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Matrix metalloproteinase-9 and -7 are regulated in experimental autoimmune encephalomyelitis

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Summary

Matrix metalloproteinases (MMPs) comprise a group of proteolytic enzymes that are implicated in the pathogenesis of inflammatory diseases of the nervous system such as multiple sclerosis. However, the exact function and expression pattern of MMPs in the inflamed nervous system are not known. In the present study we investigated the expression of 92-kDa gelatinase (MMP-9) in spinal cord from animals with adoptive transfer experimental autoimmune encephalomyelitis (AT-EAE), using a semiquantitative competitive reverse transcriptase–polymerase chain reaction assay. Increased levels of MMP-9 mRNA were found with peak values at times of maximum disease severity. Increased mRNA expression was associated with enhanced proteolytic activity of this enzyme, as demonstrated by gelatin zymography. Immunohistochemistry revealed immunoreactivity along the meninges, around blood vessels and within the parenchyma, in diseased but not in normal spinal cord. Furthermore, the expression pattern of five other MMPs was investigated. Matrilysin (MMP-7) was also found to be upregulated with maximum mRNA levels at the peak of the disease. In contrast, mRNAs for collagenase-3, 72-kDa gelatinase, and stromelysin-1 and -3 were not changed. Our findings indicate that 92-kDa gelatinase and matrilysin are selectively upregulated during AT-EAE and thus may contribute to the pathogenesis of inflammatory diseases of the CNS.

Keywords: 92-kDa gelatinase; matrilysin; experimental autoimmune encephalomyelitis; multiple sclerosis

Abbreviations: AT = adoptive transfer; BBB = blood–brain barrier; cDNA = complementary DNA; EAE = experimental autoimmune encephalomyelitis; MBP = myelin basic protein; MMP = matrix metalloproteinase; PCR = polymerase chain reaction; TACE = tumour necrosis factor-α converting enzyme; TNF = tumour necrosis factor

Introduction

The matrix metalloproteinases (MMPs) belong to a large subgroup of proteinases which includes the collagenases, gelatinases and the stromelysins, all of which contain tightly bound zinc (Birkedal-Hansen, 1995; Ries and Petrides, 1995). Several MMPs have been identified but the exact role of their involvement in various physiological and pathological processes is only incompletely understood. There is emerging evidence that MMPs might be involved in the pathogenesis of inflammatory demyelinating disorders such as multiple sclerosis and the Guillain–Barré syndrome (Opdenakker and van Damme, 1994). In a first study, Cuzner *et al*. (1978) demonstrated increased neutral proteinase activity in the CSF during exacerbations of multiple sclerosis. More recently, certain MMPs, i.e. 72-kDa gelatinase, stromelysin-1, interstitial collagenase, matrilysin (MMP-7) and 92-kDa gelatinase (MMP-9), were shown to degrade myelin basic protein (MBP) *in vitro* (Chandler *et al.*, 1995). Furthermore, evidence is emerging that MMPs might be involved in blood–brain barrier (BBB) breakdown. Thus, non-specific inhibition of MMPs was shown to protect the BBB and could suppress, and even reverse, ongoing experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis (Gijbels *et al.*, 1994; Hewson *et al.*, 1995). Among all known MMPs, the 92-kDa gelatinase might be particularly involved in BBB breakdown, as increased CSF levels of 92-kDa gelatinase in multiple sclerosis patients are associated

with a leaky BBB on magnetic resonance imaging (Rosenberg *et al.*, 1996). Moreover, in multiple sclerosis lesions increased levels of 92-kDa gelatinase were described (Cuzner *et al.*, 1996), where endothelial cells, astrocytes and microglia were all thought to be potential source of production (Maeda and Sobel, 1996). However, besides a recent report on MMP expression and its regulation by a hydroxamate-based inhibitor in actively induced EAE (Clements *et al.*, 1997), there are no data about the temporospatial regulation of 92-kDa gelatinase and other MMPs during the course of neuroinflammatory diseases.

Material and methods

Animals

Eight-week-old female Lewis rats (Charles Rivers, Sulzfeld, Germany) weighing 130–145 g were used. Animals were kept in plastic cages and given food and water *ad libitum*. All experiments were conducted according to Bavarian state regulations for animal experimentation and approved by the responsible authorities.

Induction of EAE

Adoptive transfer (AT)-EAE was induced by tail vein injection of 8×10^6 activated autoreactive T cells specific for MBP. The encephalitogenic $CD4^+$ T-cell line (line MBP.15) was isolated and activated prior to injection as described elsewhere (Jung *et al.*, 1995). Normal rats and rats injected with ovalbumin specific T cells (line OVA.2, fourth restimulation cycle) served as controls. After 2, 3, 4, 5, 7 and 11 days, groups of three animals each were sacrified and the spinal cord was flash frozen in liquid nitrogen and stored at –70°C.

Clinical scoring of EAE

Animals were weighed and clinically scored daily on a five-point scale as follows: grade 0 = no neurological signs; grade 1 = limp tail; grade 2 = mild paraparesis of the hind limbs, unsteady gait; grade 3 = moderate paraparesis, voluntary movements still possible; grade 4 = paraplegia or tetraplegia; grade 5 = moribund.

Competitive polymerase chain reaction (PCR)

For quantification of rat MMP mRNA levels, PCR using a multi-competitor DNA standard was performed (Wells *et al.*, 1996). The synthetic standard DNA contained tandem arrays of 5′ and 3′ priming sites for complementary DNAs (cDNAs) of different MMPs and β-actin.

Primer pairs for collagenase-3, MMP-7, 72-kDa and 92-kDa gelatinase, stromelysin-1 and -3, and β-actin were used according to the sequences published elsewhere (Wells *et al.*, 1996). $Poly(A)^+$ RNA was extracted from frozen spinal cord and used as a template for cDNA synthesis using AMV reverse transcriptase (Promega, Madison, Wis., USA). Threefold serial dilutions of competitive standard DNA were combined with a definite amount of sample cDNA, and PCR was performed in 50-µl reactions containing 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris–HCl at pH 9.0 and 0.1% Triton X-100 in the presence of 200 µM dNTP (Pharmacia, Freiburg, Germany), 50 pmol sense and antisense MMP primers, 1 U taq DNA polymerase (Perkin Elmer, Branchburg, NJ), and 1 µCi [^{32}P]α-dCTP (Amersham, Braunschweig, Germany). Amplification was carried out using 35 cycles (95°C for 30 s, 57°C for 30 s, 72°C for 120 s) in a Hybaid Omnigene thermal cycler (MWG Biotech, Ebersberg, Germany). Ten microlitres of the reaction products were electrophoresed on a 6% polyacrylamide gel. Gels were stained with ethidium bromide and the bands were cut out, added to 2 ml of scintillation fluid and counts per minute were measured in a liquid scintillation counter (Packard Instrument, Downers Grove, Ill., USA). MMP mRNA levels were determined by plotting the ratio of sample cDNA to standard DNA against the standard dilution using a double-logarithmic scale.

Gelatin zymography

Gelatinase activity was determined by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) zymography modified according to the method of Brown *et al.* (1990). Ten 10-µm frozen sections of spinal cord were incubated with 25 µl of Tris/glycine SDS sample buffer (Novex, San Diego, Calif., USA), degraded by ultrasound and further homogenized by vortexing for 1 h at 4°C. The samples were applied to a 10% (w/v) polyacrylamide resolving gel containing 0.1% SDS and 0.1% gelatin type A from porcine skin (Sigma, St Louis, Mo., USA). Stacking gels were 3% (w/v) polyacrylamide. After electrophoresis gels were washed in renaturing buffer (Novex) containing Triton X-100 to remove any SDS, and incubated in developing buffer (Novex) for 18 h at 37°C. Gels were stained for 6 h in 30% methanol : 10% acetic acid containing 0.5% (w/v) Coomassie Brilliant Blue G-250 and destained in the same buffer without dye. Gelatinase activity was detected as unstained bands on a blue background representing areas of gelatin digestion.

Supernatants from tumour necrosis factor (TNF)-α stimulated astrocyte cell cultures, which are known to express 92-kDa and 72-kDa gelatinase in response to cytokine stimulation *in vitro* (Gottschall and Yu, 1995), served as positive controls.

Immunohistochemistry

Frozen sections (10 µm) of spinal cord were air dried and fixed for 10 min in 4% formalin and 2 min each of 50%, 100%, then 50% acetone at room temperature. A monoclonal affinity-purified antibody against 92-kDa gelatinase was used at 1.2 µg/ml overnight at 4°C. Thereafter, a biotinylated

secondary antibody against mouse IgG and an avidin–biotinylated peroxidase complex (Dako, Hamburg, Germany) were used with 3,3′-diaminobenzidine as peroxidase substrate. Endogenous peroxidase activity was suppressed by incubating the sections with 3% H_2O_2 in methanol for 10 min prior to the secondary antibody. Sections were counterstained with haematoxylin, dehydrated, and mounted in Eukitt (Kindler, Freiburg, Germany).

Double immunofluorescence labelling was performed in an attempt to co-localize MMP-9 expression with inflammatory cells. Frozen sections prepared as above were incubated with the anti-92-kDa gelatinase antibody, which was detected using a monoclonal TRITC (trimethylrhodamine isothiocyanate)-conjugated secondary antibody against mouse IgG (Dianova, Hamburg, Germany). Afterwards the sections were double-stained by sequential incubation with either an FITC (fluorescein isothiocyanate)-conjugated monoclonal antibody specific for rat monocytes/macrophages (ED1; Serotec, Oxford, UK) or a biotinylated monoclonal antibody against the T-cell receptor (TCR-αβ; Serotec), detected by a FITC-conjugated streptavidin complex. Cross-reactivity was avoided by blocking the sections with mouse serum prior to the application of the second primary antibody.

Results

Clinical course

Weight loss and first clinical signs of the disease were detected 3 days after the injection of encephalitogenic T cells. Clinical disease severity peaked at days 4 and 5 with an average score of 3.5. First signs of clinical improvement were seen at day 6 (Fig. 1A). Control rats showed no signs of disease during the experiment.

Validity of competitive PCR

To validate the competitive PCR assay used in the present study we demonstrated that variations of sample cDNA or standard DNA resulted in strictly proportional shifts of the PCR product ratios of the respective DNAs. Plotting the ratio of sample cDNA to standard DNA PCR products against the standard dilution in a double-logarithmic scale we obtained curves with slopes close to 1. Changes in the amount of either sample cDNA or standard DNA resulted in a parallel shift of the curves, thus implying that the amplification is proportional within the given proportions of sample cDNA and standard DNA (data not shown) (Bourde *et al.*, 1996).

Expression of 92-kDa gelatinase

Quantitation of mRNA

A constitutive low level expression of 92-kDa gelatinase mRNA was found in normal rats and those injected with ovalbumin specific T cells, and up to day 2 following injection of encephalitogenic T cells. Elevated levels were

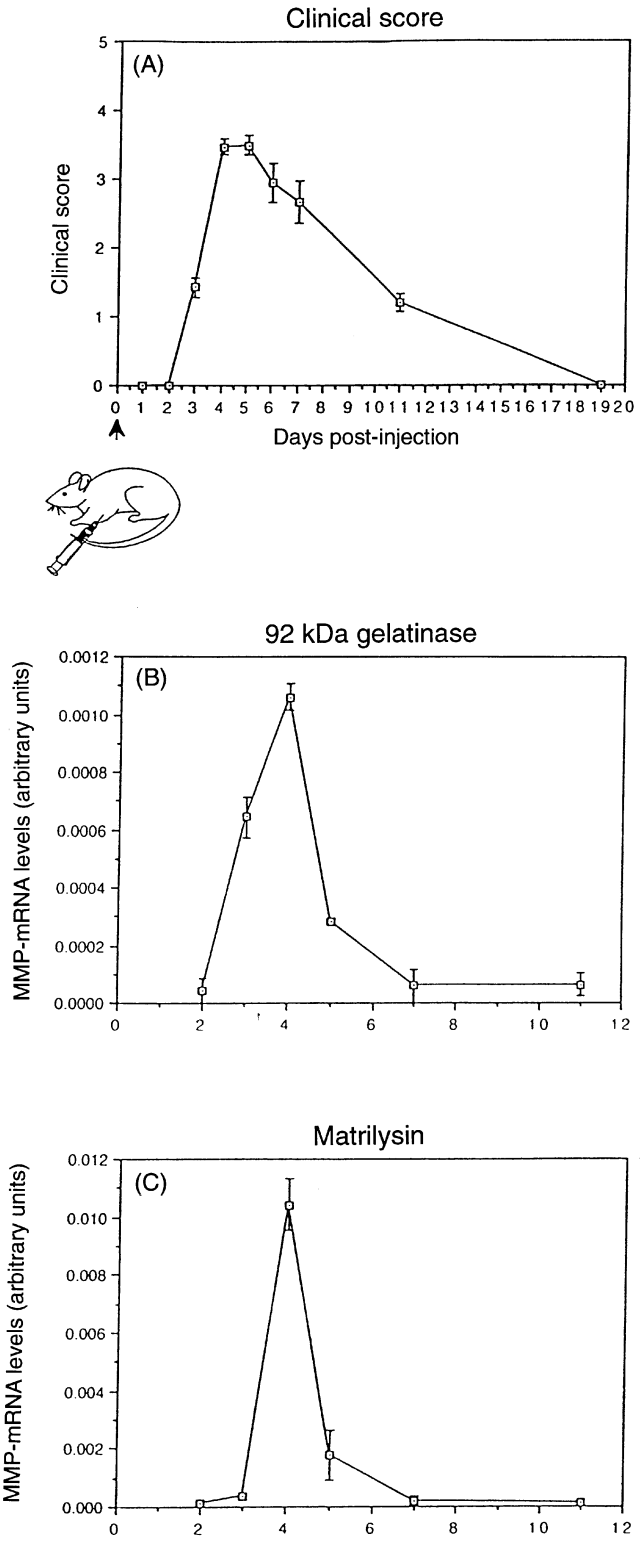

Fig. 1 MMP mRNA expression during AT-EAE. (**A**) Disease course of AT-EAE. (**B**) Expression of 92-kDa gelatinase mRNA as a fraction of β-actin, measured at different time points after injection of encephalitogenic T cells. Peak levels, tenfold above initial expression, can be found at maximal disease activity. (**C**) Expression of matrilysin mRNA as a fraction of β-actin during AT-EAE. A 100-fold increase can be detected at day 4, coincident with peak clinical severity. Values for each day represent mRNA data from three animals each, given as means ± SD.

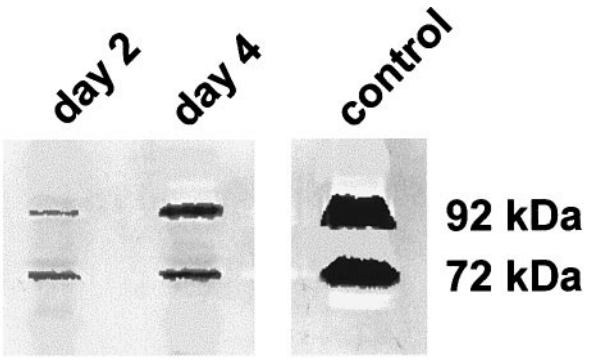

Fig. 2 Detection of proteinase activity in spinal cord from Lewis rats by SDS–substrate gel analysis. Zone of clearing represents proteinase activity. Tissue from an animal with clinical score 0, 2 days post-injection of encephalitogenic T cells, showing areas of gelatin digestion at levels of 92-kDa and 72-kDa. Tissue taken from an animal 4 days post-injection at maximal disease severity, showing an increased size of clearing zone at the 92-kDa level, indicative of increased proteinase activity. In contrast, clearing appears to be unchanged at the level of 72-kDa. Supernatants from TNF-α stimulated astrocytes served as positive controls.

found at day 3, with peak levels showing a tenfold increase above normal at day 4, coincident with peak clinical severity. Thereafter, mRNA levels declined to normal by day 7 and remained constant throughout the recovery phase of the disease (Fig. 1B).

Proteolytic activity

To demonstrate that increased expression of 92-kDa gelatinase mRNA is associated with increased enzymatic activity, zymography of spinal cord tissue taken from animals at different stages of the disease was performed. A pronounced increase of 92-kDa gelatinase activity was observed in animals with severe clinical disease compared with lower scored animals, in line with the data obtained by competitive PCR. Thus, higher expression of mRNA was accompanied by augmented gelatin digestion.

In contrast, the activity of the 72-kDa gelatinase, which is detected in the same experiment, appeared to be constitutively expressed throughout all time points, the size of the unstained bands remaining constant (Fig. 2).

Immunohistochemistry

In control animals and animals with a clinical score of 0, no immunoreactivity for the 92-kDa gelatinase could be detected. In AT-EAE, positive signals could first be seen at day 3; they increased until day 4 and 5, and decreased at day 7 to disappear by day 11. At day 3, in animals with a clinical score of 2, immunoreactivity was primarily found along the meninges and around blood vessels. Some positive signals could also be seen in the white and grey matter. Immunoreactivity was localized to cells with round nuclei, probably mononuclear invading cells, but also in the extracellular space. At the peak of the disease, around days 4 and 5, more positive cells could be detected in the white and grey matter, with less immunoreactivity in the meninges. Moreover, strong reactivity was noticeably found along the vessels. The distribution pattern of immunoreactive cells closely paralleled the density of infiltrating mononuclear cells. By day 7, the cellular and extracellular signal intensity and the number of positive cells decreased, with only a few signals along the meninges left. By day 11, all immunoreactivity had disappeared (Fig. 3). Throughout the entire course of the disease, no immunoreactive neurons were discernible. Control sections, after omission of the primary antibody, showed only background staining.

Immunofluorescence double labelling was performed in sections which revealed increased 92-kDa gelatinase immunoreactivity and it confirmed the distribution pattern of MMP-9 as described above. However, MMP-9 could not be co-localized with either $ED1^+$ monocytes/macrophages or $TCR\alpha\beta^+$ T cells (not shown).

Other matrix metalloproteinases

To investigate whether other MMPs besides MMP-9 are regulated during the course of AT-EAE we studied the mRNA expression of matrilysin, collagenase-3, 72-kDa gelatinase, and stromelysin-1 and -3. The latter four MMPs were constitutively expressed at low levels, and did not exhibit any increase in mRNA levels during the clinical course of AT-EAE. Of these, the 72-kDa gelatinase was the most abundant, expressing slightly higher levels compared with rat collagenase, stromelysin-1 and -3. The mRNA expression pattern of the 72-kDa gelatinase underlines the constitutively low proteinase activity measured by gelatin zymography.

In contrast, matrilysin was found to be strongly upregulated (100-fold) with a peak of mRNA expression 4 days after injection of encephalitogenic T cells, returning to initial levels by day 7 and remaining constantly low throughout the recovery phase of the disease (Fig. 1C).

Discussion

In the present study we investigated the expression of the 92-kDa gelatinase and other MMPs in the inflamed spinal cord of rats with EAE using a competitive PCR assay. As pointed out elsewhere (Bourde *et al.*, 1996), quantitative competitive PCR assays should follow certain theoretical requirements (Raemaykers, 1993) to be useful tools and produce meaningful results. By independent variation of standard and cDNA template concentrations and plotting the ratios of the respective PCR products in a double logarithmic manner, we obtained linear curves with slopes close to 1 and proportional parallel shifts of the curves, thus confirming the reliability of the semiquantitative competitive PCR assay used in the present study.

The time course of 92-kDa gelatinase expression in EAE, including mRNA expression, immunoreactive protein and enzyme activity, closely followed the time course of clinical

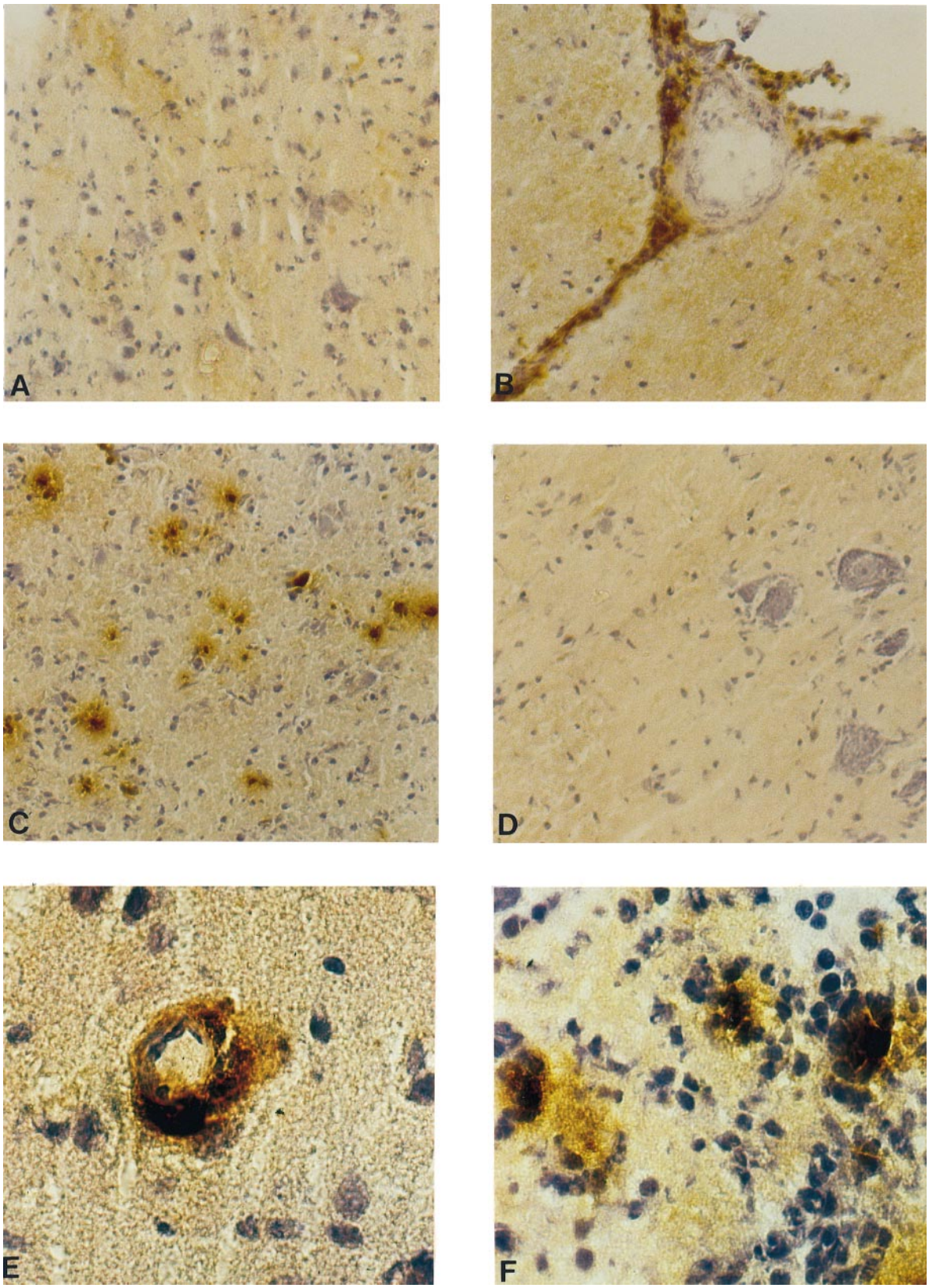

Fig. 3 Immunohistochemistry for 92-kDa gelatinase in the spinal cord of Lewis rats. Animal at day 2 post-injection with a clinical score of 0 showing no immunoreactivity (**A**), whereas at day 3 (score 2–3), positive signals for MMP-9 can primarily be found along the meninges and around blood vessels (**B**). At the peak of clinical severity, day 4, immunoreactivity can be localized to infiltrating cells within the white and grey matter (**C**), to disappear by day 11 (**D**). Higher magnifications of a vessel from an animal at day 3 (**E**) and cellular infiltrates at day 4 (**F**) clearly link positive immunoreactivity to vessels (**E**) and to infiltrating mononuclear cells (**F**). Magnifications: **A**–**D**, ×200; **E**–**F**, ×600.

disease in the present study. Furthermore, the distribution pattern of positive immunoreactivity for MMP-9 with early localization to the meninges, perivascular spaces and white matter tracts, and later the central grey matter of the spinal cord, was coincident with the topographical distribution of inflammatory infiltrates in EAE (Lassmann *et al.*, 1991). However, while some positive staining could be associated with round cellular nuclei, suggestive of infiltrating mononuclear cells, we failed to co-localize immunoreactivity for the 92-kDa gelatinase with T-cell or macrophage antigens. In addition, typical perineuronal microglial cellular profiles, presumed astrocytes and neurons themselves were unstained at all time points studied. These findings suggest a predominantly extracellular localization of enzyme secreted from an as yet unknown cellular source. However, the close association of immunoreactivity with mononuclear cellular infiltrates points to infiltrating macrophages and T cells, the prime effector cells in EAE (Huitinga *et al.*, 1990; Raine, 1991), being the predominant producers of MMP-9. This assumption is consistent with previous investigations where macrophages in multiple sclerosis plaques (Cuzner *et al.*, 1996), neutrophils (Nielsen *et al.*, 1996), monocytes (Welgus *et al.*, 1990) and T cells (Leppert *et al.*, 1995) were found to secrete 92-kDa gelatinase. In contrast, microglial cells were previously noted to express MMP-9 in multiple sclerosis lesions, and motor neurons in the spinal cord were described as a potential source of MMP-9 production in amylotrophic lateral sclerosis (Lim *et al.*, 1996). It is unclear, at present, whether technical issues, species differences or differential regulatory events during diverse biological situations account for these discrepancies. Nevertheless, we could detect proteolytic activity of the 92-kDa gelatinase in our encephalitogenic T-cell line after stimulation with interleukin-2 by zymography (data not shown), pointing to this cell population as potential producers of this MMP. However, further studies are needed to elucidate the exact cellular sources of MMPs in the pathogenesis of neuroinflammatory diseases.

The time course and cellular distribution of MMP-9 in EAE, as described in the present study, suggests biological effects in early and central stages of the disease process that are associated with inflammatory infiltration. As shown by previous *in vitro* studies, T-cell migration can be mediated by gelatinases (Leppert *et al.*, 1995), and *in vivo* gelatinase injection into the brain resulted in disruption of the BBB (Rosenberg *et al.*, 1994). The predominant localization of MMP-9 around blood vessels in the present study suggests that MMP-9 might be physiologically involved in the disruption of vascular basement membranes (Gijbels *et al.*, 1993) and the BBB, paving the way for infiltrating haematogenous cells. Since migration of autoreactive T cells from blood to the CNS is considered of paramount importance in the genesis of autoimmune inflammatory demyelination, such an action of MMPs would imply that they have a strategic role in this process (Hartung, 1995). Another potential target for MMP-9 action in the pathogenesis of neuroinflammatory disorders is the myelin sheath. As shown previously, MMP-9 and other MMPs are known to degrade MBP *in vitro* (Proost *et al.*, 1993; Chandler *et al.*, 1995) and they could therefore contribute to demyelination. However, since MBP is located at the cytoplasmic face of the myelin membrane, there is little chance that this would represent a primary mechanism of demyelination. Furthermore, little or no demyelination is seen in the Lewis rat AT-EAE model, and these animals recover spontaneously and completely without residual deficits (Wekerle *et al.*, 1994). Finally, MMP-9 may be involved in the regulation of other bioactive proteins including cytokines (Opdenakker and van Damme, 1994; Hartung *et al.*, 1992; Ries and Petrides, 1995). Thus, release of the myelinotoxic and phlogogenic cytokine TNF-α appears to be dependent on an MMP-like mechanism of action (Gearing *et al.*, 1994; Mohler *et al.*, 1994), indicating an even more complex involvement of MMPs in neuroinflammation. However, the recently described TNF-α converting enzyme (TACE) has been shown to be a disintegrin metalloproteinase with notable sequence identity to the adamalysin family of metalloproteinases, implying TACE to be more likely a novel metalloproteinase, distinct from the hitherto known 'classical' MMPs (Black *et al.*, 1997; Moss *et al.*, 1997). Further studies are needed to elucidate the contributory effects of known MMPs to the release of surface cytokines and receptors, which markedly impact on the orchestration of immunoinflammatory responses.

Recently, Cuzner *et al.* (1996) described the expression of MMP-9 in post-mortem multiple sclerosis lesions and related it to the degree of inflammation. It is obvious that such a study can provide a focused view at only one time point in the disease process of which the precise pathobiological history cannot be ascertained. Hence, it is impossible to define the exact relation to the stage of the ongoing disease. By contrast, in the present study we have investigated the temporospatial expression pattern of MMP-9 during the entire development of acute inflammation in the CNS, providing a better insight into the relationships between acute inflammation, severity of the ongoing disease and MMP expression in neuroinflammatory disorders such as multiple sclerosis.

To investigate whether other MMPs besides MMP-9 might also be regulated in experimental neuroinflammation, mRNA expression patterns of five other metalloproteinase were studied. Whereas matrilysin mRNA was clearly upregulated, with peak levels showing a 100-fold increase above the initial expression at maximum disease activity, the 72-kDa gelatinase, thought to be primarily involved in BBB disruption (Rosenberg *et al.*, 1992), was not modulated in the present study. Similarly, mRNAs for collagenase-3 and stromelysin-1 and -3 were found to be constitutively expressed at low levels but not regulated. Thus, MMPs in the brain, though all present at low levels even in normal tissue, do not respond to inflammatory stimuli in an all-or-none fashion; rather, they are differentially regulated (Kohn *et al.*, 1994) during AT-EAE. What the role of selectively upregulated matrilysin

might be in inflammation is poorly understood. A strong catalytic activity against a variety of extracellular matrix substrates such as proteoglycans, elastin, laminin and fibronectin has been described (Murphy *et al.*, 1994), and a potential role in monocyte extravasation and macrophage migration through interstitial tissue spaces was proposed (Busiek *et al.*, 1995). Increased levels of specific mRNA during EAE may suggest a potential involvement of matrilysin in such events.

The results of our present study are in accord with a recent report on MMP expression and its regulation by a hydroxamate-based inhibitor in actively induced EAE, where levels of MMP-9 and MMP-7 mRNA were found to be increased by 5- and 500-fold, respectively (Clements *et al.*, 1997). In contrast to the present findings, matrilysin mRNA levels remained increased during the recovery phase of actively induced EAE, a discrepancy which may be attributed to the difference in the animal models used.

This collective experimental evidence points to MMPs as potential targets for an effective treatment of multiple sclerosis eiher alone or in concert with other selective therapies. MMP inhibitors have, indeed, been shown to efficiently ameliorate EAE (Gijbels *et al.*, 1994; Hewson *et al.*, 1995) and to reduce BBB breakdown, inflammatory cell recruitment, and myelin damage significantly (Matyszak and Perry, 1996). Moreover, *in vitro* studies suggest an inhibitory effect of gelatinase secretion as a potential therapeutic mechanism of IFN-β-1b (Leppert *et al.*, 1996). Further knowledge of specific temporo-spatial expression patterns of selected MMPs might help to design inhibitors that could be administered at critical points in the evolution of immune mediated damage to the CNS. It is hoped that such compounds may enlarge our therapeutic armamentarium for neuroinflammatory diseases.

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References

Birkedal-Hansen H. Proteolytic remodeling of extracellular matrix. [Review]. Curr Opin Cell Biol 1995; 7: 728–35.

Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF et al. A metalloproteinase disintegrin that releases tumour-necrosis factor-α from cells. Nature 1997; 385: 729–33.

Bourde O, Kiefer R, Toyka KV, Hartung H-P. Quantification of interleukin-6 mRNA in wallerian degeneration by competitive reverse transcription polymerase chain reaction. J Neuroimmunol 1996; 69: 135–40.

Brown PD, Levy AT, Margulies IM, Liotta LA, Stetler-Stevenson WG. Independent expression and cellular processing of Mr 72,000 type IV collagenase and interstitial collagenase in human tumorigenic cell lines. Cancer Res 1990; 50: 6184–91.

Busiek DF, Baragi V, Nehring LC, Parks WC, Welgus HG. Matrilysin expression by human mononuclear phagocytes and its regulation by cytokines and hormones. J Immunol 1995; 154: 6484–91.

Chandler S, Coates R, Gearing A, Lury J, Wells G, Bone E. Matrix metalloproteinases degrade myelin basic protein. Neurosci Lett 1995; 201: 223–6.

Clements JM, Cossins JA, Wells GM, Corkill DJ, Helfrich K, Wood LM, et al. Matrix metalloproteinase expression during experimental autoimmune encephalomyelitis and effects of a combined matrix metalloproteinase and tumour necrosis factor-α inhibitor. J Neuroimmunol 1997; 74: 85–94.

Cuzner ML, Davison AN, Rudge P. Proteolytic enzyme activity of blood leukocytes and cerebrospinal fluid in multiple sclerosis. Ann Neurol 1978; 4: 337–44.

Cuzner ML, Gveric D, Strand C, Loughlin AJ, Paemen L, Opdenakker G, et al. The expression of tissue-type plasminogen activator, matrix metalloproteases and endogenous inhibitors in the central nervous system in multiple sclerosis: comparison of stages in lesion evolution. J Neuropathol Exp Neurol 1996; 55: 1194–204.

Gearing AJ, Beckett P, Christodoulou M, Churchill M, Clements J, Davidson AH, et al. Processing of tumour necrosis factor-α precursor by metalloproteinases. Nature 1994; 370: 555–7.

Gijbels K, Proost P, Masure S, Carton H, Billiau A, Opdenakker G. Gelatinase B is present in the cerebrospinal fluid during experimental autoimmune encephalomyelitis and cleaves myelin basic protein. J Neurosci Res 1993; 36: 432–40.

Gijbels K, Galardy RE, Steinman L. Reversal of experimental autoimmune encephalomyelitis with a hydroxamate inhibitor of matrix metalloproteases. J Clin Invest 1994; 94: 2177–82.

Gottschall PE, Yu X. Cytokines regulate gelatinase A and B (matrix metalloproteinase 2 and 9) activity in cultured rat astrocytes. J Neurochem 1995; 64: 1513–20.

Hartung HP. Pathogenesis of inflammatory demyelination: implications for therapy [editorial]. [Review]. Curr Opin Neurol 1995; 8: 191–9.

Hartung HP, Jung S, Stoll G, Zielasek J, Schmidt B, Archelos JJ, et al. Inflammatory mediators in demyelinating disorders of the CNS and PNS. [Review]. J Neuroimmunol 1992; 40: 197–210.

Hewson AK, Smith T, Leonard JP, Cuzner ML. Suppression of experimental allergic encephalomyelitis in the Lewis rat by the matrix metalloproteinase inhibitor Ro31–9790. Inflamm Res 1995; 44: 345–9.

Huitinga I, Van Rooijen N, de Groot CJ, Uitdehaag BM, Dijkstra CD. Suppression of experimental allergic encephalomyelitis in Lewis rats after elimination of macrophages. J Exp Med 1990; 172: 1025–33.

Jung S, Toyka K, Hartung H-P. Suppression of experimental autoimmune encephalomyelitis in Lewis rats by antibodies against CD2. Eur J Immunol 1995; 25: 1391–8.

Kohn EC, Jacobs W, Kim YS, Alessandro R, Stetler-Stevenson WG, Liotta LA. Calcium influx modulates expression of matrix metalloproteinase-2 (72-kDa type IV collagenase, gelatinase A). J Biol Chem 1994; 269: 21505–11.

Lassmann H, Zimprich F, Rössler K, Vass K. Inflammation in the nervous system. Basic mechanisms and immunological concepts. [Review]. Rev Neurol (Paris) 1991; 147: 763–81.

Leppert D, Waubant E, Galardy R, Bunnett NW, Hauser SL. T cell gelatinases mediate basement membrane transmigration in vitro. J Immunol 1995; 154: 4379–89.

Leppert D, Waubant E, Bürk MR, Oksenberg JR, Hauser SL. Interferon beta-1b inhibits gelatinase secretion and in vitro migration of human T cells: a possible mechanism for treatment efficacy in multiple sclerosis. Ann Neurol 1996; 40: 846–52.

Lim GP, Backstrom JR, Cullen MJ, Miller CA, Atkinson RD, Tökés ZA. Matrix metalloproteinases in the neocortex and spinal cord of amyotrophic lateral sclerosis patients. J Neurochem 1996; 67: 251–9.

Maeda A, Sobel RA. Matrix metalloproteinases in the normal human central nervous system, microglial nodules, and multiple sclerosis lesions. J Neuropathol Exp Neurol 1996; 55: 300–9.

Matyszak MK, Perry VH. Delayed-type hypersensitivity lesions in the central nervous system are prevented by inhibitors of matrix metalloproteinases. J Neuroimmunol 1996; 69: 141–9.

Mohler KM, Sleath PR, Fitzner JN, Cerretti DP, Alderson M, Kerwar SS, et al. Protection against a lethal dose of endotoxin by an inhibitor of tumour necrosis factor processing. Nature 1994; 370: 218–20.

Moss ML, Jin S-L, Milla ME, Burkhart W, Carter HL, Chen W-J, et al. Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor-α. Nature 1997; 385: 733–6.

Murphy G, Willenbrock F, Crabbe T, O'Shea M, Ward R, Atkinson S, et al. Regulation of matrix metalloproteinase activity. [Review]. Ann N Y Acad Sci 1994; 732: 31–41.

Nielsen BS, Timshel S, Kjeldsen L, Sehested M, Pyke C, Borregaard N, et al. 92-kDa type IV collagenase (MMP-9) is expressed in neutrophils and macrophages but not in malignant epithelial cells in human colon cancer. Int J Cancer 1996; 65: 57–62.

Opdenakker G, Van Damme J. Cytokine-regulated proteases in autoimmune diseases. [Review]. Immunol Today 1994; 15: 103–7.

Proost P, Van Damme J, Opdenakker G. Leukocyte gelatinase B cleavage releases encephalitogens from human myelin basic protein. Biochem Biophys Res Commun 1993; 192: 1175–81.

Raemaekers L. Quantitative PCR: theoretical considerations with practical implications. Anal Biochem 1993; 214: 582–5.

Raine CS. Demyelinating diseases. In: Davis RL, Robertson DM, editors. Textbook of Neuropathology. 2nd ed. Baltimore: Williams and Wilkins, 1991: 535–620.

Ries C, Petrides PE. Cytokine regulation of matrix metalloproteinase activity and its regulatory dysfunction in disease. [Review]. Biol Chem Hoppe Seyler 1995; 376: 345–55.

Rosenberg GA, Kornfeld M, Estrada E, Kelley RO, Liotta LA, Stetler-Stevenson WG. TIMP-2 reduces proteolytic opening of blood-brain barrier by type IV collagenase. Brain Res 1992; 576: 203–7.

Rosenberg GA, Dencoff JE, McGuire PG, Liotta LA, Stetler-Stevenson WA. Injury-induced 92-kilodalton gelatinase and urokinase expression in rat brain. Lab Invest 1994; 71: 417–22.

Rosenberg GA, Dencoff JE, Correa N Jr, Reiners M, Ford CC. Effect of steroids on CSF matrix metalloproteinases in multiple sclerosis: relation to blood-brain barrier injury. Neurology 1996; 46: 1626–32.

Wekerle H, Kojima K, Lannes-Vieira J, Lassmann H, Linington C. Animal models. [Review]. Ann Neurol 1994; 36 Suppl: S47–53.

Welgus HG, Campbell EJ, Cury JD, Eisen AZ, Senior RM, Wilhelm SM, et al. Neutral metalloproteinases produced by human mononuclear phagocytes. J Clin Invest 1990; 86: 1496–502.

Wells GM, Catlin G, Cossins JA, Mangan M, Ward GA, Miller KM, et al. Quantitation of matrix metalloproteinases in cultured rat astrocytes using the polymerase chain reaction with a multi-competitor cDNA standard. Glia 1996; 18: 332–40.

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