Matrix Metalloproteinases and Their Tissue Inhibitors in Cuprizone-Induced Demyelination and Remyelination of Brain White and Gray Matter

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INTRODUCTION

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases, which, together with endogenous tissue inhibitors of metalloproteinases (TIMPs), play essential roles in tissue remodeling. In addition to their functions in structural rearrangement of the extracellular matrix, these proteins regulate numerous physiological processes such as development, plasticity, cellular growth, survival, apoptosis, migration, and signaling (1, 2). Uncontrolled and abundant expression of MMPs is considered to result in harmful tissue destruction; within the central nervous system (CNS), they are implicated in the pathogenesis of inflammatory demyelinating diseases such as multiple sclerosis (MS) (3). In addition to their involvement in blood-brain barrier (BBB) damage, perpetuation of inflammation, and neurotoxicity, MMPs can directly degrade myelin proteins (4–7). Recently, MMPs and TIMPs have also been implicated in regenerative processes including axonal growth, oligodendrocyte maturation, remyelination, and maintenance of healthy myelin (8–10). Alterations in MMP/TIMP equilibrium have been found in MS (11, 12), as well as in related animal models including experimental autoimmune encephalomyelitis (EAE) (13), Theiler murine encephalomyelitis (14), and canine distemper virus encephalomyelitis (15).

In recent years, extensive cortical demyelination in MS patients has been recognized and highlighted (16). Because the extent of pathology and remyelination in the cortex (CTX) differs from that in the white matter, different mechanisms of tissue injury in the white and gray matter have been proposed (17).

Mice fed bis-cyclohexanone oxalylhydrazone (cuprizone) develop oligodendrocyte depletion and reversible demyelination in the corpus callosum (CC) and CTX (18). This model is advantageous for studying CNS remyelination without the influence of the peripheral immune system component because the BBB remains intact (19).

Because both beneficial and detrimental functions of MMPs in CNS demyelinating diseases have been proposed, a better understanding of their complex roles is needed. The aim of this study was to investigate molecular and cellular expression of MMPs and TIMPs in the brain white and gray matter within narrow time intervals during demyelination and remyelination in the cuprizone model.

Abstract

Apart from their involvement in the pathogenesis of demyelinating diseases such as multiple sclerosis, there is emerging evidence that matrix metalloproteinases (MMPs) also promote remyelination. We investigated region-specific expression patterns of 11 MMPs and 4 tissue inhibitors of metalloproteinases (TIMPs) in the cuprizone murine demyelination model. Messenger RNA (mRNA) was extracted at different time points of exposure to cuprizone from microdissected samples of corpus callosum, cortex, and ex vivo isolated microglia and analyzed using quantitative reverse transcription polymerase chain reaction. Matrix metalloproteinase 12 and TIMP-1 mRNA were significantly upregulated versus age-matched controls in both areas during demyelination and remyelination. Matrix metalloproteinases 3, 11, and 14 mRNA were upregulated only in white matter during remyelination. Matrix metalloproteinase 24 mRNA was downregulated during both demyelination and remyelination. To identify potential cellular sources of the MMPs and TIMPs, we isolated microglia and detected high MMP-12 and TIMP-2 mRNA upregulation at the peak of demyelination. By immunohistochemistry, MMP-3 protein was localized in astrocytes and MMP-12 was identified in microglia, astrocytes, and cells of oligodendrocyte lineage. These findings suggest that MMPs and TIMPs have roles in the regulation of demyelination and remyelination in this model. Moreover, differences in the expression levels of these genes between white and gray matter reveal region-specific molecular mechanisms.

Key Words: Astrocytes, Cuprizone, Demyelination, Matrix metalloproteinases, Microglia, Remyelination, Tissue inhibitors of metalloproteinases.

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MATERIALS AND METHODS

Animals and Induction of Demyelination

C57BL/6 mice were purchased from Charles River (Sulzfeld, Germany). Animals underwent weekly routine cage maintenance and were microbiologically monitored according to the recommendations of the Federation of European Laboratory Animal Science Associations (20). Food and water were available ad libitum. All research and animal care procedures were approved by the Review Board for the Care of Animal Subjects of the district government (Lower Saxony, Germany) and performed according to the International Guidelines on the Use of Laboratory Animals.

Experimental demyelination was induced in 8-week-old male mice by feeding with 0.2% cuprizone (Sigma-Aldrich, St Louis, MO), mixed into a ground standard rodent chow for 4.5 weeks. This feeding regimen was chosen because detailed analyses have demonstrated peak demyelination in the CC after 4.5 weeks of cuprizone feeding (18). Animals were then subjected to a normal diet for an additional 1.5 weeks for remyelination to occur.

Tissue Preparation

Groups of 5 (gene expression analysis) and 4 (immunohistochemistry, IHC) animals were investigated at each time point in parallel with age-matched untreated controls. At different time points (Weeks 1, 2, 3, 3.5, 4, 4.5, 5, 5.5, and 6), the mice were killed and perfused via left cardiac ventricle with ribonuclease (RNase)-free phosphate-buffered saline (PBS) for gene expression analysis or with 4% paraformaldehyde in PBS for IHC. For gene expression analysis, removed brains were embedded in Tissue Tek Compound (Sakura, Torrance, CA), snap frozen in liquid N2 and stored at −80°C until RNA extraction.

Animals and Induction of Demyelination

For the determination of demyelination and remyelination, MBP-immunostained sections from 4 animals per time point were evaluated by light microscopy (Leica Microsystems, Wetzlar, Germany). Myelin content was assessed by 3 blinded observers using a scale from 0 to 3 in the CC, or from 0 to 4 in the CTX, as previously described (18). Score 0 represents complete absence of MBP, whereas 3 and 4 represent fully expressed protein in the CC and CTX, respectively (Fig. 1).

Immunohistochemistry

Paraffin-embedded sections were dewaxed, rehydrated through graded alcohol concentrations, and boiled for 5 minutes in 10 mmol/L citrate buffer (pH 6.0) in a microwave oven. Endogenous peroxidase activity was inhibited with 3% H2O2. This step was omitted for fluorescent visualization. Microwave treatment was skipped for staining of the frozen slices (Nogo-A). Nonspecific binding was blocked for 1 hour with PBS containing normal goat or donkey serum and 0.1% Triton X-100. Tissue was incubated overnight at 4°C with the following primary reagents: anti–myelin basic protein (MBP; mouse monoclonal, 1:500; Covance, Princeton, NJ); fluorescein-coupled Ricin communis agglutinin I (RCA I, 1:500; Vector Laboratories, Burlingame, CA) for activated microglia; anti–glial fibrillary acidic protein (GFAP; rabbit anti-mouse, 1:200; Dako, Glostrup, Denmark) for astrocytes; anti–Nogo-A (rabbit anti-mouse, 1:750; Chemicon/Millipore, Billerica, MA) for mature oligodendrocytes; Olig2 (rabbit anti-mouse, 1:200; Immuno-Biological Laboratories, Takasaki, Japan) for mature oligodendrocytes and oligodendrocyte precursor cells; anti–MMP-3 (Y-16, goat anti-mouse, 1:100; Santa Cruz Biotechnology, Santa Cruz, CA) and anti–MMP-12 (M-19, goat anti-mouse, 1:50; Santa Cruz Biotechnology). For MBP and Nogo-A staining, slides were incubated for 1 hour at room temperature with the appropriate biotinylated secondary antibodies (1:500), then with peroxidase-coupled avidin-biotin complex (ABC Kit), and visualized with 3,3’-diaminobenzidine (DAB Peroxidase Substrate Kit; all from Vector Laboratories). Double immunofluorescent staining was visualized with donkey anti-goat Alexa Fluor 555 (1:500; Invitrogen, Carlsbad, CA) for MMP-3 and -12 and donkey anti-rabbit Alexa Fluor 488 (Invitrogen) for GFAP and Olig2. For the single GFAP staining, goat anti-rabbit Alexa Fluor 555 (Invitrogen) was used. All fluorescent slides were counterstained with 4’,6-diamidino-2-phenylindole nuclear dye (Invitrogen).

Evaluation of Demyelination and Remyelination

For the determination of demyelination and remyelination, MBP-immunostained sections from 4 animals per time point were evaluated by light microscopy (Leica Microsystems, Wetzlar, Germany). Myelin content was assessed by 3 blinded observers using a scale from 0 to 3 in the CC, or from 0 to 4 in the CTX, as previously described (18). Score 0 represents complete absence of MBP, whereas 3 and 4 represent fully expressed protein in the CC and CTX, respectively (Fig. 1).

RNA Isolation and Quantitative Reverse Transcription–Polymerase Chain Reaction

RNA was isolated with the RNeasy Micro Kit (CC) and the Mini Kit (CTX) according to the manufacturer’s recommendations (Qiagen, Hilden, Germany). Concentrations were measured using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). Final RNA dilutions were adjusted with RNase-free water (4 ng/µL for CC, 12 ng/µL for...
CTX, and 20 ng/μL for positive controls) and reversely transcribed using the High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems, Foster City, CA), in a MyCycler PCR Thermal Cycler (Bio-Rad Laboratories, Hercules, CA). The primer pair sequences used for the detection of MMP (MMP-2, -3, -7, -9, -10, -11, -12, -13, -14, -15, and -24), TIMP (TIMP-1, -2, -3, and -4) and housekeeping gene (β-actin, glyceraldehyde-3-phosphate dehydrogenase [GAPDH]) mRNA are described elsewhere (22). Quantitative PCR (qPCR) was performed with 1 μl of complementary DNA per 25-μL reaction mix (Brilliant SYBR Green qPCR Core Reagent Kit; Agilent Technologies, Santa Clara, CA), in a Mx3005P Real-Time PCR System (Agilent Technologies) with absolute external standards containing 10^2 to 10^8 copies/μL, as described (22). Correct product sizes were confirmed by gel electrophoresis (data not shown). The measurements obtained were analyzed using an MxPro QPCR software version 4.10 (Agilent Technologies). Data were normalized with a normalization factor calculated from geometric averaging of housekeeping genes using geNorm software version 3.5 (23).

Microglia Isolation

For ex vivo isolation of microglia, mice were anesthetized at Week 0 (controls) and Weeks 3.5 and 4.5 with ketamine/rompun and transcardially perfused with PBS. Brains were removed and placed in the ice-cold Hank’s balanced salt solution (PAA, Pasching, Germany) containing 15 mmol/L HEPES buffer and 5% glucose. The CC and CTX were dissected under a light microscope with an ultra fine needle blade (Fine Science Tools, Foster City, CA), and corresponding regions from up to 6 mice per time point were pooled to increase cell numbers. Microglia were isolated according to de Haas et al (24) in which their purity was approximately 98%. Tissue was chopped with razor blade, mechanically ground in a tissue homogenizer (Wheaton, Millville, NJ), triturated using Pasteur pipettes of decreasing diameter, and filtered through 60-μm cell strainer (Millipore Steriflip). After pelleting and resuspending, cells were centrifuged in a Percoll density gradient (GE Healthcare, Uppsala, Sweden), and layers containing microglia were collected. The high percentages of microglia in the fractions were confirmed by detecting rich CD11b+/CD45low cell populations (anti-mouse phycoerythrin/fluorescein isothiocyanate labeled antibodies; eBioscience, San Diego, CA) in a Becton-Dickinson FACSCalibur flow cytometer (data not shown). Subsequent steps of RNA isolation (final RNA dilution: 12 ng/μL), reverse transcription, and qPCR were performed as described above.

FIGURE 1. Quantification of myelin during demyelination and remyelination. (A, B) Red rectangles on the schematic drawings of the coronal mouse brain sections show the regions of the corpus callosum (CC; A) and cortex (CTX; B) examined. (C–J) Myelin amounts were analyzed by scoring myelin basic protein (MBP)-immunostained brain sections at different time points where the Score 0 stands for complete absence of MBP signal; Scores 3 and 4 represent fully expressed MBP in the CC (C) and CTX (D), respectively. Examples of scores in the CC (C, E, G, I) and CTX (D, F, H, J) are shown. (K, L) Demyelination was induced by 0.2% cuprizone diet for 4.5 weeks and remyelination took place after toxin withdrawal (Weeks 5 and 6). At Week 4.5, there was a marked loss of MBP in both the CC (K) and CTX (L), demonstrating almost complete demyelination. Significant differences compared with controls are indicated by asterisk p < 0.05 (*) and tested with Kruskal-Wallis one-way analysis of variance on ranks. Multiple comparison procedures were performed with the Dunn method. Scale bar = 200 μm.
transcription, and qPCR were performed as described above for the detection of MMP-3, -9, -12, -14, and -24; TIMP-1, -2, and -4; GAPDH, and β-actin mRNA.

Statistical Analysis

Statistical analysis was performed using PASW 18.0 (SPSS Inc., Chicago, IL) and SigmaStat 3.5 (Systat Software Inc., Chicago, IL). Measured copy numbers per 1 ng of RNA were normalized to housekeeping genes and nonspecific samples according to the melting curve were excluded. Normal distribution was tested with the Kolmogorov-Smirnov test. Statistical differences between cuprizone treated and control animals were calculated independently for each time point using paired Student t-test; values of p < 0.05, p < 0.01, and p < 0.001 were considered significant. For myelin protein expression, scoring data from the cuprizone-treated tissue were compared with those from controls and tested with Kruskal-Wallis one-way analysis of variance on ranks followed by the Dunn method for multiple comparisons; p < 0.05 was considered significant.

RESULTS

Demyelination and Remyelination

The extent of demyelination and subsequent remyelination in the white and gray matter was judged by MBP immunostaining. There was marked loss of MBP in both the CC and CTX after 4.5 weeks of 0.2% cuprizone feeding compared with control mice (p < 0.05; Fig. 1). During the subsequent normal diet period (Weeks 5 and 6), MBP was reexpressed until there seemed to be almost complete restoration of myelin (Fig. 1). In comparison to the CC, the peak of demyelination in the CTX was slightly delayed and less prominent (Figs. 1K, L), as previously described (18). After 4.5 weeks of cuprizone diet, few Nogo-A positive cells were seen. These cells reappeared after toxin removal in both analyzed areas but were constantly lower in number in the CTX versus the CC (Figs. 2T–V, X–Z). Extensive microgliosis, astrogliosis and, lack of oligodendrocytes corresponded to the maximal myelin loss, as demonstrated by MBP IHC (Figs. 1 and 2).

MMP and TIMP mRNA Expression

Relative expression of MMP and TIMP mRNA in the CC and CTX are summarized in the Table. For both regions at all time points, values for each gene are represented as ratios of arithmetic means of treated versus control mice. Changes in MMP and TIMP gene expression differed depending on the phase of the disease and the anatomic location (Figs. 3 and 4). In early phases of demyelination (Weeks 1 and 2), the most prominent upregulation was seen for MMP-3 in the CC (Fig. 3C), TIMP-4 in the CTX (Fig. 3K), and MMP-12 (Figs. 3D, E); TIMP-3 was upregulated in both areas. At the peak of demyelination (Weeks 3–4.5), elevated transcript numbers were detected for MMP-14 and TIMP-2 (Fig. 3I) in the CC and for MMP-12 and TIMP-1 in both CC and CTX (Figs. 3D, E, G, H). In this phase, MMP-15 and -24 (Fig. 3F) were significantly downregulated. During remyelination (Weeks 5 and 6), MMP-3 (Fig. 3C), -11, and -14 and TIMP-3 and -4 (Fig. 3I) were upregulated in the CC. Matrix metalloproteinase 12 expression was prominent in both areas, with an increasing tendency in the white matter while decreasing in the gray matter (Figs. 3D, E). Matrix metalloproteinase 2 and 9 were expressed only at low levels in both cuprizone-treated and control mice; MMP-9 was slightly but significantly upregulated in the CTX. Throughout the cuprizone treatment, MMP-7, -10, and -13 were minimally affected or not detected in both white and gray matter. The most remarkable relative changes in gene expression during demyelination and remyelination are schematically presented in Figure 4.

We next examined whether microglia represent the cellular source of the genes that changed their expression to the greatest extent during the peak of demyelination in the whole area of the CC and CTX. At Week 3.5, microglia demonstrated the most remarkable upregulation of MMP-12. Interestingly, at Week 4.5, the high MMP-12 expression declined more distinctively in CC microglia compared with cortical microglia. Microglial TIMP-2 expression, which could be detected already at a high level in untreated animals, increased after 4.5 weeks of cuprizone diet following, then, the TIMP-2 expression pattern of the whole callosal area (Table; Fig. 3I). Microglial MMP-24 and TIMP-4 downregulation as well as small but notable upregulation of MMP-9 during the cuprizone treatment correlated with the expression patterns of the same genes in the whole area of the CC and CTX (Figs. 3F, J, K; Table). However, MMP-14 expression levels in microglia did not change. Microglia isolated from the cuprizone-treated animals expressed a low number of MMP-3 mRNA copies that did not alter compared with controls, whereas TIMP-1 mRNA levels were below the detection limit.

Cellular Sources of MMP-3 and MMP-12

In remyelinated white matter areas (Week 6), MMP-3 was detected by IHC in astrocytes (Fig. 5C) but not in...
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activated microglia (Fig. 5A) or oligodendrocyte lineage cells (Fig. 5E). In the demyelinated CC (Week 4.5), double-fluorescent immunostaining localized MMP-12 in microglia (Fig. 5B) and in astrocytes (Fig. 5D). The scarce Olig2-positive cells did not seem to be the origin of this MMP (Fig. 5F). Compared with demyelination (Week 4.5), within the remyelinated tissue (Week 6), the amount of MMP-12 protein reduced in the CTX, whereas it was still considerable in the CC (not shown). Astrocytic MMP-12 production was noticeably higher at Week 6 (Fig. 5H) than at Week 4.5 (Fig. 5G). Interestingly, at Week 6, some of the reappearing oligodendrocytic cells colocalized with MMP-12 (Fig. 5I). Neither MMP-3 nor MMP-12 protein was detected in untreated animals (not shown).

DISCUSSION
The ability of MMPs to cleave a wide range of extracellular matrix and myelin molecules in vitro and their massive increase in demyelinating diseases originally associated

### TABLE. Relative Matrix Metalloproteinase and Tissue Inhibitor of Metalloproteinase Gene Expression During Cuprizone-Induced Demyelination and Remyelination

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*At each time point (weeks) for each gene and region (CC = corpus callosum, CTX = cortex) arithmetic means of copy numbers/1 ng of RNA from cuprizone-treated mice were compared with age-matched controls (n = 5 per group) and analyzed with paired Student t-test. Values in the table represent ratios of arithmetic means of treated versus control mice. Cuprizone was removed from the diet after 4.5 weeks. Significant differences are designated with asterisks as follows: *, p < 0.05; †, p < 0.01; ‡, p < 0.001.
MMP, matrix metalloproteinase; ND, not detected; TIMP, tissue inhibitor of metalloproteinase.

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FIGURE 2. Glial responses during cuprizone-induced demyelination and subsequent remyelination. (A, B) Red rectangles indicate analyzed parts of the corpus callosum (CC; A) and cortex (CTX; B). (C-Z) Compared with controls after 4.5 weeks of 0.2% cuprizone feeding RCA-I (green), glial fibrillary acidic protein (GFAP, red), and Nogo-A (brown) immunostaining show extensive microglial (C, D, G, H) and astrocytic (I, L, O, P) responses and loss of Nogo-A–positive oligodendrocytes (S, T, W, X) in the CC and CTX. After toxin withdrawal (Weeks 5 and 6), there is resolution of microglia (E, F, I, J) and astroglia (M, N, Q, R) and reappearance of oligodendrocytes (U, V, Y, Z) in the white and gray matter. Nuclei in the immunofluorescent staining (C–R) are counterstained with 4',6-diamidino-2-phenylindole (blue). Scale bars = CC, 100 μm; CTX, 200 μm.

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these proteases exclusively with tissue destruction and detrimental outcome. However, there is a growing body of evidence that the proteolytic functions of these molecules within pathologic conditions are more subtle and strictly controlled (25). Here, we demonstrate significant changes in the expression of several MMPs during remyelination, implying that they may be regulators of CNS tissue regeneration.

We detected high MMP-12 mRNA and protein production in the severely demyelinated tissue, preferentially in microglia from both the CC and the CTX. Matrix metalloproteinase 12 has been found to be highly expressed in active demyelinating MS lesions and, to a lower extent, within chronic active and inactive lesions (26). Moreover, there is MMP-12 upregulation during disease progression in EAE and chronic demyelination in Theiler murine encephalomyelitis (14, 27). Taken together, these data strongly imply that this MMP is somehow involved in myelin degradation. In this regard, its expression can be driven by proinflammatory...
stimuli such as tumor necrosis factor and interleukin-1β (27) and these cytokines are also expressed during demyelination in the cuprizone model (28, 29). However, despite resolution of microgliosis during remyelination (Fig. 2), MMP-12 mRNA expression in the CC stayed high, which was not the case in the CTX (Figs. 3D, E, and 4D). Interestingly, within the remyelinated tissue, we detected increased astrocytic MMP-12 protein that might explain this sustaining result (Figs. 5G, H). At the same time, a few cells of the oligodendrocyte lineage also colocalized with MMP-12 (Fig. 5I). We suggest that there may be a biphasic secretion of MMP-12 resulting in different phase-related functions. The MMP-12 produced initially might facilitate cell migration. Indeed, macrophages isolated from MMP-12 knockout mice had no migratory capacity in contrast to those from wild-type mice (30). Because we detected the highest number of RCA I- and GFAP-positive cells in both the CC and CTX at the peak of demyelination, MMP-12 may contribute to microglial and astrocytic migration toward the demyelinating sites. We further hypothesize that the subsequent secretion of astrocytic MMP-12 promotes remyelination. Accordingly, Larsen et al (31) showed that MMP-12 drives myelogenesis in vivo via releasing insulin-like growth factor-1, which has been shown to be upregulated in the CC during cuprizone treatment (32). Nevertheless, the reason for cortical decrease in MMP-12 mRNA expression during remyelination remains unclear, although it might be attributed to the lesser amount of local myelin.

Matrix metalloproteinase 3 was upregulated in the CC during early demyelination, and after returning to the basal level, its expression increased again during remyelination (Figs. 3C and 4A) being ascribed to astrocytes (Fig. 5C). The initial MMP-3 upregulation before demyelination is consistent with findings in nontoxic models such as Theiler murine encephalomyelitis and spontaneously demyelinating transgenic mouse models (14, 33), but we detected a second peak after the cessation of cuprizone. Thus, it seems unlikely that this result represents an epiphenomenon specifically related to the toxic model used. This protease is implicated in proteolytic breakdown of myelin from MS patients and has been found to be expressed in early reactive plaques (11, 34). Here, we show for the first time that MMP-3 is associated with remyelination. Its proteolytic activity has been shown to be required for releasing soluble growth factors such as insulin-like growth factor-1, fibroblast growth factor, heparin binding epidermal growth factor-like factor, and transforming growth factor-β, thereby suggesting that it could support remyelination (1, 35, 36). An additional beneficial mechanism might be that MMPs (including MMP-3 and -12), which cleave myelin proteins, may facilitate the removal of myelin debris and thus enable remyelination (6, 37).

Interestingly, we detected upregulation of MMP-14 as well as downregulation of MMP-15 and MMP-24 during demyelination (Table; Figs. 3 and 4). The same trend for MMP-14 and -24 continued during remyelination. Down-regulation of MMP-24 was also detected in isolated microglia, suggesting that this cell type is a possible source. Comparable expression patterns of these membrane-type MMPs were observed in EAE and showed no association with the expression of the proinflammatory cytokines (38). Besides cleaving a range of extracellular matrix molecules, membrane-type MMPs function as cell surface sheddases, processing pro-MMPs, and other bioactive molecules (39, 40). The functional consequences of the MMP-15 and -24 down-regulation are still not clear, but MMP-14 might facilitate cell motility.

During demyelination and remyelination, all 4 TIMPs displayed somewhat different patterns. Tissue inhibitors of metalloproteinase 1 (in both the CC and the CTX) and TIMP-2 (only in the CC) were significantly upregulated during the peak of demyelination and gradually declined during remyelination. Astrocytes are the main cellular source of TIMP-1 in the brain (41), which explains its temporal correlation with astrogliosis. By contrast, ex vivo isolated microglia showed TIMP-2 upregulation. Myelin protective properties of TIMP-1 were suggested in EAE in which TIMP-1 knockout mice and TIMP-1-overexpressing transgenic mice showed more severe and milder disease, respectively (42, 43). In the serum and cerebrospinal fluid of the MS patients, decreased TIMP-1 and TIMP-2 levels as well as elevated MMP-9 and MMP-2 levels have been found, suggesting that high MMP-9/TIMP-1 and MMP2/TIMP2 ratios play an important role in the pathogenesis and progression of MS (44, 45). Surprisingly, we did not detect high upregulation of either MMP-2 or MMP-9 mRNA in the cuprizone-treated animals, which might be explained by the intact BBB. The small increase in MMP-9 expression could possibly derive from infiltrating neutrophils detected in the CNS after 7 days of cuprizone feeding (46). With respect to the other TIMPs, there was an elevation of TIMP-3 and TIMP-4 mRNA level in the CTX early after toxin application that decreased after 3 weeks to the basal level. In the CC, there was a TIMP-3 mRNA peak during the first week of cuprizone feeding with gradual downregulation (Table). Tissue inhibitor of metalloproteinase 4 was moderately but significantly upregulated throughout remyelination (Figs. 3J and 4I). Unlike TIMP-1, -2, and -4 (which are secreted), TIMP-3 is bound to the cell surface inhibits several membrane-bound molecules, such as MMP-14, MMP-3, and TACE (47). However, we did not observe a correlation of TIMP-3 expression with that of MMP-14. Despite the fact that TIMP-4 is highly expressed in the brain, its function is still largely unknown. Tissue inhibitors of metalloproteinase
4 upregulation in the remyelinating CC may suggest a role in this process.

Matrix metalloproteinase 11 is constitutively expressed in murine astrocytes (48) and is upregulated in chronic canine distemper virus–associated brain lesions (49). Nevertheless, there is almost no information about its role and target substrates in the CNS. The possible involvement of MMP-11 in promoting remyelination can also be proposed, as it was upregulated in the CC at Weeks 5.5 and 6.

In light of differences in the pathology between the white and gray matter in the cuprizone model, MMP-3, -11, -12, and -24 and TIMP-2 and -4 deserve attention. Region-specific expression of MMPs and TIMPs in the brain has also been shown in canine distemper virus–infected mice and associated with a differential impairment of the CNS integrity (50). Possible explanations for these disparities might be the myelin amount, specific cellular interactions, or even different effector mechanisms such as alterations of glutamate reuptake regulation (51).

In conclusion, this study provides insight in the spatial and temporal expression patterns of MMPs and TIMPs during demyelination and remyelination in a toxic mouse model and highlights particularly MMP-3 and MMP-12, which are distinctively expressed by astrocytes during myelin recovery. Further studies are required to elucidate their exact mechanisms and to identify factors that promote their regenerative capacity.

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