Remodeling of the interstitial extracellular matrix in white matter multiple sclerosis lesions: Implications for remyelination (failure)

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Abstract
The extracellular matrix (ECM) provides protection, rigidity, and structure toward cells. It consists, among others, of a wide variety of glycoproteins and proteoglycans, which act together to produce a complex and dynamic environment, most relevant in transmembrane events. In the brain, the ECM occupies a notable proportion of its volume and maintains the homeostasis of central nervous system (CNS). In addition, remodeling of the ECM, that is transient changes in ECM proteins regulated by matrix metalloproteinases (MMPs), is an important process that modulates cell behavior upon injury, thereby facilitating recovery. Failure of ECM remodeling plays an important role in the pathogenesis of multiple sclerosis (MS), a neurodegenerative demyelinating disease of the CNS with an inflammatory response against protective myelin sheaths that surround axons. Remyelination of denuded axons improves the neuropathological conditions of MS, but this regeneration process fails over time, leading to chronic disease progression. In this review, we uncover abnormal ECM remodeling in MS lesions by discussing ECM remodeling in experimental demyelination models, that is when remyelination is successful, and compare alterations in ECM components to the ECM composition and MMP expression in the parenchyma of demyelinated MS lesions, that is when remyelination fails. Inter- and intralesional differences in ECM remodeling in the distinct white matter MS lesions are discussed in terms of consequences for oligodendrocyte behavior and remyelination (failure). Hence, the review will aid to understand how abnormal ECM remodeling contributes to remyelination failure in MS lesions and assists in developing therapeutic strategies to promote remyelination.
INTRODUCTION

The extracellular space of all organs and tissues is composed of a network of molecules, essential for physical support of cellular components and many cellular processes. This highly organized three-dimensional molecular network is called the extracellular matrix (ECM). The ECM composition is specific for each organ or tissue, but the majority of components is comprised of proteoglycans, hyaluronan, and fibrous (glyco)proteins, such as collagens, elastin, fibronectin, and laminin, and non-structural regulators, that is matricellular proteins such as tenascins, CCNs, SPARCs, fibulins, osteopontin, and thrombospondins (Theocharis, Skandalis, Gialeli, & Karamanos, 2016). These components act together to produce a complex and dynamic environment, involved in cell surface and transmembrane events. Next to being a physical scaffold in which cells are embedded, ECM binds to adhesion receptors on cells, such as integrins, thereby regulating numerous cellular processes, including cell migration, differentiation, proliferation, and survival (Rozario & DeSimone, 2010). Furthermore, the ECM functions as a reservoir for growth factors and other signaling molecules, thereby influencing cell behavior indirectly. It may also act as a diffusion and migration barrier. Essentially all cell types synthesize and secrete ECM molecules. Variations in the composition and structure of ECM components affect both the overall structure and bioactive properties of the ECM, thereby affecting signal transmission and thus the cellular response. The ECM is crucial for homeostasis, and is actively involved in repairing injury, whereas pathological conditions emerge from abnormalities in the ECM components.

In the central nervous system (CNS), the extracellular space takes up 17%–20% of the adult brain volume (Cragg, 1979; Nicholson & Sykova, 1998). Remarkably, while the ECM undergoes dynamic and continuous remodeling, mediated by matrix-degrading enzymes at normal and pathological conditions, ECM remodeling in the healthy adult CNS is very limited (Dityatev & Fellin, 2008; Rauch, 2007). As in all tissues, the ECM in the CNS can be classified in two major types that vary in structure, composition and regional appearance: the pericellular matrices that are in close contact with cells and the interstitial matrices that surround cells. Examples of pericellular matrices in the CNS include (a) the vascular and astroglial basement membranes that are present at the interface between endothelial cells and astrocytes, which play an important role in blood–brain barrier (BBB) maintenance (Lau, Cua, Keough, Haylock-Jacobs, & Yong, 2013; Schreibelt et al., 2007; van Horssen, Dijkstra, & de Vries, 2007) and (b) neuronal-associated perineuronal nets that emerge during synaptogenesis, which preserve neuronal health by maintaining synaptic plasticity (Frischknecht & Seidenbecher, 2008; Jacobs, & Yong, 2013; Schreibelt et al., 2007; van Horssen, Dijkstra, & de Vries, 2007).

KEYWORDS
extracellular matrix, matrix metalloproteinase, multiple sclerosis, oligodendrocyte, remyelination

Significance
Demelination and failure to remyelinate contribute to the neurological deficits that characterize the chronic progressive disease multiple sclerosis. Components of the extracellular matrix are involved in guiding each stage of the remyelination process, including oligodendrocyte progenitor cell migration, proliferation, and maturation. This review highlights the importance of extracellular matrix remodeling in successful remyelination and links dysregulated matrix metalloproteinase expression in white matter multiple sclerosis lesions to abnormal inter- and intralesional expression patterns of ECM components and remyelination failure. Hence, this review will assist in developing therapeutic strategies to overcome remyelination failure and halt disease progression.

Kwok, Dick, Wang, & Fawcett, 2011; Oohashi, Edamatsu, Bekku, & Carulli, 2015). The dispersed ECM in the CNS parenchyma is a representative example of an interstitial matrix. The interstitial ECM in healthy adult CNS contains relatively low levels of fibrous matrix proteins, like collagen, fibronectin, and laminin, which are mainly restricted to basement membranes (Ruoslaiti, 1996; van Horssen et al., 2007). Instead, the adult interstitial ECM contains high levels of glycosaminoglycans (GAGs), either covalently attached to proteins, forming mainly chondroitin sulfate proteoglycans (CSPGs), particular lecticans, such as aggrecan, versican, and neurocan, or as unbound entities in the form of hyaluronan (Kwok et al., 2011; Rauch, 2007; Ruoslaiti, 1996). Hyaluronan is the major component of healthy CNS, and is connected to CSPGs via linker proteins (Bignami, Hosley, & Dahl, 1993). Additional components of the adult ECM include the matricellular proteins of which tenasin-R and thrombospondin are most prominent in the CNS (Jones & Bouvier, 2014; Kwok et al., 2011). Notably, the perineuronal nets are enriched in hyaluronan, tenasin-R, and CSPGs. All cell types of the CNS, that is astrocytes, microglia, oligodendrocytes, and neurons, as well as endothelial cells, contribute to the pool of proteins that eventually constitute the ECM (Lau et al., 2012).

The ECM of the CNS plays an important role in many regulatory processes during development and in the homeostasis of healthy adult CNS. In response to injury, the composition of the ECM changes, resulting in a transient ECM environment that may be either stimulatory or inhibitory to repair. Different neuropathological conditions are associated with a dysregulation of alterations in the expression pattern of ECM molecules, thereby impeding the
process of repair, including regeneration. For example, a role of the dynamics and the distinct involvement of ECM components is becoming increasingly apparent in the demyelinating disease multiple sclerosis (MS), showing an association between ECM alterations and white matter MS lesion formation (Satoh, Tabunoki, & Yamamura, 2009) and progression of the neuropathological state (Bonneh-Barkay & Wiley, 2009). ECM remodeling is tightly regulated by an interplay between several proteins and enzymes of which the family of matrix metalloproteinase (MMP) is a prominent proteolytic system in the spatiotemporal regulation of the ECM (Lu, Takai, Weaver, & Werb, 2011; Page-McCaw, Ewald, & Werb, 2007). Functional dysregulation of these enzymes contributes to the pathogenesis and progression of several inflammatory demyelinating diseases, including MS (Kieseier, Seifert, Giovannoni, & Hartung, 1999). Here, we aim to unravel abnormal ECM remodeling in MS lesions and link this to remyelination failure, as to assist in developing therapeutic strategies to promote remyelination in MS.

To this end, we uncover abnormal ECM remodeling in MS lesions by describing and comparing the transient-altered expression patterns of individual ECM molecules and MMPs upon successful CNS remyelination in rodent models of toxin-induced demyelination, and upon remyelination failure in MS lesions. In addition, we discuss how the (transiently) expressed ECM proteins regulate the behavior of cells that produce myelin, that is oligodendrocytes, and how their (persistent) expression may contribute to remyelination failure. Also, the interactions between ECM molecules and MMPs and potential mechanisms leading to incorrect ECM remodeling in MS are reviewed. Before discussing this in more detail, we first present a brief overview of the pathology of MS.

2 | MULTIPLE SCLEROSIS

2.1 | Pathological hallmarks

MS is a neurodegenerative inflammatory disease of the CNS. It is one of the most common demyelinating CNS diseases with an incidence of approximately 0.1% worldwide, while the prevalence varies based on geographical and ethnic differences (Compston & Coles, 2008; Rosati, 2001). Variable patterns of the clinical course of MS are observed. The most common form is relapsing-remitting MS, which is diagnosed in about 80% of the MS patients (Lublin & Reingold, 1996). Relapsing-remitting MS is characterized by relapses followed by full or partial recovery at the onset of the disease, but incomplete recovery arises over time and the majority of patients develop secondary progressive MS with minor remissions (Compston & Coles, 2008). Primary progressive MS is diagnosed in 10%–20% of the patients (Lublin & Reingold, 1996), has an onset at a later age, and is characterized by gradual accumulation of neurological deficits, starting already at the onset of the disease (Compston & Coles, 2008).

The disease is characterized by chronic and progressive loss of myelin sheaths surrounding the axons in the brain and spinal cord. The primary causative mechanism(s) resulting in demyelination and progression of MS is (are) still unclear. However, genetic predisposition, that is mainly genes implied in (cell-mediated) immunity, and several environmental factors, such as vitamin D deficiency and viral infections, appear to play an important role in the development of MS (Compston & Coles, 2008; Correale & Gaitan, 2015; Olsson, Barcellos, & Alfredsson, 2017; Roch et al., 2017). These factors contribute to the inflammatory process occurring in MS, which is associated with disruption of the BBB. Whether this inflammation is a primary or secondary event in the pathogenesis of MS is unknown (Compston & Coles, 2008; Stys, Zamponi, van Minnen, & Geurts, 2012). An autoimmune response is initiated in the periphery through (as yet unknown) processes. Molecular mimicry might underlie such an event, resulting in activation of autoreactive T cells against self-antigens, which migrate to the CNS. Subsequently, this might initiate an immune response, thus causing damage and degeneration. In contrast, cytodegenerative processes in the CNS through (as yet equally unknown) factors may activate autoreactive T cells by presentation of self-antigens and subsequently induce a secondary immune response. In MS, these autoreactive T cells are directed to self-antigens that include specific proteins, present on the surface of mature oligodendrocytes and/or in myelin (Mallucci, Peruzzotti-Jametti, Bernstock, & Pluchino, 2015). This results in (additional) demyelination due to the destruction of myelin and/or mature oligodendrocytes.

The formation of white matter MS lesions is a dynamic process resulting in interlesional heterogeneity. More specifically, distinct lesions are histopathologically classified in inflammatory and demyelinating activity (Kuhlmann et al., 2017; van der Valk & De Groot, 2000). While different classification systems have been described, mainly three distinct lesions can be distinguished: active, mixed active/inactive, and inactive lesions (Figure 1, (Kuhlmann et al., 2017)). Active lesions are the early demyelinating phenotype and are most frequently found in acute MS patients with a very short disease duration (Frischer et al., 2015), while their proportion (approx. 25%) is similar upon longer disease duration and severity (Frischer et al., 2015; Luchetti et al., 2018). Active lesions are defined by indistinct margins, harbor inflammatory activity, and contain a hypercellular lesion center with hypertrophic astrocytes, (myelin-laden) microglia/macrophages, and lymphocytes. Mixed active/inactive lesions (also called “chronic active lesions” or “smoldering lesions”) show intralesional heterogeneity having a sharp border and consist of a hypocellular demyelinated lesion center with fibrous astrocytes. The lesion center is surrounded by a broad hypercellular inflammatory rim that contains microglia/macrophages and reactive astrocytes. The mixed active/inactive lesions are more frequently observed in progressive MS patients than in relapsing-remitting MS patients (Frischer et al., 2015; Luchetti et al., 2018). The final lesion classification is a (chronic) inactive MS lesion. These lesions have a hypocellular demyelinated center, containing mainly reactive astrocytes and show hardly signs of infiltration of microglia/macrophages or lymphocytes. Inactive lesions are also predominating in progressive MS (Frischer et al., 2015).
myelin is an insulating layer around axons that provides axonal protection and electrical isolation, and mediates saltatory conduction of action potentials. The loss of activity due to demyelination is partially compensated by a redistribution of sodium channels along the demyelinated parts of the axon, which allows for non-saltatory conduction with reduced velocity (Felts, Baker, & Smith, 1997). However, this compensatory effect is only temporal and in conjunction with the loss of axonal protection, persistent demyelination leads to axonal damage and degeneration (Compston & Coles, 2008; Funfschilling et al., 2012; Lee et al., 2012). The rate of impulse transduction is reduced or the impulses cease, which results in clinical signs and symptoms of MS that reflect the affected area of the CNS (Compston & Coles, 2008). In addition, accumulation of axonal degeneration is the main process that contributes to progression of neurological dysfunction and disease severity (Compston & Coles, 2008; Papadopoulos, Pham-Dinh, & Reynolds, 2006). Progressive axonal loss is key to the continuous and irreversible neurological decline in progressive MS (Trapp & Nave, 2008). Next to primary axon damage, a major cause of axonal loss in chronic stages of MS is secondary neurodegeneration as a consequence of remyelination failure (Irvine & Blakemore, 2008). Indeed, in addition to ensure saltatory axonal conduction, myelinating oligodendrocytes secrete metabolic and trophic factors that maintain the integrity and survival of axons (Funfschilling et al., 2012; Lee et al., 2012). Therefore, prevention of axonal degeneration might be beneficial to resolve the functional deficits and progression of MS.

A regenerative process to restore myelin is required to ensure the survival of demyelinated axons. This process is called remyelination, which re-establishes saltatory conduction, protects axons from degeneration and improves clinical features of MS (Franklin & ffrench-Constant, 2008; Franklin, Zhao, & Sim, 2002; Irvine &
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Blakemore, 2008; Jeffery & Blakemore, 1997; Smith, Blakemore, & McDonald, 1979). The myelin sheaths generated in the process of remyelination are shorter and thinner, compared to myelin sheaths produced during developmental myelination, but these newly-formed sheaths suffice for axonal protection and improved functioning (Kornek et al., 2000). Experimental demyelination in rodent models show that successful remyelination is not executed by pre-existing mature oligodendrocytes but achieved by newly-formed oligodendrocytes generated from local oligodendrocyte progenitor cells (OPCs; Zawadzka et al., 2010), while OPCs present in the adult subventricular zone contribute predominantly to remyelination of lesions in their proximity (Menn et al., 2006). In response to toxin-induced demyelination in these models, astrocytes and microglia are activated (reviewed by Franklin & ffrench-Constant, 2008; Franklin et al., 2002). These changes lead to the (transcriptional) activation of OPCs, resulting in morphological changes and enhanced gene expression of factors involved in oligodendrocyte differentiation and maturation (Arnett et al., 2004; Ferent, Zimmer, Durbec, Ruat, & Traiffort, 2013; Moyon et al., 2015; Reynolds et al., 2002; Watanabe, Hadzic, & Nishiyama, 2004). In addition to OPC activation, microglia and astrocytes recruit and mediate the migration of OPCs to the demyelinated areas, where they further proliferate (Franklin & ffrench-Constant, 2008; Franklin et al., 2002; Levine & Reynolds, 1999). The final essential step is the generation of new myelin sheaths and involves the differentiation of OPCs into mature oligodendrocytes. The process includes contact between the oligodendrocyte and the axon, upregulation of myelin-specific genes and generation and compactation of the myelin membranes (Franklin & ffrench-Constant, 2008; Franklin et al., 2002). While in experimental rodent models remyelination is executed by OPCs, carbon 14-based birth dating and single nuclei RNA sequencing of MS brain tissue reveal that remyelination in MS is most likely not executed by OPCs, but by pre-existing mature oligodendrocytes (Jakel et al., 2019; Schirmer et al., 2019; Yeung et al., 2019). Whether this is an inherent capacity of remyelination in humans, or an adaptation as of the inability of OPCs to differentiate to mature myelinating oligodendrocytes in MS lesions remains to be determined. However, in both cases, that is remyelination by newly-formed or pre-existing OLGs, new myelin membranes are formed.

A plethora of molecules play an important role in the different phases of remyelination, as well as several mediators of the inflammatory response, featuring in MS (Franklin & ffrench-Constant, 2008; Franklin et al., 2002; Gaesser & Fyffe-Maricich, 2016; Hanafy & Sloane, 2011; Miron, 2017). Although remyelination is a natural response to demyelination in most cases, this regenerative process often fails in chronic and progressive MS (Compston & Coles, 2008; Franklin & ffrench-Constant, 2008; Goldschmidt, Antel, Konig, Bruck, & Kuhlmann, 2009; Kuhlmann et al., 2008; Lucchinetti et al., 1999; Luchetti et al., 2018). In a subset of lesions, insufficient migration and/or proliferation of OPCs likely accounts for remyelination failure. However, remyelination mainly fails due to defective OPC differentiation (Franklin & ffrench-Constant, 2008; Franklin et al., 2002; Kuhlmann et al., 2008). In fact, in approx. 70% of both active and chronic MS lesions, OPCs are abundantly present (Chang, Tourtellotte, Rudick, & Trapp, 2002; Goldschmidt et al., 2009; Kuhlmann et al., 2008; Lucchinetti et al., 1999; Strijbis, Kooi, van der Valk, & Geurts, 2017; Wolswijk, 1998). Of interest in this respect is that single nuclei RNA sequencing of MS brain tissue shows that more dysregulated genes are found in mature oligodendrocytes rather than in OPCs compared to tissue of healthy subjects (Schirmer et al., 2019). This may indicate that OPCs are indeed transcriptionally relatively quiescent in MS, likely as a reflection of the inhibitory environment in MS lesions for OPC differentiation. Remyelination not always fails in MS, given the presence of partly or completely remyelinated shadow plaques. Remyelinated shadow plaques are present at all stages of MS (Frischer et al., 2015; Goldschmidt et al., 2009; Kuhlmann et al., 2017; Luchetti et al., 2018) and is thus likely executed by pre-existing oligodendrocytes (Jakel et al., 2019; Yeung et al., 2019), but the frequency of remyelinated white matter shadow plaques is lower in the progressive forms compared to relapsing-remitting MS (Luchetti et al., 2018). This is likely a reflection of remyelination failure in mixed active/inactive lesions that are dominating at later stages. Notably, although extensive remyelination is occasionally observed at late-stage progressive MS (Patani, Balaratnam, Vora, & Reynolds, 2007; Patrikios et al., 2006), it is most prominently noted in acute lesions, that is immediately upon demyelination (Prineas et al., 1989; Raine & Wu, 1993). Remyelination failure is thus thought to be a consequence of perturbations in the different phases of remyelination, that is activation, recruitment, and differentiation of OPCs, in which aging also plays an important role (Franklin & ffrench-Constant, 2008; Goldschmidt et al., 2009; Sim, Zhao, Penderis, & Franklin, 2002). The signaling and cellular environment, established by the state of the disease and lesion regulating these phases, is a crucial factor. Remyelination failure in MS is likely due to the presence of inhibitory signals or the lack of stimulatory signals in the damaged area. Indeed, various factors in the signaling environment of MS lesions are dysregulated, and together with ensuing cellular changes contribute to the failure of remyelination (Franklin & ffrench-Constant, 2008; Franklin et al., 2002; Hanafy & Sloane, 2011; Miron, 2017; Williams, Piaton, & Lubetzki, 2007). In this regard, a role of the dynamics and distinct interstitial ECM components in remyelination failure in MS lesions is becoming increasingly apparent (Lau et al., 2013; Satoh et al., 2009). In contrast, ECM remodeling benefits remyelination upon toxin-induced demyelination in rodent models, pointing to abnormal ECM remodeling in MS lesions, which is reviewed next.

### 3 | THE INTERSTITIAL ECM UPON DEMYELINATING INJURY

Dynamic remodeling of the ECM, that is transient expression and/or degradation, is an effective mechanism to regulate glial cell behavior, including OPCs upon injury (reviewed in (Pu, Stephenson, & Yong, 2018)). Analysis of mRNA expression of ECM proteins and the MS lesion proteome reveals that mixed active/inactive and inactive MS lesions have a unique ECM composition, both when compared
among each other and to control white matter and active MS lesions (Hendrickx et al., 2017; Mohan et al., 2010; Satoh et al., 2009). This hints to interlesional heterogeneity of ECM proteins in MS lesions and that dysregulated ECM remodeling may play an important role in the pathogenesis of MS, including remyelination failure. To unravel abnormal ECM composition in the distinct MS lesions, experimental toxin-induced demyelination rodent models that show robust remyelination in the absence of the complex inflammatory background in MS are most instructive to obtain insight into the natural cellular and molecular responses during demyelination of the CNS and subsequent successful recovery. These toxin-induced demyelination models include the dietary cuprizone model (mice), which shows global demyelination most prominently in the corpus callosum (Praet, Guglielmetti, Berneman, Van der Linden, & Ponsaerts, 2014; Skripuletz, Gudi, Hackstette, & Stangel, 2011; Torkildsen, Brunborg, Myhr, & Bo, 2008), and the focal lysolecithin (mice and rat) and ethidium bromide (mice) models, where demyelination is induced by local injection of the toxin in the area of interest (Blakemore, 1976; Jeffery & Blakemore, 1995; Zhao, Li, & Franklin, 2006). Notably, in the lysolecithin model the BBB is disrupted (Muramatsu et al., 2015), while the endothelial cells remain seemingly intact in the cuprizone and ethidium bromide model (Bjelobaba, Begovic-Kupresanin, Pekovic, & Lavrnja, 2018; Kondo, Nakano, & Suzuki, 1987; McMahon, Suzuki, & Matsushima, 2002). In the following, we outline for each ECM protein transient changes in its expression in the interstitial matrix, that is parenchymal ECM, upon white matter demyelination and during remyelination in experimental rodent models, and indicate its presence in the distinct white matter MS lesions (summarized in Table 1 and Figure 1). In addition, we discuss direct effect(s) of each ECM protein on OPC behavior (summarized in Figure 2) and discuss how the protein may contribute to remyelination (failure). We only briefly touch upon changes in ECM composition of basement membranes, as remyelination-predetermined OPCs in general do not face the ECM in basement membranes. For irregularities and alterations of ECM in basement membranes, we refer to two excellent reviews by van Horssen et al. (2007) and Lau et al. (2013).

### 3.1 Structural ECM proteins

CSPGs are the main proteoglycans of the CNS and consist of core proteins with covalently linked sulfated chondroitin GAG side chains. The individual secreted CSPGs, that is neurocan, aggrecan, versican, and phosphacan, differ in the composition of their core protein and/or the number and type of attached GAG chains. CSPGs can be produced by astrocytes, microglia/macrophages, neurons, and oligodendrocytes (Asher et al., 2002; Hibbits, Yoshino, Le, & Armstrong, 2012; Keough et al., 2016; Lau et al., 2012). CSPG expression, including versican and phosphacan, but not aggrecan, transiently increases upon lysolecithin- (Keough et al., 2016; Lau et al., 2012) and/or cuprizone-induced demyelination (Keough et al., 2016; Lau et al., 2012) and/or cuprizone-induced demyelination.

| TABLE 1  | Expression of interstitial ECM proteins upon toxin-induced demyelination and in distinct white matter MS lesions |
|-----------|---------------------------------------------------------------------------------------------------------------|
| **ECM proteins** | **Experimental rodent models** | **Multiple sclerosis** |
| | Demyelination | Remyelination | Active | Mixed active/inactive |
| | | | Edge | Center | Edge | Center | Inactive |
| CSPGs | ↑ | = | ↑ | ↓ | ↑ | ↓ | ↓ |
| -Neurocan | n.d. | n.d. | ↑ | ↓ | ↑ | ↓ | ↓ |
| -Aggrecan | = | n.d. | ↑ | ↓ | ↑ | ↓ | ↓ |
| -Versican | ↑ | = | ↑ | ↓ | ↑ | ↓ | ↓ |
| -Phosphacan | ↑ | = | = | = | = | = | = |
| Hyaluronan | = | = | ↑ | ↑ | ↑ | = | = |
| Collagen (V) | = | = | ↑ | = | = | = | = |
| Fibronectin | ↑ | = | ↑ | = | ↑(aggregates) | = | = |
| Vitronectin | ↑ | = | ↑ | = | = | = | = |
| Laminin | = | ↑ | = | = | = | = | = |
| Tenascins | | | | | | | |
| Tenascin-C | ↑ | = | ↓ | = | = | = | = |
| Tenascin-R | ↑ | = | ↓ | = | = | = | = |
| Thrombospondin | ↑ | n.d. | = | ↑ | = | = | = |
| Osteopontin | ↑ | = | ↑ | = | = | = | = |

**Note:** Increased (↑), decreased (↓), and similar (=) expression of interstitial ECM proteins upon toxin-induced demyelination and in distinct white matter MS lesions compared to control.

**Abbreviation:** n.d., not determined.

*Based on biochemical analysis (Stoffels et al., 2013).*

*Based on MS lesion proteome (Satoh et al., 2009) and Western blot analysis (Sikkema et al., 2018), data on localization are not available yet.*
In lysolecithin-induced lesions, CSPGs are present in microglia/macrophages at the lesion center, while astrocytes harbor CSPGs at the lesion edges (Lau et al., 2012). Depositions of CSPGs, including aggrecan, neurocan and versican, are present in astrocyte-enriched edges of active and inactive MS lesions, while their expression is downregulated in the center of active, mixed active/inactive and inactive lesions (Dahl, Perides, & Bignami, 1989; Hendrickx et al., 2017; Sobel & Ahmed, 2001). Gene expression profiling of rim and center of mixed active/inactive and inactive MS lesions revealed that neurocan mRNA is enriched at the rim of mixed active/inactive lesions (Hendrickx et al., 2017). Deposited CSPG components in active MS lesions are suggested to be phagocytosed by foamy macrophages together with myelin debris (Sobel & Ahmed, 2001), although expression of CSPGs by macrophages cannot be excluded (Lau et al., 2012). Strikingly, phosphacan expression, which is a CNS-specific CSPG, is more or less preserved in active lesions, and, in general, less reduced in inactive MS lesions compared to the other CSPGs (Sobel & Ahmed, 2001). Also, in situ hybridization studies show the presence of phosphacan mRNA in remyelinating oligodendrocytes in MS lesions (Harroch et al., 2002), and suggested to be involved in oligodendrocyte lineage progression (Karus et al., 2016). Of interest, granular aggregates of versican and aggrecan, and to a lesser extent of neurocan, but not phosphacan are evident in normal appearing white matter (NAWM; Sobel & Ahmed, 2001). This indicates that ECM alterations in MS are not limited to the lesions themselves. In vitro studies revealed that CSPG coatings, including aggrecan, neurocan, and phosphacan, inhibit OPC adhesion, process outgrowth and differentiation, likely by activating Rho-kinase-dependent signaling pathways via PTPs (Keough et al., 2016; Lau et al., 2012; Lucchinetti et al., 1999; Pendleton et al., 2013; Siebert & Osterhout, 2011). Exposure to soluble phosphacan is a ligand for oligodendroglial contactin and this complex inhibits OPC proliferation, and promotes OPC differentiation (Lamprianou, Chatzopoulou, Thomas, Bouyain, & Harroch, 2011). Thus, demyelination in experimental models leads to the upregulation of CSPGs, which may have beneficial functions at early stages.
of recovery, that is to prevent premature OPC differentiation. Moreover, CSPGs are cleared to enable remyelination. Deposition of CSPGs in MS lesion edges may lead to the formation of a barrier for OPC migration into the lesions, and loss of CSPGs in the center may preclude their beneficial actions after recovery (Keogh et al., 2016; Lau et al., 2012; Pu et al., 2018; Sobel & Ahmed, 2001).

Hyaluronan is a specialized non-sulfated GAG that functions as free, that is independent GAG molecule without protein core, or engages in non-covalent interactions with proteoglycans, including CSPGs (Sherman et al., 2002). Hyaluronan is expressed in different sizes, performing its functions in cell growth and motility and interactions between different ECM molecules to stabilize the ECM (Bignami et al., 1993). Upon lyssolecithin-induced demyelination, only a minimal accumulation of hyaluronan is noticed (Back et al., 2005), suggesting that there is no major upregulation of hyaluronan after demyelinating injury in this experimental rodent model. However, deposition of hyaluronan is associated with MS development (Nagy et al., 2019). In contrast to other glycosaminoglycans (Sobel & Ahmed, 2001), hyaluronan accumulates in the core of inflammatory demyelinating active MS lesions (Back et al., 2005). In early lesions, infiltrating T cells and microglia probably produce hyaluronan and in chronic lesions, astrocytes are the likely source (Back et al., 2005). Also, OPCs, and to a lesser extent mature oligodendrocytes, synthesize hyaluronan (Preston et al., 2013). Most interestingly, T cells and microglia produce low-molecular weight hyaluronan ranging from 200 to 400 kDa (LMW hyaluronan), whereas astrocytes produce hyaluronan with high-molecular weight, ranging from 900 to 1,000 kDa (HMW hyaluronan); Back et al., 2005). Both HMW and LMW hyaluronan are present in MS lesions (Back et al., 2005; Preston et al., 2013). HMW hyaluronan is detrimental for remyelination when injected in lyssolecithin-induced demyelinated lesions (Back et al., 2005). Treatment with soluble HMW hyaluronan or LMW hyaluronan inhibits OPC maturation through LMW hyaluronan-mediated activation of TLR2 (Back et al., 2005; Sloane et al., 2010). In fact, OPC maturation is only precluded when HMW hyaluronan is processed to a LMW form by a specific hyaluronidase (PH20) that is present in OPCs and astrocytes in lyssolecithin-induced lesions and MS lesions (Back et al., 2005; Preston et al., 2013). Hence, digestion products of HMW hyaluronan as well as unprocessed LMW hyaluronan likely prevent OPC maturation in chronic MS lesions, thereby contributing to remyelination failure (Figure 2; Preston et al., 2013).

Collagens are trimeric proteins containing long triple helical sequences that have the ability to form stable fibrils. They are arranged into networks and are involved in structuring and providing rigidity to the ECM. In the adult CNS, collagen is mainly limited to the basement membranes (e.g., collagen IV) and is hardly present in the interstitial matrix, which makes the brain a relatively soft tissue. While fibrillar collagens are not present in CNS parenchyma upon cuprizone-induced demyelination (Hibbits et al., 2012), fibrillar collagen V is closely associated with astrocytes in the interstitial matrix of active MS lesions (Mohan et al., 2010). Furthermore, genes involved in collagen synthesis are highly expressed in the rim of inactive MS lesions (Hendrickx et al., 2017). Also, the accumulation of fibrillary collagens I and II is associated with perivascular inflammation in the center of active and mixed active/inactive MS lesions, and mainly restricted to basement membranes, playing a role in limiting the enlargement of MS lesions (Mohan et al., 2010). MS proteome analyses demonstrated an enrichment of collagen IV, primarily localizing in the basement membranes in inactive lesions (Satoh et al., 2009). Although OPCs lack collagen-recognizing integrins, OPC migration is inhibited on collagen I substrates (Milner, Edwards, Streuli, & ffrench-Constant, 1996), and collagen I microspheres support OPC differentiation (Yao, Phan, & Li, 2013). Therefore, the contribution of interstitial collagen to remyelination failure via direct modulation of OPC behavior in MS lesions is likely negligible.

3.2 | Fibrous ECM glycoproteins

Fibronectins are high-molecular weight glycoproteins that are produced by a single gene (Hynes & Yamada, 1982; Pankov & Yamada, 2002). There are two types of fibronectin: plasma fibronectin and cellular fibronectin. Plasma fibronectin is a soluble compound that is generated by hepatocytes and circulates in the periphery (Owens & Cimino, 1982). Cellular fibronectin is insoluble and locally produced; in the CNS it is deposited by astrocytes, microglia/macrophage, and endothelial cells (Hibbits et al., 2012; Stoffels et al., 2013; Zhao, Fancy, Franklin, & ffrench-Constant, 2009). In normal healthy adult CNS, fibronectin is nearly absent in the interstitial ECM and only localizes to the vasculature (Sobel & Mitchell, 1989; Stoffels et al., 2013; van Horssen, Bo, Vos, Virtanen, & de Vries, 2005; Zhao, Fancy, Franklin, & ffrench-Constant, 2009). Fibronectin is a major component of the transient ECM in various tissues upon injury guiding tissue repair, where it binds many other ECM components to form matrices. In this manner, it regulates cell behavior via integrin receptors, in particular cell migration and proliferation (Pankov & Yamada, 2002; Singh, Carraher, & Schwarzbaeuer, 2010). Also, in various experimental toxin-induced lesions undergoing efficient remyelination, fibronectin is transiently expressed in white matter CNS parenchyma and upregulated in the vasculature and its level declines as remyelination proceeds (Espitia Pinzon et al., 2017; Hibbits et al., 2012; Stoffels et al., 2013; Zhao et al., 2009). In active, mixed active/inactive and inactive white matter MS lesions, however, the increased vascular and extracellular fibronectin expression persists, and the protein is present in perivascular infiltrates (Sobel & Mitchell, 1989; Stoffels et al., 2013; van Horssen et al., 2005). In addition, fibronectin is associated with inflammation-mediated aggregation in chronic lesions (Stoffels et al., 2013). Fibronectin expression is still increased in remyelinated shadow plaques, but does not aggregate (Stoffels et al., 2013). Astrocytes are the predominant source of cellular fibronectin upon lyssolecithin-induced demyelination (Stoffels, Hoekstra, Franklin, Baron, & Zhao, 2015), while in MS lesions fibronectin levels increase within demyelinated regions by both leakage from the blood circulation and production by reactive astrocytes (Sobel & Mitchell, 1989; Stoffels et al., 2013; van Horssen et al., 2005). Interestingly, white
matter astrocytes isolated from postmortem MS brain tissue show enhanced fibronectin aggregation compared to normal astrocytes that mainly deposit dimeric fibronectin (Stoffels et al., 2013). In vitro, dimeric fibronectin mediates OPC migration and proliferation (Baron, Shattil, & ffrench-Constant, 2002; Frost, Kiernan, Faissner, & ffrench-Constant, 1996; Milner et al., 1996; Stoffels et al., 2013; Tripathi, Parikh, Vora, Frost, & Pillai, 2017), while fibronectin coatings prevent myelin membrane formation, likely by perturbing (secondary) process outgrowth, myelin-directed vesicle transport, and formation of functional membrane microdomains (Baron et al., 2014; Buttery & ffrench-Constant, 1999; Lafrenaye & Fuss, 2010; Maier et al., 2005; Siskova, Baron, de Vries, & Hoekstra, 2006; Siskova et al., 2009; Stoffels et al., 2013). Similar to dimeric fibronectin, aggregates of fibronectin inhibit myelin membrane formation and myelination in mono- and co-culture systems (Qin et al., 2017; Stoffels et al., 2013). Thus, upon CNS white matter demyelination, transient expression of fibronectin precedes successful remyelination, and may be beneficial in OPC recruitment, whereas the pathological fibronectin aggregates impair remyelination in MS lesions (Figure 2).

Vitronectin is another glycoprotein that is mainly localized in the vasculature of the adult CNS. The expression of microglia/macrophages-derived vitronectin is transiently enhanced upon ethidium bromide-induced demyelination (Zhao et al., 2009). In MS, vitronectin is deposited in active lesions and at the edges of mixed active/inactive demyelinating lesions, where it is localized on the microvasculature, on demyelinated axons, and is present in a subset of hypertrophic astrocytes (Sobel, Chen, Maeda, & Hinojoza, 1995). Next to localized synthesis, part of the vitronectin acquires access into the brain, following passage across the disrupted BBB (Sobel et al., 1995). Vitronectin is absent in inactive lesions (Sobel et al., 1995). It promotes migration and proliferation of cultured OPCs (Baron, Shattil, & ffrench-Constant, 2002; Frost, Kiernan, Faissner, & ffrench-Constant, 1996), indicating that vitronectin may regulate OPC recruitment, and that the absence of the compound in chronic lesions may contribute to the reduced number of OPCs in white matter MS lesions.

Laminins are self-polymerized and heterotrimeric glycoproteins that serve as major adhesive proteins in basement membranes. Multiple genes code laminin subunits, known as α, β, and γ polypeptide chains that assemble into distinct laminin variants (Colognato & Yurchenco, 2000). In the healthy adult CNS, laminins are predominantly found at the vascular endothelial (mainly laminin-5,-8, and -10) and astrogial (mainly laminin-1 and -2) basement membranes (Sixt et al., 2001; van Horssen et al., 2005; Zhao et al., 2009). Upon CNS development, laminin-2 is localized to axons where it promotes the maturation of OPCs into oligodendrocytes, OPC proliferation, survival of oligodendrocytes, and myelination (Colognato et al., 2002; Colognato & Tzvetanova, 2011; Frost, Buttery, Milner, & ffrench-Constant, 1999; Leiton et al., 2015; Relucio, Tzvetanova, Ao, Lindquist, & Colognato, 2009; Relvas et al., 2001). Laminin-2 expression is upregulated upon CNS white matter demyelination and appears around axons at a time point that coincides with the onset of myelin membrane formation (Zhao et al., 2009), and may thus promote remyelination. In active and chronic MS lesions, the expression of laminin is mainly enhanced at the vascular and not in the lesion parenchyma (Esiri & Morris, 1991; Sobel, Hinojoza, Maeda, & Chen, 1998; van Horssen et al., 2005), indicating that beneficial effects of laminin on oligodendrocyte behavior and remyelination may be negated in MS.

### 3.3 Matricellular proteins

Matricellular proteins are non-structural regulators of the ECM that contain binding sites for other ECM proteins as well as cell surface receptors, and therefore play important roles in controlling cell behavior and ECM remodeling (Bornstein & Sage, 2002). Tenascin-C and tenascin-R are large hexameric and trimeric glycoproteins, respectively, assembled from monomers via disulfide bridges (Erickson & Inglesias, 1984; Norenberg, Hubert, & Rathjen, 1996). The proteins are expressed in the normal adult CNS (Bartsch et al., 1992; Garcia, Faissner, & ffrench-Constant, 2001; Gutowski, Newcombe, & Cuzner, 1999) and bind to CSPGs, hyaluronan, and fibronectin (Chiquet-Ehrismann, 1991, 2004; Midwood, Chiquet, Tucker, & Orend, 2016). The expression of tenascin-R is specific to the CNS and produced by oligodendrocytes, as well as by neurons during development (Fuss, Wintergerst, Bartsch, & Schachner, 1993; Pesheva, Spiess, & Schachner, 1989), while tenascin-C is a more ubiquitous ECM protein (Midwood et al., 2016). In the CNS, tenascin-C is expressed by astrocytes and oligodendrocytes (Bartsch et al., 1992; Czopka, Von Holst, Schmidt, ffrench-Constant, & Faissner, 2009; Garwood et al., 2004; Gotz, Bolz, Joester, & Faissner, 1997; Gutowski et al., 1999; Prieto, Jones, Cunningham, Crossin, & Edelman, 1990). Upon ethidium bromide-induced demyelination, both tenascin-C and tenascin-R are upregulated and produced by reactive astrocytes and recruited OPCs, respectively (Zhao et al., 2009). While tenascin-C and tenascin-R are enhanced at the gene expression level upon cuprizone-induced demyelination in one study (Zendedel et al., 2016), another study shows that tenascin-C mRNA is not strongly upregulated (Hibbits et al., 2012). In active MS lesions, both tenascin-C and tenascin-R are significantly downregulated, extending even beyond the edge of the lesion, that is at NAWM areas where the presence of macrophages is abundant (Gutowski et al., 1999). In the center of inactive MS lesions, the levels of tenascin-C and tenascin-R are almost similar to NAWM, while being reduced at the lesion rim (Gutowski et al., 1999). While tenascin-C mRNA levels are upregulated in inactive lesions compared to NAWM (Zeis, Howell, Reynolds, & Schaeren-Wiemers, 2018), tenascin-C and tenascin-R protein levels in active lesions resemble adjacent NAWM (Gutowski et al., 1999). Reactive astrocytes are likely the main producers of both tenascins in chronic lesions. Tenascin-R coatings function as a stimulator of OPC differentiation and upregulate myelin proteins (Czopka, Von Holst, Schmidt, ffrench-Constant, & Faissner, 2009; Pesheva, Gloor, Schachner, & Probstmeier, 1997), while at least in vitro the formation of myelin
membranes is retarded (Czopka et al., 2009). Tenascin-C inhibits OPC migration (Frost et al., 1996; Garcia, Faissner, & ffrench-Constant, 2001; Kiernan, Gotz, Faissner, & ffrench-Constant, 1996) as well as differentiation and myelin membrane formation (Czopka, von Holst, ffrench-Constant, & Faissner, 2010; Czopka et al., 2009; Garwood et al., 2004), while promoting OPC proliferation (Garcion et al., 2001) and survival (Garwood et al., 2004). Oligodendroglial contactin is involved in the tenascin-C-mediated inhibition of OPC differentiation (Czopka, von Holst, ffrench-Constant, & Faissner, 2010). Thus, while its degradation in active MS lesions may initially benefit OPC migration, premature re-expression by reactive astrocytes in chronic lesions may maintain OPCs in an immature state.

Thrombospondin-1 is a trimeric matricellular protein that is present in the adult CNS (Asch, Leung, Shapiro, & Nachman, 1986), expressed by astrocytes (Scott-Drew & ffrench-Constant, 1997) and involved in CNS synaptogenesis (Christopherson et al., 2005; Risher & Eroglu, 2012). Upon ethidium bromide-induced demyelination, thrombospondin-1 mRNA and protein are slightly upregulated at early demyelination (Zhao et al., 2009). Immunohistochemical analysis of thrombospondin in MS lesions has not been reported thus far. However, thrombospondin-1 mRNA levels are increased in active and inactive MS lesions (Mohan et al., 2010), while thrombospondin-1 protein levels are enriched in mixed active/inactive and inactive MS lesions (Satoh et al., 2009; Sikkema et al., 2018). Also, thrombospondin binds to fibronectin (aggregates) and collagen V (Adams & Lawler, 2011; Aho & Uitto, 1998; Sikkema et al., 2018), which are present in the interstitial ECM of MS lesions. In vitro, thrombospondin-1 coatings promote migration of an OPC-like cell line (Scott-Drew & ffrench-Constant, 1997), indicating that thrombospondin-1 may be involved in OPC recruitment.

Osteopontin is a secreted matricellular protein that binds directly to fibronectin and collagen (Giachelli & Steitz, 2000), and is expressed in grey but not in white matter in the normal adult CNS (Selvaraju et al., 2004; Shin, Cha, Chun, Chung, & Lee, 1999; Zhao, Fancy, ffrench-Constant, & Franklin, 2008). However, the protein is transiently upregulated in white matter upon cuprizone- (Selvaraju et al., 2004) and ethidium bromide-induced (Zhao, Fancy, ffrench-Constant, & Franklin, 2008) demyelination. Microglia/macrophages and astrocytes show osteopontin immune reactivity (Selvaraju et al., 2004; Zhao et al., 2008), while only microglia/macrophages harbor osteopontin mRNA (Zhao et al., 2008), and likely secrete osteopontin in the demyelinated areas. Microarray analyses showed that osteopontin mRNA levels are higher in active MS lesions than in control white matter (Chabas et al., 2001). In addition, proteomic analyses revealed that osteopontin protein is enriched in mixed active/inactive MS lesions compared to control white matter and inactive MS lesions (Satoh et al., 2009). Within active MS lesions, osteopontin is mainly present in macrophages and microvascular endothelial cells, while reactive astrocytes also express osteopontin in active and mixed active/inactive MS lesions (Diaz-Sanchez, Williams, DeLuca, & Esiri, 2006; Sinclair, Mirakhor, Kirk, Farrell, & McQuaid, 2005). Moreover, osteopontin is also present in astrocytes in NAWM at areas with high levels of microglia activation (Chabas et al., 2001; Sinclair et al., 2005) and occasionally in white matter oligodendrocytes (Chabas et al., 2001; Diaz-Sanchez et al., 2006). Contrastingly, while protein levels are increased, gene expression analysis of adjacent NAWM, rim and center of mixed active/inactive and inactive lesions revealed a downregulation in osteopontin transcript levels (Koning, Bo, Hoek, & Huitinga, 2007). In vitro studies demonstrated that soluble osteopontin induces proliferation in OPC-like cell lines and increases the expression of myelin basic protein (MBP) and myelin membrane formation in mixed cortical cultures (Selvaraju et al., 2004). Although proliferation and differentiation are seemingly opposite processes, both are necessary for successful remyelination. Similar to an integrin- and developmental stage-dependent switch in growth factor signaling (Baron, Colognato, & ffrench-Constant, 2005; Baron et al., 2002; Colognato et al., 2002), the effect of osteopontin on OPC behavior may depend on the developmental stage and to which integrin it binds. Thus, osteopontin may mediate OPC proliferation via integrin αvβ3, whereas OPC differentiation may be induced by signaling via integrin αvβ5 (Blaschuk, Frost, & ffrench-Constant, 2000). In favor of a developmental stage-dependent effect is that osteopontin is added to differentiating mixed cortical cultures (Selvaraju et al., 2004), that is when OPC proliferation is ceased. Alternatively, in mixed cultures the effect of osteopontin on OPC differentiation may be indirect. Hence, osteopontin may be beneficial to remyelination at both OPC recruitment and OPC differentiation levels (Figure 2).

The involvement of other matricellular proteins, including CCNs, SPARCs, and fibulins in ECM remodeling upon demyelination and in remyelination failure in MS, remains to be determined. Of interest is that Cyr61/CCN1 and CTGF/CCN2 associate with fibronectin aggregates (Sikkema et al., 2018), and that CTGF/CCN2 inhibits OPC differentiation (Ercan et al., 2017; Lamond & Barnett, 2013), adding another level of complexity to fibronectin aggregate-mediated perturbation of OPC maturation. In addition, regulatory T cell-derived NOV/CCN3 promotes oligodendrocyte and myelination in demyelinated toxin-induced lesions, although it is not clear whether CCN3 acts directly on cells of the oligodendrocyte lineage or via other cells (de la Vega Gallardo, Dittmer, Dombrowski, & Fitzgerald, 2018; Dombrowski et al., 2017).

## 4 DISTURBED ECM EXPRESSION IN MS LESIONS

When comparing the expression pattern of ECM molecules in the distinct MS lesions with the expression pattern of ECM molecules that are altered upon successful CNS remyelination in rodents both similarities and abnormalities are noticed (Table 1, Figure 1). In addition, inter- and intralesional heterogeneity in the localization of ECM proteins in the distinct white matter lesions is apparent. Why the expression of ECM proteins in MS lesions is abnormal, whether this affects remyelination, and what contributes to ECM heterogeneity in MS lesions are reviewed next.
4.1 | Role of abnormal ECM in remyelination failure

Similar to demyelinating CNS white matter injury in experimental models, ECM molecules that are absent in the healthy adult CNS are upregulated upon demyelination in MS lesions, indicating a “normal” initial response to demyelination in MS lesions. These interstitial ECM proteins in general contribute to OPC migration and proliferation (Figure 2), and prevent premature OPC differentiation. In experimental models where remyelination is successful, the proteins are transiently expressed and cleared at the onset of remyelination to allow for OPC differentiation, among others by the increased expression of laminin (Zhao et al., 2008). In MS lesions, the initial transient increased ECM proteins persist, while the remyelination beneficial ECM protein laminin is virtually absent in the interstitial ECM MS. This indicates that the “normal” ECM remodeling response in MS lesions is derailed and not converted to a “remyelination-favoring mode” (Table 1 and Figure 1). Another striking difference is that CSPGs, present in the interstitial ECM CNS, are upregulated upon demyelination in experimental toxin-induced demyelination rodent models, and downregulated at the center of white matter MS lesions. As CSPGs are present inside phagocytic macrophages in the center of active lesions (Sobel & Ahmed, 2001), CSPGs may be initially deposited in active MS lesions and cleared by macrophages. (Sobel & Ahmed, 2001). Whether the absence of CSPGs in inactive lesions is also the result of active clearance remains to be determined. CSPG proteins are merely inhibitory for OPC recruitment and differentiation (Figure 2), which in healthy CNS may keep the adult OPCs at their location in an immature state. Therefore, their transient degradation may be beneficial to remyelination. However, these ECM proteins are upregulated and not downregulated upon demyelination in experimental models, although their expressions at early time points, that is immediately upon induction of demyelination, have not been reported thus far. Intriguingly, with regard to ECM expression, the edges of active and mixed active/inactive MS lesions resemble demyelinated areas in experimental models more than the core of MS lesions, with the exception of tenascins (Table 1 and Figure 1). Indeed, remyelination at the rim is more pronounced than that at the MS lesion center (Raine & Wu, 1993), suggesting that the interstitial ECM at the edges of active and mixed active/inactive lesions is more permissive for remyelination than the ECM in the center of the lesions. However, the degree of remyelination depends on the net ratio of myelination-supportive (e.g., laminin) and myelination-inhibitory ECM molecules (e.g., fibronectin aggregates, hyaluronan, CSPGs). In vitro, fibronectin-derived inhibitory signals dominate over myelination-promoting laminin signals (Baron et al., 2014), while only very high amounts of laminin can overcome the inhibitory effect of CSPGs on OPC differentiation (Lau et al., 2012; Sun et al., 2017). In fact, in some studies, the effect of CSPGs on OPC behavior have been performed on a relative high laminin background (Pendleton et al., 2013; Siebert & Osterhout, 2011), further indicating that CSPGs are likely dominant over laminin. Thus, given that aggregates of fibronectin accumulate in chronic MS lesions and CSPGs at the edge of (chronic) active MS lesions, these remyelination-inhibiting obstacles have to be removed or their effects have to be bypassed to enable regeneration of myelin.

4.2 | Role of glial scar and inflammation

Astrocytes, microglia, and macrophages are the producers of ECM proteins in MS lesions. The astrocyte response in MS lesions is of dual nature, that is both astrocyte loss and astrogliosis are associated with remyelination failure (Correale & Farez, 2015; Nair, Frederick, & Miller, 2008; Williams et al., 2007). Major barriers that contribute to remyelination failure in MS are glial scars, that is astrogliosis, which parallels ECM alterations, and the ensuing inflammation. The changes in reactive astrogliosis are regulated in a context-specific manner (Sofroniew & Vinters, 2010), and given that the distinct cellular composition between the edge and center of, for example, mixed active/inactive MS lesions, this may result in a different signaling environment and different extent of gliosis, and hence differences in ECM composition. Indeed, in MS lesions, antigenic differences that reflect the state of reactive astrocytes, that is between astrocytes at the lesion edge and center, exist (Holley, Gveric, Newcombe, Czunzer, & Gutowski, 2003; Park et al., 2019), indicating that at least two distinct glial scars are formed. The glial scar around the lesions is formed as a neuroprotective response to disruption of the BBB, and mainly prevents further expansion of the lesion (Nair et al., 2008). If so, it is tempting to suggest that the glial scar formed within the lesion center is a result of the fact that the required cells for tissue repair, that is cells that express the appropriate proteases that clear the transient ECM, could not reach the lesion site. Indeed, the edge of active and inactive MS lesions is mainly composed of CSPGs (Table 1 and Figure 1) that prevent the entry of inflammatory cells, including lymphocytes and macrophages in chronic MS lesions.

Thus, the different responses of astrocytes likely result in different reactive phenotypes that are present in the distinct MS lesions (John et al., 2002; Nair et al., 2008), which may account for the distinct interlesional and intralesional local ECM profile. Major contributors to the formation of glial scars are inflammatory mediators, such as IL-1β, IL-10, TNFα, IFNγ, and TGFβ, as well as endogenous TLR ligands (Asher et al., 2000; John et al., 2004; John, Lee, & Brosnan, 2003; John, Lee, Song, Rivieccio, & Brosnan, 2005; Sofroniew & Vinters, 2010). Importantly, the different levels and distinct composition of inflammatory cues are present in the distinct lesions and at the borders of expanding active MS lesions (Cannella & Raine, 1995; Koning et al., 2007; Woodroofe, Bellamy, Feldmann, Davison, & Czunzer, 1986). A glial scar barrier is absent in cuprizone-induced demyelination models, where inflammation does not play a major role (Hibbits et al., 2012), while removing the toxin leads to the clearance of ECM molecules and enables remyelination. In contrast, glial scars are formed in immune-mediated demyelination models such as experimental autoimmune encephalomyelitis (EAE) and Theiler's murine encephalomyelitis (TME) that more closely resemble the situation in MS, including an inflammatory environment, and where remyelination is insufficient (Haist, Ulrich, Kalkuhl, Deschl, & Baumgartner, 2012; Smith & Eng, 1987). For example, similar to MS lesions, in chronic relapsing EAE but not upon toxin-induced demyelination, astrocyte-derived HMW hyaluronan (Back et al., 2005) and fibronectin aggregates...
(Stoffels et al., 2013) accumulate in lesioned areas. Also, CSPG proteins neurocan, aggregan, brevican, and versican V1 are also increased at the peak of clinical EAE severity, whereas the expression of versican V2 is reduced (Sajad, Zargan, Chawla, Umar, & Khan, 2011; Stephenson et al., 2018). Aggrecan is only present in spinal cord grey matter, while versican V1 is also present in peri-vascular cuffs and closely associated with infiltrating immune cells (Sobel & Ahmed, 2001). In TME, which is characterized by mild inflammation and insufficient OPC differentiation (Ulrich, Seeliger, Kreutzer, Germann, & Baumgartner, 2008), ECM alterations correlated with the development of astrogliosis and include among other intralesional deposition of laminin, tenascin-C, neurocan, and fibronectin, while phosphacan expression decreases and aggrecan expression remains similar (Haist et al., 2012).

Next to reactive astrocytes, other major cellular components that contribute to the dysregulated remodeling of the ECM in MS lesions are the resident microglia and the infiltrating macrophages. Microglia and macrophages are actively remodeling the ECM being producers of ECM proteins and of MMPs, that are proteases that are able to degrade and remodel the ECM (Lloyd & Miron, 2019). Given the complex beneficial and detrimental roles of microglia and macrophages in remyelination (Lloyd & Miron, 2019), and their mixed phenotype in MS lesions (Miron et al., 2013; Peferoen et al., 2015; Vogel et al., 2013), the persistent or incorrect expression of ECM molecules in MS lesions might result from altered expression patterns of microglia/macrophage-derived MMPs (Kieseier et al., 1999), which is discussed next.

5 | MMPs UPON DEMYEILING INJURY

MMPs are a family of proteolytic enzymes, also referred to as endopeptidases, that are essential for ECM remodeling in many processes. These include migration, wound healing, tissue morphogenesis, cell differentiation, neuronal growth, and several signaling processes (Lu et al., 2011; Page-McCaw et al., 2007). This family consists of 26 members, of which 24 are present in mammalian, divided into six subgroups: collagenases (MMP1, MMP8, MMP13), gelatinases (MMP2, MMP9), stromelysins (MMP3, MMP10, MMP11), matrilysins (MMP7, MMP26), membrane type (MT)-MMPs (MMP14, MMP15, MMP16, MMP17, MMP24, MMP25), and other MMPs. All newly synthesized MMPs contain a signal peptide that is cleaved during transport via the secretory pathway. There are small differences in the structure of the MMPs, but three common domains are identified (Figure 3; Page-McCaw et al., 2007).

| Secreted MMPs | Membrane-bound MMPs |
|---------------|---------------------|
| MMP7, -26     | MMP14, -15, -16, 24 |
| MMP1, -3, -8, -10, -12, -13, -19, -20, -27 | MMP17, -25 |
| MMP11, -21, -28 | MMP23 |
| MMP2, -9      | |

**FIGURE 3** Schematic structure of the matrix metalloproteinase family. Matrix metalloproteinases (MMPs) share a conserved domain structure of a N-terminal pro-peptide and catalytic domain and a C-terminal hemopexin domain that is connected via a hinge region. MMPs are secreted or are membrane type (MT-MMPs) that are linked to the plasma membrane via transmembrane domain or by a GPI anchor.
A propeptide region is located at the N terminus of the protein, which prevents proteolytic activity through interaction with the catalytic domain. This domain is removed to activate the enzyme. The catalytic domain is also located at the N terminus, in which a zinc-binding motif is inserted. The catalytic domain of gelatinases contains an additional domain, called fibronectin type II-like domain. Except for matrix metalloproteinase (MMP) 3, the C-terminal part of MMPs contains a haemopexin-like domain, which is involved in substrate recognition and anchoring, and interacts with the catalytic domain via a hinge region. The membrane-type MMPs (MT-MMPs) are membrane proteins, whereas all other MMPs are secreted into the extracellular space. Here, they are able, however, to localize at the cell surface by binding to MT-MMPs and other cell surface molecules. MMPs have overlapping substrates, and in addition, these enzymes are jointly capable of degrading virtually all ECM proteins. MMPs play also a crucial role in the shedding of growth factors, cytokines, chemokines, receptors, and cell-adhesion molecules, and activation of other MMPs (Page-McCaw et al., 2007). Obviously, given this diversity, the activity of MMPs is tightly regulated. For expression, cells have to be activated for transcription of MMPs. The secreted forms require cleavage of the propeptide for activation, while MMP activity can be inhibited by tissue inhibitors of metalloproteinases (TIMPs; Lu et al., 2011; Page-McCaw et al., 2007).

In the healthy adult CNS, MMP activity is crucial in supporting cognitive processes, such as learning and memory, due to ECM remodeling and the regulation of synaptic plasticity and long-term potentiation (Agrawal, Lau, & Yong, 2008; Huntley, 2012). Also, the physiology of axons, myelin turnover, and angiogenesis are regulated by several MMPs. In addition, differential expression of MMPs in development of the CNS is essential for neurogenesis and axonal growth as well as for the function of oligodendrocytes and myelination. MMPs also play important roles in repair processes and in pathology, and both beneficial and detrimental functions have been assigned to MMPs in the injured CNS (Javaid, Abdallah, Ahmed, & Sheikh, 2013; Yong, 2005; Yong, Power, Forsyth, & Edwards, 2001). To remodel the ECM, demyelination and subsequent remyelination require a transient alteration in the expression pattern of MMPs. In addition, MMPs may affect OPC behavior by modulating the bioavailability of several proteins, including growth factors and cytokines (Dubois-Dalcq & Murray, 2000; McCawley & Matrisian, 2001). However, uncontrolled and abundant expression of MMPs may damage the BBB, induce inflammation, and neurotoxicity, which may lead to (demyelinating) injury (Agrawal et al., 2008; Yong, 2005; Yong et al., 2001). Of interest, synergism among MMP2, MMP9, and MMP7 genes may be a susceptibility factor for MS (Rahimi et al., 2016). In the following, we provide an overview of current insight into the role of distinct MMPs in successful remyelination and their expression in the distinct MS white matter lesions (summarized in Table 2 and Figure 1). Also, the consequence for demyelinating pathology is discussed.

### TABLE 2: Expression of MMPs upon toxin-induced demyelination and in distinct white matter MS lesions and their MS relevant ECM targets

| MMP   | MS relevant ECM targets                                      | Experimental rodent models | Multiple sclerosis |
|-------|-------------------------------------------------------------|----------------------------|--------------------|
|       |                                                             | Demyelination | Remyelination |
|       |                                                             | Active       | Mixed active/inactive | Inactive |
| MMP2  | Aggrecan, versican, neurocan, collagen V, fibronectin, laminin, vitronectin, tenasin-C (large) | =            | =                  | =       |
| MMP3  | Aggrecan, versican, neurocan, phosphacan, collagen V, fibronectin, laminin, vitronectin, tenasin-C (large), osteopontin | ↑            | ↑                  | ↑       | =       |
| MMP7  | Aggrecan, fibronectin, laminin, vitronectin, tenasin-C (large, small), osteopontin | =            | ↑                  | ↑       | ↓       | ↓       |
| MMP9  | Aggrecan, collagen V, fibronectin, vitronectin, osteopontin | =            | ↑                  | ↑       | ↑       | =       |
| MMP12 | Aggrecan, fibronectin, laminin, vitronectin, osteopontin    | ↑            | ↑                  | ↑       | =       |
| MMP19 | Aggrecan, fibronectin, laminin, tenasin-C (large)           | n.d.         | n.d.               | ↑       | ↑       | =       |

Note: Increased (↑), decreased (↓), and similar (=) expression compared to control.

Abbreviation: n.d., not determined.

*Marked expression at the edge.

*Also based on biochemical analysis (Wang et al., 2018).
demyclination and subsequent remyelination. At early demyelina-
tion, MMP3 mRNA is upregulated, followed by a return to normal
levels during late stages of demyelination. A second more promi-
tent upregulation of MMP3 mRNA is observed upon remyelination,
which is also reflected at the protein level (Skuljec et al., 2011; Wang
et al., 2018). MMP3 mRNA levels, but not protein levels, are also
increased upon early demyelination in the lyssolecithin-induced
demyelination model (Wang et al., 2018). At the protein level, MMP3,
also referred to as stromelysin-1, is predominantly expressed by
astrocytes, but may also be produced by damaged neurons, mi-
croglia, and oligodendrocytes following other types of CNS injury
(Van Hove, Lemmens, Van de Velde, Verslegers, & Moons, 2012). An
(early) upregulation of MMP3 is also observed in the inflammatory
TME model (Hansmann et al., 2012; Ulrich et al., 2006), and in EAE
(Weaver et al., 2005). In these inflammatory models with insufficient
remyelination, MMP3 may aid to the disruption of the BBB. In ad-
dition, the early upregulation of MMP3 may cause the breakdown of
myelin and exacerbate demyelination (Chandler, Cossins, Lury,
& Wells, 1996; Shiraya et al., 2009). In contrast, the upregulation of
MMP3, observed at remyelination in the toxin-induced models,
may facilitate myelin regeneration. MMP3 modulates several sign-
aling pathways, such as the bioavailability of some soluble growth
factors, including IGF-1, that facilitate oligodendrocyte differentia-
tion and myelin production (D’Ercole, Ye, & O’Kusky, 2002; Dubois-
Dalcq & Murray, 2000; Fowlkes et al., 2004; McCawley & Matrisian,
2001; Zeger et al., 2007). In addition, MMP3 degrades several ECM
proteins, including CSPGs and fibronectin, and myelin debris, which
are components that inhibit differentiation and maturation of oligo-
dendrocytes (Figure 2; Kotter, Li, Zhao, & Franklin, 2006; Lau et al.,
2012; Muir et al., 2002; Stoffels et al., 2013; Van Hove, Lemmens,
Vele, Verslegers, & Moons, 2012). Hence, MMP3 may be benefi-
ciary of ECM remodeling to facilitate remyelination. MMP3 mRNA is
not upregulated in MS lesions compared to white matter of healthy
subjects (Lindberg et al., 2001). However, MMP3 protein is present in
hypertrophic astrocytes in active and chronic white matter MS
lesions, in microglia/macrophages in active lesions and on vascu-
lature in active and mixed active/inactive MS lesions (Maeda & Sobel,
1996; Wang et al., 2018). Biochemical analysis revealed that MMP3
protein levels were increased in mixed active/inactive MS lesions,
but not inactive lesions compared to control white matter (Wang
et al., 2018). Of interest, MMP3 is a potent activator of other MMPs,
such as MMP7 and MMP9 (Lu et al., 2011; Maeda & Sobel, 1996; Van
Hove et al., 2012), which are also prominently present in MS lesions
(see below).

5.2 | MMP9

The expression level of MMP12, or macrophage elastase, is signifi-
cantly altered upon cuprizone-induced demyelination. At demyeli-
nation, MMP12 mRNA is predominantly produced by microglia/
macrophages (Skuljec et al., 2011). In this phase, MMP12 may func-
tion in cellular migration of macrophages through ECM remodeling
and degeneration of myelin membranes through cleavage of MBP,
one of the major myelin components (Chandler et al., 1996; Gronski
et al., 1997; Shiplew, Wesselschmidt, Kobayashi, Ley, & Shapiro,
1996). The elevated expression pattern continued during remyelina-
tion, where MMP12 is produced by astrocytes and to some extent
by oligodendrocytes, rather than microglia/macrophages (Skuljec
et al., 2011). Upon remyelination, astrocyte-derived MMP12 may
contribute to clearance of both myelin debris and the transient-
expressed ECM proteins, and thus induce a stimulatory environment
for remyelination. Also, during CNS development, MMP12 releases
IGF-1 from IGF binding protein 6 and is required for process elon-
gation and OPC differentiation (Larsen, DaSilva, Conant, & Yong,
2006; Larsen & Yong, 2004). Studies in EAE and TME models dem-
strated an upregulation of MMP12 by microglia/macrophages and
a suggested role in disease progression and chronic phases
of demyelination, respectively (Dasilva & Yong, 2008; Hansmann
et al., 2012; Ulrich et al., 2006). In contrast, the EAE disease course
is worse in MMP12 knockout than in wild type mice, which is medi-
atied in part by modulating the Th1/Th2 effector cytokine balance
(Weaver et al., 2005) and MMP12-mediated cleavage of osteopon-
tin (Goncalves DaSilva, Liaw, & Yong, 2010). In fact, osteopontin
is linked to relapses by enhancing the survival of activated T cells
(Hur et al., 2007). This indicates that MMP12 is a protective mol-
ecule in EAE. In TME, MMP12 is likely involved in demyelination
and extravasation of macrophages, and not in BBB damage or ECM
remodeling (Hansmann et al., 2012). In MS lesions, MMP12 protein
is present in foamy macrophages and upregulated within active
demyelinating lesions, and at the rim of inactive MS lesions and to
a lesser extent in the center of mixed active/inactive and inactive
lesions (Vos, van Haastert, de Groot, van der Valk, & de Vries,
2003). In contrast to cuprizone-induced demyelination, in MS lesions,
MMP12 is not observed in astrocytes and oligodendrocytes. Hence,
the predominant macrophage expression of MMP12 in active demyelinating
MS lesions may have a dual role with regard to demyelination, that is
it may induce death of activated T cells by cleaving osteopontin, but
may also induce bystander demyelination by cleaving MBP. Notably,
MMP12 is present at lower levels in cerebrospinal fluid (CSF) of MS
patients compared to control CSF (Castellazzi et al., 2018).

5.3 | MMP9

MMP9, also called gelatinase B, is one of the most studied MMPs in
MS. While MMP9 mRNA expression is unaltered upon cuprizone-
induced demyelination compared to unlesioned white matter tis-
sue (Skuljec et al., 2011), MMP9 protein levels are upregulated
predominantly in microglia and macrophages at the onset of remy-
elination upon lyssolecithin-induced demyelination (Larsen, Wells,
Stallcup, Opdenakker, & Yong, 2003). MMP9 mRNA and protein
levels are upregulated in active and mixed active/inactive demyelinating
MS lesions (Anthony et al., 1997; Cossins et al., 1997; Czunzer et al.,
1996; Lindberg et al., 2001; Maeda & Sobel, 1996; Mohan et al.,
2010). MMP9 mRNA expression is also upregulated in an EAE model
MMP2, another member of the gelatinase family, also referred to as gelatinase A, and is in contrast to MMP3, MMP9, and MMP12, constitutively expressed in the CNS and CSF (Anthony et al., 1997; Rosenberg, 2002). MMP2 mRNA expression is not altered during demyelination and remyelination in a cuprizone-induced demyelination model (Skuljec et al., 2011), and only minor upregulation is observed in EAE (Weaver et al., 2005), in TME (Ulrich et al., 2006) and active MS lesions (Lindberg et al., 2001; Mohan et al., 2010). However, MMP2 protein is present in macrophages and infiltrating cells in the perivascular area of active MS lesions (Anthony et al., 1997; Diaz-Sanchez et al., 2006; Maeda & Sobel, 1996). MMP2-expressing macrophages are also present at the border of mixed active/inactive lesions, while its expression in chronic lesions is only perivascular (Anthony et al., 1997; Diaz-Sanchez et al., 2006). As MMP9, MMP2 is likely involved in disruption of the BBB (Rosenberg et al., 1992) and has the highest activity in MBP degradation (Chandler et al., 1995; Diaz-Sanchez et al., 2006). MMP2 appears to be more abundant in MS lesions than MMP9, and its expression is predominant in areas of damaged axons, particular at lesion borders (Diaz-Sanchez et al., 2006). Interestingly, MMP2 is also upregulated in NAWM areas adjacent to the lesions (Anthony et al., 1997; Diaz-Sanchez et al., 2006; Maeda & Sobel, 1996). Also, higher levels of MMP2 are present in serum of MS patients (Bar-Or et al., 2003; Benesova et al., 2009). Whether MMP2 may play a role in regeneration of myelin other than its potential to locally degrade only CSPGs, but not laminin, which is present in the same area (Zuo, Ferguson, Hernandez, Stetler-Stevenson, & Muir, 1998), remains to be determined.

5.5 | MMP7

MMP7, also called matrilysin, is constitutively expressed in the brain (Anthony et al., 1997; Wang et al., 2018). However, its mRNA levels are at the lower limit of detection (Clements et al., 1997; Mohan et al., 2010). Given that MMP7 has a potent activity and a broad substrate specificity, it has been suggested that MMP7 may be a regulator of ECM turnover in the healthy brain (Anthony et al., 1997). MMP7 mRNA expression is neither increased upon cuprizone-induced demyelination (Skuljec et al., 2011) nor in the TME (Ulrich et al., 2006) model, while MMP7 mRNA levels were increased upon lysolecithin-induced demyelination, and early remyelination (Wang et al., 2018). In the latter model, MMP7 is localized extracellularly and present in microglia/macrophages (Wang et al., 2018). Contrasting findings are reported in EAE models. In a mouse MOG peptide-induced EAE model MMP7 mRNA levels remain similar (Weaver et al., 2005), while MMP7 mRNA is increased by 500-fold during the course and as protein present in invading macrophages in a MOG-induced rat EAE model (Clements et al., 1997) and increased at the peak of an adoptive-transfer rat EAE model (Kieseier et al., 1998). Also, contrasting findings have been reported for MMP7 mRNA levels in MS lesions: MMP7 mRNA expression is increased in all lesion types in some studies (Cossins et al., 1997; Lindberg et al., 2001), while it was undetectable in active and chronic MS lesions in another study (Mohan et al., 2010). MMP7 protein is localized to parenchymal macrophages and occasionally observed in astrocytes in active MS lesions with a weaker expression in the center than at the edge of the lesion (Cossins et al., 1997; Wang et al., 2018). MMP7 expression in macrophages is also prominent at the lesion borders of mixed active/inactive MS lesions (Anthony et al., 1997) and not as prominent in the center (Cossins et al., 1997), while biochemical analysis reveals that proMMP7 expression is reduced in mixed active/inactive and inactive lesions (Wang et al., 2018). In remyelinated lesions, total...
mRNA levels of MMP11 and MMP14 are more than 2-fold higher in extensive qPCR analysis of 23 MMPs in control white matter, ac-  
elination (Skuljec et al., 2011). Mohan et al. (2010) performed an  
mRNA is downregulated at demyelination but upregulated at remy-  
to be downregulated at conditions of demyelination, while MMP24  
myelination and remyelination. MMP15 mRNA has been reported  
remyelination, while MMP14 mRNA is upregulated upon both de-  
tered mRNA expression patterns upon cuprizone-induced demye -  
ation (Werner et al., 2008) and macrophage recruitment (Manicone  
control white matter (Werner et al., 2008). In the adult CNS, MMP28  
analysis also shows a marked upregulation in NAWM compared to  
EAE (Werner et al., 2008). In addition, MMP28 mRNA is increased  
been shown in one demyelinated, uncharacterized MS lesion, and in  
Figure 1; Stracke et al., 2000). Upregulated MMP28 expression has  
éct of tenascin-C of which the expression is reduced in active  
expression levels of MMP7 are comparable to control white matter  
ter of healthy subjects with occasional expression in macrophages  
(Wang et al., 2018). MMP7 is not elevated in serum of MS patients,  
which is in contrast to MMP9 and MMP2 (Bar-Or et al., 2003). The  
exact role of MMP7 is not well understood, but like the other MMPs,  
a role in extravasation of monocytes into the tissue and migration  
of macrophages through remodeling of basement membrane ECM,  
such as proteoglycans, fibronectin, laminin, and elastin, can be fore-  
seen. In addition, MMP7 potentially induces bystander demyelina-  
tion and axonal loss, although likely to a lesser extent than MMP2  
and MMP9 (Anthony et al., 1997). However, when expressed in the  
CNS parenchyma in a timely manner, MMP7 may also be beneficial  
in clearing the transiently expressed ECM components and myelin  
debris (Chandler et al., 1995).

5.6 | Other MMPs

Other MMPs that have been analyzed at the protein level in MS le-  
sions are MMP1 (Maeda & Sobel, 1996), MMP19 (van Horssen et al.,  
2006), and MMP28 (Werner, Dotzla, & Smith, 2008). MMP1 is ex-  
pressed in the majority of macrophages in active MS lesions (Maeda  
& Sobel, 1996). MMP19 is constitutively expressed by microglia in  
the healthy adult CNS, and highly expressed in macrophages in the  
parenchyma and perivascular areas in active MS lesions and in the  
rims of mixed active/inactive lesions, and occasionally by reactive  
astrocytes (van Horssen et al., 2006). Also, MMP19 mRNA levels are  
increased in active and inactive MS lesions (Mohan et al., 2010). While  
the function of MMP19 in MS pathology remains to be established,  
it is interesting to note that MMP19 specifically degrades the large  
isoform of tenascin-C of which the expression is reduced in active  
MS lesions and mixed active/inactive lesions borders (Table 1 and  
Figure 1; Stracke et al., 2000). Upregulated MMP28 expression has  
been shown in one demyelinated, uncharacterized MS lesion, and in  
EAE (Werner et al., 2008). In addition, MMP28 mRNA is increased  
in inactive MS lesions (Mohan et al., 2010). Moreover, Western blot  
analysis also shows a marked upregulation in NAWM compared to  
control white matter (Werner et al., 2008). In the adult CNS, MMP28  
is mainly expressed in neurons and is a negative regulator of myelination  
(Werner et al., 2008) and macrophage recruitment (Manicone et al., 2009), justifying more research on this protein.

In addition to the marked altered expression patterns of MMP3  
and MMP12, several other MMPs, including MT-MMPs, show al-  
tered mRNA expression patterns upon cuprizone-induced demyel-  
ation (Skuljec et al., 2011). Thus, MMP11 mRNA is upregulated at  
remyelination, while MMP14 mRNA is upregulated upon both de-  
myelination and remyelination. MMP15 mRNA has been reported  
to be downregulated at conditions of demyelination, while MMP24  
mRNA is downregulated at demyelination but upregulated at remy-  
elination (Skuljec et al., 2011). Mohan et al. (2010) performed an  
extensive qPCR analysis of 23 MMPs in control white matter, ac-  
tive and inactive MS lesions. In addition to the discussed MMPs, the  
mRNA levels of MMP11 and MMP14 are more than 2-fold higher in  
active lesions than in control white matter and MMP11, MMP14,  
and MMP17 mRNAs are increased in inactive lesions. In contrast,  
the mRNA levels of two MMPs (MMP15 and MMP23) and one MMP  
(MMP23) were downregulated by at least 50% in active and inactive  
lesions, respectively (Mohan et al., 2010). The cellular localization  
of these MMPs and their significance for ECM remodeling, remyelin-  
ation, and MS lesion pathology remains to be determined. Of note,  
the expression levels of most of these MMPs are also altered in TME  
and EAE models (Ulrich et al., 2006; Weaver et al., 2005).

6 | MMP DYSFUNCTION IN MS LESIONS

MMPs benefit remyelination by clearing the transiently expressed  
ECM proteins upon demyelination (Figure 4a). In MS lesions, ECM  
substrates are not appropriately degraded by MMPs, among others  
by the absence of MMPs in the lesion center and the inability of  
MMPs to process substrates at the lesion rim (Figure 4b). In addition,  
the interference of MMPs with basement membranes contributes to  
demyelination. In the following, we reflect on MMP dysfunction and  
how this may contribute to abnormal ECM expression in MS lesions.

6.1 | Altered MMP expression

The expression pattern of MMPs mainly reflects the inflammatory  
activity of the lesions. In fact, the distribution patterns of MMPs  
closely resemble each other, mainly localizing in macrophages, often  
in close association with vasculature, predominantly present in ac-  
tive lesions. Concomitantly, and similar to the intralesional heteroge-  
eity of ECM proteins, a more pronounced expression at the rim of  
mixed active/inactive MS lesion compared to the core of the lesion  
is usually observed. Furthermore, MMPs in MS lesions are only oc-  
casionally present in astrocytes, with the exception of MMP3, which  
is primarily localized to astrocytes (Figure 4b). Likely, most MMPs  
in MS lesions are implicated in disruption of the BBB by affecting  
its permeability upon enzymatic cleavage of ECM molecules in  
the basement membrane, such as type IV collagen, fibronectin and  
laminin, which enables the inflammatory cells, that is lymphocytes  
and monocytes to infiltrate the CNS (Leppert, Lindberg, Kappos, &  
Leib, 2001). Also, most MMPs present in inflammatory lesions de-  
grade MBP, which may enhance demyelination and, in turn, contrib-  
utes to axonal damage (Anthony et al., 1998). However, since MBP  
is an intracellular, peripheral protein, prior myelin degeneration  
is obviously required to allow proteolytic accessibility of the protein.  
In fact, the potential of MMPs to degrade MBP may indirectly  
facilitate clearance of remyelination-inhibiting myelin debris (Kotter  
et al., 2006).

Functional in vivo evidence for a role MMP-mediated remodel-  
ing upon demyelination is currently lacking. Therefore, it is unclear  
whether the mainly microglia/macrophage-derived MMPs in MS  
are involved in the dynamic turnover of interstitial ECM upon de-  
ymyelination. As remyelination fails, among others by the persistent
FIGURE 4 Schematic representation of interstitial extracellular matrix (ECM) remodeling upon toxin-induced demyelination in experimental rodent models and in chronic demyelinated MS lesions. Upon demyelination, ECM remodeling, that is transient changes in the expression of ECM proteins and the proteolytic enzymes matrix metalloproteinases (MMPs), regulates oligodendrocyte behavior and thereby remyelination. (a) MMPs and their (ECM) targets in experimental toxin-induced demyelination rodent models of successful remyelination. MMPs are expressed by microglia/macrophages and astrocytes and benefit remyelination by (1) degrading the transiently expressed ECM proteins, (2) clearing myelin debris, and (3) by releasing insulin-like growth factor (IGF) from IGF-binding proteins. (b) MMPs and their (ECM) targets in chronic-demyelinated MS lesions. Note that MS lesions are characterized by intralesional heterogeneity in the expression of MMPs and ECM proteins, that is in the rim and center of the lesion. MMPs are mainly expressed by microglia/macrophages and contribute to demyelination by interfering with blood–brain barrier integrity. In addition, MMPs fail to degrade ECM proteins at the rim of MS lesions, and are absent at the lesion center, resulting in the accumulation of ECM proteins that inhibit oligodendrocyte progenitor cell differentiation. Of relevance, functional in vivo evidence for a role of most MMPs in ECM remodeling is currently lacking. Therefore, the indication of their ability to clear (green scissors) or not to clear (red scissors) their targets is based on their presence, their ability to degrade the indicated substrate, and the transient (cleared) or persistent (not cleared) presence of the indicated substrate. Note the distinct cellular expression of MMPs and ECM proteins in experimental rodent models and MS lesions. Thrombospondin-1 expression is increased in chronic demyelinated MS lesions, while data on (cellular) localization are not available. A-can, aggrecan; Fn, fibronectin; HA, hyalurunan; N-can, neurocan; OPN, osteopontin; P-can, phosphocan; Tn-C, tenascin-C; Tn-R, tenascin-R; TSP, thrombospondin-1; V-can, versican; Vn, vitronectin
presence of interstitial ECM components (Table 1, Figures 1 and 2), a dysfunction in MMP expression and/or their activation are more likely relevant parameters in this regard. As discussed above, the composition of both ECM molecules and ECM-degrading MMPs differ between demyelinating injury in experimental rodent models and the distinct white matter MS lesions (summarized in Tables 1 and 2). The tightly and temporal (cellular) expression of several MMPs regulate the clearance of the transiently expressed ECM molecules to enable regeneration of myelin (Figure 4a). In contrast, in MS lesions, where remyelination fails, a different and more persistent pattern of MMPs and ECM molecules is observed (Figure 4b; Agrawal et al., 2008; van Horsen et al., 2007). For example, the mRNA levels of gelatinases MMP2 and MMP9 are not (MMP2) or transiently (MMP9) upregulated upon demyelination and remyelination models (Larsen et al., 2003; Skuljec et al., 2011), but these enzymes are highly expressed in active and mixed active/inactive MS lesions (Anthony et al., 1997; Cossins et al., 1997; Cuzner et al., 1996; Lindberg et al., 2001; Maeda & Sobel, 1996). The disturbed ECM in MS lesions may regulate the expression of these MMPs. Fibronectin increases the expression levels and proteolytic activity of MMP2 and MMP9 by T-lymphocyte cell lines (Esparza et al., 1999). Also, fibronectin and vitronectin induce the expression of MMP9 in microglia (Milner et al., 2007), and thrombospondin-1 regulates MMP2 and MMP9 activity (Bein & Simons, 2000; Rodriguez-Manzaneque et al., 2001; Tarabottelli et al., 2000) and influences the balance between MMPs and inhibitors (TIMP) in tumor cells (John, Hu, Rothman, & Tuszynski, 2009). Moreover, gelatinases, and MMP9 in particular, are not the most efficient proteases in proteolysis of ECM proteins (Fosang et al., 1992; Imai, Shikata, & Okada, 1995; Muir et al., 2002; Murphy, Cockett, Ward, & Docherty, 1991; Siri et al., 1995). Rather, spatial temporal regulation of MMP2 and MMP9 expression may be more important. Thus, MMP9 cleaves membrane-spanning CSPG NG2, allowing (morphological) oligodendrocyte differentiation (Larsen et al., 2003), while incorrect localization of MMP9, as mediated by fibronectin, perturbs oligodendrocyte process branching (Siskova et al., 2009). Also, MMP2, present at the tips of the axonal extensions, eliminates the CSPG-mediated inhibition of laminin through specific proteolytic cleavage of CSPGs rather than laminin, which is beneficial to regeneration (Zuo et al., 1998). However, MMP2 and MMP9 are also potent inducers of BBB disruption and axonal injury (Anthony et al., 1997; Diaz-Sanchez et al., 2006), and may induce bystander demyelination via degradation of MBP (Chandler et al., 1995). Hence, MMP2 and MMP9 may contribute more to a demyelinating MS pathology than to interstitial ECM remodeling for remyelination.

6.2 | MMPs are present at the rim of chronic MS lesions

MMP7 and MMP3 are more potent proteases to degrade the interstitial ECM, present in MS lesions (Imai et al., 1995; Muir et al., 2002; Murphy et al., 1991; Siri et al., 1995). Therefore, these MMPs are potentially able to reintroduce a permissive environment for remyelination and improve the neuropathological conditions in MS. Indeed, both MMPs are upregulated at remyelination following demyelination in experimental rodent models (Skuljec et al., 2011; Wang et al., 2018). MMP3 degrades the CSPGs, aggrecan, versican, neurocan, and phosphacan, while MMP7 cleaves aggrecan (Fosang et al., 1992; Muir et al., 2002). However, while CSPG expression is reduced in the center of active MS lesions, where MMP3-expressing astrocytes and MMP7-expressing macrophages are located, there is still accumulation of CSPGs at the lesions borders, where MMP7 expression is more prominent. Therefore, it may be hypothesized that MMP3 and MMP7 are actively degrading CSPGs in the center of active lesions, while these MMPs are not active at the lesion edges. Indeed, immunohistochemistry identifies and spatially localizes MMP, but it does not distinguish active from inactive MMP. Moreover, in MS lesions, most of the MMPs reside within cells and often no extracellular staining is noticed, while for interstitial ECM remodeling MMPs have to be secreted and activated. Activation of MMPs by other proteases is spatially focused and regulated by other MMPs or plasmin, which is locally generated by tissue-type plasminogen activator (t-PA; Lu et al., 2011; Page-McCaw et al., 2007). While absent in control white matter, in MS lesions t-PA is expressed in macrophages and its expression pattern is comparable to MMP expression, that is present in active and absent in chronic lesions (Cuzner et al., 1996). Another tightly regulated mechanism for MMP actions is the regulation of its activity by TIMPs (Lu et al., 2011; Page-McCaw et al., 2007). Upon toxin-induced demyelination, increased mRNA levels of TIMP1 and TIMP2 are observed at demyelination, followed by a gradual decline during remyelination (Skuljec et al., 2011; Wang et al., 2018). TIMP3 is transiently upregulated in the first week during demyelination, whereas TIMP4 is upregulated during remyelination (Skuljec et al., 2011). Lindberg et al. (2001) show that the mRNA levels of these TIMPs are not significantly altered at all MS lesion types. In contrast, Mohan et al. (2010) show that both TIMP1 and TIMP3 mRNA are upregulated in active and inactive lesions, while TIMP3 mRNA are downregulated in inactive lesions. Immunohistochemistry of TIMPs may reveal the expression patterns and cellular localization of TIMPs, and whether the delicate balance between MMP and their inhibitors is disturbed in MS lesions, that is interfere with interstitial ECM remodeling. Also, an in situ MMP activity assay on MS lesions may provide insight in localized MMP activity levels. Of note, inhibiting MMP activity reduced the clinical symptoms in an EAE model by reducing the breakdown of the BBB and demyelinating pathology (Gijbels, Galardy, & Steinman, 1994; Liedtke et al., 1998). Hence, although modulation of MMP activity can be an advantageous approach, it remains to be determined whether MMP activity within the CNS is actually inhibited in this model. In contrast to CSPGs, tenascin expression is reduced in active lesions and the lesion border of inactive lesions, indicating proteolytic activity at these sites. Upon alternative splicing, a large and small isoform of tenascin-C are generated. The small variant is more resistant to degradation, but cleaved by MMP7, while MMP3, MMP7, MMP12, and MMP19 degrade large tenascin-C. Also, other, yet
undefined MMPs, or even non-related proteases, such as cathepsin B (Bever & Garver, 1995; Mai, Sameni, Mikkelsen, & Sloane, 2002), may clear tenascins in active MS lesions and at the border of mixed active/inactive lesions.

6.3 | MMPs are absent in the center of chronic MS lesion

MMPs are hardly present in inactive and in the center of mixed active/inactive lesions, which may be the main reason why the ECM proteins, fibronectin and osteopontin, persist. While loss of function studies demonstrated that fibronectin (Stoffels et al., 2015) and osteopontin (Zhao et al., 2008) are redundant for remyelination, the persistence of otherwise transient ECM proteins in MS lesions, such as fibronectin (aggregates), results in a gain of function, that is they perturb OPC differentiation. Why MMPs are not upregulated in chronic MS lesions is not known. It may be hypothesized that the cellular source of MMPs might determine whether these enzymes perform beneficial or detrimental roles in MS lesions. This is supported by observations that MMP12 is initially produced by microglia/macrophages, while the protease localizes to astrocytes and oligodendrocytes, as observed at successful remyelination, which is beneficial for remyelination. Also, the CSPG-enriched barrier at the lesion border may prevent the migration of cells that produce a suitable MMP to the lesion center. CSPGs are reduced in the lesion center, indicating that they are cleared, but not resynthesized to reestablish the interstitial ECM in adult healthy CNS. Indeed, mRNA levels of CSPGs are reduced in inactive MS lesions (Mohan et al., 2010), which may be due to the lack of sufficient and/or the presence of misactivated microglia/macrophages, the main producers of CSPGs, in the center of lysolecithin-induced lesions (Lau et al., 2012). In this regard, it will be of interest to analyze the ECM composition of remyelinated lesions. In remyelinated lesions, MMP7 levels are comparable to control white matter (Wang et al., 2018), while fibronectin levels are still increased (Stoffels et al., 2015). Of note, astrocyte-derived HMW hyaluronan, which is highly expressed in chronic MS lesions, is degraded by the hyaluronidase family rather than by MMPs (Wang et al., 2018; Wang, Wang, & Li, 2017). Similarly, thrombospondin-1 is also not cleaved by MMPs (Iruela-Arispe, 2008).

7 | CONCLUDING REMARKS AND PERSPECTIVES

Upon demyelinating injury, remodeling of the interstitial ECM is essential in guiding oligodendrocyte behavior to succeed successful remyelination. Remodeling of the interstitial ECM upon demyelination in MS lesions, including the expression patterns of ECM molecules and MMPs, is dependent on the nature of and the localization within lesions, and differs from ECM remodeling upon demyelinating injury in experimental rodent models that show successful recovery (Figure 4). More specifically, the transient upregulation of ECM proteins, as a natural response to demyelination, persists in MS lesions, among others by dysregulation of MMP expression, their localization and, likely, their activation. Several MMPs are upregulated in a seemingly uncontrollable manner (MMP2 and MMP9), while others (MMP3, MMP7, and MMP12) that perform beneficial functions for remyelination upon demyelination in CNS white matter in rodent models are absent or incorrectly located in MS lesions. Also, and in contrast to demyelination in experimental models, MMPs are mainly expressed by microglia/macrophages and hardly, with the exception of MMP3, by astrocytes (Figure 4). In addition, the phenotype of astrocytes, producers of ECM molecules in the CNS, is changed as a consequence of the ensuing spatially focused inflammation, leading to the formation of at least two glial scars: one at the border of active and inactive lesions, which is enriched in CSPGs, and the other within the center of MS lesions that is devoid of CSPGs, but enriched in fibronectin aggregates, osteopontin, and hyaluronan.

Taking the effect on OPC behavior into account, the persistent presence of mainly OPC differentiation-inhibiting ECM proteins and the absence of myelination-promoting laminin in MS lesions contribute to remyelination failure. As a therapeutic strategy, it is essential to clear or bypass these dominant OPC differentiation-inhibiting ECM proteins, as targeted upregulation of remyelination-promoting laminin may not be efficient to enable regeneration of myelin. Therefore, the ECM environment in MS lesions should also be taken into account when determining the effectiveness of potential remyelination-inducing compounds that are based on stimulating endogenous OPCs or when a cell therapy with exogenous remyelinating cells is considered as a strategy to promote remyelination in MS lesions.

Local activation of MMPs, that is in the rim and/or lesions center with an appropriate substrate specificity, may be a means to degrade ECM depositions and reinstall a permissive environment for remyelination, as is the case in recovery upon demyelination in experimental models. However, the dual role of MMPs, such as their ability to degrade vascular basement membrane ECM components and to induce axonal injury and myelin loss, complicates the development of treatment strategies, aimed at correct interstitial ECM remodeling in favor of remyelination. Thus, MMPs contribute to damage at early lesions stages, while their absence at later stages is detrimental to interstitial ECM remodeling, which, in turn, is essential for the regeneration of myelin. In fact, as microglia/macrophages have also receptors for ECM proteins, the dysregulated interstitial ECM in MS lesions also affects microglia/macrophage activation (Austin et al., 2012; Ebert et al., 2008; Milner et al., 2007; Rolls et al., 2008; Sikkema et al., 2018), which may not only affect MMP expression, but also indirectly contribute to OPC differentiation and therefore remyelination (Lloyd & Miron, 2019; Miron et al., 2013). Hence, means to overcome the dysregulated activation of microglia/macrophages in white matter MS lesions (Peferoen et al., 2015; Vogel et al., 2013) may indirectly also reinstall correct ECM remodeling.
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