vascular endothelial cells, neuron, reactive astrocytes and the microglia, and accumulated inflammatory cells. In the CNS, the high numbers of MMPs lead to the perpetuation of neuroinflammation, which contributes to myelin degradation and axonal damage.
BBB = blood-brain barrier; MBP = myelin basic protein; MAG = myelin-associated glycoprotein.

The MMPs or matrixins represent a large family of zinc-dependent proteolytic enzymes that are known for their capacity to degrade extracellular matrix (ECM) components.[19] MMPs are a family of proteases, classified into subfamilies based on their substrate preferences. Currently, there are 23 known MMPs, including: gelatinases (MMP-2 and −9); collagenases (MMP-1, -8, -13 and -18); stromelysins (MMP-3, -10 and -11); matrilysins (MMP-7 and -26); membrane type (MT) MMPs (including MMP-14, -15, -16, -17, -24 and -25), and a group of unnamed members (MMP-11, -12, -19, -20, -21, -23a, -23b, -27 and -28). All MMPs are secreted as proenzymes and require extracellular activation.[20–23] Recent findings indicate that MMPs are involved in different physiological and pathological processes, such as placental development, morphogenesis, reproduction, wound repair, inflammation, angiogenesis, neurological disorders, and cancer cell invasion and metastasis.[24]

MMPs affect a variety of extracellular proteins in the CNS, including cytokines, chemokines, antimicrobial peptides and immune regulatory proteins. Using quantitative reverse transcriptase polymerase chain reaction (RT-PCR), Bar-Or *et al.* systematically evaluated the expression of 23 MMP members in subsets of leukocytes isolated from the blood of normal populations. They found a specific pattern of MMP expression in different cellular populations: MMP-11, -26 and -27 were

Table 1: Some important matrix metalloproteinases in the central nervous system and their relationship with the pathogenesis of multiple sclerosis

MMP	MW in kDa		Cell source	Functions	TIMPs
	Pro-enzyme	Active form			
MMP-1	57 and 52	42	Astrocytes, monocytes, macrophages and microglia	•Unknown •Activator for MMP-2 and -9 •Breaks down the interstitial collagens (types I, II and III)	TIMP-1
MMP-2	72	63	Astrocytes, microglia, monocytes, macrophages and endothelial cells	•BBB disruption, which facilitates immune cell transmigration into the CNS •Fragmentation of the MBP •Demyelination •Axonal injury •Activator for MMP-1 and -9	TIMP-2 and TIMP-4
MMP-3	57	54	Astrocytes, monocytes, macrophages, endothelial cells and microglia	•BBB disruption •Activates microglia or 'white matter' brain cells •Activator for MMP-1, -7 and -9 •Normal functioning as neuronal migration •Neurite outgrowth and CNS development •Stem cell migration •Neuroprotection •Remyelination in the injured CNS	TIMP-1 and TIMP-3
MMP-7	29	20	Microglia, monocytes and macrophages	•Cleaves NR1 (an obligate sub-unit of the NMDA receptor) •Activator for MMP-1, -2 and -9	TIMP-1
MMP-9	92	67	Astrocytes, microglia, monocytes, macrophages, endothelial cells, neurons, neutrophils and CCR2+ CCR5+ T cells	•Increases the permeability of the BBB •Facilitates the infiltration of leukocytes into the CNS •Myelin sheath degradation •Neuronal damage	TIMP-1
MMP-12	~54	48	Macrophages, microglia and astrocytes	•Unknown •Potentially partakes in myelinogenesis	TIMP-1
MMP-14	66	~54	Monocytes	•Activator for MMP-2 •Receptor for TIMP-2 and/or the pro-MMP-2/TIMP-2 complex	TIMP-2 and TIMP-4

MMP = matrix metalloproteinase; MW = molecular weight; kDa = kilodaltons; TIMP = tissue inhibitors of metalloproteinases; BBB = blood-brain barrier; CNS = central nervous system; MBP = myelin basic protein; NMDA = N-methyl-d-aspartate.

plentiful in B cells, while MMP-15, -16, -24 and -28 showed up more often in T cells. The majority of MMP members are reported in monocytes: MMP-1, -3, -9, -10, -14, -19 and -25. In addition, MMP-2 and -17 were mainly represented in monocytes, although B lymphocytes had significant amounts of these MMPs.[25,26]

Growing evidence implies that the normal, mature CNS contains low or non-detectable levels of most MMPs; the principal cells that express these MMPs are perivascular and parenchymal microglia. On the other hand, studies on the serum, cerebrospinal fluid (CSF) and brain tissue of MS patients have shown an increase of MMP-1, -2, -3, -7, -9, -12 and -14 activity.[27–29] Some data suggest that microglial-derived MMPs may mediate the turnover of the CNS's ECM under normal conditions in microglial nodules, but in many neuroinflammatory conditions, such as encephalitis, meningitis, brain tumours, cerebral ischaemia, Guillain-Barré syndrome and MS, these enzymes are significantly upregulated.[30–32]

In neuroinflammatory conditions, MMPs are expressed in the CNS by a variety of cell types, including vascular endothelial cells, meninges, resident cells such as neuronal cells, astrocytes, microglial cells and accumulated inflammatory cells [Figure 1]. It has been shown that astrocytes can release MMP-1, -2, -3 and -9, whereas the microglia secretes MMP-7, -9, -12 and -19.[33,34] Most microvessel endothelial cells in the CNS express MMP-3 and -9 but not -1 or -2. Also, the majority of macrophages in active MS and necrotic lesions are positive for MMP-l, -2, -3, -7, -9 and -19, whereas chronic MS lesions have fewer MMP-positive macrophages.[32,35] Neurons may also release MMPs. It is reported that in normal adult rat brains, MMP-9 (but not -2) is highly expressed by neurons and localised in neuronal cell bodies and dendrites.[36]

In the damaged sites of the CNS, there are complex and dynamic regulations of MMP expression by different cell types.[32] The imbalance between MMP activity and the inhibitory action of tissue inhibitors of metalloproteinases (TIMPs) are implicated in MS development [Table 1], as one of the MMP roles may be to facilitate the transmigration of circulating leukocytes into the CNS.[25] Therefore, it is possible that the MMPs attack the basal lamina macromolecules that line the blood vessels, disrupting the BBB's integrity.[31]

Moreover, MMPs can be involved in the fragmentation of MBP and myelin-associated glycoprotein (MAG), and injury to the myelin. In the CNS, the MBP gene is expressed in the oligodendrocytes and is named classic-MBP; in the immune cells it is named golli-MBP.[37] Therefore, MMP-mediated proteolysis of the MBP isoforms is a source of immunogenic peptides in autoimmune MS.[38] Consequently, through the remodelling of the blood vessels, MMPs cause hyalinosis and gliosis, and they attack the myelin, disrupting the myelin sheath and axons. Excessive proteolytic activity is detected in the CSF and blood of patients with acute MS. Moreover, MMPs are induced in immunological and non-immunological forms of demyelination.[31]

Regarding the high expression of many MMPs by monocytes which facilitate the transmigration of the leukocytes, Bar-Or *et al.* showed that monocytes migrate more rapidly through the BBB than T or B lymphocytes, *in vitro*.[25] Although data about the role of MMPs in monocyte trafficking are limited, Lucchinetti *et al.*[18] suggested that the frequency of macrophages/microglia in MS is approximately 10 times higher compared to lymphocytes. Also, in active MS lesions, macrophages were found to be positive for MMP-2, -7, -9, -12 and -19.[39,40] Since monocytes constitute a major cell population in acute MS lesions, it may be possible that MMP secretion facilitates their entrance into the CNS. Findings on MMP secretion by blood monocytes can be useful in improving our understanding of the immunopathogenesis of MS.[41]

To summarise, it seems that monocytes are key contributors to the neuroinflammatory process in MS through a mechanism that involves the high expression of different MMPs, such as MMP-1, -2, -3, -7, -9, -14 and decreased expression of TIMP-1 and TIMP-2.[25,42]

Matrix Metalloproteinase-2

Matrix metallopeptidase-2 (also known as gelatinase A and a 72 kilodalton [kDa] type II collagenase) plays an important role in inflammation and immunity in addition to its physiological function in degrading and remodelling the ECM. The expression of MMP-2 is upregulated in many human diseases as well as in animal models of inflammatory and immune diseases. Bar-Or *et al.*, following analyses of all MMPs in leukocytes, implicated monocytes as major inflammatory cells in MS. They found higher levels of monocyte-expressed MMP-2 and -14 in MS patients compared to normal subjects.[25] Moreover, another report suggested that MMP-2 can be expressed not only in monocytes but also in the astrocytes, microglia and macrophages.[43]

Although the correlation between the frequency of macrophages and reactive glial cells with axon injuries in the acute plaques is well-established, little is known about the precise role of MMP-2 in the immunopathogenesis of MS.[44,45] Anthony *et al.*[46] and Maeda *et al.*[32] showed that MMP-2 was upregulated not only in plaques but also in the seemingly normal white matter adjacent to the acute plaques. In these acute plaques, the myelin was degraded and engulfed by the reactive glial cells and macrophages, leading to demyelination.[35] In addition, Newman *et al.* showed that a microinjection of MMP-2 into rat subcortical white matter led to axonal injury.[47]

It seems that MMP-2 plays a key role in the BBB disruption, which facilitates immune cell transmigration into the CNS and the development of MS.[38,43,48] Therefore, the downregulation of MMP-2 may inhibit the BBB disruption and migration of the inflammatory cells to the CNS. Although little is known about the involvement of MMP-2 and its tissue inhibitor TIMP-2 in MS, it has been shown that TIMP-2 is elevated in the monocytes of MS patients. However, it should be noted that there are no data regarding the functionality of TIMP-2 in MS patients.

TIMP-2 is an inhibitor of activated MMPs; it inhibits the cell surface activation of pro-MMP-2 by MMP-14.[25,49] MMP-14 acts as a receptor for TIMP-2 (but not TIMP-1) and the pro-MMP-2/TIMP-2 complex, thereby facilitating the activation of pro-MMP-2. In addition, the serum MMP-2:TIMP-2 ratio may represent a useful indicator for monitoring the MS patient during a recovery phase.[45] Benesová *et al.* observed in 2009 that there was a significant elevation in MMP-2 serum levels and the MMP-2:TIMP-2 ratio in the PPMS and SPMS groups compared to the RRMS group. This increase was also associated with the level of disability in the patient and the severity of the disease.[43] Thus, it seems that MMP-2, -14 and TIMP-2 can be considered interesting targets for potential therapeutic interventions to inhibit the entry of monocytes into the CNS, and to alleviate injuries of the CNS in MS patients.[25]

Matrix Metalloproteinase-3

Also known as stromelysin−1, MMP-3 is an enzyme involved in ECM remodelling. MMP-3 is an activator of other MMPs, including MMP-1, -7 and -9.[50] It has been demonstrated that MMP-3 contributes to different pathological conditions, such as rheumatoid arthritis (RA), asthma, cancer and neurological disorders, including MS, Parkinson's disease (PD) and Alzheimer's disease (AD).[51] In MS, the increased circulatory level of MMP-3 correlates with disease activity in RRMS. This may contribute to the breakdown of the BBB at the time of relapse.[52] Moreover, the release of MMP-3 into the ECM activates microglial or brain cells in the white matter.[53] Some evidence strongly suggests that the distinctive signal of neuronal apoptosis is the release of the active form of MMP-3 that activates the microglia and subsequently exacerbates neuronal degeneration.[54] It has been shown consistently that the inhibition of MMP-3 leads to the suppression of inducible nitric oxide synthase, (iNOS), proinflammatory cytokines and the inflammatory transcription factors nuclear factor kappa B (NF-κB), activator protein 1 (AP1) and mitogen-activated protein kinases (MAPKs) in the microglia.[53]

Interestingly, it has been reported that MMP-3 can be involved in normal CNS-remodelling and in the remyelination process, as shown by the strict spatiotemporal MMP-3 upregulation in the injured CNS, which may contribute to stem cell migration, neuroprotection and remyelination in the injured sites.[51] An involvement of MMP-3 in remyelination during the regenerative period after a CNS injury was demonstrated for the first time in the murine cuprizone-induced demyelination model. It was reported that not only during the early demyelination stage, but also during the stage of remyelination, MMP-3 was highly expressed in astrocytes of the *corpus callosum*. Although the exact mechanism is unknown, MMP-3 might be involved in remyelination through enhancing insulin growth factor (IGF) secretion, which is essential for the proliferation and differentiation of myelin-forming cells.[55–57] In addition, MMP-3 might stimulate remyelination by removing and cleaving the myelin debris, which inhibits the oligodendrocyte precursor cell differentiation.[51,58]

Matrix Metalloproteinase-7

Also known as matrilysin, MMP-7 is increased in the serum, CSF and brain tissues of MS patients.[27] It has been shown that the messenger ribonucleic acid (mRNA) levels of MMP-7 and -9 are elevated in all stages of lesion formation in MS patients.[59] Moreover, MMP-7 expression was found to be upregulated in microglia/macrophages within acute MS lesions. In chronic MS lesions, the expression of MMP-7 was confined to the macrophages within the perivascular cuffs, whereas only a low level of MMP-7 expression was detected in normal brain tissue.[46] In a study by Cossins *et al.*, MMP-7 immunoreactivity was weakly detected in microglial-like cells in normal brain tissue sections, and was very strongly detected in the parenchymal macrophages in active demyelinating MS lesions. MMP-7 immunoreactivity was not detected in macrophages in the spleen or tonsils, indicating that it is specifically induced in infiltrating macrophages in active demyelinating MS lesions.[35] Elevated levels of MMP-7 and -9 have also been detected in cases of EAE. In one study of adoptive transfer EAE, mRNA for MMP-7 was increased, with maximum levels at the peak of the disease.[60]

Matrix Metalloproteinase-9

Also known as gelatinase B and type IV collagenase, MMP-9 is secreted from neutrophils, macrophages and a number of transformed cells in zymogen form.[50] Upon activation, MMP-9 acts on many inflammatory processes and is involved in the progression of cardiovascular disease, RA, chronic obstructive pulmonary disease and MS.[24] MMP-9 is also important in cytokine and protease modulation; it degrades the serine protease inhibitor α1-antitrypsin, which may lead to the destruction of the lungs.[24] Different studies have indicated the increased expression of MMP-9 in MS.[61] In one study, zymography methods showed that MMP-9 levels were high in the CSF of MS patients and in patients with infections of the CNS and other inflammatory diseases.[28] In patients with MS, high MMP-9 levels were associated with the immunoglobulin G (IgG) index. Hence, MMP-9 is an unspecific laboratory marker of inflammation. For instance, the expression of MMP-9 as well as other MMPs (for instance, MMP-2, -7 and -12) is increased in MS brain sections as measured by immunohistochemical analysis.[32,35,46,62] In addition, high MMP-9 levels in serum or leucocytes were detected by immunochemical methods.[63] Long-term follow-up studies have enabled patterns to be recorded with MRI; additionally, enhanced MMP-9 steady-state mRNA levels were measured in MS.[59]

In the CNS, MMP-9 can be expressed in the vascular endothelial cells, meninges, microglia, astrocytes and accumulated inflammatory cells, as well as in inflamed MS plaques and in the seemingly normal white matter or cerebral-infarction tissue.[64] It has been also observed that MMP-9 was strongly expressed by the neutrophils in patients up to one week after an infarction—at that point a large number of macrophages were expressing MMP-9.[46] In Wistar rat EAE models, an elevated expression of MMP-9 may play a role in some pathological changes, similar to MMP-2.[65] For example, it increases the permeability of the BBB, facilitates the infiltration of leukocytes into the CNS and causes myelin sheath degradation and neuronal damage.[24] Also, in MMP-9-deficient mice, the chemotaxis of neutrophils to intradermally-injected granulocyte chemotactic protein-2 was decreased.[66] It was found that young MMP-9 knockout mice are partially resistant to the development of EAE.[61,67] However, when both gelatinases (MMP-2 and -9) were genetically knocked out, a complete resistance against myelin oligodendrocyte glycoprotein peptide-induced EAE was observed.[68] The latter study is important as it shows that many proteinases act in these cascades or networks.[69]

Indeed, investigations of MMP-2 indicate that it is able to activate MMP-9 *in vitro*.[70] Both enzymes thus reinforce each other and they also share a number of substrates, including denatured collagen or gelatin.[71] In addition, decreased serum MMP-8 and -9 levels were correlated with a decreased number of contrast-enhanced T2-weighted MRI lesions in MS patients.[72] In the same study, MS patients treated with interferon β 1b (IFN-β1b) showed a reduction in serum MMP-8 and -9 in parallel with the disease stabilisation. The authors concluded that the serial measurement of MMPs and other inflammatory mediators may serve as sensitive markers for measuring therapeutic response to IFN-β1b during the first year of treatment.

As mentioned earlier, it is suggested that MMP-9 has a role in MS by mediating T-cell migration through the BBB. During a relapse course, the CCR2+CCR5+ T cells are abundant in the CSF of MS patients. These T cells have the potent ability to produce osteopontin and MMP-9, both of which have an important role in the MS pathology.[73] Also, in MS patients in the relapse phase, these subtypes of T cells are reactive to MBP, as this ability can be evaluated by IFN-γ production. Other findings suggested that the CCR6- subtype (but not the CCR6+ subtype within CCR2+CCR5+ T cells) is very abundant in the CSF during a MS relapse and can produce higher levels of MMP-9 and IFN-γ.[73,74]

As TIMP-1 is a tissue inhibitor of MMP-9, a considerable elevation in the MMP-9:TIMP-1 ratio and in MMP-9 serum levels was observed in MS patients in the RRMS and SPMS groups.[43] Fainardi *et al.* have suggested that a shift in the MMP-9:TIMP-1 ratio towards MMP-9 proteolytic activity can be the consequence of MS immune downregulation.[75] Moreover, using immunohistochemistry, TIMP-1 was found to be upregulated in chronic plaques.[62]

The concentrations of these metalloproteinases inhibitors in the CSF and plasma were low in patients with MS, whereas during treatment with interferon β (IFN-β), their concentrations increased.[48,76,77] Moreover, the levels of active MMP-9 in the serum and CSF of MS patients may represent indicators for the monitoring of disease activity. In particular, the serum active MMP-9:TIMP-1 ratio seems to be a very suitable and easily measurable biomarker of the continuous inflammation in MS.[75] Furthermore, in a MS clinical trial, erythropoietin induced the expression of TIMP-1 in the endothelial cells, which helped to maintain the BBB integrity. The protective effects of erythropoietin were associated with an increase in the number of astrocytes expressing TIMP-1 in the brain and spinal cord in cases of EAE.[78] It has also been demonstrated that there is a significant association between the gene polymorphism of MMP-9 and MS susceptibility and severity.[74,79,80]

Matrix Metalloproteinase-12

MMP–12 is a macrophage-specific MMP with a broad substrate specificity and is expressed in MS lesions at various stages.[39] Moreover, the transient expression of MMP-12 has also been reported in the microglial cells and astrocytes of MS patients.[39] It has also been demonstrated that in active demyelinating lesions, phagocytic macrophages express MMP-12. Moreover, in inactive lesions and chronic active demyelinating lesions, lower ratios of phagocytic cells were MMP-12-positive.[39]

Out of all of the MMPs that could be measured in the spinal cord tissue at the peak of the disease, MMP-12 is significantly upregulated.[81] In contrast to previous data, Weaver *et al*. demonstrated that an elevation in MMP-12 expression was related to protection against EAE. In this study, MMP-12-null mice had significantly severe EAE when compared to the control mice. In addition, *in vitro* findings showed that the lymph node and spleen cells of the MMP-12-null mice had a significantly higher Th1 to type 2 T helper cell (Th2) cytokine proportion compared with similar cells in the control mice. Evaluations of the main transcription factors of T cell polarisation also showed that MMP-12-null cells had reduced *GATA-3* and increased the T-bet expression, a situation that is in favor of Th1 bias.[82]

Matrix Metalloproteinase-25

MMP-25 is a member of the MT MMPs, which is expressed almost exclusively in peripheral blood leukocytes and in anaplastic astrocytomas and glioblastomas, but not in meningiomas.[83]

It was previously shown that the gene expression of the majority of MMPs was upregulated in the spinal cords of Swiss/Jackson laboratory (SJL) mice with severe EAE. Here, four of the six MT MMPs (MMP-15, -16, -17 and -24) were downregulated and the two remaining MT MMPs (MMP-14 and -25) were upregulated in whole tissue.[84] MMP-25 proteolysis can inactivate a MS regulator known as αB-crystallin. Therefore, MMP-25 functions and their restricted cell/tissue expression patterns play an important role in demyelinating diseases such as MS.[37,85] MMP-25 cleaves golli-MBP isoforms in the immune cells, therefore stimulating specific clones of the autoreactive T cells.[37] It is possible that the transmission of these autoreactive T cells, through the disrupted BBB, and the appearance of MBP in the neuronal cells can lead to inflammation and self-reactive responses. The activation of different elevated MMPs and the dysregulation of TIMPs enhances inflammation, autoreactive responses, MBP cleavage and demyelination and, consequently, the development of MS.[37,85]

Matrix Metalloproteinase-based Therapeutic Approaches to Multiple Sclerosis

In view of the above, it seems that the direct inhibition of proteolysis or the induction of a balance between the endogenous proteinases and their natural inhibitors could be possible approaches to MS treatment. However, further proof is needed to demonstrate the pathogenic role of extracellular proteolysis.[86] Generally, the role of MMP involvement in pathology is that of matrix degradation, so MMP inhibition may be of therapeutic benefit.

The first disease targeted with MMPs was RA but the range of potential applications has broadened to include the treatment of cancer.[87] However, the multiple roles of MMPs in the CNS make them a therapeutic target for the treatment of neurological disorders. Further complications are that the various MMP members induce or compensate for one another, and most MMP inhibitors are non-selective for particular MMP members. There is also the likelihood that, while MMP inhibitors may protect against certain of the detrimental effects of some MMPs, they will also block the useful actions of these MMPs, thus slowing disease recovery.[88] The first synthetic MMP inhibitor was developed in the early 1980s as a pseudopeptide derivative based on the structure of the collagen molecule at the site of the initial cleavage by interstitial collagenase.[89] A number of synthetic inhibitors of MMP activity have been developed and have been shown to decrease the incidence and severity of EAE. These inhibitors include GM6001,[90,91] Ro-31-9790,[92] BB-1101,[93] UK221 and D-penicillamine.[94]

A semi-synthetic tetracycline derivative, minocycline, also has a MMP inhibitory function.[95] In EAE mice models, it has been shown that minocycline decreases the expression and function of MMP-9 in T cells and attenuates the disease severity and neuropathology.[96] In the clinical trial of minocycline in RRMS, it significantly decreased gadolinium-enhanced MRI activity within the first two months of treatment; this effect was associated with the role of MMPs in reducing disruption of the BBB.[97] However, the decreased function of MMP-9 during treatment would also support this mechanism.[98] The beneficial effect of IFN-β, which is the current drug in MS therapy, is also associated with the reduced production of MMP-9 by T cells, the decreased ability of T cells to transmigrate a basement membrane barrier and the reduced gadolinium-enhanced MRI activity.[99–101] These results imply that IFN-β exerts alleviating effects in part through the suppression of the MMPs' functions. Clinical studies support the possibility that IFN-β regulates MMP and TIMP-1 levels in patients with MS.[48,102,103] However, there is no justified data regarding the anti-MMP effects of glatiramer acetate, another current drug in MS therapy.[101] Interestingly, it has been shown that MMP-9 could disrupt IFN-β and that this was inhibited by minocycline through antagonism of the MMP-9 functioning.[104] Thus, it seems that the combination of IFN-β and minocycline leads to promising results in MS therapy.[105] It has been shown consistently that the combination of IFN-β and minocycline in the treatment of EAE leads to a greater alleviation of the EAE severity score and histological outcomes, compared to either medication alone.[106] However, because several MMPs are elaborated, it seems that a non-selective MMP inhibitor might be more efficient than those that target only specific MMPs or subclasses of the MMP family. It is suggested that current MMP inhibitors should only be used in short treatment courses, so that they do not inhibit the MMP-mediated repair processes that subsequently ensue after an injury. Thus, careful selection of the time frames and doses of MMP inhibitors is recommended and patients can benefit from their utilisation in neurological conditions.[88]

Another strategy to treat MS is targeting the upstream molecules which regulate the expression of MMPs and TIMPs. For instance, the ECM metalloproteinase inducer EMMPRIN (also known as CD147) induces MMPs and this constitutes a new therapeutic target.[107] It is reported that EMMPRIN is upregulated on the peripheral leukocytes before the onset of EAE. In brain sections of EAE cases, EMMPRIN expression was localised with MMP-9 protein and activity. The increased EMMPRIN levels were also characteristic of brain samples from MS subjects, particularly in plaque-containing areas. As EMMPRIN regulates leukocyte trafficking by increasing MMP activity, it may be a novel therapeutic target in the treatment of MS.[107] Additionally, in keeping with this concept to interfere with the proteinase/inhibitor balances in MS, statins have also been studied as they have been shown to reduce the expression level of MMP-9.[108,109]

The effect of intravenous IgG in MS is disputed; however, it has been reported in animal models that intravenous IgG might be effective as a prophylaxis, preventing disease development, and exerting its function by suppressing the activation of MMPs-2 and -9 by blocking MMP activities at the interface between the blood stream and CNS.[110]

Another interesting strategy is the control of MMP regulator cytokines and chemokines; by using neutralising antibodies or pharmacological antagonists against cytokines, chemokines and/or their receptors may bring considerable change in the therapeutic landscape for MS.[86,111]

Conclusion

In MS disease, increased MMP activity and reduced TIMP levels contribute to a loss of the BBB integrity and infiltration of inflammatory immune cells to the CNS. Therefore, MMPs and their TIMPs play a key role in the immunopathogenesis of MS, and are suggested as potential targets to treatments. Hence, more research in MMPs/TIMPs domain and their roles in immunopathogenesis of disease might be recommended as a therapeutic toll for controlling MS.

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