Acta Neuropathol (1997) 94 : 590–598

REGULAR PAPER

Judy A. Cossins · John M. Clements · Janice Ford
Karen M. Miller · Rod Pigott · Wim Vos
Paul Van Der Valk · Corline J. A. De Groot

Enhanced expression of MMP-7 and MMP-9 in demyelinating multiple sclerosis lesions

Received: 19 December 1996 / Revised: 22 May 1997 / Accepted: 4 June 1997

Abstract The pathology of multiple sclerosis (MS) is characterised by breakdown of the blood-brain barrier accompanied by infiltration of macrophages and T cells into the central nervous system (CNS). Myelin is degraded and engulfed by the macrophages, producing lesions of demyelination. Some or all of these mechanisms might involve proteinases, and here we have studied the cellular localisation and distribution of two matrix metalloproteinases (MMPs), MMP-7 (matrilysin) and MMP-9 (92-kDa gelatinase), in the normal human CNS and active demyelinating MS lesions. Cryostat sections of CNS samples were immunostained with antisera to MMP-7 and MMP-9. In addition, non-radioactive in situ hybridisation (ISH) was performed using a digoxygenin-labelled riboprobe to detect the expression of MMP-7. MMP-7 immunoreactivity was weakly detected in microglial-like cells in normal brain tissue sections, and was very strong in parenchymal macrophages in active demyelinating MS lesions. This pattern of expression was confirmed using ISH. MMP-7 immunoreactivity was not detected in macrophages in spleen or tonsil, indicating that it is specifically induced in infiltrating macrophages in active demyelinating MS lesions. MMP-9 immunoreactivity was detected in a few small blood vessels in normal brain tissue sections, whereas many blood vessels stained positive in CNS tissue sections of active demyelinating MS lesions. The up-regulation of MMPs in MS may contribute to the pathology of the disease.

Key words Immunohistochemistry · In situ hybridisation · Multiple sclerosis · MMP-7 · MMP-9

J. A. Cossins · J. M. Clements · J. Ford · K. M. Miller · R. Pigott
British Biotech Pharmaceuticals, Watlington Road,
Oxford OX4 5LY, UK

C. J. A. De Groot (✉) · P. Van Der Valk · W. Vos
Graduate School Neurosciences Amsterdam,
Department of Pathology, Division of Neuropathology,
Free University Academic Hospital, P.O. Box 7057,
1007 MB Amsterdam, The Netherlands
Fax: 31-20-4442964

Introduction

The underlying cause of multiple sclerosis (MS) is unknown. A characteristic feature of the disease is the ongoing development and disappearance of areas of demyelination (lesions) in the white matter of the central nervous system (CNS) [26]. Initial events leading to the formation of lesions include an inflammatory response and damage to the blood-brain barrier (BBB), allowing mononuclear cells to enter the CNS. Monocytes become activated to parenchymal macrophages which engulf and degrade myelin, and oligodendrocyte death occurs. Glial cells become activated, and later remyelination and astrogliosis occur and the lesion becomes inactive. There is evidence that several cytokines are involved in MS. For example, tumour necrosis factor (TNF-α) is present in astrocytes, macrophages and endothelial cells [38, 43] within MS lesions, and is up-regulated in the cerebral spinal fluid (CSF) and blood mononuclear cells immediately before and during relapse [2, 28, 32]. It is also able to damage the BBB [40] and can damage oligodendrocytes and myelin in vitro [6, 37].

In addition to cytokines, there is increasing evidence that matrix metalloproteinases (MMPs) might be involved in demyelinating diseases such as MS [24]. MMPs comprise a large family of at least 14 structurally related enzymes that are involved in tissue remodelling during development, as well as wound healing [3, 25], tumour growth and metastasis [33] and inflammation [42]. MMPs can process TNF-α to a mature soluble form [12], and MMP-9 (92-kDa gelatinase), which can degrade collagen types IV and V, has also been implicated as an intermediate in TNF-α-induced breakdown of the BBB [36]. Activated T cells secrete MMP-2 and MMP-9, which might aid in their transmigration from the vasculature into the CNS [23, 35]. MMPs can also degrade myelin in vitro [8, 15, 29] and might participate in lesion formation in MS.

The role of MMPs in experimental autoimmune encephalomyelitis (EAE), the animal model for MS, has been well studied. Elevated levels of MMP-9 have been

found in the CSF during EAE [15], and inhibition of MMPs can reverse EAE [16]. In addition, there is an increase in mRNA expression of MMP-7 and MMP-9 in rat spinal cord during EAE [9]. Elevated levels of MMP-9 have also been found in the CSF of MS patients [14] and also in MS lesions [13], but MMP-7 expression in MS has not been reported. We have, therefore, investigated the expression of MMP-7 and MMP-9 in tissue sections of active demyelinating MS lesions, using immunohistochemistry and non-radioactive in situ hybridisation (ISH).

Materials and methods

MS tissue

Human brain and spinal cord tissue was obtained at autopsy (with short post-mortem intervals) from four MS patients and two patients without neurological complications (for details see Table 1). The autopsies were performed under the management of the Netherlands Brain Bank, Amsterdam (coordinator Dr. R. Ravid). In all MS cases, tissue samples were taken from lesions located in the brain and/or spinal cord. Tissue samples from the two control cases were taken from the subcortical white matter or corpus callosum. The clinical diagnosis of MS was confirmed neuropathologically. Human spleen and tonsil samples were obtained from the Tissue Bank of the Department of Pathology, Free University Academic Hospital, Amsterdam. Tissue samples were snap frozen in liquid nitrogen and stored at 196° C. Haematoxylin-eosin (H & E)-stained sections were always prepared from the tissue samples, and tissue sections from MS lesions were stained for 5–10 min with the neutral lipid marker Oil Red O (ORO) to delineate areas of myelin breakdown and demyelination.

Antibodies

Polyclonal anti-MMP-7 antibody RP21 was raised in rabbits against a peptide sequence AEYSLFPNSPKWTSKVC. Anti-MMP-9 mouse monoclonal antibody (mAb) 4H3 (IgG1) was raised against recombinant protein [8] and generated by conventional methods. Mouse anti-human KP1 mAb (IgG1), which recognises the monocyte/macrophage-specific antigen CD68 [30], mouse anti-human leukocyte antigen (LCA, IgG1), rabbit anti-cow glial fibrillary acidic protein (GFAP) which is an astrocytic marker, biotinylated rabbit anti-mouse IgG $F(ab')_2$ and biotinylated swine anti-rabbit IgG $F(ab')_2$ were obtained from Dakopatts (Copenhagen, Denmark). Mouse anti-human HLA-DR/DQ (OKIa, IgG1) was obtained form Ortho (Beerse, Belgium). Horseradish peroxidase (HRP)-linked goat anti-rabbit and monoclonal anti-digoxin, which cross-reacts with digoxygenin, were purchased from Sigma (St. Louis, Mo.).

Table 1 Details of control and MS autopsy tissue (*MS* multiple sclerosis)

	Case no.	Age	Sex	Post-mortem delay	Cause of death/ neuropathology
MS	S115	57	F	5 h 45 min	Aspiration pneumonia/ definitive MS
	S116	35	F	5 h 45 min	Cachexia/definitive MS
	S136	46	M	8 h 00 min	Cachexia/definitive MS
	S276	56	M	5 h 30 min	Respiratory failure/ definitive MS
Controls	S281	89	F	6 h 00 min	Aspiration pneumonia
	S283	82	M	5 h 30 min	Respiratory failure

Western blotting

Recombinant MMPs were purified [8] and separated on pre-cast 4–20% TRIS-glycine gels (Novex, San Diego, Calif.) by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) [22], and blotted onto nitrocellulose using conventional methods. The nitrocellulose was incubated in blocking buffer, which was 0.01 M phosphate-buffered saline (PBS) containing 0.5% powdered milk and 0.1% Tween 20, for 1 h at room temperature (RT). The filter was washed in wash buffer (PBS containing 0.1% Tween 20) and then incubated with 10 μg/ml RP21 diluted in blocking buffer for 2 h at RT. The filter was washed as before and incubated in blocking buffer containing HRP-linked goat anti-rabbit diluted 1:1000 for 1 h at RT. The filter was washed and peroxidase activity was demonstrated using PBS containing 3,3′-diaminebenzidinetetrahydrochloride (DAB: Sigma), 0.05% H_2O_2, 0.02% $CoCl_2$ and 0.02% $(NH_4)_2Ni(SO_4)_2$.

RNA probe for ISH of MMP-7

cDNA encoding full-length human MMP-7 was cloned into pGEM4Z, and the construct linearised by digestion with *Hin*dIII or *Eco*RI for antisense and sense probes, respectively. Digoxygenin-labelled RNA probes were generated using the DIG RNA Labelling Kit (Boehringer Mannheim, Germany), and the RNA was then ethanol precipitated, resuspended in 40 μl tissue culture grade water (Sigma) and stored at –20° C. Relative labelling efficiency of the two probes was analysed by Northern blot and by dot blots of probes at decreasing concentrations, using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

Immunohistochemistry

Serial 7-μm-thick cryostat sections of MS lesions and control tissue were collected onto glass slides. The sections were either fixed with 4% paraformaldehyde in PBS for 15 min or with acetone for 10 min. All washes were carried out for 15 min with PBS containing 0.1% saponin, and antibodies were diluted in PBS containing 0.1% saponin and 1% bovine serum albumin. Sections were immunostained by the streptavidin-biotin complex (sABC) procedure as described previously [10]. Briefly, the slides were washed and then pre-incubated with 1% H_2O_2 + 0.02% NaN_3 in PBS for 30 min. Sections were pre-incubated with either 2% normal rabbit serum (for MMP-9, KP1, LCA and OKIa staining) or 2% normal swine serum (for MMP-7 and GFAP staining) for 10 min. Primary antibodies were diluted [RP21 was used at 5 μg/ml, 4H3 was used at 9 μg/ml, KP1 was diluted 1:400, leukocyte common antigen (LCA) 1:50, HLA-DR/DQ (OKIa) 1:100 and GFAP was diluted 1:1000], and incubated for 1 h at RT, followed by washing. Slides were incubated with biotinylated secondary antibodies [biotinylated rabbit anti-mouse IgG $F(ab')_2$ was used 1:500 and biotinylated swine anti-rabbit IgG $F(ab')_2$ was used 1:300] for 30 min at RT and then washed. HRP-linked sABC (sABC-HRP: Dakopatts) was used for 1 h at RT, and peroxidase activity was demonstrated with 0.5 mg/ml DAB in 0.05 M TRIS-HCl pH 7.6 containing 0.03% H_2O_2 for 5 min. Controls were set up omitting the primary antibodies. A further control was also included in which RP21 was pre-absorbed with purified MMP-7.

In situ hybridisation

All solutions were purchased as RNase-free solutions or were pretreated with 0.02% diethyl pyrocarbonate. 3-Aminopropyltriethoxysilane (APES)-coated slides were made by incubation with acetone for 5 min at RT, incubation with acetone containing 2%

APES (Sigma) for 5 min at RT and then incubation with acetone for 5 min at RT. Slides were washed with RNase-free distilled water and dried at 37° C. Prior to use, the slides were activated by incubation with 3% glutaraldehyde for 5 min, washed with distilled water and air-dried. Serial 6-µm-thick cryostat sections were collected on APES-coated slides. The sections were fixed according to Woodroofe and Cuzner [43]. Briefly, sections were fixed in freshly prepared 4% paraformaldehyde in PBS containing 0.02% diethyl pyrocarbonate (DEPC) for 5 min, washed in PBS and incubated with freshly prepared 0.1 M triethanolamine + 0.25% acetic anhydride for 10 min. Sections were rinsed in 70% ethanol, 96% ethanol and 100% ethanol, incubated with chloroform for 5 min and then rinsed in 96% ethanol. Sections were air dried ready for hybridisation. Probes were diluted in 50% formamide + 10% dextran sulphate + 0.5 × SSC + 1 µg/ml surgle-stranded (ss)DNA and 100 µl probe added to each slide, which was then covered with a coverslip. Slides were heated to 63° C for 7 min and hybridised at 55° C for 3 h. They were washed with 0.05 × SSC at 60° C for 1 h, with several changes of buffer. Slides were washed for 1 min in PBS containing 0.25% tween, and were used for immunological detection as previously described [20]. Briefly, slides were incubated with monoclonal anti-digoxin (diluted 1:250 in PBS containing 0.5% blocking reagent: Boehringer Mannheim) for 1 h, washed in 0.005% Tween-20 in PBS for 15 min and incubated with biotinylated rabbit anti-mouse IgG $F(ab')_2$ diluted 1:500 in 0.5% blocking reagent in PBS for 30 min. Slides were washed as before, and incubated with sABC-HRP diluted 1:200 in 0.5% blocking reagent for 1 h. Slides were rinsed in PBS and peroxidase activity was detected with 50 mM TRIS-HCl pH 7.6 containing 0.2 mg/ml DAB, 0.12% $NiSO_2(NH_2)_2SO_4$ and 0.03% H_2O_2. Slides were counterstained with haematoxylin and mounted with coverslips using DePex (BDH, Poole, UK). Hybridisation with the sense probe or pretreatment of sections with RNase served as negative controls.

Results

Specificity of polyclonal antibody RP21

Because RP21 is polyclonal it is potentially cross-reactive. We, therefore, tested it for recognition of purified MMP-1, -2, -3, -7 -9 and -12 by Western blot. Figure 1 shows that RP21 recognised MMP-7 but showed insignificant recognition of the other MMPs tested.

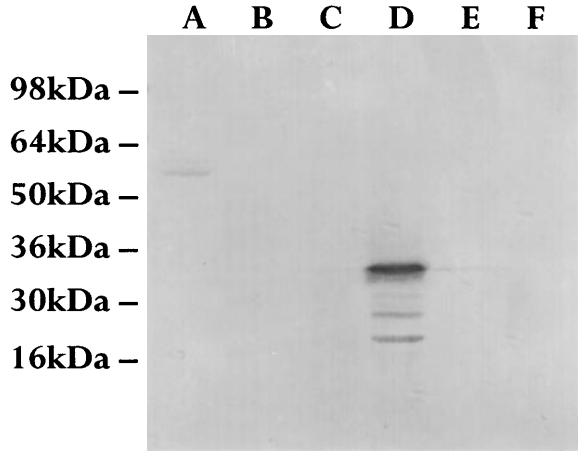


Fig. 1 Western blot demonstrating that RP21 polyclonal antisera is specific for MMP-7 *(lane D)* but shows minimal cross-recognition of MMP-1 *(lane A)*, -2 *(lane B)*, -3 *(lane C)*, -9 *(lane E)* and -12 *(lane F)* (*MMP* matrix metalloproteinase)

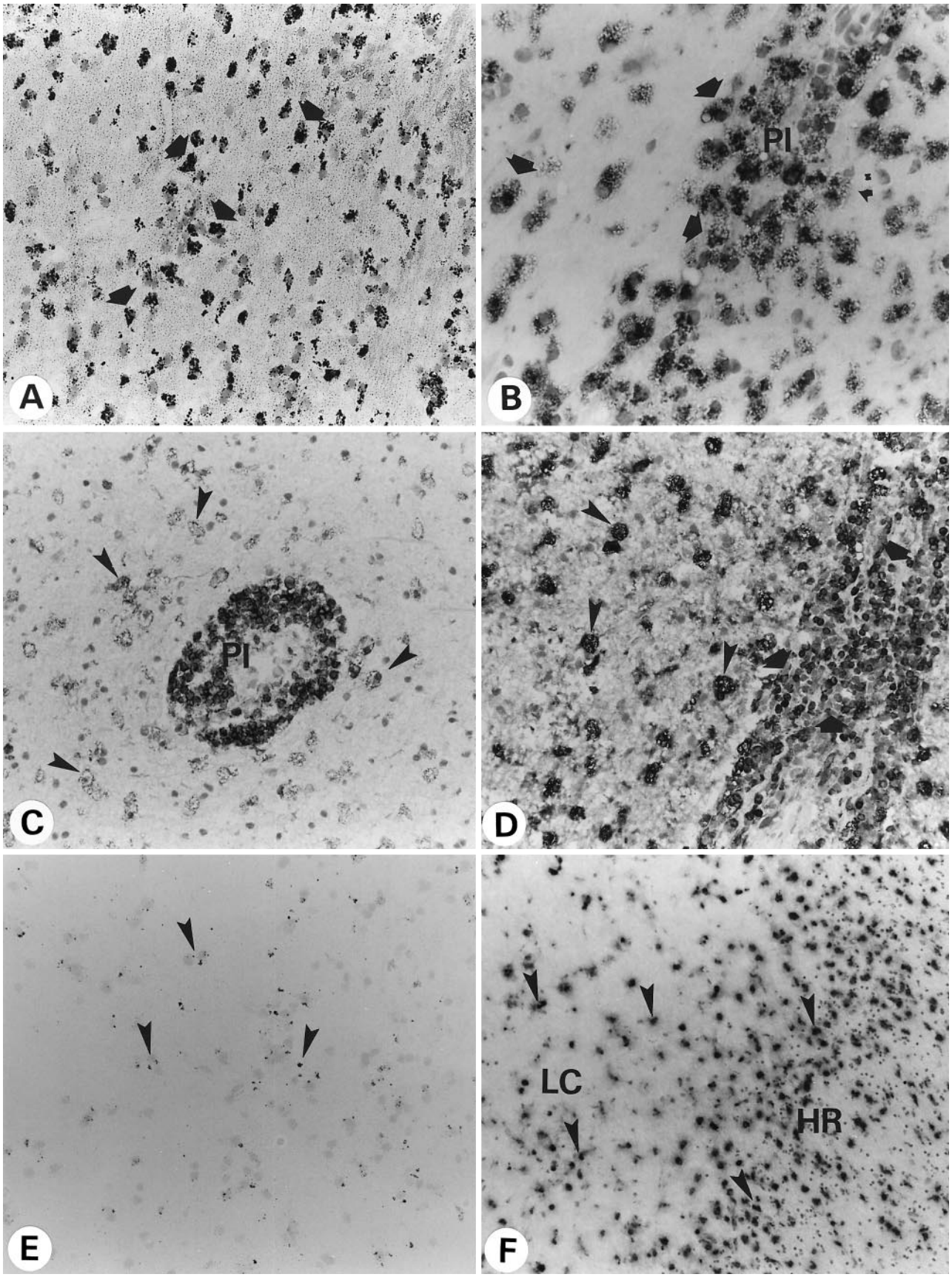
Neuropathological evaluation

To evaluate MS lesion activity and histological characterisation of the investigated tissue samples, cryostat sections from all MS samples were histochemically stained with H&E, ORO and immunohistochemically with KP1, LCA, HLA-DR/DQ (OKIa) and GFAP antibodies. Conforming the classification described by Bo et al. [4, 5] four MS lesions from the brain and two from the spinal cord were identified containing abundant phagocytic macrophages and were classified as active demyelinating (cases S136, S115 and S116) (Fig. 2A–D), and two MS lesions from the brain had a hypocellular centre and hypercellular rims and were classified as chronic active demyelinating (case S276) (Fig. 2E,F). In normal control brain no inflammatory cells were detected.

MMP expression

In normal white matter derived from control cases, a weak immunoreactivity for MMP-7 was detected only in microglial-like cells distributed throughout the white matter (Fig. 3A). In active demyelinating MS lesions, however, a very strong immunoreactivity for MMP-7 was found in parenchymal macrophages distributed throughout the centre of the lesion (Fig. 3B). Immunoreactivity for MMP-7 was detected in microglial-like cells located at the edge of the demyelinated regions, and immunoreactivity became weaker with distance away from the leading edge of the lesion (Fig. 3B). In adjacent sections the parenchymal macrophages that were distributed throughout the lesion site were immunoreactive for KP1, confirming their macrophage phenotype (Fig. 3C). MMP-7-immunoreactive cells were also found in perivascular cuffs (Fig. 3D) in active demyelinating lesions. Finally, a small population of cells that were KP1 negative and had the morphology of reactive astrocytes stained weakly positive for MMP-7 in active demyelinating MS lesions at the edge of the lesion (not shown). In adjacent sections the identity of astrocytes was confirmed by GFAP staining (not shown). In chronic active demyelinating MS lesions a few parenchymal macrophages, still present in the lesion, were weakly immunoreactive for MMP-7 (not shown). No significant immunoreactivity was observed in any of the above tissues when RP21 antisera had been preabsorbed with purified recombinant MMP-7 (not shown). In addition, no significant MMP-7-immunoreactive cells could be detected in tonsil or spleen, indicating that antibody RP21 does not aspecifically bind to macrophages (not shown).

No significant MMP-9 immunoreactivity was found in normal white matter of control cases (Fig. 4A). In active demyelinating MS lesions the number and intensity of positively stained cerebral blood vessels was significantly increased (Fig. 4B). The expression of MMP-9 was detected in leukocytes within the lumen of the blood vessels, and also within endothelial cells (Fig. 4C). Perivascular infiltrates were not immunoreactive for MMP-9 (see also

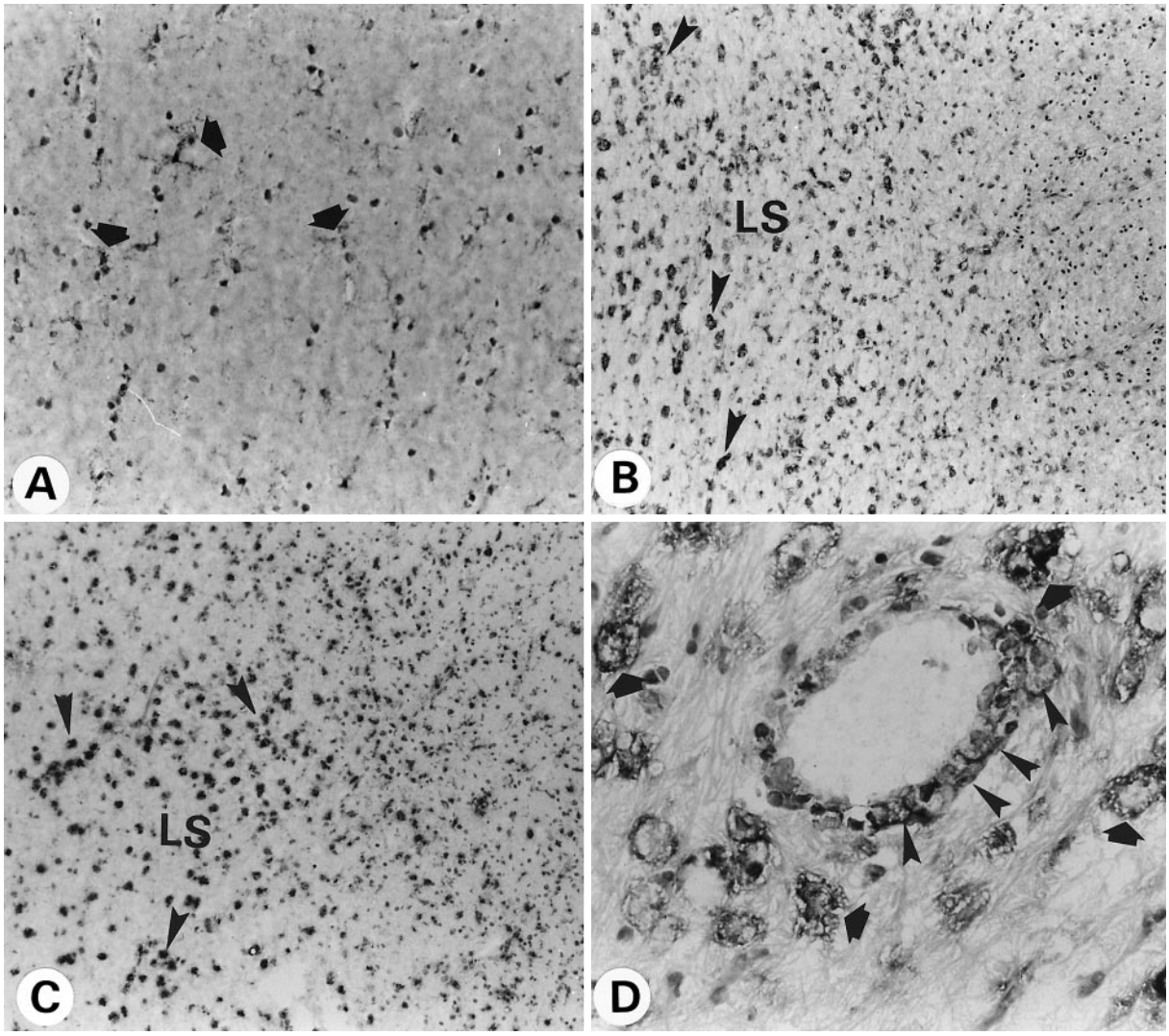

A
B
PI
C
PI
D
E
F
LC
HR

◀ **Fig. 2A–F** Cryostat sections from active (cases S115/S136) and chronic active demyelinating MS lesions (case S276). **A** ORO staining of an active demyelinating MS lesion. Degraded lipid was detected in the parenchymal macrophages (*arrows*). **B** Immunohistochemical staining of an active demyelinating MS lesion with a macrophage-specific mAb KP1 (CD68). Strong immunoreactivity for KP1 was detected in perivascular and parenchymal (foamy) macrophages (*arrows*; *PI* perivascular infiltrate). **C** Immunohistochemical staining of an active demyelinating MS lesion with a leukocyte-specific mAb LCA (CD45). A perivascular cuff containing lymphocytes with strong immunoreactivity for LCA (*arrows*). Moderate immunoreactivity for LCA was detected in parenchymal macrophages (*arrowheads*). **D** Immunohistochemical staining of an active demyelinating MS lesion with an MHC class II -specific mAb OKIa (anti-HLA-DR/DQ). Strong immunoreactivity for OkIa was detected in perivascular infiltrates (*arrows*) and in parenchymal macrophages (*arrowheads*). **E** ORO staining of a chronic active demyelinating MS lesion. Residual ORO reactivity in parenchymal macrophages in the lesion center was detected. **F** Immunohistochemical staining of a chronic active demyelinating MS lesion with KP1 mAb. Fewer KP1-positive parenchymal macrophages were detected in the lesion center, whereas in the hypercellular rim abundant KP1 immunoreactivity was present in macrophages (*arrowheads*; *LC* lesion center, *HR* hypercellular rim) (*MS* multiple sclerosis, *ORO* Oil Red O, *LCA* leukocyte common antigen). **A–E** × 200, **F** × 100

Fig. 4C). MMP-9 immunoreactivity was found in red pulp macrophages in spleen, whereas only blood vessels were immunolabelled in tonsil (not shown).

To confirm the immunohistochemical staining results of MMP-7, we also carried out non-radioactive ISH on

Fig. 3A–D Cryostat sections from normal white matter (case S283) and an active demyelinating MS lesions (case S136). **A** Immunohistochemical staining of normal white matter with an MMP-7-specific pAb. Weak immunoreactivity was detected in microglial-like cells (*arrows*). **B** Immunohistochemical staining of the edge of an active demyelinating MS lesion with an MMP-7-specific pAb. Strong immunoreactivity was detected in parenchymal macrophages within the MS lesion site (*LS*; *arrowheads*). The immunoreactivity for MMP-7 in the microglial-like cells became weaker with distance from the lesion site. **C** Immunohistochemical staining of the edge of an active demyelinating MS lesion with strong KP1-positive parenchymal macrophages within the MS lesion site (*arrowheads*). The immunoreactivity for KP1 in the microglial-like cells became weaker with distance from the lesion site. **D** Strong immunoreactivity for MMP-7 was detected in parenchymal macrophages within a perivascular cuff (*arrowheads*) and in the surrounding tissue (*arrows*) within an active demyelinating MS lesion (*pAB* polyclonal antibody). **A–C** × 200, **D** × 400



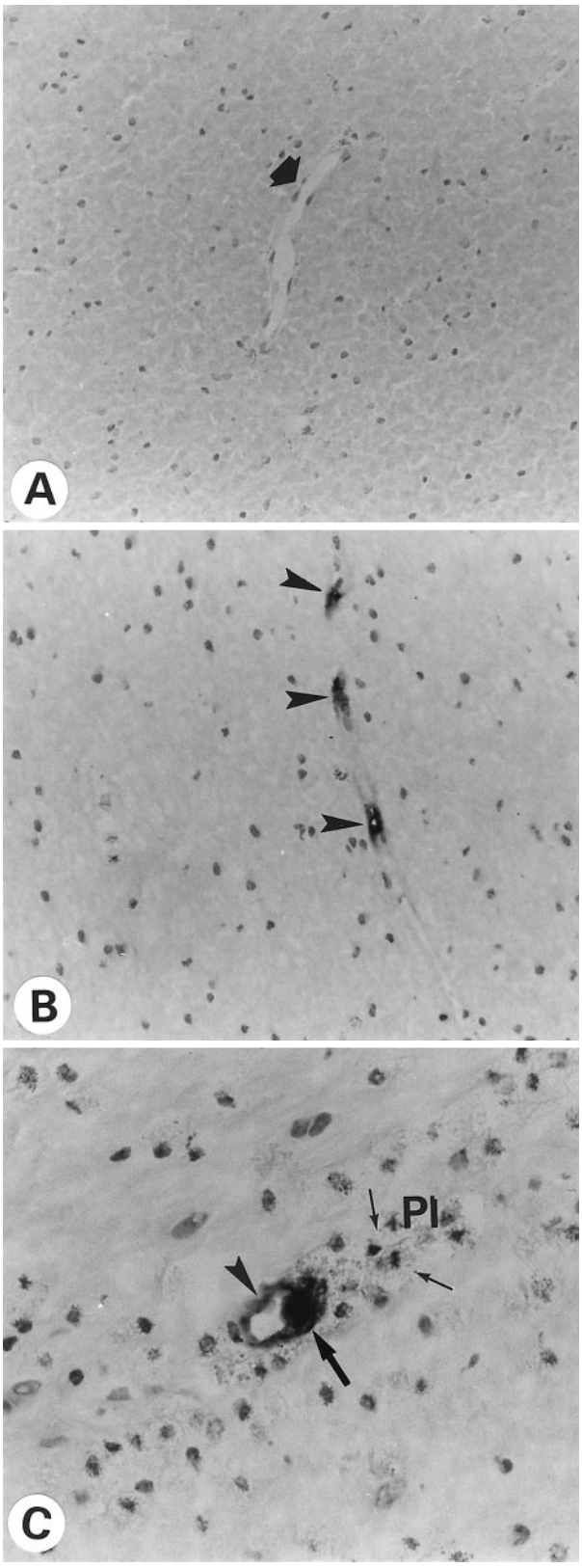


Fig. 4A–C Cryostat sections from normal white matter (case S283) and an active demyelinating MS lesions (case S136). **A** Immunohistochemical staining of normal white matter with an MMP-9-specific mAb. No staining of cerebral blood vessels was detected (*arrow*). **B** Immunoreactivity for MMP-9 was detected in small blood vessels in an active demyelinating MS lesion (*arrowheads*). Strong immunoreactivity for MMP-9 of a blood vessel wall (*arrowhead*) containing an MMP-9-positive leukocyte (*thick arrow*) present within the lumen. Foamy macrophages within the perivascular infiltrate are MMP-9-negative. **A,B** × 200, **C** × 400

control brain tissue and on active demyelinating MS lesions to analyse mRNA expression. In normal white matter of control cases and in white matter outside MS lesions a punctate labelling of microglial-like cells could be detected (Fig. 5A,B). In active demyelinating MS lesions expression of MMP-7 mRNA was found in parenchymal macrophages (Fig. 5C) and in perivascular infiltrates containing foamy macrophages (Fig. 5D). No significant staining was observed either when the sense probe was used (Fig. 5E, F) or when the tissue was pretreated with RNase (not shown), indicating that the mRNA MMP-7 labelling is specific.

Discussion

In this study we have made the novel observation that there was a very strong immunoreactivity for MMP-7 in parenchymal macrophages distributed through active demyelinating MS lesions compared with normal white matter CNS tissue. We have previously observed an increase in MMP-7 mRNA expression of over 500-fold in rat spinal cord during EAE, with MMP-7 immunoreactivity largely restricted to the invading mononuclear cells within perivascular lesions in the spinal cord [9], thus supporting the data presented here. The expression of MMP-7 by parenchymal macrophages in active demyelinating MS lesions has not been previously reported. It is known to be expressed in peripheral blood monocytes [39] and also in infiltrating monocytes in cystic fibrosis [7]. From their results, Busiek et al. [7] suggested a role for MMP-7 in migration of activated monocytes within regions of acute inflammation. Our results clearly support this, especially since we detected only low immunoreactivity for MMP-7 within macrophages in tissue where there is little or no inflammation, such as chronic active MS lesions, spleen and tonsil.

Microglial-like cells were weakly positive for MMP-7 in control white matter brain tissue. However, within active demyelinating MS lesions microglial-like cells stained more strongly, particularly at the leading edges of the lesion. This immunoreactivity became weaker with distance away from the leading edge. The regulation of MMP-7 in vivo is unknown, but the gene contains an activator protein-1 (AP-1) binding site and a sequence homologous to transforming growth factor beta (TGF-β-inhibitory element [27]. TGF-β is associated with recovery in EAE [21] and MS [32], and can inhibit chronic relapsing EAE [31]. Low levels of immunoreactivity were also

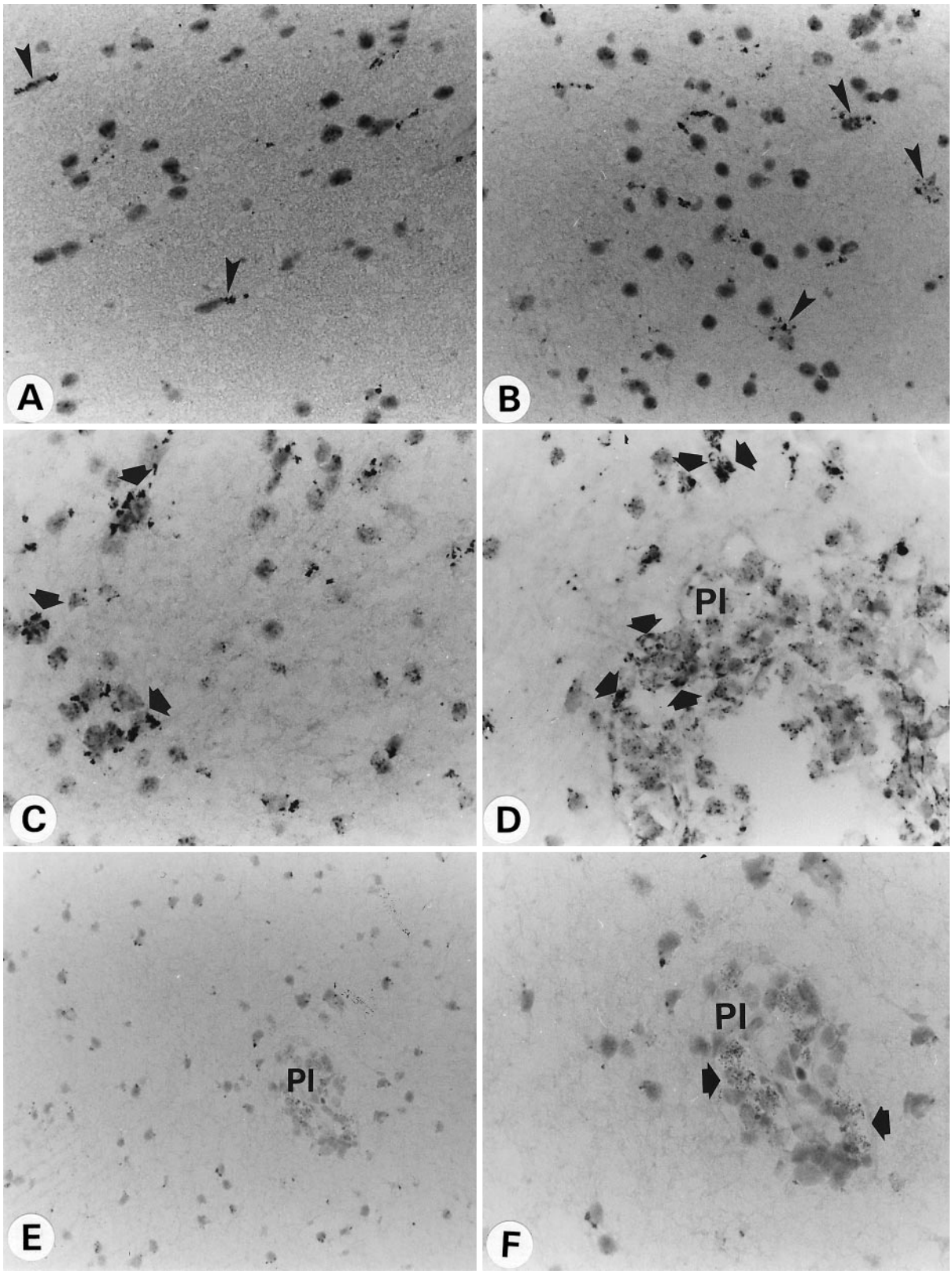


Fig. 5A–F Cryostat sections from normal white matter (case S283) and an active demyelinating MS lesions (case S136). **A** MMP-7 mRNA expression in normal white matter using an MMP-7 antisense probe in non-radioactive ISH. Punctate staining was detected in microglial-like cells (*arrowheads*). **B** MMP-7 mRNA expression near an active demyelinating MS lesion. Punctate staining was detected in microglial-like cells (*arrowheads*). **C** MMP-7 mRNA expression in an active demyelinating MS lesion. Punctate staining was detected in parenchymal macrophages (*arrows*) and **D** in perivascular infiltrates containing macrophages. **E** Non-radioactive ISH of an active demyelinating MS lesion using an MMP-7 sense probe. No significant staining was detected. **F** A higher magnification of **E** showing no staining of perivascular (foamy) macrophages using the sense probe (*ISH* in situ hybridisation). **A–C, E** × 200, **D, F** × 400

observed in cells with the morphology of reactive astrocytes at the edge of active demyelinating MS lesions but not in control brain tissue. The identity of these cells as astrocytes was confirmed by GFAP staining of adjacent sections.

The anti-MMP-7 antisera that we used was polyclonal and we carried out additional controls to show that the above observations were not due to cross-reactivity of the antisera. Firstly, RP21 did not recognise five other purified MMPs (MMP-1, -2, -3, -9 and -12) by Western blot. Secondly, pre-absorbtion of RP21 with purified recombinant MMP-7 completely removed the immunoreactivity described above. These data indicate that the immunoreactivity presented here is due to specific recognition of MMP-7 by RP21.

We observed an increase in the intensity and number of blood vessels that were positively stained for MMP-9 in active demyelinating MS lesions compared with control tissue white matter. The expression of this enzyme appears to be within monocytes, granulocytes, and possibly neutrophils, which express high levels of MMP-9 and neutrophil collagenase [18] and store them in intracellular granules: the enzymes can be activated upon release from the cells [11]. Staining of endothelial cells was also very strong in MS lesions compared with control tissue white matter. Others have seen expression of this enzyme by endothelial cells in vitro [17] and in normal and MS white matter [24]. MMP-9 is thought to be an intermediate in TNF-α-induced opening of the BBB [36] and might be involved in migration of activated T cells into the CNS [23]. Thus, up-regulation of the enzyme, particularly in blood vessels adjacent to perivascular cuffs, is consistent with MMP-9 being involved in breakdown of the BBB in MS.

The lack of MMP-9 immunoreactivity within parenchymal macrophages in active demyelinating MS lesions was surprising because macrophages are known to express it [41] and others have detected it in macrophages, microglia and astrocytes in MS [13, 24]. These contradictions might be due to differential recognition by the antibodies of alternatively glycosylated forms of MMP-9. Another explanation might be that the polyclonal antibodies used by others are cross-reactive.

MMP-7 expression in microglial-like cells in normal brain tissue sections and outside active demyelinating lesions, and in parenchymal macrophages in active demyelinating lesions, was confirmed using non-radioactive ISH. We did not observe staining of astrocytes using ISH, even though these cells were weakly immunoreactive for MMP-7. A possible explanation is that the level of MMP-7 mRNA is too low to be detected by this method.

Our results show the enhanced expression of MMP-7 and MMP-9 in active demyelinating MS lesions, although the activation state of these enzymes and whether they are complexed with their natural inhibitors, tissue inhibitors of MMPs (TIMPs), is unknown. In comparison with other known MMPs, MMP-7 does not have a hemopexin-like domain which is involved in substrate specificity and TIMP binding and as a consequence it interacts poorly with TIMPs [1]. MMP-7 also has potent and a broad spectrum of proteolytic activity against a number of extracellular substrates, including proteoglycans [19] which are a major constituent of the extracellular matrix (ECM) of the CNS [34], myelin [8] and TNF-α [12]. It could, therefore, play a crucial role in the development of MS lesions by one (or more) of several mechanisms, namely degradation of the CNS ECM, proteolysis of myelin, activation of TNF-α and generation of encephalitogenic peptides. MMP-9 might be involved in breakdown of the BBB and migration of T lymphocytes into the CNS. Therefore inhibition of one or both of these enzymes might be of therapeutic value in MS.

References

1. Baragi VM, Fliszar CJ, Conroy MC, Ye Q-Z, Shipley JM, Welgus HG (1994) Contribution of the C-terminal truncated domain of metalloproteinases to binding by TIMP: C-terminal truncated stromelysin and matrilysin exhibit equally compromised affinities compared to full-length stromelysin. J Biol Chem 269:12692–12697
2. Beck J, Rondot P, Catinot L, Falcoff E, Kirchner H, Wiotzerbin J (1988) Increased production of interferon gamma and tumour necrosis factor precedes clinical manifestations in multiple sclerosis: do cytokines trigger off exacerbations? Acta Neurol Scand 78:318–323
3. Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, Engler JA (1993) Matrix metalloproteinases: a review. Crit Rev Oral Biol Med 4:197–250
4. Bö L, Mork S, Kong PA, Nyland H, Pardo CA, Trapp BD (1994) Detection of MHC class II-antigens on macrophages and microglia, but not on astrocytes and endothelia in active multiple sclerosis lesions. J Neuroimmunol 51:135–146
5. Bö L, Dawson TM, Wesselingh S, Mork S, Choi S, Kong PA, Hanley D, Trapp BD (1994) Induction of nitric oxide synthase in demyelinating lesions of multiple sclerosis brains. Ann Neurol 36:778–786
6. Brosnan CF, Selmaj K, Raine CS (1988) Hypothesis: a role for tumour necrosis factor in immune-mediated demyelination and its relevance to multiple sclerosis. J Neuroimmunol 18:87–94
7. Busiek DF, Baragi V, Nehring LC, Parks WC, Welgus HG (1995) Matrilysin expression by human mononuclear phagocytes and its regulation by cytokines and hormones. J Immunol 154:6484–6491
8. Chandler S, Coates R, Gearing A, Lury J, Wells G, Bone E (1995) Matrix metalloproteinases degrade myelin basic protein. Neurosci Lett 201:223–226
9. Clements JM, Cossins JA, Wells GMA, Corkill DJ, Helfrich K, Wood LM, Pigott R, Stabler G, Ward GA, Gearing AJH, Miller KM (1997) Matrix metalloproteinase expression during experimental autoimmune encephalomyelitis and effects of a combined matrix metalloproteinase and tumour necrosis factor-α inhibitor. J Neuroimmunol 74:85–94
10. De Groot CJA, Theeuwes JWM, Dijkstra CD, Van Der Valk P (1995) Postmortem delay effects on neuroglial cells and brain macrophages from Lewis rats with acute experimental allergic encephalomyelitis: an immunohistochemical and cytochemical study. J Neuroimmunol 59:123–134
11. Desrochers PE, Mookhtiar K, Van Wart HE, Hasty KA, Weiss SJ (1992) Proteolytic inactivation of α_1-proteinase inhibitor α_1-antichymotrypsin by oxidatively activated human neutrophil metalloproteinases. J Biol Chem 267:5005–5012
12. Gearing AJH, Beckett P, Christodoulou M, Churchill M, Clements J, Davidson AH, Drummond AH, Galloway WA, Gilbert R, Gordon JL, Leber TM, Mangan M, Miller K, Nayee P, Owen K, Patel S, Thomas W, Wells G, Wood LM, Woolley K (1994) Processing of tumour necrosis factor-α precursor by metalloproteinases. Nature 370:555–557

13. Gijbels K, Steinman L (1994) Gelatinase B producing cells in multiple sclerosis lesions. J Cell Biochem Suppl 18D:143
14. Gijbels K, Masure S, Carton H, Opdenakker G (1992) Gelatinase in the cerebrospinal fluid of patients with multiple sclerosis and other inflammatory neurological disorders. J Neuroimmunol 41:29–34
15. Gijbels K, Proost P, Masure S, Carton H, Billau A, Opdenakker G (1993) Gelatinase B is present in the cerebrospinal fluid during experimental autoimmune encephalomyelitis and cleaves myelin basic protein. J Neurosci Res 36:432–440
16. Gijbels K, Galardy RE, Steinman L (1994) Reversal of experimental autoimmune encephalomyelitis with a hydroxamate inhibitor of matrix metalloproteases. J Clin Invest 94:2177–2182
17. Hanemaaijer R, Koolwijk P, Le Clercq L, De Vree WJA, Van Hinsbergh VWM (1993) Regulation of matrix metalloproteinase expression in human vein and microvascular endothelial cells. Biochem J 296:803–809
18. Hibbs MS, Hasty KA, Seyer JM, Kang AH, Mainardi CL (1985) Biochemical and immunological characterisation of the secreted forms of human neutrophil gelatinase. J Biol Chem 260:2493–2500
19. Imai K, Yokohama Y, Nakanishi I, Ohuchi E, Fujii Y, Nakai N, Okada Y (1995) Matrix metalloproteinase 7 (matrilysin) from human rectal carcinoma cells. J Biol Chem 270:6691–6697
20. Jiwa NM, Kanavaros P, Bruin PC de, Valk P van der, Horstman A, Vos W, Mullink H, Walboomers JMM, Meijer CJLM (1993) Presence of Epstein-Barr virus harbouring small and intermediate-sized cells in Hodgkins disease. Is there a relationship with Reed-Sternberg cells? J Pathol 170:129–136
21. Khoury SJ, Hancock WW, Weiner HL (1992) Oral tolerance to myelin basic protein and natural recovery from experimental autoimmune encephalomyelitis are associated with downregulation of inflammatory cytokines and differential upregulation of transforming growth factor beta, interleukin 4, and prostaglandin E expression in the brain. J Exp Med 176:1355–1364
22. Laemelli UK, Favre M (1973) Maturation of the head of bacteriophage T4. J Mol Biol 80:575–599
23. Leppert D, Waubant E, Galardy R, Bunnett NW, Hauser SL (1995) T cell gelatinases mediate basement membrane transmigration in vitro. J Immunol 154:4379–4389
24. Maeda A, Sobel RA (1996) Matrix metalloproteinases in the normal human central nervous system, microglial nodules, and multiple sclerosis lesions. J Neuropathol Exp Neurol 55:300–309
25. Matrisian LM (1992) The matrix-degrading metalloproteinases. BioEssays 14:455–463
26. McDonald WI, Miller DH, Barnes D (1992) The pathological evolution of multiple sclerosis. Neuropathol Appl Neurobiol 18:319–334
27. McDonnell S, Wright JH, Gaire M, Matrisian LM (1994) Expression and regulation of stromelysin and matrilysin by growth factors and oncogenes. Biochem Soc Trans 22:58–63
28. Merrill JE, Strom SR, Ellison GW, Meyers LW (1989) In vitro study of mediators of inflammation in multiple sclerosis. J Clin Immunol 9:84–96
29. Proost P, Van Damme J, Oppendakker G (1993) Leukocyte gelatinase B cleavage releases encephalitogens from human myelin basic protein. Biochem Biophys Res Commun 193: 1175–1181
30. Pulford KA, Rigney EM, Micklem KJ, Jones M, Stross WP, Gatter KC, Mason DY (1989) KP1: a new monoclonal antibody that detects a monocyte/macrophage associated antigen in routinely processed tissue sections. J Clin Pathol 42:414–421
31. Racke MK, Dhib-Jalbut S, Canella B, Albert PS, Raine CS, McFarlin DE (1991) Prevention and treatment of chronic relapsing experimental allergic encephalomyelitis by transforming growth factor-β1. J Immunol 146: 3012–3017
32. Rieckmann P, Albrecht M, Kitze B, Weber T, Tumani H, Broocks A, Luer W, Helwig A, Poser S (1995) Tumour necrosis factor-α messenger RNA expression in patients with relapsing-remitting multiple sclerosis is associated with disease activity. Ann Neurol 37:82–88
33. Ries C, Petrides PE (1995) Cytokine regulation of matrix metalloproteinase activity and its dysfunction in disease. Biol Chem Hoppe-Seyler 376:345–355
34. Romanic AM, Madri JA (1994) Extracellular matrix-degrading proteinases in the nervous system. Brain Pathol 4:145–156
35. Romanic AM, Madri JA (1994) The induction of 72-kD gelatinase in T cells upon adhesion to endothelial cells is VCAM-1 dependent. J Cell Biol 125:1165–1178
36. Rosenberg GA, Estrada EY, Dencoff JE, Stetler-Stevenson WG (1995) Tumour necrosis factor-α-induced gelatinase B causes delayed opening of the blood-brain barrier: an expanded therapeutic window. Brain Res 703:151–155
37. Selmaj K, Raine CS (1988) Tumor necrosis factor mediates myelin damage in organotypic cultures of nervous tissue Ann NY Acad Sci 540:568–570
38. Selmaj K, Raine CS, Canella B, Brosnan CF (1991) Identification of lymphotoxin and tumour necrosis factor in multiple sclerosis lesions. J Clin Invest 87:949–954
39. Shapiro SD (1994) Elastolytic metalloproteinases produced by human mononuclear phagocytes. Am J Respir Crit Care Med 150:S160–S164
40. Sharief MK, Thompson EJ (1992) In vivo relationship of tumour necrosis factor-α to blood-brain barrier damage in patients with active multiple sclerosis. J Neuroimmunol 38:27–34
41. Wilhelm SM, Collier IE, Marmer BL, Eisen AZ, Grant GA, Goldberg GI (1989) SV-40-transformed human lung fibroblasts secrete a 92 kDa type IV collagenase which is identical to that secreted by normal lung macrophages. J Biol Chem 264:17213–17221
42. Woessner JF Jr (1992) Role of cellular proteinases and their protein inhibitors in inflammation. In: Whicher JT, Woessner JF Jr (eds) Immunology and medicine, biochemistry of inflammation. Kluwer, Dordrecht, pp 57–89
43. Woodroofe MN, Cuzner ML (1993) Cytokine mRNA expression in inflammatory multiple sclerosis: detection by non-radioactive in situ hybridization. Cytokine 5:583–588