Expression and regulation of matrix metalloproteinase-12 in experimental autoimmune encephalomyelitis and by bone marrow derived macrophages in vitro

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Abstract

Several matrix metalloproteinase (MMP) members contribute to pathology in multiple sclerosis and experimental autoimmune encephalomyelitis (EAE). As the role of MMP-12 in EAE has been understudied, we examined its expression in EAE and in vitro. MMP-12 transcripts increased with EAE, and protein was localized to a subset of macrophages/microglia. The temporal expression of MMP-12 largely corresponded to that of cytokines, and we demonstrate that IL-1β and TNF-α promoted MMP-12 expression in cultured macrophages. We postulate that cytokine — MMP-12 interactions are important in the disease process of EAE.

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1. Introduction

Multiple sclerosis (MS) is an autoimmune neurological disease primarily affecting young adults. Experimental autoimmune encephalomyelitis (EAE), a commonly used animal model of MS, has provided many clues to the general understanding of MS, but the etiology of the disease remains uncertain. Whatever the initial trigger(s) of MS, a major feature of the disease is the infiltration of activated leukocytes through the blood-brain barrier (BBB) into the central nervous system (CNS) to produce neuropathology (Sospedra and Martin, 2005; Frohman et al., 2006).

Matrix metalloproteinases (MMPs) are a subgroup of the metzincin proteases (Parks et al., 2004). It is now well established that infiltrating leukocytes secrete MMPs and that these MMPs help degrade the basement membrane surrounding cerebral vascular vessels (Cossins et al., 1997; Kouwenhoven et al., 2002; Agrawal et al., 2006; Toft-Hansen et al., 2006). Besides participating in BBB breakdown, MMPs can also produce demyelination and axonal injury, promote neuroinflammation via the proteolysis of zymogens, and participate in mediating cell death (Kieseier et al., 1999; Yong et al., 2001; Opdenakker et al., 2003). Thus, inhibiting the activity of MMPs has been a goal of providing for novel therapeutics in MS (Kieseier et al., 1999; Rosenberg, 2001; Hu et al., 2007; Yong et al., 2007).

The MMPs constitute a family of 25 members, and several of these are found elevated in serum, leukocytes, cerebrospinal fluid and the CNS tissues of patients with MS, or in animals afflicted with EAE. These include MMP-2, -3, -8, -9, -10, -11, -12, -13, -14 and -25 (Anthony et al., 1997; Cossins et al., 1997; Pagenstecher et al., 1998; Kouwenhoven et al., 2001; Lindberg et al., 2001; Weaver et al., 2005). The contribution of MMPs to disease evolution and progression has been supported by EAE studies where a variety of inhibitors of metalloproteinase activity, including GM6001 (Gijbels et al., 1994), BB1101...
(Chandler et al., 1997; Liedtke et al., 1998), Ro31-9790 (Hewson et al., 1995) and minocycline (Brundula et al., 2002) can either prevent disease manifestation or suppress ongoing disease activity. The MS medication, interferon-β, reduces MMP activity and levels in leukocytes and in the serum of patients (Leppert et al., 1996; Stuve et al., 1996; Galboiz et al., 2001; Avolio et al., 2005).

It is uncertain if all the MMP members found elevated in MS or EAE contribute to disease activity. Conversely, it is also possible that an MMP member is elevated to help suppress disease activity (Parks et al., 2004; Hu et al., 2007). In this regard, we have previously determined that MMP-12 null mice have a more severe EAE course than wildtype controls (Weaver et al., 2005). Thus, while most MMP members may serve detrimental roles in MS and EAE, as supported by the data that metalloprotease inhibitors reduce EAE disease activity, there is at least one MMP member, MMP-12, which may function to dampen inflammation. Since the profile of MMP-12 expression during EAE has not been described previously, it would be important to address its temporal expression and cellular sources during EAE, and to determine how its levels are regulated during neuroinflammation. In this manuscript, we describe the expression and regulation of MMP-12 in EAE and in tissue culture studies.

2. Materials and methods

2.1. Disease induction and EAE analysis

EAE was induced in female C57BL/6 mice (Charles River, Montreal, QC), aged 8–9 weeks, by injecting subcutaneously (s.c.) 50 μg MOG₃₅₋₅₅ in Complete Freund’s Adjuvant (CFA) (Fisher, Michigan USA). Intraperitoneal (i.p.) pertussis toxin (0.3 μg/200 μl, List Biological labs, Hornby, ON) was administered on days 0 and 2.

Animals were assessed daily using a 15-point disease score scale (Giuliani et al., 2005; Weaver et al., 2005) that replaced the more commonly used 5-point scale since the 15-point scale differentiates individual limb disability, rather than grouping both fore- or hind-limbs together, thus allowing for better control for disease variability amongst mice; also, fewer mice were killed to perform these experiments as outlined in our animal care directives.

2.2. RNA extraction from thoracic spinal cords of EAE C57BL/6 mice

Animals were killed via an overdose of ketamine/xylazine (Bimed-MTC animal Health Inc., Cambridge, ON) and bled by an incision to the atrium of the heart (N of 5 per time point). Immediately after bleeding the thoracic spinal cord was removed and placed into 1 ml of TRIZol® (GibcoBRL), homogenized via syringe, flushed frozen in liquid nitrogen and stored at −80 °C until further RNA processing.

Total RNA was isolated from thoracic spinal cords of mice using a standard extraction protocol for TRIZol® as described by the manufacturer (Invitrogen, Missisauga, ON). Only samples with an A₂₆₀/A₂₈₀ ratio > 1.60 were used for subsequent analysis.

2.3. Generation of cDNA from RNA isolated from thoracic spinal cords of EAE C57BL/6 mice

A total of 1 μg of RNA was used from each sample evaluated and cDNA was made. RNA was treated with RQ1 RNase-Free DNase at 37 °C for 45 min and 65 °C for 10 min. Following DNase treatment the cDNA master mix (5× First strand buffer, 0.1 M DTT, 5 mM dNTPs, Random primers (dN)₆, 40 U/ml RNAsin/RNase out and 200 U/ml Superscript reverse transcriptase) was added to each sample. Samples were incubated using the following protocol: 37 °C for 1 1/2 h, 70 °C for 5 min and then 4 °C. cDNA was diluted to a final volume of 100 μl and stored at −20 °C until further use.

2.4. Real-time PCR of cDNA from thoracic spinal cords of EAE C57BL/6 mice

Real-time PCRs were done using SYBR green and relative fold expression values were calculated based on a housekeeping gene and using the comparative C_T method for relative quantization using 2^-ΔΔC_T. For each sample 5 μl of cDNA was added to 20 μl of the PCR master mix (40 mM Tris–HCl pH 8.4, 100 mM KCl, 6 mM MgCl₂, 400 μM dGTP, 400 μM dTTP, 400 μM dCTP, 400 μM dATP, 10% Glycerol, 0.1% Tween 20, SYBER Green 1 (1/50,000), Fluorescein (1/10,000), 5 μM primer mix, and Taq polymerase) and was incubated using the following conditions: 95 °C for 5 min, 45 cycles of
95 °C for 30 s, 55–60 °C for 30 s and 72 °C for 30 s, 72 °C for 5 min followed by a melt curve gradient. The primers are listed in Table 1. Since our experience is that levels of 18S remained unchanged across groups in tissue culture experiments, while GAPDH was constant across mouse spinal cords for in vivo experiments, these were used as housekeeping genes for normalization in tissue culture and spinal cord samples, respectively.

2.5. Histology and immunofluorescence of tissue from control and EAE mice

Animals were killed via an overdose of ketamine/xylazine and perfused with PBS followed by 4% paraformaldehyde (PFA) through the heart (N of 4 per group). Immediately after perfusion spinal cords were removed and placed in 4% PFA overnight followed by cryoprotection (10% sucrose followed by 30% sucrose), divided into thoracic and lumbar sacral section before being imbedded in tissue tek (Sakura Finetek, Torrance, CA) and flash frozen. Cords were cut on a cryostat at 10 μm thickness and placed on superfrost+ slides (Fisher, Michigan USA) before being processed for histology (hematoxylin–eosin and luxol fast blue) as previously described (Giuliani et al., 2005) and for immunofluorescence.

For immunofluorescence, slides containing cryosections were warmed up to RT before being incubated with 0.25% Triton-X in PBS for 30 min. Slides were then washed three times in PBS and blocked with 10% fetal calf serum, 10% goat serum, 2% horse serum and 1% Vector M.O.M. mouse IgG blocking reagent (Vector laboratories Inc., Burlingame, CA) in modified Eagle’s medium for 1 h. Slides were incubated with their primary antibody for 1 h at 37 °C. Primary antibodies and their dilutions were: rabbit anti-Iba-1 (Wako, Richmond, VA; 1/500) and mouse anti-F4/80 (AbD serotec, Hornby, ON; 1/500), both monocytoid cell markers; mouse anti-MBP (Covance, Berkeley, CA; 1/500), a myelin marker; goat anti-MMP-12 (W15) (Santa Cruz, Santa Cruz, CA; 1/250) and rabbit anti-MMP-12 (Biomol, Plymouth Meeting, PA; 1/500). Following a washing step, slides were then incubated with Alexa 488 Goat anti rabbit IgG antibody or Cy3 goat anti mouse IgG antibody (1/400) for 1 h at RT. Slides were washed, incubated with Hoechst (Sigma, Oakville, ON 500 ng/ml) and coverslipped. The slides were examined using a Leica microscope equipped with fluorescent filters using 40× and 100× objective. Images were captured using a Spot digital camera (Diagnostic instruments Inc. (Sterling Heights, MI) and a computer equipped with Image-pro plus software (Media cybernetics, Silver springs, Maryland).

2.6. Differentiation and Stimulation of bone marrow derived macrophages (BMDM) from C57BL/6 mice

To analyze MMP-12 expression by microglia or macrophages in culture, it would be desirable to use both subsets. Given the difficulty of obtaining large numbers of microglia for culture studies, we utilized BMDM as a model of macrophages infiltrating into the CNS. Animals were killed with an overdose of ketamine/xylazine and the tibia, femur and pelvis bones were removed and cleaned. Marrow was flushed from the bones using complete bone marrow growth medium (DMEM, 10% fetal bovine serum, 2% penicillin/streptomycin and 10% L929 conditioned medium from week 1 and week 2 cultures). Cells were spun at 1000 rpm for 10 min, resuspended in fresh growth medium, counted and then plated at a density of 3.5 × 10^5 cells/well in 24 well culture plates (VWR, Mississauga, ON). Cells were incubated at 37 °C at 10% CO2 for 5 days before feeding with fresh growth medium. On the 6th day, the growth medium was replaced and cultures were ready for use on the 7th day. Confluent cultures (>80%) were used for experiments in which the cells were stimulated with lipopolysaccharide (LPS, 1 μg/ml, Calbiochem, San Diego, CA), TNF-α (10 ng/ml, R and D, Burlington, ON), IL-1β (10 ng/ml, R and D, Burlington, ON), or IFN-γ (100 U/ml, Genzyme, Cambridge, MA) for 6 or 24 h. These concentrations represent optimal ones as determined by prior concentration response curves in preliminary experiments. Cultures were harvested for RNA and real-time PCR was performed as described above using primers outlined in Table 1. The housekeeping gene, 18S was used since it was determined experimentally to be the best housekeeping gene for these cultures; in contrast, GAPDH expression changed in these cultures with stimulation paradigm, which has been described by others (Messmer and Brune, 1996; Foss et al., 1998).

In other experiments, parallel cultures were fixed with 4% PFA for 10 min, washed with PBS containing 0.1% Triton, blocked for non-specific binding for 30 min, and then incubated with Iba-1 polyclonal antibody overnight at 4 °C. Cells were then washed in PBS three times before being incubated with a Alexa 488 Goat anti rabbit IgG antibody (1/400) for 2 h at room temperature, washed in PBS three times and coverslipped. The slides were examined using a Leica microscope equipped with fluorescent filters using 40× objective and image were captures as outlined above.

2.7. Casein zymography of lumbar sacral protein homogenates from EAE C57BL/6 mice

Protein was isolated from lumbar spinal cord using a previously described protocol (Szklarczyk et al., 2002). Protein
homogenates were measured for total protein content by the Bradford assay and 20 µg/sample was run on reducing 12% SDS-acrylamide gel containing 1 mg/ml alpha-casein (Worthington Biochemical Corporation, Lakewood, NJ). After separation the gel was incubated in rinse buffer (2.5% Triton-X-100, 50 mM Tris–HCl, pH 7.5 and 5 mM CaCl$_2$) for 6 h on a shaker at room temperature, in order to remove the SDS, thus allowing the proteins to renature within the gel. The gel was then placed in incubation buffer (50 mM Tris–HCl, pH 7.5, 5 mM CaCl$_2$, and 1.25 µM ZnCl$_2$) for 48 h at RT, which allows in gel proteolytic degradation of casein. Finally, the gel was stained with Coomassie blue for 4 h and destained (10% Acetic Acid: 30% Methanol: 60% deionized distilled water). Images of gels were captured by scanning. Zones of proteolytic activity were evident as clear bands against a dark blue background but for presentation purposes were converted to black bands on a grey background and then densitometry processing was achieved using Genetools 3.07 (SynGene, Frederick, MD, USA). We have normalized the loading of each sample by virtue of total protein content of the homogenates and represent the data as a ratio between control and EAE samples.

2.8. Statistical analysis

Statistical analysis was performed using Prism 5 (version 5.0) (GraphPad Software Inc., San Diego, CA). Statistical differences between groups with respect to gene expression were evaluated using repeated measures One-way ANOVA and Tukey’s Multiple Comparison Tests.

3. Results

3.1. Expression of MMP-12 and its possible inflammatory regulators in EAE

To evaluate the expression of MMP-12 in EAE, we first examined mRNA expression in animals killed at different time points after disease induction (Fig. 1A). MMP-12 transcript was undetectable in normal control spinal cord and was upregulated 5 days after MOG immunization and before symptoms manifested. MMP-12 transcript level continued to rise at onset of clinical signs and was maximal when animals showed signs of hind limb paresis with disease progression. MMP-12 mRNA expression then declined at peak and post-peak stabilization of EAE, but was still significantly elevated in comparison to control animals.

MMP-12 protein level, determined by casein zymography, was not found in control or pre-symptomatic mice but could be detected clearly at disease onset. Protein expression was subsequently reduced at disease progression and peak disease although still increased from controls (Fig. 1B).

To provide clues to the regulators of MMP-12 expression in EAE, we used real-time PCR to determine the expression levels of IL-1β, TNF-α and IFN-γ, and evaluated their relative time course of expression to that of MMP-12. We find that all three inflammatory cytokines were increased from onset of clinical signs of EAE, and that they remained elevated from controls with EAE progression and at peak disease (Fig. 1A). In contrast, levels of these inflammatory molecules were reduced to control levels at post-peak stabilization of disease. We found general correspondence between expression of MMP-12 with specific inflammatory cytokines including IL-1β, TNF-α and IFN-γ during EAE onset, progression and peak.

Since the use of CFA and pertussis toxin can induce inflammatory reactions (Toft-Hansen et al., 2006; Tonon et al., 2006; Chen et al., 2007), we repeated our analysis using animals that were injected with CFA and pertussis toxin without MOG35–55. We found that MOG35–55 was integral in mediating the changes in RNA expression (Fig. 1). Without MOG35–55 (Fig. 1; PBS+CFA+PT group), TNF-α, IFN-γ, IL-1β and MMP-12 mRNA expression did not change in comparison to levels from naïve mice. Thus, MOG35–55 in conjunction with CFA and pertussis toxin was needed in order to attain high levels of inflammatory cytokines and MMP-12.

3.2. Cellular source of MMP-12 in EAE

We used immunohistochemistry to delineate the cellular sources of MMP-12 in EAE. We found that MMP-12 expression occurred in areas of inflammation, manifested by increased cellular density either by Hoechst staining or by hematoxylin/eosin analysis. Whereas there is no MMP-12 expression in control tissues, an increase in MMP-12 positive cells was detected in EAE and these cells were co-labeled for Iba-1 and F4/80 (Figs. 2A and 3, respectively), signifying their macrophage/microglia origin. The Iba-1 reactive cells in EAE tissue were more numerous and were ameboid in shape compared to ramified ones in normal control tissue. We found that during EAE areas of demyelination also expressed MMP-12. In particular, the loss of MBP staining depicting demyelination (● in Fig. 2B) co-localized to areas of MMP-12 staining. MMP-12 staining did not co-localize with GFAP staining (data not shown).

Overall, our data shows that MMP-12 is expressed by a sub-population of monocytoid cells in EAE, even though we could not differentiate further between microglia and macrophages.

3.3. Regulation of MMP-12 expression in BMDM cells by inflammatory molecules

Given that MMP-12 is expressed by monocytoid cells in EAE, and since certain inflammatory molecules are increased in conjunction with MMP-12, we used cells in culture to determine whether there was a direct relation between these inflammatory molecules and MMP-12 expression. We selected IL-1β, TNF-α, and IFN-γ for testing since they were distinctly elevated in EAE. We first isolated bone marrow derived macrophages (BMDM) and found these to be over 98% pure as determined by Iba-1 immunohistochemistry (data not shown). MMP-12 RNA expression in BMDM increased after 6 h of IL-1β treatment although this was not further potentiated with 24 h of stimulation. TNF-α stimulation of BMDM increased MMP-12 expression after 24 h, but not at 6 h (Fig. 4(i)). In contrast, IFN-γ did not elevate MMP-12 levels. While none of the treatments...
altered cellular morphology to any significant extent (data not shown), all agents elevated iNOS expression (Fig. 4(ii)), which we used here as a positive control to verify treatment-induced macrophage activation (Saha and Pahan, 2006). Thus MMP-12 expression is stimulation specific and could be driven by the IL-1β and TNF-α cytokine milieu during EAE.

4. Discussion

MS is a devastating neurological disease that involves the infiltration of T cells, dendritic cells, monocytes/macrophages and B lymphocytes across the blood-brain barrier (Sospedra and Martin, 2005; Frohman et al., 2006). The infiltration of
leukocytes across the blood-brain barrier requires activity of MMPs (Cossins et al., 1997; Kouwenhoven et al., 2002; Agrawal et al., 2006; Toft-Hansen et al., 2006) and, in accordance, there is ample literature that MMPs are upregulated in MS leukocytes and CNS tissues (Kieseier et al., 1999; Yong et al., 2001; Opdenakker et al., 2003). In animals afflicted with EAE, the breakthrough of leukocytes into the CNS parenchyma is correlated with increased MMP activity (Agrawal et al., 2006; Toft-Hansen et al., 2006). The many MMP members that are elevated in MS and EAE (Anthony et al., 1997; Cossins et al., 1997; Pagenstecher et al., 1998; Kouwenhoven et al., 2001; Lindberg et al., 2001; Weaver et al., 2005) are thought to be undesirable since a number of metalloproteinase inhibitors or MMP deletion via knockouts attenuate EAE disease activity (Gijbels et al., 1994; Hewson et al., 1995; Liedtke et al., 1998; Brundula et al., 2002).

Although MMPs in general would seem to have detrimental roles in MS, it is possible that some of the MMPs in MS or EAE are elevated to serve beneficial actions. Particularly, during development, MMPs have important physiologic functions. In the developing mouse CNS, MMP-9 mRNA expression has been observed in progenitor cells in close proximity to developing structures (Canete Soler et al., 1995; Canete-Soler et al., 1995). In the mouse cerebellum, MT5-MMP (MMP-24) has been associated with dendritic tree formation of Purkinje cells and they are localized to the growth cones of neurons (Hayashita-Kinoh et al., 2001; Sekine-Aizawa et al., 2001). Within the CNS MMPs are critical in regulating the conversion of pro-brain derived neurotrophic factor (BDNF) and pro-nerve growth factor (NGF) to their mature forms which are pivotal to neuron growth and survival (Lee et al., 2001; Bruno and Cuello, 2006). MMPs are also important to oligodendrocyte

![Image](https://example.com/image.png)

**Fig. 2.** MMP-12 expression in the spinal cord occurs in areas of inflammation and demyelination during EAE progression. A: In control normal spinal cord tissue, MMP-12 expression is absent and Iba-1 labeled microglia are ramified in morphology. During disease, MMP-12 positive cells appear in areas of inflammation (increased density of Hoechst-positive cells) and some MMP-12 expression correlate with Iba-1 immunoreactive cells with amoeboid morphology (yellow color in the overlay panel). B: In normal mice, MBP-stained myelin is distributed evenly around the lateral column of a coronal slice of the spinal cord and MMP-12 expression is absent. During EAE, MMP-12 positive cells are present in the demyelinated area (loss of MBP (*) in areas of inflammation). These results are representative of 4 mice per group with 2–7 lesions analyzed per animal across all groups.
MMP-12 expression in the spinal cord occurs in cells of inflamed lesions. In control normal tissue, there are no leukocytes accumulated within the tissue. Correspondingly, there is no expression of either F4/80 or MMP-12 within this tissue. During EAE, there is an influx of leukocytes within the tissue as observed with LFB/H&E (arrows). Cells within these lesions express F4/80 and MMP-12. Interestingly some but not all of the F4/80 positive cells express MMP-12 (arrow) and may reflect a specific monocytoid population. Furthermore, MMP-12 did not co-localize to CD3-positive T cells (data not shown). These results are representative of 4 mice per group with 2–7 lesions analyzed per animal across all groups.
development and differentiation. Specifically, MMP-9 and MMP-12 transcripts are elevated during myelination in mice and their deficiency in the early postnatal periods result in delayed myelin formation (Larsen et al., 2006). Ulrich et al. (2005) also found increased MMP-12 transcripts corresponding with myelination in the rhombencephalon and spinal cord in rodents. In correspondence with useful functions of MMP-12 in normal physiology, we described that MMP-12 null mice have a higher disease severity score than wildtype controls, implying a useful role for this MMP (Weaver et al., 2005). Thus, given the rarity of a beneficial MMP in EAE or MS, we have sought to understand better the expression and regulation of MMP-12 in EAE.

Herein, we have found that MMP-12 mRNA levels increased before symptoms appeared. MMP-12 expression became increasingly prominent with the onset of paresis, and it peaked during disease progression. Although levels were reduced somewhat thereafter, MMP-12 transcript expression remained high with paralysis at peak disease and during stabilization (Fig. 1A). When we examined the protein level of MMP-12 expression we found that only the active enzyme was expressed with the onset of disease symptoms and remained high with paresis but that MMP-12 expression decreased as disease progressed. The protein data thus differs from what we observed with the RNA levels and this could be due to MMP-12 being an enzyme that occurs in two forms, inactive (approximately 54 kDa) and an active (approximately 22 kDa) form. Our protein data only describes the active isoform of MMP-12 whereas the RNA data encompasses the production of the inactive form. Overall, MMP-12 transcripts remain high through EAE progression but this is not necessarily reflected in the protein expression data.

When we examined the cellular contributors of MMP-12 expression within the lesions of EAE animals we found that MMP-12 expression was predominantly in areas of cellular infiltration and demyelination. In EAE, MMP-12 expression was primarily in cells of the mononuclear phagocyte system, as inferred by co-expression in Iba-1 positive macrophage/microglia and F4/80 positive macrophages (Figs. 2A and 3). Work by others have also shown that during EAE, macrophages are the primary source of MMP-12 RNA expression (Toft-Hansen et al., 2004). MMP-12 protein expression has also been primarily detected at the active edge of MS lesions, in foamy macrophages (Vos et al., 2003). In our study, we find that not all macrophage/microglia cells expressed MMP-12. There appeared to be a population of MMP-12 reactive macrophage/microglia cells in areas of hypercellularity and their function remains to be explored. It is possible that MMP-12 expression in macrophage/microglia represents a stage of differentiation in which certain circulating monocytes differentiate into MMP-12 expressing tissue specific cells. It has been previously documented that peripheral blood monocytes do not express
MMP-12 (Shipley et al., 1996), and that as monocytes differentiate into macrophages, they alter their proteinase profiles (Campbell et al., 1991). As such, during EAE, a population of MMP-12 expressing macrophage/microglia could function differently than other populations of non-MMP-12 expressing macrophage/microglial cells.

We also found that MMP-12 expression occurs in areas of demyelination (Fig. 2B). The exact role of MMP-12 in these lesions remains unclear although it has been suggested that MMP-12 positive macrophages may aid in the migration of cells though the parenchyma or vessels (Toft-Hansen et al., 2006). MMP-12 is known to cleave a number of ECM components found in the basement around cerebral vessels, including collagen, laminin, fibronectin, vitronectin and proteoglycans (Chandler et al., 1997; Giambernardi et al., 1998; Lang et al., 2001). The need for MMP-12 in cellular migration has been demonstrated by Shipley et al. (1996), since macrophages from MMP-12 null mice have very little migratory capacity in vitro and in vivo when compared to controls. The role for MMP-12 within demyelinating lesion is further complicated by the fact that MMP-12 can cleave myelin basic protein which could facilitate epitope spreading (Proost et al., 1993; Sternlicht and Werb, 2001). Alternatively, MMP-12 may serve useful roles in helping to polarize CD4+ T cells into those that are Th2 biased (Weaver et al., 2005). Moreover, given the role of MMP-12 in developmental myelin formation (Larsen et al., 2006), it is possible that the earlier upregulation of MMP-12 may subserve activities related to the inflammatory process while the later rise of MMP-12 transcripts may be more important for repair processes; these possibilities are speculative at this point and require further studies.

While the exact roles for MMP-12 in EAE lesions remain to be fully elucidated, it is apparent that MMP-12 expression occurs in conjunction with other cytokotks during disease progression. This coincidental rise does not equate to a causal relationship but in support of a functional interaction in vivo, we found that BMDM expression of MMP-12 mRNA in culture could be increased by the addition of IL-1β and TNF-α to the medium. Nonetheless, complex in vivo situations are not reproduced in culture and we caution the extrapolation of in vitro results to the condition in vivo. In studies by others, IL-1β and TNF-α induce monocyte activator protein-1 (AP-1) activity by increasing AP-1 binding to the MMP-12 promoter (Wu et al., 2001). Xie et al. (2005) showed that MMP-12 activity and gene expression in smooth muscle cells from human bronchial biopsy was regulated by IL-1β and TNF-α through enhancing AP-1 activation. Furthermore, Wu et al. (2001) found that the combination of treatment with GM-CSF and IL-1β resulted in a further increase of MMP-12 expression compared to treatment with GM-CSF alone. TNF-α has been shown to induce MMP-12 mRNA production in chondrosarcoma-derived HTB-94 cells (Kerkela et al., 2001; Kerkela et al., 2002).

Finally, MMP expression often occurs in conjunction with that of tissue inhibitors of metalloproteinases (TIMPs) (Weaver et al., 2005) so the evaluation of protease alone without naturally occurring inhibitors is an underestimation of the overall proteolytic cascade. However, there are potentially many inhibitors of MMP-12 besides the four TIMPs, and the net activity of MMP-12 in the presence of its inhibitors is not possible to measure with current technology at this point.

In summary, our results demonstrate that during EAE, MMP-12 is temporally upregulated with disease progression in conjunction with the expression of cytokotks that are known factors in inflammation. The expression of MMP-12 in areas of cellular infiltration corresponded to areas of demyelination and macrophage/microglial activation suggestive of a role for MMP-12 in these areas. The fact that cytokotks such as TNF-α and IL-1β can regulate the expression MMP-12 in BMDM cultures furthers the idea that MMP-12 might play an important role in the progression of EAE. In this study we are the first to systematically examine the temporal and spatial role of MMP-12 in EAE. These results should aid in better understanding MMP biology in EAE.

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References


