On the role of macrophages, microglia and the extracellular matrix in remyelination

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Chapter 1

Remodelling of the extracellular matrix in multiple sclerosis lesions: Implications for remyelination

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submitted for publication
Abstract
The extracellular matrix (ECM) provides protection, rigidity and structure towards 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 central nervous system (CNS) homeostasis. Remodelling of the ECM, i.e., transient changes in ECM proteins, for example regulated by matrix metalloproteinases (MMPs), is also an important process that modulates cell behaviour, thereby facilitating recovery after injury to the CNS. Importantly, failure of ECM remodelling plays a role in the pathogenesis of CNS diseases, including multiple sclerosis (MS). MS is a neurodegenerative demyelinating CNS disease, with an inflammatory response against protective myelin sheaths, surrounding the axons, representing a major contribution towards demyelination. Remyelination of denuded axons improves the neuropathological conditions of MS, but this regeneration process fails over time, leading to chronic progression of the disease. In this review, we compare ECM composition and MMP expression in the parenchyma of distinct white matter MS lesion stages to physiological alterations in ECM composition and MMP expression upon CNS white matter demyelination. These issues will be discussed in terms of consequences for oligodendrocyte behaviour and remyelination failure.
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 (glycol)proteins, such as collagens, elastin, fibronectin and laminin, and non-structural regulators, i.e., matricellular proteins such as tenascins, CCNs, SPARCs, fibulins, osteopontins, and thrombospondins (Theocharis et al., 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 et al., 2010). Furthermore, the ECM functions as a reservoir for growth factors or other signalling molecules, thereby influencing cell behaviour 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 the ECM components affect both the overall structure and bioactive properties, and thereby signal transmission and thus the cellular response. The ECM is crucial to normal 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 ECM takes up 17-20% of the adult brain volume (Cragg, 1979, Nicholson & Syskova, 1998). Remarkably, while the ECM undergoes dynamic and continuous remodelling, mediated by matrix-degrading enzymes at normal and pathological conditions, ECM remodelling in the healthy adult CNS is very limited (Rauch, 2007, Dityatev &
Fellin, 2008). 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 interstitial matrices that surround cells, and the pericellular matrices that are in close contact with cells. Examples of pericellular matrices in the CNS include (i) 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 maintenance (van Horssen et al. 2007, Lau et al., 2013) and (ii) neuronal-associated perineuronal nets that emerge during synaptogenesis, which preserve neuronal health by maintaining synaptic plasticity (Frischknecht & Seidenbecher, 2008, Kwok et al., 2011, Oohashi et al., 2015). The dispersed ECM in the CNS parenchyma is a representative example of an interstitial matrix. The interstitial ECM in healthy adult CNS contains relative low levels of fibrous matrix proteins, like collagen, fibronectin and laminin, which are mainly restricted to basement membranes (Ruoslahti, 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 sulphate proteoglycans (CSPGs), particular lecticans, such as aggrecan, versican and neurocan, or as unbound entities in the form of hyaluronan (Rauch, 2007, Kwok et al., 2011, Ruoslahti, 1996). Hyaluronan is the major component of healthy CNS, and is connected to CSPGs via linker proteins (Bignami et al., 1993). Additional components of the adult ECM include the matricellular proteins of which tenascin-R and thrombospondin are most prominent in the CNS (Kwok et al., 2011, Jones & Bouvier, 2014). The perineuronal nets are enriched in hyaluronan, tenascin-R, and CSPGs. All cell types of the CNS, i.e., astrocytes, microglia, oligodendrocytes and neurons, as well as endothelial cells and neurons, 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 regeneration. Different neuropathological conditions are associated with a dysregulation of alterations in the expression pattern of ECM molecules, thereby impeding the process of regeneration. For example, a role of the dynamics and the distinct involvement of ECM components is becoming apparent in the demyelinating disease multiple sclerosis (MS), showing an association between ECM alterations and white matter MS lesions stages (Satoh et al., 2009) and progression of the neuropathological state (Bonneh-Barkay & Wiley, 2009). ECM remodelling 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 (Page-McCaw et al., 2007, Lu et al., 2011). Functional dysregulation of these enzymes contributes to the pathogenesis and progression of several inflammatory demyelinating diseases, including MS (Kieseier et al., 1999). Here, we review the role of interstitial ECM remodelling in remyelination, i.e., the regeneration of myelin membranes, and ECM dysregulation in MS. More specifically, the expression patterns of ECM molecules and MMPs upon successful CNS remyelination in MS are compared and discussed in terms of how they regulate the behaviour of cells that produce myelin, i.e., oligodendrocytes. Also, the interactions between ECM molecules and MMPs and potential mechanisms leading to incorrect ECM remodelling in MS, will be reviewed. Before discussing this in more detail, we will first present a brief overview of the pathology of MS.
Pathological hallmarks

MS is a neurodegenerative inflammatory disease of the CNS. It is one of the most common central demyelinating diseases with an incidence of approximately 0.1% worldwide, while the prevalence varies greatly, based on geographical and ethnical differences (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 (Lubin et al., 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 et al., 1996), has an onset at a later age, and is characterized by gradual accumulation of 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, i.e., 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 et al., 2017). These factors contribute to the inflammatory process occurring in MS, which is associated with disruption of the blood-brain barrier. Whether this inflammation is a primary or secondary event in the pathogenesis of MS is unknown (Compston & Coles, 2008, Stys et al., 2012). An autoimmune response might be initiated in the periphery through (as yet unknown) processes. Molecular mimicry might underlie such an event, resulting in activation of auto-reactive T-cells against self-antigens, which
migrate to the CNS. Subsequently, this might initiate an immune response, thus causing damage and degeneration. On the other hand, cytodegenerative processes in the CNS through (as yet, equally unknown) factors may activate auto-reactive 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 et al., 2004). This results in (additional) demyelination due to the destruction of myelin and/or mature oligodendrocytes, the glial cells that produce the myelin in the CNS.

The formation of white matter MS lesions is a dynamic process. More specifically, distinct lesions are histopathologically classified in inflammatory and demyelinating activity (van der Valk & de Groot, 2000, Kuhlmann et al., 2017). While different classification systems have been described, mainly three distinct lesions stages can be distinguished: active, chronic active and chronic inactive lesions. Active lesions are the early demyelinating phenotype, and are most frequently found in relapsing-remitting MS (Frischer et al., 2015). 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. Chronic active lesions have a sharp border and consist of a hypocellular demyelinated lesion center with fibrous astrocytes that is surrounded by a broad hypercellular inflammatory rim that contains microglia/macrophages and reactive astrocytes. The final lesion stage is a chronic inactive MS lesion. These lesions contain a hypocellular demyelinated center, containing mainly reactive astrocytes and no signs of infiltration of microglia/macrophages or lymphocytes. The chronic active lesions are mainly observed in progressive MS, in which also chronic inactive lesions are predominating (Frischer et al., 2015).
Failure of remyelination

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 is partially compensated for by a redistribution of sodium channels along the demyelinated parts of the axon, which allows for non-saltatory conduction with reduced velocity (Felts et al., 1997). However, this compensatory effect is only temporal and in conjunction with a lack of axonal protection, the loss of myelin sheaths leads to axonal damage and degeneration (Compston & Coles, 2008, Fünfschilling et al., 2008, Lee et al. 2012). The rate of impulse transduction is reduced or worse, 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 (Papadopoulos et al., 2006, Compston & Coles, 2008). 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 (Fünfschilling et al., 2008, Lee et al. 2012). Therefore, prevention of axonal degeneration might be beneficial to resolve the functional deficits and progression of MS.

Thus, 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 (Smith et al., 1979, Irvine & Blakemore, 2008, Jeffery & Blakemore, 1997, Franklin, 2002, Franklin & ffrench-Constant, 2008). The myelin sheaths generated in the process of
remyelination are shorter and thinner, compared to myelin sheaths produced during myelination, but these newly formed sheaths suffice for axonal protection and improved functioning (Kornek et al., 2000). Remyelination is executed by local oligodendrocyte progenitor cells (OPCs) (Zawadzka et al., 2010), while OPCs present in the adult subventricular zone (SVZ) contribute predominantly to remyelination of lesions in their proximity (Menn et al., 2006). In response to damage, astrocytes and microglia are activated (Franklin, 2002, Franklin & ffrench-Constant, 2008). These changes lead to activation of OPCs, resulting in morphological changes and enhanced gene expression of factors involved in oligodendrocyte differentiation and maturation (Reynolds et al., 2002, Ferent et al., 2013, Watanabe et al., 2004, Arnett et al., 2004, Moyon et al., 2015). In addition to OPC activation, microglia and astrocytes recruit and mediate migration of OPCs to the demyelinated areas, where they further proliferate (Levine & Reynolds, 1999, Franklin, 2002, Franklin & ffrench-Constant, 2008). 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 compaction of the myelin membranes (Franklin, 2002, Franklin & ffrench-Constant, 2008). 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, 2002, Franklin & ffrench-Constant, 2008, Hanafy & Sloane, 2011, Gaesser & Fyffe-Maricich, 2016, 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, Kuhlmann et al., 2008, Goldschmidt et al., 2009). 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, 2002, Franklin & ffrench-Constant, 2008, Kuhlmann et al., 2008). In fact, in approx. 70% of both active and chronic MS lesions, OPCs are abundantly present (Wolswijk, 1998, Luchinetti et al., 1999, Kuhlmann et al., 2008, Goldschmidt et al., 2009, Chang et al., 2012, Strijbis et al., 2017). However, remyelination not always fails in MS, given the presence of partly or complete remyelinated shadow plaques. Although remyelination is more frequent at early stages of MS, extensive remyelination is occasionally observed at late-stage progressive MS (Patrikios et al., 2006, Patani et al., 2007). However, in contrast to the demyelinated MS lesion stages, and likely as a reflection of remyelination failure at later stages, the frequency of remyelinated white matter shadow plaques (approx. 20%) is similar in relapsing-remitting MS and the progressive forms, and is present at all stages of white matter lesion development (Goldschmidt et al., 2009, Frischer et al., 2015, Kuhlmann et al., 2017).

Remyelination failure is thus thought to be a consequence of perturbations in the different phases of remyelination, i.e., activation, recruitment and differentiation of OPCs, in which ageing also plays an important role (Sim et al., 2002, Franklin & ffrench-Constant, 2008, Goldschmidt et al., 2008). The signalling and cellular environment, established by the state of the disease and lesion stage regulating these phases, is a crucial factor. Remyelination failure in MS is likely due to the presence of inhibitory signals in the damaged area or the lack of stimulatory signals. Indeed, various factors in the signalling environment of MS lesions are dysregulated, and together with ensuing cellular changes contribute to the failure of remyelination (Franklin, 2002, Williams et al., 2007, Franklin & ffrench-Constant, 2008, Hanafy & Sloane, 2011, Miron, 2017). In this regard, a role of the dynamics and distinct interstitial ECM components in remyelination failure in MS lesions, is becoming increasingly apparent (Satoh et al., 2009, Lau et al., 2013).
The interstitial ECM in MS lesions: role in remyelination failure

Dynamic remodelling of the ECM, i.e., transient expression and/or degradation, is an effective mechanism to regulate glia cell behaviour, including OPCs, upon injury. Analysis of mRNA expression of ECM proteins and the MS lesion proteome reveals that chronic active and chronic inactive MS lesions have a unique ECM composition, both when compared among each other and in control white matter and active MS lesions (Satoh et al., 2009, Mohan et al., 2010). Thus, dysregulated ECM remodelling plays an important role in the pathogenesis of MS, i.e., the composition and expression of ECM proteins is persistently altered upon MS lesions progression. Experimental toxin-induced demyelination models show robust remyelination and are most instructive to obtain insight into cellular and molecular responses during demyelination of the CNS, and subsequent successful recovery in the absence of the complex inflammatory background in MS. In the following, we provide a detailed overview of ECM proteins, present or absent in the interstitial matrix, i.e., parenchymal ECM, upon white matter demyelination at healthy conditions and in the distinct white matter MS lesion stages (summarized in table 1). We will

<table>
<thead>
<tr>
<th>ECM protein</th>
<th>healthy demyelination</th>
<th>healthy remyelination</th>
<th>active</th>
<th>chronic active</th>
<th>chronic inactive</th>
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<td>hyaluronan</td>
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<td>↑(LMW)</td>
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<td>collagen (V)</td>
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<td>fibronectin</td>
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<td>- tenascin-R</td>
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<td>thrombospondin</td>
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<td>osteopontin</td>
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</table>
ECM remodelling in MS lesions

- increased (↑), decreased (↓) and similar (=) expression compared to control; n.d. is not determined; * based on MS lesion proteome (Satoh et al., 2009), data on localization are not available yet; ** based on biochemical analysis discuss their direct effects on OPC behaviour (summarized in Fig. 1) and how this may contribute to remyelination (failure). We only briefly touch upon alterations 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).

![Fig 1. Effect of ECM proteins on OPC behaviour relevant for remyelination.](image)

Successful remyelination upon CNS demyelination involves the activation of adjacent oligodendrocyte progenitor cells (OPCs), followed by recruitment (migration and proliferation) to and maturation (process arborization, differentiation and myelin membrane formation) in the demyelinated area. The effect of ECM proteins present in toxin-induced CNS white matter and/or distinct white matter MS lesions are shown. ECM proteins depicted in green increase and ECM proteins depicted in red decrease the indicated OPC behaviour.
Structural CNS ECM proteins

CSPGs are the main proteoglycans of the CNS and consist of core proteins with covalently linked sulphated chondroitin GAG side chains. The individual secreted CSPGs, i.e., neurocan, aggrecan, versican and phosphocan, 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 et al., 2012, Lau et al., 2012, Keough et al., 2016). CSPG expression, including versican and phosphocan, but not aggrecan, transiently increases upon lysolecithin- (Lau et al., 2012, Keough et al., 2016) and/or cuprizone-induced demyelination (Hibbits et al., 2012). Remarkably, in lysolecithin-induced lesions, CSPGs are present in microglia/macrophages at the lesion center, while astrocytes harbour 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 chronic inactive MS lesions, while their expression is downregulated in the center of active, chronic active and in chronic inactive lesions (Dahl et al., 1989, Sobel & Ahmed, 2001). The ECM 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 chronic inactive MS lesions compared to the other CSPGs (Sobel & Ahmed, 2001). Also, in situ hybridization studies showed the presence of phosphacan mRNA in remyelinating oligodendrocytes in MS lesions (Harroch et al., 2002). 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), indicating 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 signalling pathways via PTPσ (Siebert & Osterhout, 2011, Lau et al., 2012, Pendleton et al., 2013, Keough et al., 2016), as well as OPC migration in vivo (Siebert et al., 2011). Exposure to soluble CSPGs impairs process outgrowth, but not differentiation (Lau et al., 2012, Pendleton et al., 2013). Moreover, soluble phosphacan is a ligand for oligodendroglial contactin and this complex inhibits OPC proliferation, and promotes OPC differentiation (Lamprianou et al., 2011). Thus, demyelination of normal CNS leads to upregulation of CSPGs, which may have beneficial functions at early stages of recovery, i.e., to prevent premature OPC differentiation. Moreover, CSPGs are cleared in order 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 (Sobel & Ahmed, 2001, Lau et al., 2012, Keough et al., 2016).

Hyaluronan is a specialized nonsulfated GAG that functions as free, i.e., 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 lysolecithin-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 to normal CNS. However, and 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). Most interesting, T cells and microglia produce low molecular weight hyaluronan
ranging from 200-400 kDa (LMW-hyaluronan), whereas astrocytes produce hyaluronan with high molecular weight, ranging from 900-1000 kDa (HMW-hyaluronan) (Back et al., 2005). In fact, HMW-hyaluronan and astrocyte-derived hyaluronan, but not LMW-hyaluronan coatings inhibit OPC maturation in vitro, and showed detrimental effects to remyelination, when injected in lysolecithin-induced demyelinated lesions (Back et al., 2005). Soluble HMW-hyaluronan also inhibits OPC maturation (Back et al., 2005). Hence, the accumulated astrocyte-derived HMW-hyaluronan likely prevents OPC maturation in MS lesions, and thereby contributes to remyelination failure.

Collagens are trimeric proteins that contain long triple helical sequences that have the ability to form stable fibrils. They are arranged into networks, and are involved in structuring of 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 lesions (Mohan et al., 2010). Also, fibrillary collagen I and II accumulation is associated with perivascular inflammation in the center of active and chronic active 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 to the basement membranes in chronic inactive lesions (Satoh et al., 2009). Although OPCs lack collagen-recognizing integrins, OPC migration is inhibited on collagen I substrates (Milner et al., 1996), and collagen I microspheres support OPC differentiation (Yao et al., 2013). Therefore, the
ECM remodelling in MS lesions

contribution of interstitial collagen to remyelination failure via direct modulation of OPC behaviour in MS lesions, is likely negligible.

**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 and circulating in the periphery, generated by hepatocytes (Owens & Cimino, 1982). Cellular fibronectin is insoluble and locally produced; in the CNS it is deposited by astrocytes, microglia/macrophage and endothelial cells (Zhao et al., 2009, Hibbits et al., 2012; Stoffels et al., 2013). In normal healthy adult CNS, fibronectin is nearly absent in the interstitial ECM and only localizes to the vasculature (Sobel & Mitchell, 1989, van Horssen et al., 2005, Zhao et al., 2009, Stoffels et al., 2013). 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 behaviour, in particular cell migration and proliferation, via integrin receptors (Singh et al., 2010, Pankov & Yamada, 2002). Also, in various experimental toxin-induced lesions undergoing efficient remyelination, fibronectin is transiently expressed in the white matter CNS parenchyma and upregulated in the vasculature. Its level declines as remyelination proceeds (Zhao et al., 2009, Hibbits et al., 2012, Stoffels et al., 2013, Espitia Pinzon et al., 2017). In active, chronic active, and chronic inactive white matter MS lesions, however, the increased vascular and extracellular fibronectin expression persists, and the protein is present in perivascular infiltrates (Sobel & Mitchel, 1989, van Horssen et al., 2005, Stoffels et al., 2013). In addition, fibronectin is associated with inflammation-mediated aggregation in chronic lesions (Stoffels et al., 2013). In remyelinated shadow plaques, fibronectin expression is still increased, but does not aggregate (Stoffels et al., 2013). Astrocytes are the predominant source of
cellular fibronectin upon lysolecithin-induced demyelination (Stoffels et al., 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, van Horssen et al., 2005, Stoffels et al., 2013). Interestingly, white matter astrocytes, isolated from MS patients, 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 (Milner et al., 1996, Frost et al., 1996, Baron et al., 2002, Tripathi et al., 2017), while fibronectin coatings prevent myelin membrane formation, likely by perturbing (secondary) process outgrowth, myelin-directed vesicle transport and formation of functional membrane microdomains (Buttery & ffrench-Constant, 1999, Maier et al., 2005, Siskova et al., 2007, 2009, Maier et al., 2005, Lafrenaye & Fuss, 2010, Stoffels et al., 2013, Baron et al., 2014). Similar to dimeric fibronectin, aggregates of fibronectin inhibit myelin membrane formation and myelination in co-culture systems (Stoffels et al., 2013, Qin et al., 2017). 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.

Vitronectin is another glycoprotein that is mainly localized in the vasculature of the adult CNS. The expression of vitronectin, derived from microglia/macrophages, 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 chronic active demyelinating lesions, where it is localized on the microvasculature, on demyelinated axons, and is present in a subset of hypertrophic astrocytes (Sobel et al., 1995). Next to localized synthesis, part of the vitronectin acquires access into the brain, following passage across the disrupted blood–brain barrier (Sobel et al. 1995).
Vitronectin is absent in chronic inactive lesions (Sobel et al., 1995). It promotes migration and proliferation of cultured OPCs (Frost et al., 1996, Baron et al., 2002), 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 $\alpha$, $\beta$, and $\gamma$ 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 astroglial (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 maturation of OPCs into oligodendrocytes, OPC proliferation, survival of oligodendrocytes and myelination (Frost et al., 1999, Colognato et al., 2002, Colognato & Tzvetanova, 2011, Relucio et al., 2009, 2012, Leiton et al., 2015). Laminin-2 expression is not immediately upregulated upon CNS white matter demyelination, but it may promote remyelination at later time points (Zhao et al., 2009). 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 et al., 1998, van Horssen et al., 2005), indicating that beneficial effects of laminin on oligodendrocyte behaviour and remyelination may be negated in MS.

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 behaviour and ECM remodelling (Bornstein & Sage, 2002). Tenascin-C and tenascin-R are large
hexameric and trimeric glycoproteins, respectively, assembled from monomers via disulphide bridges (Erickson & Inglesias, 1984, Norenberg et al., 1996). The proteins are expressed in the normal adult CNS (Bartsch et al., 1992, Gutowski et al., 1999, García et al., 2001) and bind to CSPGs, hyaluronan and fibronectin (Chiquet-Ehrismann, 1991; Midwood et al., 2016). Tenascin-R expression is restricted to the CNS, where it is produced by oligodendrocytes, and during development by neurons as well (Pesheva et al., 1989, Fuss et al., 1993), while tenascin-C is a more ubiquitous ECM protein (Midwood et al., 2016). In the CNS, it is expressed by astrocytes and oligodendrocytes (Prieto et al., 1990, Bartsch et al., 1992, Gutowski et al., 1999, Gotz et al., 1997, Garwood et al., 2004, Czopka et al., 2009). 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), in another it has been shown that tenascin-C mRNA is not strongly upregulated (Hibitts et al., 2012). In active MS lesions, both tenascin-C and tenascin-R are significantly downregulated, extending even beyond the edge of the lesion, i.e., at NAWM areas where the presence of macrophages is abundant (Gutowski et al., 1999). In the center of chronic 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). In chronic inactive lesions the expression of tenascin-C and tenascin-R resembles 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 (Pesheva et al., 1997, Czopka et al., 2009), 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, Kiernan et al., 1996, García et al., 2011) as well as differentiation and myelin membrane
ECM remodelling in MS lesions

formation (Garwood et al., 2004, Czopka et al., 2009, Czopka et al., 2010), 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 et al., 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 et al., 1986), expressed by astrocytes (Scott-Drew and ffrench-Constant, 1997) and involved in CNS synaptogenesis (Christopherson et al., 2005). 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 chronic active and chronic inactive MS lesions (Satoh et al., 2009, chapter 3). Also, thrombospondin binds to fibronectin (aggregates) and collagen V (Aho & Uiitio, 1998, Adams & Lawler, 2011, chapter 3), which are present in the interstitial ECM of chronic 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 white matter in the normal adult CNS (Shin et al., 1999, Selvaraju et al., 2004, Zhao et al., 2008). The protein is however transiently upregulated in white matter upon cuprizone- (Selvaraju et al., 2004) and ethidium bromide-induced (Zhao
et al., 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 is higher in active MS lesions than in control white matter (Chabas et al., 2001), and proteomic analyses revealed that osteopontin protein is enriched in chronic active MS lesions compared to control white matter and chronic inactive MS lesions (Satoh et al., 2009). Within active MS lesions, osteopontin is mainly present in macrophages and microvasculcular endothelial cells, while reactive astrocytes also express osteopontin in active lesions and chronic active MS lesions (Sinclair et al., 2005, Diaz-Sanchez et al., 2006). 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). Remarkably, while protein levels are increased, gene expression analysis of adjacent NAWM, rim and center of chronic active and chronic inactive lesions revealed a downregulation in osteopontin transcript levels (Koning et al., 2007). In vitro studies demonstrated that soluble osteopontin induced proliferation of OPC-like cell lines, increased MBP expression and enhanced myelin membrane formation in mixed cortical cultures (Selvaraju et al., 2004). Hence, osteopontin may be beneficial to remyelination at both the level of OPC recruitment and OPC differentiation. Of note, given that mixed cultures were applied in the studies of Selvaraju et al., the effect on OPC differentiation may be indirect.

The involvement of other matricellular proteins, including CCNs, SPARCs, and fibulins in ECM remodelling upon demyelination and in remyelination failure in MS, remains to be determined. Of interest is that Cyr61/CCN1 and
ECM remodelling in MS lesions

CTGF/CNN2 associate with fibronectin aggregates (chapter 3), and that CTGF/CNN2 inhibits OPC differentiation (Lamond & Barnett, 2013, Ercan et al., 2017), adding another level of complexity to fibronectin aggregate-mediated perturbation of OPC maturation.

The interstitial ECM in MS lesions: role of glial scar and inflammation

When comparing the expression pattern of ECM molecules at the distinct MS lesion stages with the expression pattern of ECM molecules, altered upon successful CNS remyelination (table 1), both similarities and abnormalities are noticed. Similar to demyelinating injury in the normal CNS, 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 (Fig. 1), and prevent premature OPC differentiation. In normal CNS, the proteins are cleared at the onset of remyelination to allow for OPC differentiation, among others by the expression of laminin. In MS lesions, the transient increased ECM proteins persist, while laminin is virtually absent in the interstitial ECM MS, indicating that the ‘normal’ ECM remodelling response in MS lesions is derailed and not converted to a ‘remyelination-favoring mode’. Another striking difference is that CSPGs, present in the interstitial ECM CNS, are upregulated upon demyelination in the healthy CNS, and downregulated at the center of MS lesions. CSPG proteins are merely inhibitory for OPC recruitment and differentiation (Fig. 1), which in healthy CNS may keep the adult OPCs at their location in an immature state. Therefore, their transient degradation may be beneficial for remyelination. However, these ECM proteins are upregulated and not downregulated upon demyelination in the healthy CNS, although their expression at early time points, i.e., immediately upon induction of demyelination, has not been examined thus far. Intriguingly, with regard to ECM expression, the edges of active and chronic active MS lesions, resemble demyelinated areas in normal
CNS more than the core of MS lesions, with the exception of tenascins. 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 chronic active lesions is more permissive for remyelination than the ECM in the center of the lesions.

Astrocytes are the main producers of ECM proteins, and the astrocyte response in MS lesions is of dual nature. Both astrocyte loss and astrogliosis are associated with remyelination failure (Williams et al., 2007, Nair et al., 2008, Correale & Farez, 2015). Major barriers that contribute to remyelination failure in MS are glial scars, i.e., astrogliosis, which parallels ECM alterations, and the ensuing inflammation. The changes of 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 e.g., chronic active MS lesions, this may result in a different signalling environment and different extent of gliosis, and hence differences of ECM compositions. Indeed, in MS lesions, antigenic differences that reflect the state of reactive astrocytes, i.e., between astrocytes at the lesion edge and center, exist (Holley et al., 2003), 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 blood-brain barrier, 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, i.e., cells that express the appropriate proteases that clear the transient ECM, could not reach the lesion site. Indeed, the edge of active and chronic inactive MS lesions is mainly composed of CSPGs (table 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 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., 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 (Woodroofe & Cuzner, 1993, Cannella & Raine, 1995, Koning et al., 2007). 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 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 (Smith & Eng, 1987, Haist et al., 2012). 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. In TME, which is characterized by mild inflammation and insufficient OPC differentiation (Ulrich et al., 2008), ECM alterations correlated with the development of astrogliosis, and includes among others 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 remodelling of the ECM in MS lesions, are the resident microglia and the infiltrating macrophages. Microglia and macrophages, next
to secreting ECM molecules, are main producers of MMPs, i.e., proteases that are able to degrade and remodel the ECM. Given that the phenotype of microglia and macrophages is not appropriate for remyelination (Vogel, et al., 2013, Miron et al., 2013, Peferoen et al., 2015), the persistent or incorrect expression of ECM molecules in MS lesions stages might result from altered expression patterns of MMPs. Indeed, MS is one of the CNS diseases in which altered expression levels of MMPs play a role in the pathogenesis of the disease (Kieseier et al., 1999).

**MMPs in MS lesions: dual role in demyelination and remyelination**

MMPs are a family of proteolytic enzymes, also referred to as endopeptidases, that are essential for ECM remodelling in many processes. These include migration, wound healing, tissue morphogenesis, cell differentiation, neuronal growth and several signaling processes (Page-McCaw et al., 2007, Lu et al., 2011). 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 MMPs are synthesized with a signal peptide, which is cleaved during transport via the secretory pathway. There are small differences in the structure of the MMPs, but three common domains are identified (Fig. 2, Page-McCaw et al. 2007). 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 matrilysins, 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 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, 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) (Page-McCaw et al., 2007, Lu et al., 2011).

In the healthy adult CNS, MMP activity is crucial in supporting cognitive processes, such as learning and memory, due to ECM remodelling and the regulation of synaptic plasticity and long-term potentiation (Agrawal et al., 2008, Huntley, 2012). Also, the physiology of axons, myelin turnover and angiogenesis are regulated by several MMPs. In addition, differential expression of MMPs in the development of the CNS is essential for neurogenesis and axonal growth as well as for the function of oligodendrocytes and myelinogenesis. 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 (Yong et al., 2001, Yong, 2005, Javaid et al., 2013).

Demyelination and subsequent remyelination require a transient alteration in the expression pattern of MMPs to remodel the ECM. 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 blood-brain barrier, induce inflammation, and neurotoxicity, which may lead to (demyelinating) injury (Yong et al., 2001, Yong, 2005, Aggrawal et a.l, 2008). Of interest, synergism between MMP2, MMP9 and MMP7 genes may be a susceptibility factor for MS (Rahimi et al., 2016). In the following, we will provide an overview of current insight into the role of MMPs in (re)myelination and their expression in the distinct MS white
matter lesions. The consequences for demyelinating MS pathology, ECM remodelling and remyelination failure (table 2) will be discussed.

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

<table>
<thead>
<tr>
<th>MMP</th>
<th>MS relevant ECM targets</th>
<th>healthy demyelination</th>
<th>remyelination</th>
<th>active chronic</th>
<th>active chronic</th>
<th>inactive chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP2</td>
<td>aggrecan, versican, neurocan, collagen V, fibronectin, laminin, vitronectin, tenascin-C (large)</td>
<td>=</td>
<td>=</td>
<td>↑*</td>
<td>↑*</td>
<td>=</td>
</tr>
<tr>
<td>MMP3</td>
<td>aggrecan, versican, neurocan, phosphacan, collagen V, fibronectin, laminin, vironectin, tenascin-C (large), osteopontin</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑**</td>
<td>=</td>
</tr>
<tr>
<td>MMP7</td>
<td>aggrecan, fibronectin, laminin, vitronectin, tenascin-C (large, small), osteopontin</td>
<td>=</td>
<td>↑</td>
<td>↑*</td>
<td>↓**</td>
<td>↓**</td>
</tr>
<tr>
<td>MMP9</td>
<td>aggrecan, collagen V, fibronectin vitronectin, osteopontin</td>
<td>=</td>
<td>=</td>
<td>↑</td>
<td>↑*</td>
<td>=</td>
</tr>
<tr>
<td>MMP12</td>
<td>aggrecan, fibronectin, laminin, vitronectin, osteopontin</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑*</td>
<td>=</td>
</tr>
<tr>
<td>MMP19</td>
<td>aggrecan, fibronectin, laminin, tenascin-C (large), osteopontin</td>
<td>n.d.</td>
<td>n.d.</td>
<td>↑</td>
<td>↑*</td>
<td>=</td>
</tr>
</tbody>
</table>

- increased (↑), decreased (↓) and similar (=) expression compared to control; n.d. is not determined; * marked expression at the edge; ** based on biochemical analysis.

**MMP3**

Škuljec et al. (2011) investigated the expression profile of eleven MMPs (MMP2, MMP3, MMP7, MMP9, MMP10, MMP11, MMP12, MMP13, MMP14, MMP15 and MMP24) upon cuprizone-induced demyelination and subsequent remyelination. At early demyelination, MMP3 mRNA is upregulated, followed by a return to normal levels during late stages of demyelination. A second more prominent upregulation of MMP3 mRNA is observed upon remyelination (Škuljec et al., 2011, chapter 2). MMP3 mRNA levels, but not protein levels, are also increased upon early remyelination in the lysolecithin-induced demyelination model (chapter 2). At the protein level, MMP3, also referred to as stromelysin-1, is predominantly expressed by astrocytes, but, following other types of CNS injury, may also be produced by damaged neurons, microglia and oligodendrocytes (van Hove et al., 2012). An (early) upregulation of MMP3 is also observed in the inflammatory TME model.
(Ulrich et al., 2006, Hansmann et al., 2012), and in EAE (Weaver et al., 2005). In these inflammatory models with insufficient remyelination, MMP3 may aid to the disruption of the blood-brain barrier. In addition, the early upregulation of MMP3 may cause the breakdown of myelin and exacerbate demyelination (Chandler et al., 1995, Shiryaev et al., 2009). In contrast, the upregulation of MMP3, observed at remyelination in the toxin-induced models, may facilitate myelin regeneration. MMP3 modulates several signaling pathways, such as the bioavailability of several soluble growth factors, including IGF-1, that facilitate oligodendrocyte differentiation and myelin production (Dubois-Dalcq & Murray, 2000, McCawley & Matrisian, 2001, D’Ercole et al., 2002, Fowlkes et al., 2004, 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 oligodendrocytes (Fig. 1, Muir et al., 2002, Kotter et al., 2006, Lau et al., 2012, van Hove et al., 2012, Stoffels et al., 2013). Hence, MMP3 may be beneficiary for ECM remodelling to facilitate remyelination. MMP3 mRNA is not upregulated in the MS lesion stages compared to white matter of healthy subjects (Lindberg et al., 2001). MMP3 protein is however present in hypertrophic astrocytes in active and chronic lesions, in microglia/macrophages and on vasculature in active and chronic active lesions (Maeda & Sobel, 1996, chapter 2). Biochemical analysis revealed that MMP3 protein levels were increased in chronic (active) MS lesions, but not chronic inactive lesions (chapter 2) compared to control white matter. Of interest, MMP3 is a potent activator of other MMPs, such as MMP7 and MMP9 (Lu et al., 2011, van Hove et al., 2012), which are also prominently present in MS lesions (see below).

**MMP12**

The expression level of MMP12, or macrophage elastase, is significantly altered in cuprizone-treated mice. At demyelination, MMP12 mRNA is
predominantly produced by microglia/macrophages. In this phase, MMP12 may function in cellular migration of macrophages through ECM remodelling and degeneration of myelin membranes through cleavage of myelin basic protein (MBP), one of the major myelin components (Shipley et al., 1996, Chandler et al., 1996, Gronski et al., 1997). The elevated expression pattern continued during remyelination, where MMP12 is produced by astrocytes and to some extent by oligodendrocytes, rather than microglia/macrophages (Škuljec et al., 2011). Upon remyelination, MMP12, expressed by astrocytes, may contribute to clearance of 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 elongation and OPC differentiation (Larsen & Yong, 2004, Larsen et al., 2006). Studies in EAE and TME models demonstrated an upregulation of MMP12 by microglia/macrophages and a suggested role in disease progression and chronic phases of demyelination, respectively (Dasilva & Yong, 2008, Ulrich et al., 2006, Hansmann et al., 2012). On the other hand, the disease course is worse in MMP12 knockout than in wild type mice, which is mediated in part by modulating the Th1/Th2 effector cytokine balance (Weaver et al., 2005) and MMP12-mediated cleavage of osteopontin (Goncalves DaSilva et al., 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 molecule in EAE. In TME, MMP12 is likely involved in demyelination and extravasation of macrophages, and not in blood-brain barrier damage or ECM remodelling (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 chronic inactive MS lesions and to a lesser extent in the center of chronic active and inactive lesions (Vos et al., 2003). In contrast, upon cuprizone-induced demyelination, 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, i.e., it may induce death of activated T-cells by cleaving osteopontin, but may also induce bystander demyelination by cleaving MBP.

**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 tissue (Škuljec et al., 2011), MMP9 mRNA and protein are upregulated in active and chronic active demyelinating MS lesions (Maeda & Sobel, 1996, Cuzner et al., 1996, Cossins et al., 1997, Anthony et al., 1997, Lindberg et al. 2001, Mohan et al., 2010). MMP9 mRNA expression is also upregulated in an EAE model for MS (Clements et al., 1997, Kieseier et al., 1998, Weaver et al., 2005), but not in the TME model (Ulrich et al., 2006). It is hypothesized that MMP9 is important for the infiltration of inflammatory cells into the CNS. Thus, the level of MMP9 is significantly increased in cerebrospinal fluid (CSF) and serum of MS patients with active disease, compared to healthy individuals (Waubant et al., 1999, Bar-Or et al., 2003, Fainardi et al., 2006, Benesova et al., 2009). Injection of recombinant MMP9 into the CNS parenchyma resulted in breakdown of the blood-brain barrier, neuronal and myelin loss (Anthony et al., 1998). The localization of MMP9 in cells present in perivascular areas, such as endothelial cells and the infiltrating cell population, including lymphocytes and macrophages, is also in favor of its contribution to the disruption of the blood-brain barrier in MS (Cossins et al., 1997, Lindberg et al., 2001). In addition, resident CNS cells, such as microglia/macrophages and astrocytes in active MS lesions also express MMP9, mainly around the perivascular areas but also in the CNS parenchyma (Cuzner et al., 1996, Maeda & Sobel, 1996). In chronic active lesions, MMP9 is more prominently localized at the edge of the lesions (Anthony et al., 1997), while this protease is occasionally also present in
astrocytes in chronic inactive lesions. These data further suggest that MMP9 is upregulated and exert its functions in active demyelinating lesions during inflammation. In addition to the stimulation of inflammatory cell infiltration through blood-brain barrier disruptions, MMP9 derived from leukocytes cleaves MBP and may be involved in degradation of myelin membranes (Proost et al., 1993, Gijbels et al., 1993). On the other hand, MMP9 has also beneficial roles for remyelination (Uhm et al., 1998, Oh et al., 1999, Larsen et al., 2003, Siskova et al., 2009). Localization of MMP9 at the tips of the extending processes of oligodendrocytes is essential for their outgrowth, i.e., process elongation and arborization is reduced in absence of MMP9 (Uhm et al., 1998, Oh et al., 1999) and upon its mislocalization (Siskova et al., 2009). In addition, MMP9 produced by macrophages and microglia in the remyelination phase degrades NG2, a membrane bound CSPG present on OPCs that inhibits maturation of oligodendrocytes (Larsen et al., 2003). Hence, MMP9 plays a dual role in MS. The activity of this enzyme in active demyelinating lesions may be detrimental and contribute to the pathogenesis and progression of MS, but beneficial functions of MMP9 are observed for remyelination, in which it facilitates oligodendrocyte maturation via ECM remodelling.

**MMP2**

MMP2, is 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). mRNA expression of MMP2 is not altered during demyelination and remyelination in a cuprizone-induced demyelination model (Škuljec 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 (Maeda & Sobel, 1996, Anthony et al., 1997, Diaz-Sanchez et al., 2006). MMP2-expressing
Macrophages are also present at the border of chronic active lesions, while its expression in chronic lesions was only perivascular (Anthony et al., 1997, Diaz-Sanchez et al., 2006). As MMP9, MMP2 is likely involved in disruption of the blood-brain barrier (Rosenberg et al., 1992) and has the highest activity in MBP degradation (Chandler et al., 1995). MMP2 appears to be more abundant in MS lesions than MMP9, and its expression is predominant in areas where damaged axons are abundant, particular at lesion borders (Diaz-Sanchez et al., 2006). Interestingly, MMP2 is also upregulated in NAWM areas adjacent to the lesions (Maeda & Sobel, 1995, Anthony et al., 1997, Diaz-Sanchez et al. 2006). 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 et al., 1998), remains to be determined.

**MMP7**

MMP7, also called matrilysin, is constitutively expressed in the brain (Anthony et al., 1997, chapter 2). 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 (Škuljčec 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 (chapter 2). In the latter model, MMP7 is localized extracellularly and present in macrophages (chapter 2). Contrasting findings are reported in EAE models. In a mouse MOG-peptide induced EAE model MMP7 mRNA levels remain similar (Weaver et al., 1995), 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 at all lesions stages in one study (Lindberg et al., 2001), while it was undetectable in active and chronic MS lesions (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, chapter 2). MMP7 expression in macrophages is also prominent at the lesion borders of chronic active 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 chronic active and chronic inactive lesions (chapter 2). In remyelinated lesions, total expression levels of MMP7 are comparable to control white matter of healthy subjects with occasional expression in macrophages (chapter 2). 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 remodelling of basement membrane ECM, such as proteoglycans, fibronectin, laminin and elastin, can be foreseen. In addition, MMP7 potentially induces bystander demyelination and axonal loss, although likely to a lesser extent than MMP2 and MMP9 (Anthony et al., 1998). 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).

Other MMPs

Other MMPs that have been analyzed at the protein level in MS lesions are MMP1 (Maeda & Sobel, 1996), MMP19 (van Horssen et al., 2006) and
MMP28 (Werner et al., 2008). MMP1 is expressed in the majority of macrophages in active MS lesions (Maeda and 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 the rim of chronic active lesions, and occasionally by reactive astrocytes (van Horsse et al., 2006). Also, MMP19 mRNA levels are increased in active and chronic 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 (Stracke et al., 2000) of which the expression is reduced in active MS lesions and chronic active lesions borders (table 1). 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 chronic inactive MS lesions (MMP28). Moreover, Western blot analysis shows also a marked upregulation in NAWM as compared to control white matter (Werner et al., 2008). Interestingly, MMP28 mRNA levels are upregulated in active MS lesions and downregulated in chronic inactive MS lesions (Mohan et al., 2010). 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 altered expression patterns upon cuprizone-induced demyelination. Thus, MMP11 mRNA is upregulated at remyelination, while MMP14 mRNA, is upregulated upon both demyelination and remyelination. MMP15 mRNA has been reported to be downregulated at conditions of demyelination, while MMP24 mRNA is downregulated at demyelination but upregulated at remyelination (Škuljic et al., 2011). Mohan et al., 2010 performed an extensive qPCR analysis of 23 MMPs
in control white matter, active and chronic 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 (Mohan et al., 2010). In contrast, the mRNA levels of 2 MMPs (MMP15 and MMP23) and 1 MMP (MMP23) were downregulated by at least 50% in active and inactive lesions, respectively (Mohan et al., 2010). Validation at the protein level, their cellular localization, and the significance for ECM remodelling, remyelination and MS lesion pathology remains, however, to be largely established. Of note, the expression levels of most of these MMPs are also altered in TME and EAE models (Weaver et al., 2005, Ulrich et al., 2006).

**MMPs in MS: interstitial ECM remodelling for remyelination?**
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 active lesions. Concomitantly, a more pronounced expression at the rim of chronic active MS lesion compared to the core of the lesion is usually observed. Furthermore, MMPs in MS lesions are only occasionally present in astrocytes, with the exception of MMP3, which is primarily localized to astrocytes. Likely, most MMPs in MS lesions are implicated in disruption of the blood-brain barrier 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, i.e., lymphocytes and monocytes to infiltrate the CNS (Leppert et al., 2001). Also, most MMPs present in inflammatory lesions degrade MBP, which may enhance demyelination and which, in turn, contributes 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 remodelling upon demyelination is currently lacking, and therefore it is unclear whether the mainly macrophage-derived MMPs in MS are involved in the dynamic turnover of interstitial ECM upon demyelination. As remyelination fails, among others by the persistent presence of interstitial ECM components (table 1, Fig. 1), 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 to the normal CNS and the distinct white matter MS lesion stages (summarized in tables 1 and 2). The tightly and temporal (cellular) expression of several MMPs regulate the clearance of the transiently expresses ECM molecules to enable regeneration of myelin. In contrast, in MS lesions, where remyelination fails, a different and more persistent pattern of MMPs and ECM molecules is observed (van Horssen et al., 2007, Aggrawal et al., 2008). For example, the mRNA levels of gelatinases MMP2 and MMP9 are not upregulated upon demyelination and remyelination in the normal CNS (Škuljec et al., 2011), but these enzymes are expressed in active and chronic active MS lesions (Maeda & Sobel, 1996, Cuzner et al., 1996, Cossins et al., 1997, Anthony et al., 1997, Lindberg et al. 2001). 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). Moreover, gelatinases, and MMP9 in particular, are not the most efficient proteases in proteolysis of ECM proteins (Murphy et al., 1991, Fosang et al., 1992, Imai et al., 1995, Siri et al., 1995, Muir et al., 2002). Rather, spatial temporal
regulation of MMP2 and MMP9 expression may be more important. Thus, MMP9 cleaves membrane-bound 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, eliminate the CSPG-mediated inhibition of laminin through specific proteolytic cleavage of CSPGs rather than laminin, which is beneficial for regeneration (Zuo et al., 1998). However, MMP2 and MMP9 are also potent inducers of blood-brain barrier disruption and axonal injury (Anthony et al., 1998, 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 in interstitial ECM remodelling for remyelination.

MMP7 and MMP3 are more potent proteases to degrade the interstitial ECM, present in MS lesions (Murphy et al., 1991, Imai et al., 1995, Siri et al., 1995, Muir et al., 2002). 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 normal CNS (Škuljec et al., 2011, chapter 2). 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 remodelling 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). (Page-McCaw et al., 2007, Lu et al., 2011). While absent in control white matter, in MS lesions t-PA is expressed in macrophages and its expression pattern is comparable to MMP expression, i.e., 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 (Page-McCaw et al., 2007, Lu et al., 2011). Upon toxin-induced demyelination, increased mRNA levels of TIMP1 and TIMP2 are observed at demyelination, followed by a gradual decline during remyelination (Škuljec et al., 2011, chapter 2). TIMP3 is transiently upregulated in the first week during demyelination, whereas TIMP4 is upregulated during remyelination (Škuljec et al., 2011). Lindberg et al. (2001) showed that the mRNA levels of these TIMPS are not significantly altered at all MS lesion stages. In contrast, Mohan et al. (2010) showed that both TIMP1 and TIMP3 mRNA are upregulated in active and chronic inactive lesions, while TIMP3 mRNA are downregulated in chronic 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, i.e., interfere with interstitial ECM remodelling. 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 blood-brain barrier and demyelinating pathology (Gijbels et al., 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 tenascin expression, 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 et al., 2002), may clear tenascins in active MS lesions and at the border of chronic active lesions.

Strikingly, MMPs are hardly present in chronic inactive and in the center of chronic active 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, i.e., 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. Strikingly, 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 chronic 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 a. 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 (chapter 2), while fibronectin levels are still increased (Stoffels et al., 2013). Of note, astrocyte-derived HMW-hyaluronan, which is highly expressed in chronic MS lesions, is degraded by the hyaluronidase family rather than by MMPs.

**Concluding remarks**

Taken together, remodelling of the interstitial ECM in MS, including the expression patterns of ECM molecules and MMPs, is dependent on the nature of and the localization within MS lesions, and differs from ECM remodelling upon demyelinating injury to the normal CNS and subsequent successful recovery. 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 in the normal injured CNS, are absent or incorrectly located in MS lesions. In addition, the phenotype of astrocytes, the main producers of ECM molecules, 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 chronic inactive lesions, which is enriched in CSPGs, and one within the center of MS lesions that is devoid of CSPGs, but enriched in fibronectin aggregates, osteopontin and HMW-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, also contribute to remyelination failure. As a therapeutic strategy, it is essential to clear these OPC differentiation-inhibiting ECM proteins, as targeted upregulation of remyelination-promoting laminin may not be efficient. *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). In fact, in some studies, the effect of CSPGs on OPC behaviour have been performed on a relative high laminin background (Siebert & Osterhout, 2011, Pendelton et al., 2013), further indicating that CSPG-effects are likely dominant over laminin. Thus, given that aggregates of fibronectin accumulate in chronic MS lesions and CSPGs at the edge of (chronic) active lesions, these remyelination-inhibiting obstacles have to be removed. Furthermore, 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. Local activation of MMPs, i.e., with an appropriate substrate specificity, may be a means to degrade ECM depositions and reinstate a permissive environment for remyelination, as is the case in recovery upon demyelination to the normal CNS. 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 remodelling in favor of remyelination. Thus, MMPs contribute to damage at early lesions stages, while their absence at later stages is detrimental to interstitial ECM remodelling, 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 affect microglia/macrophage activation (Milner et al., 2007, Austin et al., 2012, Rolls et al., 2008, chapter 3), which may not only affect MMP expression, but also indirectly contribute to OPC differentiation and therefore remyelination (Miron et al., 2013). Hence, means to overcome the dysregulated activation of microglia/macrophages in MS lesions (Vogel et al., 2013, Peferoen et al., 2015) may indirectly also reinstall correct ECM remodelling.
Scope of this thesis
Fibronectin, a relevant extracellular matrix molecule (ECM), may cluster and form fibronectin aggregates (aFn), which inhibits remyelination. Failure of the latter is a hallmark in the etiology of the disease Multiple Sclerosis (MS). To device means for overcoming the inhibitory effects of this ECM molecule, a detailed understanding of the underlying mechanism(s) is essential. Potential options include interference of aggregated fibronectin with the differentiation and maturation of precursor cells of oligodendrocytes (OPCs), the myelin-producing cells in the brain. Alternatively, elimination of the aggregated fibronectin clusters by metalloproteases (MMPs) might reactivate OPC maturation and if so, activation of these proteases in vivo might serve as an attractive therapeutic approach. Aberrant ECM remodelling in demyelinated MS lesions, its role in remyelination failure and challenges and options to overcome this, are outlined and summarized in chapter 1. In the work presented in chapter 2 of this thesis, MMP3, MMP7 and MMP9 were studied to clarify whether they are able to cleave remyelination-impairing aFn and whether their expression levels are altered in MS lesions. In addition, cellular sources of these MMPs were examined, including astrocytes, microglia, bone marrow-derived macrophages (BMDMs), OPCs and oligodendrocytes (chapter 2). Since beneficial environmental conditions for remyelination require an appropriate phenotype of the microglia and infiltrating macrophage, it was of next interest to examine whether aFn could interfere with the ability of microglia and BMDMs to convert to such a remyelination-promoting phenotype (chapter 3). Apart from aFn, other ECM molecules, such as plasma fibronectin and chondroitin sulfate proteoglycans (CSPGs) may induce both pro-inflammatory and regenerative features in microglia and BMDMs, interfering with OPC maturation. In chapter 4, we therefore investigated whether aFn, pFn and CSPGs affect pro-inflammatory and regenerative features of microglia and BMDMs and whether this could modulate the
differentiation of OPCs, as a potential underlying mechanism in the failure of remyelination. In our work, we observed that especially aFn, might modulate microglia and BMDMs to give rise to an intermediate phenotype. Therefore, in chapter 5, the cytokines IFNγ and IL-4, as stimulators of the pro-inflammatory and pro-regenerative phenotype respectively, were applied in demyelinated organotypic forebrain slice cultures to assess whether these cytokines modulate the ability of aFn to trigger a microglia/BMDM phenotype and overcome failure of remyelination. Finally, in chapter 6, the main conclusions and future perspectives are summarized.

References


Chapter 1


ECM remodelling in MS lesions


Chapter 1


ECM remodelling in MS lesions


