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The glycosyltransferase EXTL2 and its regulation of remyelination and neuroinflammation

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

GRADUATE PROGRAM IN NEUROSCIENCE

CALGARY, ALBERTA

APRIL 2019

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ABSTRACT

The extracellular matrix is an area that is poorly studied in the context of central nervous system (CNS) regeneration and inflammation. Previous works have demonstrated the chondroitin sulfate proteoglycans (CSPGs) to be a major group of matrix constituents that potently inhibit the growth of axons both during development and following injury. However, there are gross changes that occur in the composition of the matrix in injury, the full implications of which have yet to be elucidated. We use the lysolecithin-induced model of demyelination in mice to investigate the roles that CSPGs serve in remyelination and inflammation with respect to microglia and macrophages. We find that CSPGs are a mediator of microglia/macrophage-mediated inflammation in the spinal cord, and loss of a regulatory enzyme, exostosin-like 2 (EXTL2), results in exacerbated neuroinflammation following injury. In culture, bone marrow-derived macrophages from EXTL2$^{-/-}$ animals produce more matrix metalloproteinase and tumor necrosis factor alpha when stimulated with CSPGs. The supernatant from these cells are also more neurotoxic to cultured neurons. Overall, this work highlights CSPGs as an important factor that influences inflammation in the CNS.

PREFACE

Portions of the introductory text in Chapter 1 are used with permission from Pu et al. (2018) of which I am the primary author.

Chapter 2 is being prepared as a manuscript for publication.

ACKNOWLEDGMENTS

I would like to thank my supervisor, Dr. V. Wee Yong, without whom this project would not have been possible. I am truly grateful for Wee's guidance and endless patience as I learned how to form hypotheses, develop ideas, and turn them into data. Wee is dedicated to the success of his trainees and has been a wonderful mentor, and I will always be thankful for the training I have received in his lab. His advice is something I will take with me and apply in each step of my scientific career. I could not have had a better training opportunity, nor lab experience anywhere else.

I would also like to thank my committee members, Drs. Shalina Ousman and Jeff Biernaskie for their mentorship and scientific discussions during our meetings. Their insight and knowledge have contributed greatly to the progress of my work and my development as a scientist.

Thank you to my fellow lab members and colleagues Khalil Rawji, Nathan Michaels, Erin Stephenson, Dennis Brown, Brian Lozinski, Manoj Mishra, Jeff Dong, Reza Mirzae, Susobhan Sarkar, Helvira Melo, Claudia Silva, Rigel Chan, Fiona Yong, Candice Poon, Runze Yang, and the summer students for their friendship and support. They have made days in the lab a joy and have given me invaluable advice over the past two years. Without their vast expertise and knowledge, this project would not have developed as well as it did. I would like to thank Erin Stephenson and Cristina Santamaria-Plaza for our collaboration on our ECM project. They have been a pleasure to work with and I am grateful for the time and effort they have put in to make this project a success.

I would like to thank the administrative staff Tanna Giroux and Trisha Lichtenberger. Their kindness and willingness to help with any administrative problems has allowed me to

progress through this program with no problems. I would also like to thank Trisha for involving me in community outreach events like MS Connect. I'm grateful for having had the opportunity to share my work with the public and gain valuable experience.

Lastly, I would like to thank my parents for their interest in my work and support over the past two years.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

Symbol/Abbreviation	Definition
α	alpha
β	beta
γ	gamma
µ	micro
2AG	2-arachydonoylglycerol
AMPAR	alpha-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid receptor
APC (CC1)	adenomatous polyposis coli
B3GalT6	galactosyltransferase II
B3GaT3	glucuronosyltransferase I
B4GalT7	galactosyltransferase I
BBB	blood-brain barrier
BDNF	brain-derived neurotrophic factor
BM	basement membrane
BMDM	bone marrow-derived macrophage
BSA	bovine serum albumin
CCL	C-C motif chemokine ligand
CCN3	cellular communication network factor 3
CD	cluster of differentiation
ChABC	chondroitinase ABC
ChPF	glucuronosyltransferase II
CNS	central nervous system
CS	chondroitin sulfate
CSGalNAcT	chondroitin sulfate N-acetylgalactosaminyltransferase
CSPG	chondroitin sulfate proteoglycan

CXCL	C-X-C motif chemokine ligand
CX3CR1	C-X3-C motif chemokine receptor 1
DAMP	danger-associated molecular pattern
DMEM	Dulbecco's modified eagle's medium
DNase	deoxyribonuclease
DRG	dorsal root ganglion
EAE	experimental autoimmune encephalomyelitis
ECM	extracellular matrix
EGF	epidermal growth factor
ELISA	enzyme-linked immunosorbent assay
EXTL2	exostosin-like 2
FBS	fetal bovine serum
FGF	fibroblast growth factor
GABA	gamma-aminobutyric acid
GAG	glycosaminoglycan
GAGE	generally applicable gene set enrichment
GalNAc	N-acetylgalactosamine
GlcA	glucuronic acid
GPCR	G protein-coupled receptor
HA	hyaluronic acid/hyaluronan
HS	heparan sulfate
HSPG	heparan sulfate proteoglycan
IFNγ	interferon gamma
IGF	insulin-like growth factor
KOR	kappa opioid receptor
LAR	leukocyte-common antigen receptor
Iba1	ionized calcium-binding adaptor molecule 1

IL-1β	interleukin 1 beta
LINGO-1	leucine rich repeat and Immunoglobin-like domain-containing protein 1
LPC	lysophosphatidylcholine/lysolecithin
LPS	lipopolysaccharide
mAChR	muscarinic acetylcholine receptor
MAP2	microtubule-associated protein 2
MBP	myelin basic protein
mRNA	messenger ribonucleic acid
MMP	matrix metalloproteinase
MS	multiple sclerosis
NCAM	neural cell adhesion molecule
NF200	neurofilament heavy chain 200 kilodalton
NG2	neuron-glial antigen-2
NGF	nerve growth factor
NMDAR	N-methyl-D-aspartate receptor
OCT	optimal cutting temperature compound
OPC	oligodendrocyte precursor cell
PBS	phosphate buffered saline
PCA	principal component analysis
PDGF	platelet-derived growth factor
PDGFRα	platelet-derived growth factor receptor alpha
PLP	proteolipid protein
PNN	perineuronal net
PTPσ	protein tyrosine phosphatase sigma
RNA	ribonucleic acid
RPTP	receptor protein tyrosine phosphatase

SCI	spinal cord injury
Shh	sonic hedgehog
siRNA	small interfering ribonucleic acid
T3	triiodothyronine
TGFβ	transforming growth factor beta
TLR	toll-like receptor
TNFα	tumor necrosis factor alpha
UDP	uracil diphosphate
XYLT1	xylosyltransferase 1

CHAPTER 1 – Introduction

1.1 Multiple Sclerosis

Multiple sclerosis (MS) is a CNS demyelinating disease, in which aberrant immune activation and penetration into the CNS parenchyma causes stripping of myelin from axons, leaving axons vulnerable to secondary insult or death of the soma. The perturbation of myelin integrity, in conjunction with axonal disruption is thought to underlie many neurological deficits clinically observed in MS (Brück, 2005). One of the most currently active areas of MS research is the pursuit of remyelination strategies: therapeutic approaches to the regeneration of myelin. All currently approved treatments for MS are targeted towards dampening the immune system in a variety of ways, in an attempt to halt the progression of disease or conversion from clinically isolated syndrome (a single incidence of clinical presentation) to relapsing-remitting MS (wherein patients experience cycles of exacerbation of symptoms followed by recovery) to secondary progressive MS (where there is continuous neurodegeneration in the absence of overt inflammation). However, a pathological consequence of MS is neurodegeneration, likely contributed to by the loss of myelin and damage to their underlying axons. Therefore, there is a significant need for treatments aimed at neuroprotection and regenerating the lost myelin.

1.2 Oligodendrocyte Precursor Cells and their Biology

Mature oligodendrocytes arise from oligodendrocyte precursor cells (OPCs) derived from progenitors in the ventricular zone during embryogenesis. The initial population of OPCs throughout the fetal brain and spinal cord is a late developmental event, beginning at E12, and appears to take on a ventral to dorsal pattern. In the brain, an initial wave of OPCs forms and migrates from the medial ganglionic eminence and anterior entopeduncular area of the ventral

forebrain (Kessaris et al., 2006). These OPCs will populate the entire cortex, followed by subsequent waves of OPCs originating from lateral and caudal ganglionic eminences later in fetal development, and eventually from a population of cortical progenitors near time of birth (Kessaris et al., 2006). A similar pattern has been demonstrated in the spinal cord, where the ventral wave of OPCs is sonic hedgehog (Shh) signaling dependent, and the dorsal wave is Shh-independent (Crawford et al., 2016). Interestingly, in the fetal forebrain, ventrally-derived OPCs appear to be largely replaced by dorsally-derived OPCs by P10 (Kessaris et al., 2006). In contrast, others have reported a more restricted spatial and functional patterning in both the brain and spinal cord (Yue et al., 2006; Tripathi et al., 2011).

Although developmental myelination involves the direct differentiation of OPCs into myelinating oligodendrocytes, a population of self-renewing OPCs remain distributed evenly in the adult CNS. Adult OPCs are highly active in their surveillance of their environment and maintenance of homeostatic density in the CNS. Time lapse microscopy through a cranial window shows constant extension and retraction of processes, and continuous migration of OPCs in the adult cortex (Hughes et al., 2013). OPCs also maintain stringent control of their density in the cortex despite undergoing proliferative, apoptotic, or differentiation processes (Hughes et al., 2013). The density of OPCs plays a major role in the regulation of myelination. OPCs require a sufficiently high spatial constraint in order to differentiate, and this effect is replicated using inert microbeads (Rosenberg et al., 2008).

OPCs arising from or residing in various regions of the brain and spinal cord will exhibit functional differences. Surprisingly, dorsally-derived OPCs in the spinal cord exhibit greater proliferation and recruitment to a lysolecithin-induced lesion in the ventral funiculus. Therefore, this population of OPCs appears to a major player in remyelination (Crawford et al., 2016).

Interestingly, the same study reports that dorsal OPCs are simultaneously more susceptible to impairment in differentiation in aging conditions (Crawford et al., 2016). Transplantation studies where white matter OPCs were grafted into grey matter niches, and vice versa, revealed functional differences between white and grey matter-derived OPCs. White matter-derived OPCs were able to efficiently differentiate into oligodendrocytes in both white and grey matter niches, whereas grey matter-derived OPCs did not exhibit the same competency (Viganò et al., 2013). Despite this, cortical grey matter lesions in MS have been reported to have greater remyelination efficiency (Albert et al., 2007a; Chang et al., 2012; Strijbis et al., 2017), and white matter OPCs appear to be more susceptible to inflammatory factors such as IFNγ (Lentferink et al., 2018). Important to note, however, is that the contributions of intrinsic functional differences in white and grey matter OPCs, and the role the microenvironment of each niche plays, has yet to be clarified.

OPCs and oligodendrocytes are electrophysiologically responsive: they express α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), N-methyl-D-aspartate receptor (NMDAR), and γ-aminobutyric acid (GABA) receptors, indicating they are responsive to neurotransmitter signaling in the CNS. AMPAR and NMDAR are thought to regulate, in part, activity-dependent myelination, where axonal glutamate will promote OPC survival, "tethering" of OPCs to the axon, and differentiation of OPCs into oligodendrocytes (Lundgaard et al., 2013; Wake et al., 2011), although NMDAR-deficient OPCs function and myelinate normally (De Biase et al., 2011). In myelin-producing oligodendrocytes, NMDARs are present on the inner tongue of the compact myelin sheath and respond to glutamate released as a result of axonal activity. Calcium influx through NMDARs promotes the shuttling of lactate from the

oligodendrocyte to the periaxonal space, supporting the increased metabolic demand in the axon during neuronal activity (reviewed in Micu et al., 2017).

1.3 CNS Myelination

In central nervous system (CNS) myelination, OPCs differentiate into immature oligodendrocytes, which subsequently extend processes to contact multiple axons. These processes then begin to enwrap the axons and the inner tongue of myelin continually spirals around the axon, pushing under previously wrapped layers (Snaidero et al., 2014).

Several other major changes are associated with myelination, including activation of directed mRNA transport, cytoskeletal rearrangements (Zuchero et al., 2015), electrochemical changes (Baraban et al., 2017; Cheli et al., 2015) and local changes in protein and lipid composition of the myelinic plasma membrane (Fitzner et al., 2006). Finally, multiple layers of the oligodendrocyte membrane form compact myelin. MBP is essential in the compaction of the phospholipid bilayers (Weil et al., 2016). Myelin sheaths form internodes along the axon, clustering ion channels into nodal regions and segregating distinct paranodal and juxtaparanodal regions. The internodal length and thickness of the myelin sheath is determined by several intrinsic and external factors. Oligodendrocytes originating from the spinal cord produce longer sheaths both *in vitro* and *ex vivo* than those originating from the cortex (Bechler et al., 2015). Oligodendrocytes exhibit preference for wrapping inert microfibres or axons above a specific diameter, demonstrated to be independent of axonal activity (Lee et al., 2012); however, optogenetically stimulating neurons in the premotor cortex induces myelin thickening (Wake et al., 2011; Gibson et al., 2014). Varying types of experiences also have differential effects on myelination, where exercise and environmental enrichment promotes oligodendrocyte

development and social isolation reduces prefrontal cortex myelination in mice (Krityakiarana et al., 2010; Tomlinson et al., 2018; Tomlinson et al., 2016).

Axonal health and function in both central and peripheral nervous systems is highly dependent on myelin. In the CNS, the ensheathment of axons by oligodendrocyte myelin increases conduction velocity by allowing saltatory conduction. The formation of myelin internodes along axons clusters ion channels to nodes of Ranvier while insulating other segments of the axon, increasing resistance and allowing for more efficient action potential propagation. Importantly, oligodendrocytes have also been shown to provide metabolic support to axons, as implied by targeted impairment of oligodendroglial peroxisome function, where impairment of normal peroxisome functioning in oligodendrocytes resulted in axonal degeneration, demyelination, and premature death of mutant mice (Kassmann et al., 2007). This hypothesis is further supported by studies using mice lacking the lactate transporter monocarboxylate transporter-1 (Lee et al., 2012). Furthermore, there may still be forms of axonal support provided by oligodendrocytes that have yet to be fully elucidated. Mice lacking 2',3'-cyclic nucleotide phosphodiesterase exhibit normal myelination in development, but in adulthood have severe motor deficits and premature death due to axonal loss (Lappe-Siefke et al., 2003). Swelling of myelinated axons was also seen in white matter. Proteolipid protein (PLP)-deficient mice show similar axonal swellings and neurodegeneration in adolescence, despite phenotypically normal myelin development, and a similar phenomenon was observed in patients with null mutations in the PLP gene (Griffiths et al., 1998; Garbern et al., 2002). Together, these suggest that myelin serves essential functions aside from insulation of axons.

1.4 Remyelination and Remyelination Failure in MS

Several lines of evidence suggest a compounding effect of inhibitory factors that ultimately impair the regenerative capacity of the CNS. The microenvironment of the CNS, including extracellular matrix composition and deposition, the inflammatory state of the lesional milieu, the presence of active immune cells, and aging-related deficits in both immune and CNS compartments cumulatively limit remyelination.

As mature oligodendrocytes are not thought to regenerate new myelin, remyelination requires the replenishment and maturation of OPCs (Gensert and Goldman, 1997; Jarjour and Kennedy, 2004) within the injured area. OPCs are a population of surveillant, self-renewing glial progenitors labeled by antibodies to neuron-glial antigen 2 (NG2) (Dawson et al., 2003; Tripathi et al., 2010), platelet-derived growth factor receptor alpha (PDGFRα) (Rivers et al., 2008; Zhu et al., 2014), and the transcription factors Olig1 and Olig2 (Dai et al., 2005; Othman et al., 2011). OPCs homeostatically form a tiled pattern in the adult CNS; however, they will constitutively survey the microenvironment and respond to injury by migrating to the injury site and proliferating (Hughes et al., 2013). OPCs will subsequently terminally differentiate into oligodendrocytes, which then extend processes to contact and ensheath axons (Sherman and Brophy, 2005; Emery, 2010).

Restoration of myelin to denuded axons has been shown to preserve axonal health and restore axonal function to a limited but significant extent. Promoting remyelination is an appealing goal not only to protect the demyelinated axons from functional destabilization and secondary damage (Irvine and Blakemore, 2008), but also to restore electrophysiological (Blight and Young, 1989) and functional (Duncan et al., 2009) properties as demonstrated in animal

models. It is speculated that remyelination may even reverse some early deficits in MS (Alizadeh et al., 2015).

Remyelination has been documented in subsets of MS patients; however, the mechanisms differentiating plaques that are able to remyelinate and plaques that do not have yet to be elucidated. Remyelination has been observed in demyelinated white matter (Lassmann et al., 1997; Patrikios et al., 2006; Prineas et al., 1993; Prineas & Connell, 1979) and more recently in cortical grey matter (Albert et al., 2007; Chang et al., 2012) MS plaques. However, remyelination is often restricted to lesion borders (Prineas and Connell, 1979). Moreover, remyelinated internodes do not regain their original thickness (Ludwin and Maitland, 1984), and internodes are generally shorter (Blakemore and Murray, 1981) than in normal appearing white matter. Interestingly, acute demyelinated plaques appear to remyelinate more efficiently than chronic ones (Ozawa et al., 1994; Patrikios et al., 2006). There are several possible explanations as to why remyelination is limited both spatially and temporally in MS, such as failure of OPCs to migrate and repopulate the lesion, or failure of OPCs to mature and differentiate into oligodendrocytes due to several inhibitory factors (Wolswijk, 1998; Kuhlmann et al., 2008) including the chondroitin sulfate proteoglycans (Lau et al., 2012; Keough et al., 2016).

Research in the past few decades have identified a plethora of factors that promote or inhibit remyelination in various animal models of CNS injury. Receptors interacting with either secreted ligands or cell-derived ligands can exert profound influence on oligodendrocyte proliferation and maturation. For example, epidermal growth factor (EGF) (Aguirre et al., 2007; Palazuelos et al., 2015), fibroblast growth factor (FGF) (Murtie et al., 2005), platelet-derived growth factor (PDGF) (Allamargot et al., 2001), brain-derived neurotrophic factor (BDNF) (Miyamoto et al., 2015), nerve growth factor (NGF) (Takano et al., 2000), and insulin-like

growth factor (IGF) (Pang et al., 2007) have been shown in *in vitro* and mouse studies to have remyelination-promoting effects. Inflammation-related molecules have also been identified to enhance OPC proliferation, such as TNFα (Arnett et al., 2001), IL-1β (Mason et al., 2001) and complement factor C5 (Weerth et al., 2003). General inflammation in the microenvironment also seems to stimulate remyelination (Arnett et al., 2003; Foote & Blakemore, 2005; Mei et al., 2016). Estrogen, progesterone, prolactin, triiodothyronine (T3), and the endocannabinoid 2-arachidonoylglycerol have also been implicated in remyelination-enhancing roles (Ibanez et al., 2004; Gregg et al., 2007; Khalaj et al., 2013; Kumar et al., 2013; Itoh et al., 2016; Feliu et al., 2017; Remaud et al., 2017). Several studies have now shown roles for axon-derived neuregulins in both PNS and CNS remyelination (Fricker et al., 2011; Kataria et al., 2018; Lundgaard et al., 2013; Michailov et al., 2004; Stassart et al., 2013; Viehover et al., 2001) and axonal glutamate signaling (Gautier et al., 2015). OPCs express the nicotinic acetylcholine receptor and, when stimulated with nicotine, increase their intracellular free calcium (Rogers et al., 2001), although it is still unclear whether cholinergic signaling exerts any effect on OPC proliferation or maturation. Several GPCRs expressed by OPCs are also required for successful and efficient remyelination (Mei et al., 2016; Mogha et al., 2016). Other signaling pathways known to be involved, although with some conflicting reports, include sonic hedgehog (Ferent et al., 2013; Sanchez & Armstrong, 2017; Wang et al., 2008), Notch (Aparicio et al., 2013; Hammond et al., 2014; John et al., 2002; Jurynczyk et al., 2005), muscarinic acetylcholine receptors (Cui et al., 2006; De Angelis et al., 2012; Mei et al., 2016; Welliver et al., 2018), TLRs (Srivastava et al., 2018), and the kappa opioid receptor (Mei et al., 2016). Nogo and LINGO-1 are two surface receptors expressed by OPCs, shown to potently inhibit remyelination (Mi et al., 2009; Mi et al., 2013; Sozmen et al., 2016; Sun et al., 2015), with function-blocking anti-LINGO-1 antibodies

being extensively studied in MS patients (Mellion et al., 2017; Tran et al., 2014; ClinicalTrials.gov NCT03222973). More developmental myelination-modulating factors have been identified but have yet to be investigated in the context of CNS injury. The above is summarized in Table 1.1.

Several drug screens have also been performed to identify clinically available pharmaceutical compounds that promote OPC differentiation and MBP expression *in vitro* and possess remyelinating or neuroprotective properties *in vivo*. These drug screens have identified, among many candidates, tamoxifen (Gonzalez et al., 2016), benztropine (Deshmukh et al., 2013), clemastine (Mei et al., 2014), the imidazole class of antifungals (Najm et al., 2015; Hubler et al., 2018), quetiapine (Zhang et al., 2012; Zhornitsky et al., 2013), and omeprazole (Zhu et al., 2019). A double-blind clinical trial has been recently completed documenting positive results with the use of clemastine fumarate for relapsing remitting MS patients (Green et al., 2017).

Spatial and mechanical properties of the tissue microenvironment modulate the proliferation, migration, and differentiation of OPCs. A softer ECM will be more conducive to OPC differentiation compared to a stiffer, less elastic ECM, which favors Schwann cell myelination (Urbanski et al., 2016). Mechanical stimulation of the tissue can also alter the signaling mechanisms through which ECM molecules act. Static stretch delivered to murine neural stem and progenitor cells revealed a decreased preference towards oligodendrocyte lineage differentiation (Arulmoli et al., 2015). Additionally, static tension applied to OPCs inhibited proliferation but promoted differentiation (Hernandez et al., 2016; Jagielska et al., 2017). OPC differentiation and myelination appear to also depend heavily on spatial packing, as

OPCs plated at higher densities with either other cells or inert beads in culture exhibit greater MBP expression overall (Rosenberg et al., 2008).

Overall, several intrinsic and extrinsic factors must work in concert to overcome the many inhibitors of remyelination and orchestrate successful remyelination. Aside from cell-derived molecules described above, the extracellular matrix is also highly modified in injury, and is abundant in molecules that are able to potently inhibit OPC proliferation, migration, differentiation, and myelination.

1.5 The Extracellular Matrix Plays a Role in Remyelination Failure

The extracellular matrix can be largely separated into three compartments in the CNS: the parenchymal basement membrane (BM), the interstitial matrix, and the perineuronal nets (PNNs) (Lau et al., 2013). The BM is primarily comprised of the heparan sulfate proteoglycan (HSPG) family member perlecan, laminin-2, type IV collagens, and fibronectin. The PNNs and interstitial matrix differ from the BM in that they are principally composed of chondroitin sulfate proteoglycans (CSPGs). CSPGs form PNNs by directly interacting with hyaluronan through the G1 domain, and indirectly interacting with tenascin R via link proteins. These structures are found in close association with neuronal soma and dendrites, in high abundance starting at the closing of critical periods. PNNs are thought to buffer ions and offer protection from oxidative stress, stabilize existing synaptic connections and reduce plasticity of synapses to prevent aberrant connections, and to some extent, modulate the activity of neurons (reviewed in Testa et al., 2018).

There is extensive disruption and remodeling of the ECM following injury. The dynamics of several ECM constituents are correlated with gliosis and inflammation, or regenerative stages

in murine demyelination. I describe major ECM members present in the injured area and their currently known effects on remyelination below.

1.5.1 Heparan sulfate proteoglycans (HSPGs)

Sections 1.5.1 through 1.5.6 are adapted from Pu et al., 2018, coauthored with Erin L. Stephenson and V. Wee Yong.

HSPGs are one of the major constituents of the ECM. Family members include glypicans (1-6), syndecans (1-4), perlecan (also known as HSPG2), agrin, collagen XVIII, CD44, neuropilin-1, betaglycan and serglycin. Syndecans, glypicans, CD44, neuropilin-1 and betaglycan are membrane-anchored, whereas perlecan, agrin, and collagen XVIII are found extracellularly in basement membranes (BM). Serglycin is found in secretory vesicles (Sarrazin et al., 2011). HSPGs structurally parallel CSPGs, similarly composed of a core protein with attached GAGs. HS GAGs are repeating disaccharides of N-acetylglucosamine and glucuronic acid (Kjellén and Lindahl, 1991), and differ from CSPGs such that CSPGs contain N-acetylgalactosamine in place of N-acetylglucosamine.

Similar to CSPGs, many functions of HSPGs are exerted through GAG chains and are regulated by sulfation patterns of GAGs. In development, HSPGs act as co-receptors and reservoirs for fibroblast growth factor-2 (FGF2), a growth factor critical for proliferation and differentiation of neural stem cells (Nurcombe et al., 1993). HSPGs may induce the dimerization and improved activation of FGF receptor by binding to multiple FGF proteins (Spivak-Kroizman et al., 1994). The positive effect of HSPGs on FGF signaling may be conferred by the specific 6-O-sulfation pattern on heparan sulfate GAGs, as there is no promotion of mitosis in a lymphoblastic cell line in the absence of this sulfation pattern (Pye et al., 1998). In addition, HSPGs modulate NCAM, slit-1 and -2, Wnt, and transforming growth factor-β (TGFβ)

signaling. In the case of Wnt, HSPG interaction with Wnt prevents the aggregation of the protein and maintains the morphogenic gradient for stable signaling (Fuerer et al., 2010). Increasing bioavailability of various factors by stabilizing connections between the ligand and its receptor is a mechanism of signaling modulation common to other binding partners of HSPGs (Häcker et al., 2005). HSPGs also bind other ECM molecules such as laminin, fibronectin, tenascins, hyaluronan and thrombospondin (Bandtlow and Zimmermann, 2000). They serve roles in axonal guidance and distribution of morphogens Wnt, Engrailed, slits, and netrins (Properzi et al., 2008).

HSPGs are upregulated following injury in peripheral nerve and CNS nigrostriatal lesions associated with fibroblasts and macrophages (Properzi and Fawcett, 2004). For example, perlecan is upregulated in injured neurons and reactive astrocytes of the mouse hippocampus following intra-cerebroventricular kainate injections (Shee et al., 1998). De Yébenes and colleagues (De Yébenes et al., 1999) showed that perlecan was correspondent with astrocytes and microglia *in vitro*, and both perlecan mRNA and immunoreactivity increased 3 days following stab wound in the mouse hippocampus.

While little work has been done investigating the direct effect of HSPGs on oligodendrocyte function following injury, HSPGs may influence oligodendrogenesis by modulating the injured microenvironment. Oligodendrocytes differentially express HSPGs at various stages of differentiation (Bansal et al., 1996; Stringer et al., 1999). Increased syndecan-2 and -4 and glypican mRNA correlated with differentiation of progenitors into mature cells, and syndecan-1 and -3 exhibited the inverse expression, facilitating FGF2-mediated oligodendrocyte progenitor proliferation (Winkler et al., 2002) and myelin sheath thickness (Furusho et al., 2012). The interaction between HSPGs and FGF2 and platelet-derived growth factor (PDGF) has been

well-documented (Gutiérrez and Brandan, 2010; Mundhenke et al., 2002; Smith et al., 2009; Zhang et al., 2001). FGF2 and PDGF are both mitogens for OPCs, and FGF2 is also an inhibitor of OPC differentiation (Armstrong et al., 2002; Baron et al., 2000; Butt and Dinsdale, 2005; Murtie et al., 2005; Woodruff et al., 2004). Importantly, OPCs express FGF2 and PDGF ligands and receptors in response to demyelination (Armstrong et al., 2002; Frost et al., 2003; Redwine and Armstrong, 1998). Thus, it is possible that HSPGs deposited within a lesion accumulate FGF2 and PDGF, resulting in suppression of OPC differentiation and maturation. Currently, more work is required to directly correlate HSPG to oligodendrocyte function *in vivo*.

1.5.2 Hyaluronan

Hyaluronan is an unsulfated, linear GAG chain comprised of alternating units of GlcA and GlcNAc (Necas et al., 2008). It is secreted natively as a high molecular weight polymer primarily from astrocytes, and is able to bind the N-terminus of lectican core proteins with the assistance of link proteins (Bignami et al., 1993; Spicer et al., 2003). Hyaluronan is an essential component of the perineuronal net, connecting to multiple CSPGs in order to create an organized network (Kwok et al., 2011).

Hyaluronan possesses different functions depending on its molecular weight. Under inflammatory conditions, there is an increase in synthesis of high molecular weight hyaluronan that is subsequently digested into low molecular weight hyaluronan fragments by secreted hyaluronidases and (matrix metalloproteinases) MMPs (Noble, 2002). Presently, controversy surrounds the debate on which form of hyaluronan is more pro-inflammatory or beneficial to remyelination.

There is support for the hypothesis that low molecular weight hyaluronan acts as danger associated molecular patterns (DAMPs), able to initiate pro-inflammatory signaling in

macrophages/microglia, resulting in the release of inflammatory cytokines such as MIP1α, CCL2, IL-12 and TNFα (Mckee et al., 1996; Jiang et al., 2005). The pro-inflammatory effects of low molecular weight hyaluronan are thought to be exerted through CD44, TLR2 and TLR4 signaling. Function-blocking anti-CD44 antibody given to alveolar macrophages inhibited increases in pro-inflammatory cytokine production (Mckee et al., 1996), and use of TLR2 and TLR4 knockout mice has revealed that both TLRs, in addition to the intracellular adaptor MyD88, are also critical in hyaluronan signaling in macrophages/microglia following spinal cord injury (Jiang et al., 2005). High molecular weight hyaluronan also decreases inflammatory signaling in cultured microglia through competitive binding to TLRs or CD44, as both are also receptors for low molecular weight hyaluronan and LPS (Austin et al., 2012). High molecular weight hyaluronan was reported to contribute to the pathogenesis of experimental autoimmune encephalomyelitis (EAE) through facilitating lymphocyte adhesion and rolling (Winkler et al., 2013). In addition, inhibition of hyaluronan synthesis in EAE mice resulted in skewing of reactive T cells toward T_H2 lineage and increased the number of $Foxp3^+$ Tregs in the spinal cord (Kuipers et al., 2016). In contrast, Muto et al. showed that injection of low molecular weight hyaluronan reduced LPS-mediated sickness syndrome in mice, suggesting a potential anti-inflammatory role for low molecular weight hyaluronan depending on context (Muto et al., 2009).

High molecular weight hyaluronan from astrocytes accumulates in chronic demyelinated MS lesions; these authors postulated that the high molecular weight hyaluronan inhibits OPC maturation, and ultimately leads to deficient remyelination *in vivo* (Back et al., 2005). However, others suggest that high molecular weight hyaluronan reduces proliferation and deposition of CSPGs by cultured astrocytes (Struve et al., 2005; Khaing et al., 2011), giving rise to a more

permissive environment for remyelination. Data from Sloane et al. has identified TLR2 to be the primary receptor mediating inhibition of OPC maturation by hyaluronan (Sloane et al., 2010). Importantly, TLR2 appears to only be stimulated by low molecular weight hyaluronan (Termeer et al., 2002; Scheibner et al., 2006), implicating this form of hyaluronan to be the repressive species for OPC maturation. Digestion products of the hyaluronidase PH20 expressed by OPCs and reactive astrocytes have also been reported to inhibit OPC maturation and remyelination in lysolecithin-induced demyelination (Preston et al., 2013). It is important to note, however, that a more recent study reports that PH20 mRNA and protein are undetectable in the murine CNS, and infusion of recombinant human PH20 failed to inhibit OPC differentiation in culture (Marella et al., 2017). More studies are required to clarify the individual effects of high or low molecular weight hyaluronan on remyelination.

1.5.3 Collagens

Collagens, in addition to several other functions, are essential structural proteins of the ECM. The general structure is three polypeptide α chains forming varying lengths of triple helix motifs. Several forms of the α chains exist and are able to assemble into homotrimers or heterotrimers, conferring further diversity to collagen family members. Currently there are 28 known collagens discovered in vertebrates, termed collagen I through collagen XXVIII. Collagens can be categorized based on the number of triple helix domains: fibrillar collagens contain one triple helical domain, while fibril-associated collagens have interrupted triple-helices (FACITs), and other collagen subfamilies contain multiple triple helical domains (Gordon and Hahn, 2010; Kadler et al., 2007; Ricard-Blum, 2011).

Under homeostatic conditions in the CNS, collagens I, III, and IV are present in the glia limitans externa and the vascular basement membrane (Rutka et al., 1988). However, following

mouse spinal cord contusion and Theiler`s virus-induced demyelination, collagens I and IV are deposited into the lesioned area and become a major component of the fibrous scar tissue (Haist et al., 2012; Klapka and Müller, 2006; Okada et al., 2007). Traumatic spinal cord injury in mice have also shown that collagens are secreted primarily from lesion-infiltrating cells, such as perivascular fibroblasts (Soderblom et al., 2013), reactive astrocytes (Hara et al., 2017; Liesi and Kauppila, 2002), and meningeal fibroblasts (Matthews et al., 1979).

Work done on the influence of collagen on oligodendrocytes and remyelination has yielded conflicting results. OPCs plated on collagen are less migratory when compared to control culture (Milner et al., 1996). Premyelinating oligodendrocyte growth cones avoided collagen IV in culture (Fox et al., 2006). In contrast, collagen I purified and formed into microspheres have been used as a delivery medium for OPCs onto a dorsal root ganglion (DRG) culture. Collagen microspheres were shown to support OPC proliferation and differentiation, and OPCs were able to successfully myelinate cultured DRG neurons (Yao et al., 2013), implying an important scaffolding function. Similarly, collagen I and IV are commonly used in oligodendrocyte cell cultures (Sundberg et al., 2010; Yamashita et al., 2017), suggesting at minimum a lack of repressive function in oligodendrocytes. Interestingly, oligodendrocytes lack collagen-binding integrin receptors (Milner et al., 1996), and the role of other collagen receptors on oligodendrocytes in the context of myelination/remyelination is unclear.

1.5.4 Laminins

Laminins are high molecular weight fibrous glycoproteins abundantly present in the endothelial basement membrane of the blood-brain barrier (Engel and Furthmayr, 1987). Laminins' myriad functions are exerted through interactions with collagen IV, HSPGs, nidogen, dystroglycan, and laminin receptors of the integrin family (Leiton et al., 2015; Novak and Kaye,

2000). Their largely structural role is mediated in part by associating with collagen IV networks through nidogen-1 (Takagi et al., 2003) and perlecan (Iozzo, 2005). In injury, laminins are secreted by reactive astrocytes (Bernstein et al., 1985; Liesi et al., 1984) and deposited into the injury site, particularly into expanded perivascular spaces (Risling et al., 1993).

Laminin-2 is generally a permissive substrate for OPC survival and maturation. It enhances myelin membrane formation and oligodendrocyte sensitivity to PDGF in developmental myelination (Barros et al., 2011; Buttery and Ffrench-Constant, 1999; Colognato et al., 2002; Frost et al., 1999); however, this phenomenon is unexplored in the context of injury, and may be detrimental to remyelination as PDGF signaling results in the arrest of terminal oligodendrocyte differentiation (Baron et al., 2000). Blockade of the mouse α6β1 integrin, a known laminin receptor, decreased oligodendrocyte survival in culture (Corley et al., 2001; Frost et al., 1999). Concurrently, overexpression of a dominant negative β1 integrin results in hypomyelination of spinal cord and optic nerve axons. Transgenic mice expressing a dominant-negative β1 integrin subjected to cuprizone-induced demyelination exhibited reduced percentages of remyelinated axons in the corpus callosum; however, remyelinated axons had g-ratios comparable to those in wild-type mice (Lee et al., 2006), indicating a possible role of the laminin-β1 integrin interaction in OPC proliferation or recruitment. Indeed, high expression of α6β1 integrin in OPCs and low expression in mature oligodendrocytes support this finding (O'Meara et al., 2011). Studies using knockout of the laminin-2 α2 subunit show laminin promotes OPC survival in the subventricular zone at birth, and loss of the laminin α2 gene results in delayed oligodendrocyte maturation and dysmyelination at postnatal day 21 (Relucio et al., 2012). There is also *in vitro* evidence suggesting laminin-2 promotes OPC proliferation through

stimulating cleavage of dystroglycan by MMPs. Blocking dystroglycan cleavage resulted in significantly decreased OPC proliferation (Leiton et al., 2015).

Interestingly, Benninger and colleagues (Benninger et al., 2006) report that β1 integrin signaling is not essential for developmental myelination, and conditional ablation of the β1 integrin in oligodendroglial cells of mice given a focal demyelinating lesion resulted in unimpaired remyelination. Unlike the dominant-negative β1 integrin mice described above (Lee et al., 2006), assessment of developmental myelination in the optic nerve showed no difference in the conditional knockout mice compared to wild-type.

1.5.5 Fibronectin

Fibronectin exists as a protein dimer, serving as a scaffold for cell adhesion and migration. During embryogenesis fibronectin is essential as defects in fibronectin signaling causes embryonic lethality (George et al., 1993). While not expressed in the developed CNS normally, following injury, fibronectin leaks in from plasma and is also produced locally by reactive astrocytes, microglia/macrophages and endothelial cells (Milner et al., 2007; Stoffels et al., 2013b; Zhao et al., 2009). Fibronectin can signal through the αv integrins on OPCs, which are upregulated in demyelinated lesions (Stoffels et al., 2013a).

Whether fibronectin is inhibitory or beneficial to remyelination is still debated. Fibronectin has been shown to increase MMP9 activity in conditioned media from primary OPC cultures; in the presence of fibronectin, MMP9 activity is constrained to the cell bodies of OPCs. Due to this mislocalization of MMP9 activity, OPCs exhibited less branching and fewer secondary processes on fibronectin compared to laminin (Šišková et al., 2009). These results are corroborated by immature oligodendrocytes plated on fibronectin. Oligodendrocytes appear morphologically immature and a majority of cells fail to form myelin membranes; however,

MBP expression was normal compared to control cultures (Qin et al., 2017). There is also data suggesting fibronectin impedes the morphological differentiation of OPCs by inhibiting reorganization of the actin cytoskeleton that would normally allow myelin sheet-directed vesicular transport (Šišková et al., 2006). Fibronectin aggregates have also been observed in both chronic EAE and chronically demyelinated MS lesions, and injection of astrocyte-derived fibronectin aggregates into experimentally demyelinated lesions resulted in reduced OPC differentiation and remyelination (Stoffels et al., 2013a). However, Milner et al. (Milner et al., 1996) show fibronectin promotes migration of OPCs to a similar extent *in vitro* compared to laminin. In addition, knockout of astrocyte-derived fibronectin resulted in reduced OPC proliferation following demyelination (Stoffels et al., 2015).

1.5.6 Other ECM molecules that regulate properties of oligodendrocytes and remyelination

Several other ECM components have also been shown to change in response to CNS injury and to influence oligodendrocyte function in myelination/remyelination. Following injury, vitronectin is upregulated by macrophages/microglia and reactive astrocytes (Zhao et al., 2009). *In vitro*, vitronectin has been observed to promote oligodendrocyte migration (Milner et al., 1996) and to promote the differentiation of oligodendrocytes from embryonic stem cells (Gil et al., 2009).

Dystroglycan serves as an adaptor, linking laminin to intracellular actin networks (Chen et al., 2003). There are several reports of dystroglycan regulating myelination. Remyelination of the rat sciatic nerve is improved with interaction of β-dystroglycan, a cleavage product of the full dystroglycan protein, with the laminin α2 chain (Masaki et al., 2003). siRNA depletion of dystroglycan eliminates the differentiation of oligodendrocytes potentiated by IGF1 and laminin *in vitro* (Eyermann et al., 2010). Blockade of dystroglycan function results in failure of

oligodendrocytes to produce myelin membrane sheets or to initiate myelination in co-culture with dorsal root ganglion neurons (Colognato et al., 2007).

Decorin, a member of the small leucine-rich proteoglycans, is known to suppress the expression of phosphacan, brevican, neurocan and NG2 (Davies et al., 2004), and thus would reduce an inhibitory CSPG environment and facilitate repair processes. This effect may be in part due to the negative regulation of TGFβ by decorin (Yamaguchi et al., 1990), as TGFβ has been shown to induce CSPG expression (Gato et al., 2002; Schachtrup et al., 2010; Schönherr et al., 1991). Minor et al. (Minor et al., 2008) also suggest that decorin directly affects neurons and promotes axonal growth on CSPGs and myelin. In addition, decorin treatment following stab wound to the cerebral cortex in rats decreases Sema3A protein within the lesion area (Minor et al., 2011). The recent report that Tregs promote remyelination is attributed to their production of the secreted ECM-associated signaling protein CCN3 (Dombrowski et al., 2017).

The role of thrombospondin, an ECM glycoprotein, in the context of remyelination has yet to be elucidated. While thrombospondin-1 has been shown to promote OPC migration in the normal CNS (Scott-Drew and ffrench-Constant, 1997), others report that knockout of thrombospondin-1 increases the differentiation of mature oligodendrocytes (Lu and Kipnis, 2010). Osteopontin, another glycoprotein, stimulates MBP synthesis and myelin sheath formation when applied to oligodendrocytes *in vitro*. In the same study, myelinating mixed cultures taken from osteopontin null mice contain less MBP than wild-type cultures (Selvaraju et al., 2004).

A growing body of evidence implicates tenascin C, commonly upregulated following CNS injury (Jones and Bouvier, 2014), as a negative regulator of myelinogenesis. Astrocytes plated on tenascin C have fewer myelinated fibers in myelinating cultures (Nash et al., 2011).

OPCs derived from tenascin C knockout mice exhibit accelerated rate of maturation and earlier morphological differentiation (Garwood et al., 2004). Moreover, oligodendrocytes plated on tenascin C fail to express MBP (Czopka et al., 2009).

1.6 Chondroitin Sulfate Proteoglycans (CSPGs)

CSPGs are a class of ECM proteoglycans that are widely expressed within the CNS; they can be synthesized by all neural cell types (Dyck and Karimi-Abdolrezaee, 2015) or brought into the CNS by infiltrating immune cells (Stephenson et al., 2018). CSPGs are comprised of a central core protein with varying number of associated glycosaminoglycan (GAG) chains bound to serine residues. A distinct tetrasaccharide linker mediates the joining of the core protein and GAGs. Chondroitin sulfate GAGs are formed from repeating disaccharides of N-acetylgalactosamine (GalNAc) and glucuronic acid (GlcA) (Prydz and Dalen, 2000). In addition, GAGs may also be further modified through sulfation at various carbon positions. Functions of CSPGs vary depending on core protein, number of chondroitin sulfate GAGs, and sulfation patterns of GAG chains (Sugahara et al., 2003; Galtrey and Fawcett, 2007). Members of the CSPG family are distinguished by their core proteins. Family members include the four lecticans (*i.e.*, aggrecan, brevican, neurocan, and versican), RPTP/phosphacan, and NG2 (Siebert et al., 2014).

During development, CSPGs provide guidance cues for axonal growth cones (Laabs et al., 2005), and, in late development are found in perineuronal nets surrounding neural soma (Carulli et al., 2006; Kwok et al., 2011; Lau et al., 2013; Wang & Fawcett, 2012). As developmental critical periods close, perineuronal nets envelop soma and proximal dendrites to limit synaptic plasticity and stabilize existing synapses (Bandtlow and Zimmermann, 2000; Rhodes and Fawcett, 2004; Dyck and Karimi-Abdolrezaee, 2015). In the adult CNS, the N-

terminal domains of lectican core proteins interact with hyaluronan through link proteins, and the C-terminal domain interacts with tenascins to form a structural network.

1.7 Role of CSPGs in MS

CSPGs are a substantial component of the fibrotic scar following several types of CNS injury, including traumatic injuries (Jones et al., 2003; Buss et al., 2009), ischemic insults (Huang et al., 2014; Deng et al., 2015), and MS. Several CSPG species are present at the border of active MS plaques, including versican, aggrecan, and neurocan (Sobel and Ahmed, 2001). Despite the ubiquity of CSPGs in CNS injury, the function of these CSPGs in the fibrotic scar is largely unclear. It is suggested that the CSPG/astrocytic scar acts as a barrier to further damage in the acute phase of injury. Importantly, preventing the acute deposition of CSPGs immediately following injury using xyloside results in a worsened injury. Therefore, the temporal kinetics of CSPG deposition should be further explored to guide the development of therapies.

Modulation of CSPGs such as by the use of proteases to remove the glycosaminoglycans of CSPGs have shown varying successes in the neuronal regeneration field (Barritt et al., 2006; Bradbury et al., 2002; Caggiano et al., 2005; Ikegami et al., 2005; Massey et al., 2006; Siebert et al., 2011; Yick et al., 2000) and the non-permissive effects have been identified in several animal models of demyelination and MS (discussed below). However, the direct impact of CSPGs in remyelination in MS remains largely unexplored.

1.8 CSPGs in Oligodendrocyte Biology

While substantial literature exists characterizing the non-permissive properties of CSPGs with regards to axonal regeneration, the effects of CSPGs on oligodendrocytes and OPCs are less well described. Recent studies have highlighted the suppression of myelinogenic processes by

CSPGs. *In vitro* studies show that CSPGs impede process outgrowth and differentiation of OPCs; most OPCs failed to extend processes at 1-2 days post-plating and lacked formation of membranous sheets at 6 days post-plating (Siebert and Osterhout, 2011). Similarly, there is data to suggest inhibitory effects of CS GAGs on oligodendrocyte differentiation (Karus et al., 2016). Pendleton et al. (Pendleton et al., 2013) have published congruent results showing inhibitory effects of CSPGs on oligodendrocyte growth. Our group has found impaired adhesion and process outgrowth when neonatal or adult mouse OPCs were plated on CSPGs. While the acquisition of oligodendrocyte maturity marker myelin basic protein (MBP) appeared to be unaffected by CSPGs, OPCs adhered poorly to a CSPG-coated substrate. These results were further corroborated by human OPCs plated on CSPGs, which also exhibited significantly reduced process outgrowth (Lau et al., 2012). Support for CSPGs being inhibitory for myelin repair *in vivo* comes from studies where the inhibition of CSPG synthesis and deposition after demyelination enhanced remyelination post injury (Lau et al., 2012; Keough et al., 2016).

Interestingly, NG2 is highly expressed on OPCs in homeostasis and upregulated in response to injury (Jones et al., 2002). Expression of NG2 is likely contributing to proliferation of the OPC pool following injury, as NG2 knockout in OPCs results in a reduced mitotic index and fewer mature oligodendrocytes following lysolecithin injection (Kucharova and Stallcup, 2015). This effect may be attributed to NG2 maintaining expression of PDGFRα on the cell surface, resulting in increased sensitivity to PDGF (Nishiyama et al., 1996). There is also evidence suggesting that CS-GAGs modified by different sulfation patterns will differentially affect regenerative processes. 4-sulfated CS is non-permissive while 6-sulfated CS is permissive to axon growth, but CS-E is also non-permissive (Brown et al., 2012; Lin et al., 2011; Wang et al., 2008). This may be an important distinction because most CSPGs deposited in the glial scar

after CNS injury is 4S-GAGs (Wang et al., 2008). Selective elimination of 4S-GAGs was shown to have therapeutic effects in mouse optic nerve crush 3 days after injury (Pearson et al., 2018). Together, these data may imply a need to consider temporal kinetics as well as composition of CSPGs in order to develop effective therapeutics.

There is currently limited knowledge on the mechanisms by which CSPGs inhibit oligodendrocyte differentiation and maturation. Several CSPG receptors have been identified on neurons, including the Nogo receptors 1 and 3 (Dickendesher et al., 2012), and CD44 (Kawashima et al., 2000). Interestingly these receptors do not appear to be highly expressed by oligodendrocyte precursor cells (Zhang et al., 2014).

A possible oligodendrocyte receptor for CSPGs may be the protein tyrosine phosphatase sigma (PTPσ). Cell cultures using oligodendrocytes derived from PTPσ$^{-/-}$ mice as well as knockdown of PTPσ *in vivo* show that the inhibitory effects of the CSPG aggrecan is ameliorated by loss of PTPσ signaling (Pendleton et al., 2013), although whether the same effect extends to other CSPG members remains to be elucidated. Others have utilized pharmacological inhibitors to perturb PTPσ signaling and demonstrated improved axonal regeneration as well as remyelination following spinal cord injury (Dyck et al., 2018; Luo et al., 2018). The downstream effects are hypothesized to be generated by activation of a pathway involving protein kinase C, the GTPase RhoA, and Rho-associated kinase (ROCK), culminating in the depolymerization of the actin cytoskeleton and altering polarity of the cell (Amano et al., 2010).

1.9 CSPGs and Inflammation

Aside from the growth-inhibitory effects of CSPGs on regenerative processes such as remyelination, we and others have also demonstrated that they promote a pro-inflammatory phenotype in macrophages and microglia (Figure 1.1). When presented with a purified CSPG

mixture, cultured bone marrow-derived macrophages (BMDMs) secrete cytokines such as colony stimulating factors, interleukins (IL) 1α, 1β, and 6, TNFα, several chemokines, and select MMPs, including MMP2, 3, 8, 9, and 12 (Stephenson et al., 2018). MMP2 and 9 have been previously reported to play key roles in leukocyte infiltration into the CNS (Agrawal et al., 2008). BMDMs will also migrate towards CSPGs (Kang et al., 2017; Stephenson et al., 2018), indicating chemoattractive properties of CSPGs.

CSPGs are thought to interact with macrophages through binding to cell surface receptors, including toll-like receptors (TLRs) 2, 6, and 4 (Kim et al., 2009; Zhang et al., 2015), CD44 (Kawashima et al., 2002; Rolls et al., 2008), β1 integrins, leukocyte-common antigen receptor (LAR), and PTPσ. MyD88 is recruited downstream of TLR signaling, resulting in the activation of NFκB. CSPGs have also been shown to signal through PTPσ expressed by microglia/macrophages in spinal cord injury, and blockade of this interaction decreased the presence of pro-inflammatory microglia/macrophages in the lesioned area (Dyck et al., 2018).

In addition to their interaction with other ECM components, highly-sulfated CSPGs also interact with and sequester cytokines and chemokines. Several CXC- and CC-family chemokines bind to CSPGs, including CXCL4, 8, 10, and 12, and CCL2, 5, 8, 11, 20, and 21 (Hirose et al., 2001; Hirose et al., 2002; Kawashima et al., 2002). Interferon-γ (IFNγ), IL-1α, IL-1β, 2, 4, 6, 7, 8, and 12 have also been shown to bind to CSPGs (Clarke et al., 1995; Fernandez-Botran et al., 1999; Frevert et al., 2003; Garnier et al., 2003; Hurt-Camejo et al., 1999; Lortat-Jacob et al., 1997; Ramsden & Rider, 1992). The cytokine/chemokine-CSPG interaction appears to be mediated by the highly negatively-charged GAGs. The binding of these cytokines and chemokines generates an immobilized gradient and facilitates the recruitment of immune cells to the site of inflammation.

Interestingly, previous studies in peripheral systems have indicated that CSPGs may be therapeutic rather than exacerbating inflammation. In combination with the pro-inflammatory stimulating agents IFNγ and lipopolysaccharide (LPS), addition of 6-sulfated CS-C GAGs reduced nitrite levels in culture supernatant, suppressed IL-6 and TNFα, and enhanced IL-10 production (Tan and Tabata, 2014). Treatment of diet-induced obese mice with a mixture of CS GAGs reduced levels of cytokines IL-1β and TNFα and chemokines CXCL1, CCL5, and CCL17 in serum, and fewer cultured macrophages stimulated with both TNFα and CS GAGs migrated in a transwell migration assay (Melgar-Lesmes et al., 2016). This effect appears to extend into the CNS compartment, as astrocyte activation induced by LPS was prevented by addition of CS GAGs (Cañas et al., 2010) and intravenous injection of CS disaccharides alleviated severity of EAE and experimental autoimmune uveitis (Rolls et al., 2006a). However, these studies were performed using CS GAGs or disaccharide subunits of CS GAGs, while our studies supporting a pro-inflammatory nature were done using a mixture of native CSPGs, suggesting there are key functional differences closely tied with structure of the molecule itself. This may be a consideration for future studies, as using proteases and chondroitinases has been a key approach to clearing CSPGs.

Further possible pro-regenerative roles of CSPGs have also been discussed. CSPGs enhance expression of insulin-like growth factor 1 (IGF1) and BDNF (Rolls et al., 2008) by microglia/macrophages, both of which function as trophic factors for OPCs (Hsieh et al., 2004; Van't Veer et al., 2009; VonDran et al., 2011). Furthermore, inhibition of CSPG synthesis immediately following spinal cord contusion injury attenuates IGF1 production and causes an increase in TNFα levels; the authors suggested there was a switch of microglia to an inflammatory phenotype in the absence of CSPGs (Rolls et al., 2008).

1.10 Biosynthesis of CSPGs

CSPG biosynthesis is initiated by the addition of a linkage tetrasaccharide to the core protein (Figure 1.2a). The tetrasaccharide is highly conserved across proteoglycan species, comprised of xylose, two galactose monomers, and glucuronic acid. The transfer of xylose from xylose-UDP to the core protein acceptor is catalyzed by xylosyltransferase 1 (XYLT1). The first and second galactoses are transferred from UDP-galactose to the proteoglycan by galactosyltransferase I (B4GalT7) and II (G3GalT6), respectively. Finally, glucuronosyltransferase I (B3GaT3) attaches the final monomer GlcA. The GAG is then produced by repeated addition of sugar units to the growing chain. The first sugar added to the linkage region determines whether the GAG will then become chondroitin sulfate, dermatan sulfate, heparan sulfate, or keratan sulfate. In the case of chondroitin sulfate, GalNAc will be added, with alternating GlcA.

The polymerization of GAG chains requires several transferase enzymes. Chondroitin sulfate GalNAc-transferase I (CSGalNAcT1) adds the initial GalNAc unit in a β1,4 manner. glucuronosyltransferase II (ChPF) will attach the following GlcA in a β1,3 linkage. Hereafter, alternating activity of chondroitin sulfate GalNAc-transferase II (CSGalNAcT2) and glucuronosyltransferase II will produce an elongated GAG chain (Prydz and Dalen, 2000).

CSPGs may also be modified following the polymerization of GAGs. Several sulfation patterns of CS-GAGs also contribute to function of the molecule. The modifications are carried out by four sulfotransferases: chondroitin sulfate 4-O-sulfotransferase, chondroitin 6-O-sulfotransferase, uronyl 2-O-sulfotransferase, and N-acetylgalactosamine 4-sulfate 6-O-sulfotransferase. CS-GAGs can thus be categorized based on sulfation pattern, where CS-A is 4-sulfated, CS-C is 6-sulfated, CS-D is 6 sulfated on GalNAc and 2-sulfated on GlcA, and CS-E

bears 4- and 6-sulfation on GalNAc (Dyck and Karimi-Abdolrezaee, 2015) (Figure 1.3). Different sulfation patterns have been proposed to serve different functions; for example, an increase in the ratio of 4-sulfation (CS-A) to 6-sulfation (CS-C) is reported to underlie the closing of the critical period for ocular dominance in the mouse visual cortex (Miyata et al., 2012). Evidence from multiple groups also suggest that CS-E plays a more significant role in the inhibition of neurons than CS-GAGs of other sulfation patterns (Karumbaiah et al., 2011; Brown et al., 2012).

1.11 Exostosin-like 2 (EXTL2)

Exostosin-like 2 (EXTL2) is a glycosyltransferase thought to be involved in CSPG and heparan sulfate proteoglycan (HSPG) biosynthesis. Mouse studies have revealed EXTL2 is developmentally expressed from embryonic day 7 and expression is maintained through development and adulthood. While ubiquitously expressed in tissues, EXTL2 is particularly abundant in the brain, heart, and testis (Nadanaka et al., 2013b).

EXTL2 exhibits both α1,4-N-acetylgalactosaminotransferase and α1,4-N-acetylglucosaminotransferase activities (Kitagawa et al., 1999). EXTL2 is through to catalyze the addition of the first N-acetylglucosamine (GlcNAc) or GalNAc onto the linkage region of the GAG chain (Figure 1.2b). Possession of both catalytic capabilities implies roles in both CS-GAG and heparan sulfate (HS) GAG synthesis. While normal CS-GAG synthesis is initiated by the addition of β1,4-GalNAc to the linkage region, *in vitro* data suggests the pentasaccharide product of EXTL2 (GlcNAcα1–4GlcAβ1–3Galβ1–3Galβ1–4Xylβ1-O-Ser) is unable to be utilized as an acceptor for further polymerization of the CS-GAG chain (Kitagawa et al., 1999), implying that EXTL2 may serve as a chain terminator and regulator of CSPG synthesis. Further studies show

that the enzymatic activity of EXTL2 is augmented by phosphorylation of the xylose residue of the linkage region by the xylose kinase FAM20B (Nadanaka et al., 2013b).

Loss of function studies in various models of pathology in peripheral systems utilizing EXTL2-null mice, discussed below, has given further clarification of the role of this enzyme. Although loss of EXTL2 produces profound phenotypes in mouse models of pathology and overproduce HS- and CS-GAGs (Nadanaka et al., 2013; Nadanaka et al., 2013b), normal EXTL2-null animals have no reported defects; mice develop normally and are fertile. However, mice lacking EXTL2 function produce greater amounts of CS-GAGs both homeostatically and in injury. In carbon tetrachloride-induced liver injury, CSPG production is higher in $EXTL2^{-/-}$ animals compared to wild-type, and fewer hepatocytes incorporate BrdU, indicating depressed proliferation of hepatocytes in response to injury and impaired recovery from liver damage (Nadanaka and Kitagawa, 2014). $EXTL2^{-/-}$ animals also exhibit increased calcification of the aorta in a mouse model of chronic kidney disease. The authors report that $EXTL2^{-/-}$ vascular smooth muscle cells (VSMCs) cultured in high phosphate conditions similarly exhibit increased calcification compared to wild-type VSMCs; however, treatment with either chondroitinase or heparitinase significantly reduced calcium deposition (Purnomo et al., 2013).

As EXTL2 also possesses α1,4-N-acetylglucosaminotransferase activity, a process required for the initiation and elongation of heparan sulfate GAGs, loss of EXTL2 has also been explored in the context of HSPGs. siRNA and RNA interference strategies have been employed in the context of Sanfilippo C syndrome and other mucopolysaccharidoses, for which knockdown of EXTL2 activity in fibroblasts derived from patients inhibited HSPG production (Canals et al., 2015; Kaidonis et al., 2009). However, siRNA knockdown of EXTL2 in a human embryonic kidney cell line resulted in increased heparan sulfate GAG chain length (Katta et al.,

2015). Thus, the question of whether EXTL2 augments or inhibits HSPG production has yet to be definitively answered. Due to the limited literature existing on EXTL2 and its functions, more work is required to fully elucidate the role of EXTL2 in both CSPG and HSPG biology.

A genome-wide association study of MS patients by Dr. Stephen Sawcer group (University of Cambridge, during sabbatical in Calgary) in collaboration with the Yong lab found single nucleotide polymorphisms enriched in MS versus control DNA in a region of chromosome 1 containing EXTL2 (unpublished). Presently, there is limited knowledge on how dysfunction of EXTL2 in the CNS may affect CSPG biosynthesis in the context of CNS injury, and whether it may be a viable target for future MS therapies. CSPGs are difficult to remove from the lesion microenvironment once deposited, and while targeted therapies exist, such as chondroitinase ABC, delivery of the treatment is another challenge. Chondroitinase ABC has seen varying success within the traumatic spinal cord injury field (Barritt et al., 2006; Bradbury et al., 2002; Caggiano et al., 2005; James et al., 2015; Massey et al., 2006; Starkey et al., 2012; Yick et al., 2000), however it requires focal yet sustained delivery into the tissue, which would be difficult for chronic, diffuse lesions such as MS. Understanding the regulatory mechanisms of CSPG biosynthesis may provide more insight into how CSPG deposition in response to injury may be limited.

1.12 The Lysolecithin Model of Murine Demyelination

The lysolecithin model of toxic demyelination involves stereotactic injection of lysophosphatidylcholine (LPC; lysolecithin) into white matter tracts of the CNS, such as the corpus callosum and dorsal or ventral funiculi of the spinal cord (Keough et al., 2015). While the mechanism of myelin disruption is still unclear, there is evidence to suggest that lysolecithin non-specifically disrupts lipids (Plemel et al., 2018).

This model is ideally suited to investigate remyelination and inflammation as the temporal progression of the injury is well-defined. There is an observable disruption of myelin, as determined by staining of hyperintense MBP^+ debris at 4 hours post-injection. OPCs and oligodendrocytes density is decreased acutely as well, with lowest densities at 24 hours. Astrogliosis, defined by an increase in GFAP intensity within the lesion, occurs by 72 hours (Plemel et al., 2018). A significant area of demyelination is identifiable by 7 to 10 days. Substantial remyelination of the lesion is visible by 21 to 28 days (Figure 1.4). Another advantage of the lysolecithin model is the spatial confinement of the injury, allowing efficient identification of the injured area and subsequent analysis.

CSPGs have been shown to be deposited within the demyelinated lesion using immunofluorescent staining of chondroitin sulfate glycosaminoglycan (CS-GAG) side chains (Lau et al., 2012). Previous work from our lab indicates that among the lectican CSPG members, the versican V1 isoform is most significantly upregulated following injection of lysolecithin, with minimal changes in aggrecan (Keough et al., 2016), neurocan, and brevican (unpublished observations). The distribution of versican V1 in the lysolecithin model is closely correlated with astrocyte reactivity; however, other likely sources include reactive microglia or infiltrating macrophages (Lau et al., 2012). The clearance of CSPGs from the lesion is also correlated to the remyelination period.

Lysolecithin injection has been utilized previously to extensively investigate several aspects of remyelination. This includes stereotactic injection into the corpus callosum, another area of high myelin density (Sahel et al., 2015; Warford et al., 2018), the dorsal spinal column (Garay et al., 2011; Kucharova et al., 2011), and the cerebellum (Woodruff and Franklin, 1999). The injection of lysolecithin is also not limited to mice; other model species such as cats

(Blakemore et al., 1977), and rats (Kotter et al., 2001). Lysolecithin has also been applied to *in vitro* models to induce demyelination (Miron et al., 2010).

Other models of demyelination have also been used to study the biology of remyelination. The cuprizone model, in which cuprizone is supplied through chow over several weeks, induces demyelination seen in the corpus callosum. Remyelination will occur with the withdrawal of cuprizone in chow (Kipp et al., 2009). Compared to the lysolecithin model, the cuprizone model requires a more protracted time period of demyelination. In contrast, stereotactic injection of inflammatory agents such as TNFα and IFNγ (Merkler et al., 2006) induces a similar focal demyelinating lesion, however the peripheral immune response is more reflective of MS pathology, and involves T and B cells, as opposed to the limited engagement of T cells in lysolecithin-induced demyelination (Ousman and David, 2000).

1.13 Gaps of Knowledge

Although loss of EXTL2 has been associated with deficient repair in peripheral systems such as the liver, EXTL2 and its role in CNS injury has yet to be described. Whether loss of EXTL2 results in an increase in CSPG, particularly versican, deposition into a demyelinated lesion in the CNS has not been previously determined. Furthermore, the effects of more extensive CSPG deposition on the progression of injury, particularly with respect to inflammation and remyelination, need to be clarified. Literature on how CSPGs influence neuroinflammation remains limited.

1.14 Statement of Aims

To investigate the role of EXTL2 in CSPG production and subsequent effects on remyelination and neuroinflammation during an acute demyelinating injury, studies will be conducted along the following aims:

1. Characterize the lysolecithin-induced lesion at days 7, 14, and 21 post-injection with respect to CSPG deposition and cellular representation.
2. Elucidate potential differences in inflammation and remyelination in EXTL2 null mice compared to wild-type mice.
3. Determine potential mechanisms by which loss of EXTL2 affects inflammation or the repair process.

1.15 Hypothesis

I hypothesize that a loss of function of the Extl2 gene will result in overproduction and increased deposition of CSPGs into the ECM of a lysolecithin-induced demyelinating injury. This would inhibit OPC differentiation, migration, and maturation into oligodendrocytes to a greater extent than in wild-type animals, and will also exacerbate the microglial/macrophage response, ultimately impairing remyelination.

Signaling Molecule	Effect	References
Growth factors		
EGF	+	Aguirre et al., 2007; Palazuelos et al., 2015
FGF	+	Murtie et al., 2005
PDGF	+	Allamargot et al., 2001
BDNF	+	Miyamoto et al., 2015
NGF	+	Takano et al., 2000
IGF	+	Pang et al., 2007
Cytokines		
TNFa	+	Arnett et al., 2001
C5	+	Weerth et al., 2003
IL-1b	+	Mason et al., 2001
IFNg	-	Agresti et al., 1996
Endocrine factors		
Estrogen	+	Itoh et al., 2016; Khalaj et al., 2013; Kumar et al., 2013
Progesterone	+	Ibanez et al., 2004
Prolactin	+	Gregg et al., 2007
T3	+	Remaud et al., 2017
2-AG	+	Feliu et al., 2017
Cell surface molecules		
Neuregulins	+	Kataria et al., 2018; Lundgaard et al., 2013; Viehover et al., 2001
GPCRs	+	Mei et al., 2016; Mogha et al., 2016
Nogo	-	Sozmen et al., 2016
LINGO-1	-	Mi et al., 2009; Mi et al., 2013; Sun et al., 2015
Neurotransmitter		
Glutamate	+	
Other		
Shh	+	Ferent et al., 2013; Sanchez & Armstrong, 2017
	-	Wang et al., 2008
Notch	-	Hammond et al., 2014; John et al., 2002; Jurynczyk et al., 2005
mAChR	+	Cui et al., 2006; De Angelis et al., 2016; Mei et al., 2016
	-	Welliver et al., 2018
TLRs	-	Srivastava et al., 2018
KOR	+	Mei et al., 2016

Table 1.1 **Summary of currently known factors influencing remyelination following CNS injury.** EGF = endothelial growth factor; FGF = fibroblast growth factor; PDGF = platelet-derived growth factor; BDNF = brain-derived neurotrophic factor; NGF = nerve growth factor; IGF = insulin-like growth factor; TNFα = tumor necrosis factor alpha; IFNγ = interferon gamma; T3 = triiodothyronine; 2-AG = 2-arachidonoylglycerol; GPCRs = G-protein coupled receptors; LINGO-1 = leucine rich repeat and immunoglobulin-like domain-containing protein 1; Shh = sonic hedgehog; mAChR = muscarinic acetylcholine receptor; TLRs = toll-like receptors; KOR = kappa opioid receptor

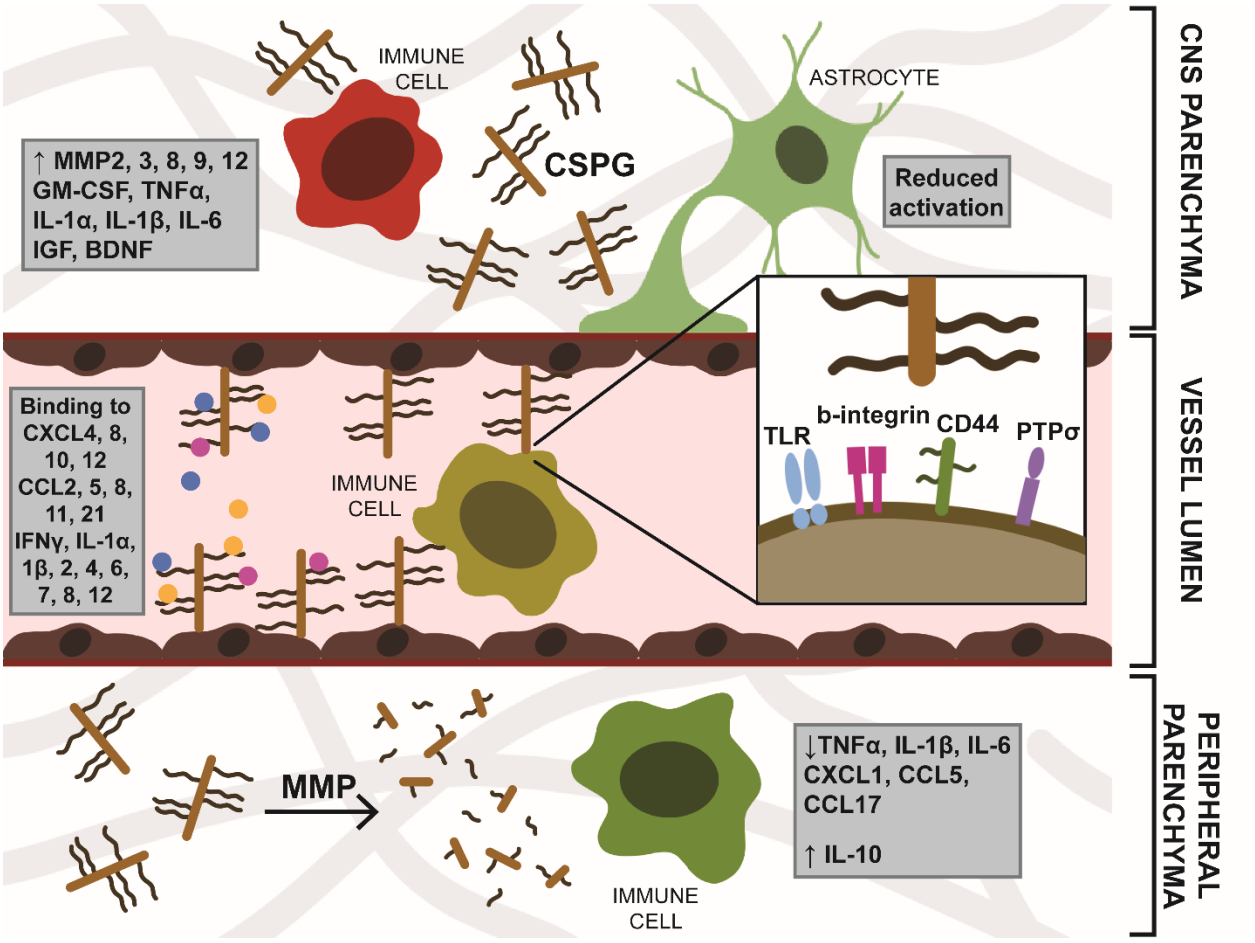

IMMUNE CELL
ASTROCYTE
CSPG
↑ MMP2, 3, 8, 9, 12
GM-CSF, TNFα,
IL-1α, IL-1β, IL-6
IGF, BDNF
Reduced activation
CNS PARENCHYMA
VESSEL LUMEN
PERIPHERAL PARENCHYMA
Binding to CXCL4, 8, 10, 12 CCL2, 5, 8, 11, 21 IFNγ, IL-1α, 1β, 2, 4, 6, 7, 8, 12
IMMUNE CELL
TLR
b-integrin
CD44
PTPσ
MMP
↓TNFα, IL-1β, IL-6
CXCL1, CCL5, CCL17
↑ IL-10
IMMUNE CELL

Figure 1.1

Figure 1.1 **CSPGs are implicated in peripheral and CNS inflammation**. Within the lumen of blood vessels, CSPGs expressed by endothelial cells bind to cytokines and chemokines, and interact with various receptors on the surface of immune cells. In peripheral systems, CS disaccharides may promote an anti-inflammatory phenotype in macrophages, whereas in the CNS parenchyma, intact CSPG promotes the production of several pro-inflammatory factors. Contrastingly, CSPGs in the CNS may also reduce activation of astrocytes.

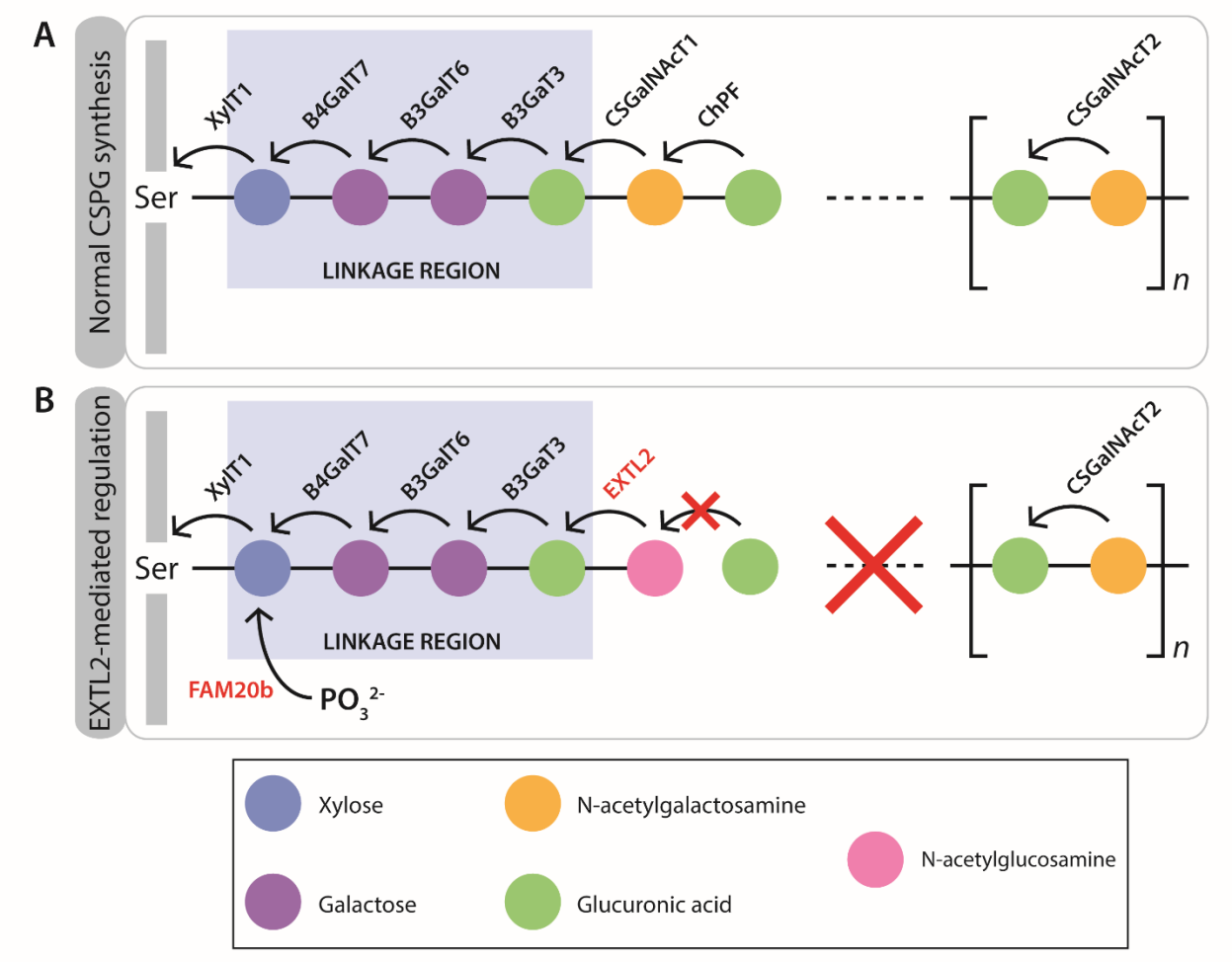

A
Normal CSPG synthesis
XylT1
B4GalT7
B3GalT6
B3GaT3
CSGalNAcT1
ChPF
CSGalNAcT2
Ser
LINKAGE REGION
n
B
EXTL2-mediated regulation
XylT1
B4GalT7
B3GalT6
B3GaT3
EXTL2
CSGalNAcT2
Ser
LINKAGE REGION
FAM20b
PO3 2-
n
Xylose
N-acetylgalactosamine
N-acetylglucosamine
Galactose
Glucuronic acid

Figure 1.2

Figure 1.2 **Normal biosynthesis of CSPGs and EXTL2-mediated downregulation of CSPG synthesis.** The initiation and elongation of GAG chains from the CSPG core protein requires the sequential actions of several enzymes (A). EXTL2 transfers a glucuronic acid onto the linkage region through a β1,4 linkage, which is thought to prevent subsequent elongation of the GAG chain (B). XylT1 = xylosyltransferase 1; B4GalT7 = β-1,4-galactosyltransferase 7; B3GalT6 = β-1,3-galactosyltransferase 6; B3GaT3 = β-1,3-glucuronyltransferase 3; CSGalNAcT1 = chondroitin sulfate N-acetylgalactosaminyltransferase 1; ChPF = chondroitin polymerizing factor; CSGalNAcT2 = chondroitin sulfate N-acetylgalactosaminyltransferase 2.

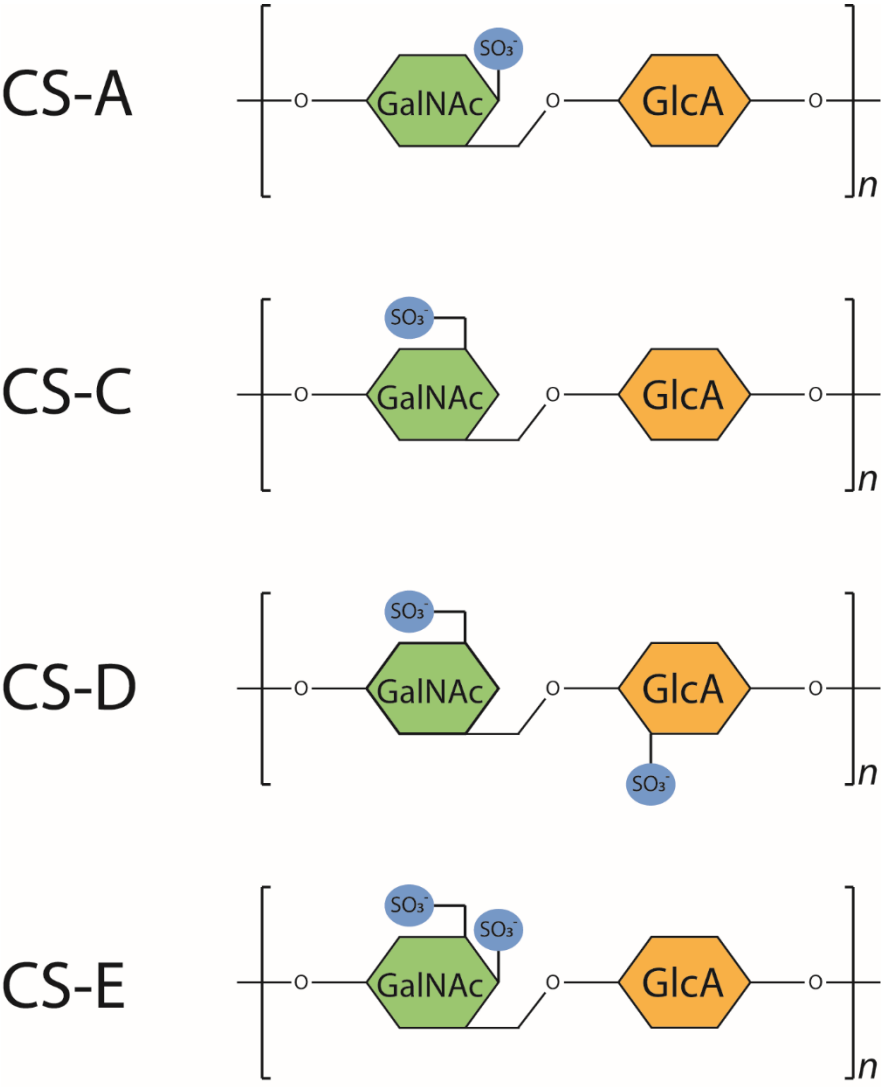

CS-A
SO3-
GalNAc
O
GlcA
O
O
n
CS-C
SO3-
GalNAc
O
GlcA
O
O
n
CS-D
SO3-
GalNAc
O
GlcA
O
O
SO3-
n
CS-E
SO3-
SO3-
GalNAc
O
GlcA
O
O
n

Figure 1.3

Figure 1.3 **Types of chondroitin sulfate glycosaminoglycans and associated sulfation patterns.** CS-A is 4-sulfated on the N-acetylgalactosamine, CS-C is 6-sulfated on the N-acetylgalactosamine, CS-D is both 4-sulfated on N-acetylgalactosamine and 6-sulfated on glucuronic acid, CS-E is doubly sulfated on both C4 and C6 on N-acetylgalactosamine.

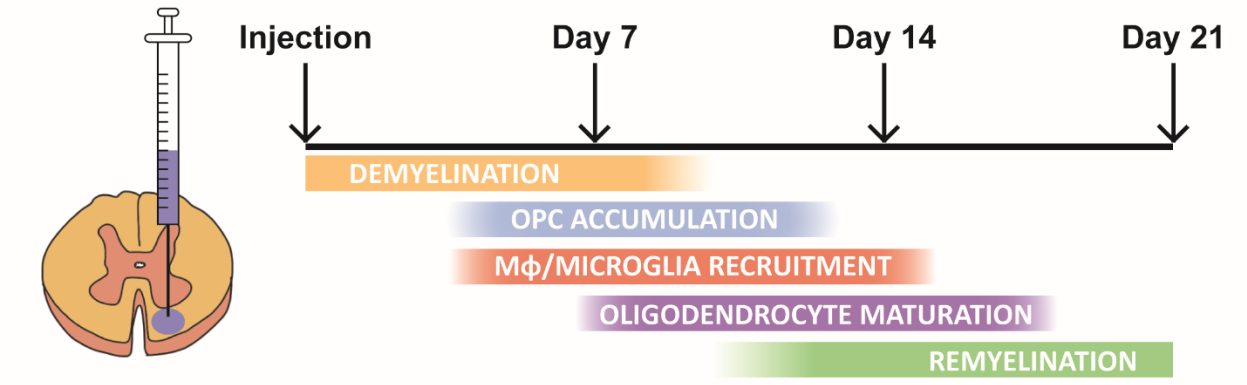

Injection
Day 7
Day 14
Day 21
DEMYELINATION
OPC ACCUMULATION
MΦ/MICROGLIA RECRUITMENT
OLIGODENDROCYTE MATURATION
REMYELINATION

Figure 1.4

Figure 1.4 **The lysolecithin model of demyelination.** Lysolecithin is injected stereotactically into the ventral white matter between T3 and T4. Both OPC and immune (microglia/macrophage) recruitment can be observed as early as day 3, and OPC maturation and differentiation occurs later and can be seen at day 7 and on. Subsequent remyelination occurs from 2-3 weeks following injury. The microglia/macrophage response is eventually resolved.

CHAPTER 2 – The glycosyltransferase EXTL2 regulates CSPG synthesis and neuroinflammation in a murine model of demyelination

2.1 Abstract

Chondroitin sulfate proteoglycans (CSPGs) have been identified to be a potent inhibitor of both axonal growth and myelin generation. More recently, they have also been highlighted as a modulator of macrophage infiltration into the central nervous system in experimental autoimmune encephalomyelitis, and they play key roles in modulation of inflammation in the periphery. In this study we show that CSPGs also modulate the state of neuroinflammation in an acutely demyelinated injury in the ventral spinal cord of mice. Loss of the glycosyltransferase exostosin like 2 (EXTL2), an enzyme that normally suppresses CSPG biosynthesis, results in excessive deposition of CSPGs in the injured area. EXTL2$^{-/-}$ mice also exhibit exacerbated axonal damage and myelin disruption compared to wildtype mice. More microglia/macrophages are recruited to the lesion site and inflammation is protracted compared to that in wild-type mice. In tissue culture, activated bone marrow derived macrophages from EXTL2$^{-/-}$ mice overproduce tumor necrosis factor α (TNFα) and matrix metalloproteinases (MMPs), and are more phagocytic. Furthermore, activated EXTL2$^{-/-}$ macrophages are more detrimental to neurons but not oligodendrocytes. These results support the view that CSPGs accumulating in lesions hamper recovery and an emerging role for CSPGs as a modulator of neuroinflammation.

2.2 Introduction

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) with focal areas of demyelination induced by aberrant immune activation. Associated with demyelination and inflammation are a multitude of changes in the composition of the CNS extracellular matrix (ECM) (reviewed in Burnside & Bradbury, 2014; Pu et al., 2018). Previous reports have highlighted the deposition of chondroitin sulfate proteoglycans (CSPGs) at the borders of chronic active MS lesions (Sobel and Ahmed, 2001). CSPGs are a class of large, glycosylated proteins present both on cell surfaces and in the extracellular matrix. Lecticans, a subset of ECM CSPGs, homeostatically comprise a significant portion of perineuronal nets as well as the neural interstitial matrix, serving roles in development, plasticity, and neuronal function (Lau et al., 2013; Suttkus et al., 2014). Lectican CSPG members are versicans, aggrecan, neurocan, and brevican. In demyelinating injury, they are locally upregulated by several cell types and deposited near the injury site (Asher et al., 2000, 2002; Beggah et al., 2005; Hallmann et al., 2015; Jones et al., 2003; Jones et al., 2002; Properzi et al., 2005). CSPGs are potent inhibitors for regenerative processes such as axonal regeneration and remyelination (Deng et al., 2015; Karimi-Abdolrezaee et al., 2012; Siebert & Osterhout, 2011; Wang et al., 2008). Following an ischemic insult in perinatal rats, chondroitinase ABC (ChABC)-mediated CSPG digestion improved remyelination and improved cognitive recovery (Deng et al., 2015). ChABC treatment in models of spinal cord injury (SCI) yielded improvements in remyelination and promoted axon sprouting (Barritt et al., 2006; Siebert & Osterhout, 2011; Siebert et al., 2011). Removal of CSPGs by locally applied ChABC has also improved functional recovery in a rat SCI model (Bradbury et al., 2002). Perturbing CSPG signaling through antagonism of receptors leukocyte-common antigen related (LAR) and protein tyrosine phosphatase σ also

increased the population of alternatively-activated macrophages following SCI (Dyck et al., 2018).

CSPGs have been recently identified by our group as a novel regulator of neuroinflammation in a mouse model of MS. In particular, the V1 isoform of versican (in which the core protein consists only of the GAGβ segment) is particularly abundant in inflammatory CNS lesions, and a mixture of lecticans stimulates bone marrow-derived macrophages to produce inflammatory cytokines and proteases (Stephenson et al., 2018). Others have reported that the genetic loss of versican in lung macrophages attenuates their pro-inflammatory response to polyinosine-polycytidylic acid (Kang et al., 2017).

As CSPGs potently influence reparative processes in the CNS, it is important to explore approaches of overcoming this impediment to regeneration. The biosynthetic process of CSPGs has largely been elucidated (Prydz and Dalen, 2000), and a number of pharmacological inhibitors of CSPG production have been examined for therapeutic effects, such as xylosides and fluorinated analogues that inhibit activity of CSPG biosynthesis enzymes (Lau et al., 2012; Keough et al., 2016). In addition, other inhibitors of CSPG signaling, proteases such as MMPs and ADAMTSs able to cleave CSPGs, and chondroitinase ABC, a bacteria-derived enzyme able to cleave the sugar chains from the core proteins of CSPGs have been explored in models of traumatic CNS injury (Bradbury et al., 2002; Barritt et al., 2006; Massey et al., 2006; Siebert et al., 2011; Karimi-Abdolrezaee et al., 2012; Tauchi et al., 2012; Dyck et al., 2018). However, finding an appropriate approach to removing deposited CSPGs in diffuse or heterogeneous pathology is difficult, and the mechanisms regulating CSPG production are not well studied. Understanding the regulatory mechanisms of CSPG biosynthesis may provide more insight into how CSPG deposition in response to injury may be limited. Exostosin like 2 (EXTL2) is a bi-

functional glycosyltransferase thought to limit the production of CSPGs by forming an unusable substrate for synthesis enzymes (Kitagawa et al., 1999). In collaboration with Dr. Stephen Sawcer, a genome-wide association study of MS patients found an enrichment of single nucleotide polymorphisms in a gene region containing EXTL2 (unpublished data). Thus, we asked whether investigating the biology of EXTL2 and its effects on CSPG deposition and repair following injury could provide insight to endogenous targets for limiting CSPG production.

In this study we used mice with constitutive knockout of EXTL2 ($EXTL2^{-/-}$) in the lysolecithin model of acute demyelination (Keough et al., 2015) to investigate the effects of dysregulation of CSPG production *in vivo*. We show increased CSPG deposition and persistence following lysolecithin-induced injury in $EXTL2^{-/-}$ mice. Interestingly, $EXTL2^{-/-}$ mice exhibit an exacerbated injury and increased neuroinflammation. We therefore propose that elevated CSPG deposition in the lesion may contribute to the recruitment and activation of microglia/macrophages that lead to injury.

2.3 Methods

2.3.1 Animals

All experiments were performed in accordance with the ethical guidelines of the Animal Care Committee at the University of Calgary. Female wild-type C57Bl/6 mice aged 6 to 8 weeks were purchased from Charles River and *Extl2*$^{-/-}$ mice were originally obtained from Dr. Kitagawa, Kobe Pharmaceutical University (Kitagawa et al., 1999; Nadanaka et al., 2013b) and bred at the University of Calgary Animal Health Unit. The Extl2-null mice were generated by truncating exon 3 and removal of exon 4 of the *Extl2* gene (Nadanaka et al., 2013b). All experiments were completed on female mice aged 8 to 12 weeks.

2.3.2 Lysolecithin-induced demyelination

Experimental demyelination was produced in the ventral spinal cord by stereotactic injection of the toxin lysolecithin. A 10 μL glass Hamilton syringe with a 34-gauge needle was pre-loaded with a 1% solution of lysolecithin. Animals were anesthetized with a mixture of ketamine/xylazine (100 mg/kg and 10 mg/kg, respectively) administered through an intraperitoneal injection. The analgesic buprenorphine (0.05 mg/kg) was injected subcutaneously prior to surgery. Ophthalmic lubricant was applied to the eyes to prevent eyes from drying during surgery. A small area on the back was shaved with a razor and the surgical field was disinfected with 70% ethanol and iodine solution. A midline incision was made, and underlying muscle and adipose tissue separated using retractors. Connective tissue between the T3 and T4 vertebra was cut to reveal the spinal cord. The dura mater was opened using a 30-gauge needle. The tip of the Hamilton 34-gauge needle was placed at the surface of the spinal cord, lateral to midline. A baseline measurement was made, and 1.3 mm was subtracted from the baseline measurement to obtain the final desired measurement for targeting the ventral white matter. The needle was depressed and injection of lysolecithin was completed with one rotation of the micromanipulator every 5 seconds for 2 minutes, for a final volume of 0.5 μL. Animals were sutured and placed in a recovery chamber and monitored until awake. Post-operative buprenorphine was administered at 0.05 mg/kg 16 hours following surgery. Sham operations were not performed as no abnormal pathology is present in saline-injected animals (Keough et al., 2016).

2.3.3 Tissue harvesting and processing

Animals were sacrificed at days 7, 14, and 21 days post-injection of lysolecithin. Mice were given a lethal dose of ketamine/xylazine anesthetic. Animals were perfused through the left ventricle of the heart with 15 mL of phosphate-buffered saline (PBS) for Western blot analysis,

or 15 mL of 4% paraformaldehyde (PFA) in addition to PBS for immunohistochemistry. The cervical and upper thoracic section of the spinal cord was harvested. Spinal cords were laid on strips of thick card stock to maintain structural integrity. For Western blot, spinal cords were immediately flash frozen in liquid nitrogen and stored at -80^{o}C until use. For immunohistochemistry, spinal cords were post-fixed overnight in 4% PFA at 4^{o}C, then transferred into 30% sucrose solution for 72 hours. Spinal cords were then frozen into O.C.T. blocks using dry ice and blocks were stored at -20^{o}C until sectioning on the cryostat.

2.3.4 Immunohistochemistry

Spinal cords frozen in OCT blocks were cut into 20 μm-thick sections and mounted on glass slides. Slides containing tissue sections were stored at -20^{o}C until use. Staining of tissue sections was done in a humidity chamber to prevent evaporation. All washes were completed using PBS containing 0.25% Tween-20. Blocking of tissue sections was done using horse serum blocking solution (0.01 M PBS, 10% horse serum, 1% bovine serum albumin (BSA), 0.1% cold fish skin gelatin, 0.1% Triton-X100, and 0.05% Tween-20) for 2 hours at room temperature. Blocking solution was removed prior to application of primary antibodies. Primary antibodies were diluted in 0.01 M PBS containing 1% BSA, 0.1% cold fish skin gelatin, and 0.5% Triton-X100. Primary antibodies were left on sections overnight at room temperature. Secondary antibodies were diluted in the same dilution buffer as primary antibodies and left for 2 hours at room temperature. Slides were mounted with Fluoromount G® (SouthernBiotech). Fluorescence imaging was done using the Leica TCS SP8 confocal laser scanning microscope.

2.3.5 Chondroitinase ABC digestion

Chondroitinase ABC (ChABC) digestion was done for immunohistochemical staining of V0/V1, as the removal of the GAG chains facilitate the binding of antibody to the core protein.

Slides were thawed to room temperature and washed once with PBS. ChABC was diluted in PBS and applied to slides at a concentration of 0.2 units/mL. Slides were incubated in a heated chamber at 37°C for 30 minutes. Slides were washed with PBS before proceeding with staining.

2.3.6 BMDM isolation and culture

C57Bl/6 wild-type or EXTL2$^{-/-}$ female mice were euthanized by overdose of ketamine/xylazine. Femurs and tibia were dissected from the body and soaked briefly in ethanol. Bone marrow was harvested from bones and centrifuged at 1200 rpm for 10 minutes. Cells were re-suspended in PBS and counted. Cells were centrifuged again at 1200 rpm for 10 minutes. BMDMs were cultured at 37°C, 8.5% CO_2 in 100 mm plastic, uncoated dishes in Dulbecco's modified eagle's medium (DMEM) containing 10% FBS, 1% Penicillin-Streptomycin (Gibco), 1% sodium pyruvate (Gibco), 1% GlutaMAX™ (Gibco), and 10% L929 conditioned media containing mouse macrophage-colony stimulating factor. Cells were cultured for 8-10 days before harvesting. Media was removed from plates and cold PBS was applied to the cells. Cell scrapers were used to dissociate cells from dishes. Cells were re-plated at a density of 50,000 cells/100 μL/well in a 96-well plate in the same media, except lacking L929 conditioned media. Plates were incubated at 37°C in 5% CO_2. A media switch to 1% FBS in DMEM with supplements was performed at 24 hours post-plating, and treatments were added to wells in 1% FBS in DMEM.

2.3.7 Enzyme-linked Immunosorbent Assay

Supernatant from BMDM cultures (as described above) was harvested at 24 hours following the initiation of stimulation. Supernatant was frozen at -80°C until use. Samples were thawed to room temperature and ELISA was performed according to manufacturer instructions (Invitrogen).

2.3.8 Mouse MMP 5-plex Luminex Assay

BMDM supernatant was harvested at 24 hours following the initiation of stimulation. Supernatant was frozen at -80^{o}C until the assay was performed. Supernatant was thawed at room temperature, and 50 µL of supernatant was transferred into 0.7 mL Eppendorf tubes. Tubes were stored on ice until used. Samples were processed by Eve Technologies (Calgary) for a panel of 5 MMP members.

2.3.9 Phagocytosis assay

pH-sensitive pHRodo® Red dye conjugated to *S. aureus* particles (Invitrogen) was added in combination with NucBlue® (Hoechst 33342, Invitrogen) to live cultured wild-type and EXTL2$^{-/-}$ BMDMs at manufacturer-specified concentrations. Cells were incubated in Live Cell Imaging Solution (Invitrogen) for the duration of the live imaging (45 minutes). Images were taken on the ImageXpress Micro XLS Widefield High-Content Analysis System every 15 minutes. Cells were kept in 37^{o}C incubators at 5% CO_2 between imaging sessions.

2.3.10 OPC isolation and harvest

P0-2 CD-1 mouse pups were sacrificed, and brains were isolated. Under a dissection microscope, meninges and choroid plexus tissue were removed from the cortical and ventricular surfaces, respectively. Cortices were dissected away from the hippocampus and cerebellum and cut into 2-3 mm^2 pieces. A cocktail of papain, DNase, and L-cysteine was used for tissue digestion. The digestion cocktail was added to tissue in a 50 mL tube and the tube was placed in a 37^{o}C water bath for 30 minutes, with occasional inversion. More DNase was added if tissue became gel-like. Cells were centrifuged at 1200 rpm for 10 minutes and re-suspended in growth medium. Dissociated cells were plated in T-75 culture flasks pre-coated with poly-L-lysine (10 µg/mL, Sigma). The mixed glial culture was maintained in DMEM containing 10% FBS, 1%

GlutaMAX™, 1% sodium pyruvate, and 1% Penicillin-Streptomycin. Flasks were kept at 37ºC, 8.5% CO_2 for 9 days. Half media changes were performed every 3 days. On day 9, flasks were shaken overnight in a 37ºC, 5% CO_2 incubator at 220 rpm to dislodge OPCs and microglia from astrocytes. The supernatant containing OPCs and microglia was plated on 100 mm plastic, uncoated 100 mm dishes for 30 minutes to allow microglia to adhere. Media containing enriched OPCs was harvested and plated in oligodendrocyte differentiating media (described below) at 5,000-10,000 cells/100 µL/well in a 96-well plate pre-coated with poly-L-lysine and other extracellular matrix molecules when appropriate.

Oligodendrocyte differentiating medium was DMEM containing 2% (v/v) B27 supplement (Gibco), 1% (v/v) oligodendrocyte supplement cocktail (see below), 1% (v/v) GlutaMAX™, 100 µM sodium pyruvate, 1% (v/v) Penicillin-Streptomycin (Gibco), 50 µg/mL holo-transferrin (Sigma), 5 µg/mL N-acetyl-L-cysteine (Sigma), 50 ng/mL ciliary neurotrophic factor (PeProTech), and 0.005% (v/v) Trace Elements B (Corning).

Oligodendrocyte supplement cocktail was made from 100 mL DMEM with 1% BSA, 0.06% progesterone (Sigma), 16.1% putrescine (Sigma), 0.005% sodium selenite (Sigma), 0.4% 3,3',5-triiodo-L-thyronine (Sigma).

2.3.11 Cell staining and imaging

All washing was done using PBS. Plates were placed on an orbital shaker during all incubations.

For OPCs, blocking was done using 10% normal goat serum (NGS) in PBS or Odyssey® Blocking Buffer (Licor) for 1 hour at room temperature. Primary antibody against O4 (Millipore) was diluted in blocking buffer and incubated for 2 hours at room temperature or overnight at

4°C. An anti-mouse IgM antibody conjugated to Alexa Fluor® 488 (Jackson ImmunoResearch) as well as nuclear yellow was added in blocking buffer for 1 hour at room temperature. Plates were washed with PBS and imaged using the ImageXpress Micro XLS Widefield High-Content Analysis System (Molecular Devices).

For BMDMs, for detection of intracellular molecules, cells were permeabilized using 0.025% Triton-X100 for 5 minutes at room temperature. No permeabilization was required for surface molecules. Blocking was done using Odyssey® Blocking Buffer (Licor) for 30 minutes at room temperature. Primary antibodies were diluted in the same blocking buffer incubated overnight at 4°C. Secondary antibodies were diluted in the same blocking buffer and incubated for 1 hour at room temperature. Nuclear yellow was added in PBS and incubated for 10 minutes. Plates were washed with PBS and imaged using the ImageXpress Micro XLS Widefield High-Content Analysis System.

2.3.12 Image analysis

For immunohistology, images were processed and analyzed using ImageJ. All images were blinded prior to analysis. Analyses were performed on single z-plane images of 20 µm tissue sections near the lesion epicenter. For percent of lesion area analyses, lesion area was demarcated using MBP staining. A disruption in MBP density was determined to be lesional. The lesion area was isolated and area of signal of interest was determined using differential thresholding. The area occupied by the signal was normalized to total area of the lesion or represented as an absolute area. For axonal counts in the lesion, the lesion area was demarcated using MBP. Differential thresholding was used to isolate individual axons present inside the lesional area and was automatically counted. The number of axons within the lesion was normalized to total lesion area to obtain density. For oligodendrocyte-lineage cell counting and

scoring, the same approach to demarcating the lesion area was used. Images containing $Olig2^{+}$ nuclei were overlaid with either PDGFRα staining or CC1 staining. Co-localization of either PDGFRα or CC1 with Olig2 was determined to be an OPC or mature oligodendrocyte, respectively.

For immunofluorescence images: images were analyzed on MetaeXpress High-Content Image Acquisition and Analysis Software (Molecular Devices). Appropriate thresholds were determined for true signals. OPC cultures were analyzed using the Neurite Outgrowth program, and BMDM cultures were analyzed using the Cell Scoring program.

2.3.13 Statistics

All statistical analysis was performed with GraphPad Prism 8.0. Student's t-test, ordinary one-way ANOVA, and two-way ANOVA statistical tests were used with an alpha of 0.05.

2.4 Results

2.4.1 Increased deposition and persistence of the CSPG member versican V1 and CS-A glycosaminoglycan following injury in the EXTL2$^{-/-}$ spinal cord

Based on previous literature suggesting that EXTL2 is a negative regulator of CSPG synthesis (Nadanaka et al., 2013b) (see Chapter 1), we first asked whether loss of EXTL2 results in increased CSPG deposition into an injured area. We induced lysolecithin lesions in the ventral columns of spinal cords of wild-type and EXTL2$^{-/-}$ animals and evaluated versican V1 deposition into the lesion at 14 and 21 days post-lesion (dpl). Eriochrome cyanine staining shows a demarcated area with distinctive loss of myelin in the lesioned area (Figure 2.1a), corresponding to an increase in V1 immunoreactivity (Figure 2.1b). In both wild-type and EXTL2$^{-/-}$ spinal cords, upregulation of V1 was limited to the lesion site (Figure 2.1c). By 14 dpl, there was a

greater accumulation of V1 in EXTL2$^{-/-}$ lesions compared to wild-type (Figure 2.1d). Interestingly, by day 21 V1 was largely cleared from the wild-type lesion, whereas it remained in the lesion of EXTL2$^{-/-}$ animals (Figure 2.1e).

2.4.2 EXTL2$^{-/-}$ mice exhibit no overt differences in OPC repopulation and maturation following injury

We next asked whether the extensive deposition and persistence of CSPGs in the EXTL2$^{-/-}$ animals impacted the remyelination process. As there is extensive literature on the inhibitory nature of CSPGs on OPCs, we expected a deficiency or delay in remyelination in the EXTL2$^{-/-}$ mice. In the spinal cord lysolecithin model, oligodendrocyte progenitor cells (OPCs) begin repopulation of the lesion 3-4 days following injury, with subsequent accumulation of OPCs and eventual differentiation into mature oligodendrocytes and remyelination (Keough et al., 2015). We analyzed spinal cord sections stained for the transcription factor Olig2 that defines cells of the oligodendrocyte lineage, the surface receptor PDGFRα that is selective to OPCs, and APC (adenomatous polyposis coli, also called CC1, labeling mature oligodendrocytes) to determine the number of oligodendrocyte-lineage cells present within the lesion at days 14 and 21 post-injury (Figure 2.2a,e). The density of oligodendrocytes in the lesions was calculated by isolating the lesion area and counting the number of Olig2^{+} nuclei and normalizing counts to the respective lesion area. Percentage of OPCs or mature oligodendrocytes were calculated using the total number of Olig2^{+} nuclei. Unexpectedly, we found no significant differences in the density of Olig2^{+} oligodendrocyte-lineage cells within the lesion (Figure 2.2b,f), nor in proportions of Olig2^{+}PDGFRα^{+} OPCs (Figure 2.2c,g) and Olig2^{+}APC^{+} oligodendrocytes (Figure 2.2d,h) at any of the assessed time points. No deficiencies in OPC recruitment to the lesion, nor maturation into oligodendrocytes, was apparent.

2.4.3 Exacerbated lesion area and decreased axonal density in EXTL2$^{-/-}$ lesions

In our characterization of the EXTL2$^{-/-}$ phenotype following injury, we found an exacerbated injury area demarcated by loss of MBP immunoreactivity (Figure 2.3a). Analysis of the total area of MBP disruption in both wild-type and EXTL2$^{-/-}$ lesioned spinal cords showed a larger area of demyelination in EXTL2$^{-/-}$ spinal cords at day 14 (Figure 2.3b) and a similar but non-statistically significant trend at day 21 (Figure 2.3c) following injection of lysolecithin compared to those of wild-type animals. We then hypothesized that there should be a corresponding loss of axons in the demyelinated area. To identify axons, we used an antibody against the 200 kD neurofilament (NF200), which reliably labels axons in the ventral column (Figure 2.4a). Qualitatively, there was close correlation between the area of axonal loss to area of demyelination (Figure 2.4a). Although total axon counts within wild-type and EXTL2$^{-/-}$ lesions were not significantly different, at day 14 we observed a decreased axonal density in the EXTL2$^{-/-}$ lesions, corroborating our finding of a larger area of demyelination (Figure 2.4b,c). Overall, there is more severe damage to in the EXTL2$^{-/-}$ spinal cord with injection of lysolecithin.

2.4.4 Increased representation of microglia and macrophages within lesions of EXTL2$^{-/-}$ animals

Given our previous report of CSPGs being a stimulator of macrophages in EAE (Stephenson et al., 2018) and our previous finding of worsened injury in the EXTL2$^{-/-}$ spinal cord, we investigated whether there were correlating pro-inflammatory phenotypes in lesional microglia/macrophages in EXTL2$^{-/-}$ animals. Macrophages and microglia are labeled by Iba1 (ionized calcium-binding adaptor molecule 1) (Figure 2.5a). We determined that there was a greater area of lesion covered by Iba1 immunoreactivity at day 14 (Figure 2.5b), indicating an increase in macrophages/microglia density within the lesion, in the EXTL2$^{-/-}$ animals. Macrophages/microglia persisted for longer in the EXTL2$^{-/-}$ lesion; more Iba1 immunoreactivity

was still observed at day 21 (Figure 2.5c), although the origin of these cells have not been determined in this study.

2.4.5 No differences in the surface marker expression of CD16/32 and CD206 in lesional Iba1$^+$ cells

To further resolve whether differences in polarization exist in the Iba1$^+$ cells in the wild-type and EXTL2$^{-/-}$ lesions, we probed tissue sections using immunofluorescence staining for M1-like pro-inflammatory and M2-like anti-inflammatory surface markers, CD16/32 and CD206 (Kigerl et al., 2009), respectively (Figure 2.7a,d); the pro-inflammatory enzyme inducible nitric oxide synthase (iNOS) was attempted but did not result in reliable staining. We assessed the mean fluorescence intensity of each of the markers within the lesion, internally normalized to the background fluorescence (Figure 2.6). Mean fluorescence intensity was assessed within the area of positive immunoreactivity. For both CD16/32 and CD206, intensity was not significantly different between wild-type and EXTL2$^{-/-}$ lesions (Figure 2.7b,c,e,f), indicating that expression levels of these receptors are similar and no profound alterations in microglia/macrophage phenotype in EXTL2$^{-/-}$ animals.

2.4.6 EXTL2$^{-/-}$ BMDMs secrete more TNFα and differentially regulate MMPs in response to CSPG stimulation in vitro

Due to limitations of assessing the activity of Iba1$^+$ cells in the lesion using fixed tissue sections, we instead used cultured murine-derived BMDMs *in vitro* to examine functional or phenotypic differences between wild-type and EXTL2$^{-/-}$ macrophages upon stimulation. As peripherally-derived macrophages constitute a significant portion of immune cells present in the lysolecithin-induced lesion (unpublished observations from the Yong lab), we reasoned that BMDMs are an appropriate population of cells to study as a model for macrophages in the

lesion. We hypothesized that excessive deposition and prolonged persistence of CSPGs in EXTL2$^{-/-}$ animals underlies the protracted neuroinflammatory response, which consequently contributes to exacerbated injury. BMDMs were harvested from both wild-type C56Bl/6 and EXTL2$^{-/-}$ mice and stimulated with a commercial CSPG mixture for 24 hours (Figure 2.8). Due to a lack of readily available versican V1, we instead used this commercial mixture that contains versican. Cultured BMDMs were stained for the pro-inflammatory marker inducible nitric oxide synthase (iNOS) (Figure 2.9a), and number of cells with enhanced iNOS expression was quantified. While unstimulated wild-type and EXTL2$^{-/-}$ BMDMs both expressed iNOS at similar frequencies, CSPG stimulation resulted in a marked increase in iNOS^{+} EXTL2$^{-/-}$ BMDMs (Figure 2.9b).

Multiplex analysis to detect the presence of secreted MMP members was performed on supernatant harvested from stimulated wild-type and EXTL2$^{-/-}$ BMDMs. We found that EXTL2$^{-/-}$ BMDMs secreted more MMP2 and 8 relative to wild-type BMDMs (Figure 2.10a,b). Upon stimulation with CSPGs, only pro-MMP9 levels were increased in wild-type BMDMs (Figure 2.10d). No significant differences in MMP3 secretion were detected in any group (Figure 2.10c). Unexpectedly, wild-type BMDMs decreased secretion of MMP12 in response to CSPG stimulation, and EXTL2$^{-/-}$ BMDMs exhibited a baseline decrease in MMP12 production and did not alter MMP12 production with stimulation (Figure 2.9e). Absolute quantities of assessed MMPs in each group are presented in Appendix A. We also selected TNFα, an inflammatory cytokine and characterized neurotoxic factor (Chen et al., 2012), to be a readout of macrophage activation. Assessment of the BMDM conditioned media using enzyme-linked immunosorbent assay (ELISA) analysis revealed markedly increased amounts of TNFα in the supernatant of

EXTL2$^{-/-}$ BMDMs compared to wild-type BMDM supernatant in response to CSPG stimulation (Figure 2.10f).

2.4.7 EXTL2-/- BMDMs are more phagocytic and exhibit a larger capacity to phagocytose following CSPG stimulation

Phagocytic activity is a key feature of microglia and macrophages following demyelinating injury; debris clearance is essential in facilitating repair. To further characterise the activity of EXTL2$^{-/-}$ BMDMs in comparison to wild-type BMDMs, we conducted an *in vitro* phagocytosis assay using bacteria particle-conjugated pHRodo™. Fluorescence at 585 nm is seen when particles are engulfed by cells and the local environment becomes acidic. Calcein AM and Nuclear Blue were used to label the cytosol and nuclei of cells, respectively. Cells were stimulated with CSPGs for 24 hours prior to the application of the pHRodo conjugate. Phagocytosis was assessed at 15 minutes after addition of the pHRodo (Figure 2.11a). At baseline, we observed an increase in the number of phagocytic EXTL2$^{-/-}$ BMDMs compared to wild-type. Interestingly, this effect was further augmented in the CSPG-stimulated cells (Figure 2.11b).

2.4.8 Conditioned media from EXTL2$^{-/-}$ BMDMs are more detrimental to cultured neurons

Given that in the presence of CSPGs, EXTL2$^{-/-}$ BMDMS exhibited a more pro-inflammatory phenotype in culture, we next asked whether the pro-inflammatory nature of these macrophages may underlie the exacerbated damage observed in EXTL2$^{-/-}$ animals following lysolecithin injection. To exclude the possible contribution of contact-mediated cell death, we harvested conditioned media from cultured wild-type and EXTL2$^{-/-}$ BMDMs stimulated with CSPGs. Conditioned media was applied directly to established primary neuron cultures in a 1:1 ratio with neuron culture media. Cells were treated for 24 hours and analyzed (Figure 2.12).

MAP2 expression and morphological characterization of neurite outgrowth were used as indicators of neuronal survival and health. When BMDMs are not stimulated by CSPGs, the conditioned media does not cause loss of $MAP2^+$ cells in culture (Figure 2.13a). However, conditioned media taken from CSPG-stimulated $EXTL2^{-/-}$ BMDMs were more detrimental to cultured neurons, decreasing absolute number of $MAP2^+$ cells per field (Figure 2.13a).

2.4.9 Conditioned media from $EXTL2^{-/-}$ BMDMs affect cultured OPCs in a similar way as wild-type BMDM conditioned media

To determine whether the same conditioned media that we previously showed to be detrimental to the survival of neurons *in vitro* exhibited similar effects in cultured OPCs, we used primary OPC cultures derived from CD-1 neonatal mice. OPCs were collected from mixed glial cultures and conditioned media was immediately applied at the time of seeding in a 1:1 ratio of OPC media (see Methods) and conditioned media (Figure 2.14). Cells were allowed to grow for 24 hours before fixing and assessment of process outgrowth using the oligodendrocyte marker O4 (Figure 2.15a). Surprisingly, $EXTL2^{-/-}$ and wild-type BMDM conditioned media had the same effect on OPC growth. The conditioned media of both wild-type and $EXTL2^{-/-}$ BMDMs stimulated with CSPGs similarly impaired outgrowth, while conditioned media harvested from unstimulated BMDMs did not seem to have any inhibitory effects (Figure 2.15b). Interestingly, this supports our previous observations that repopulation of the lesion by OPCs and subsequent maturation into APC-expressing oligodendrocytes was not impaired in $EXTL2^{-/-}$ mice.

2.5 Discussion

In the present study we show that dysregulation of CSPG production following CNS injury profoundly impacts the inflammatory state of a demyelinated lesion. Loss of the enzyme EXTL2, a regulatory mechanism serving to limit CSPG synthesis, resulted in more extensive

CSPG deposition into a lysolecithin-induced demyelinated injury, a larger area of injury with an associated decrease in axonal density within the lesion, and greater accumulation of macrophages and microglia. To our knowledge, this is the first study to explore the role of EXTL2 within the CNS.

Although we report here that several aspects of microglia/macrophage activation and function are altered with the loss of EXTL2, there are several questions that remain unanswered. While we observed greater CSPG production and deposition into the lesion, the cellular sources of these CSPGs have yet to be confirmed. Multiple cell types are known to produce CSPGs, including astrocytes, OPCs, neurons, and microglia/macrophages. As our mouse line is a global and constitutive knockout of EXTL2, it would be of value to determine the contribution of various cell types through using conditional knockouts. Using inducible OPC, macrophage/microglia, or astrocyte-specific knockout models of EXTL2 would facilitate the investigation of whether EXTL2 and CSPG signaling confer different roles in different cell populations. Tamoxifen-inducible PDGFRα-driven Cre recombinase would excise EXTL2 from OPCs (O'Rourke et al., 2016), whereas CX3CR1-driven Cre labels macrophages and microglia. GFAP-driven Cre has been previously characterized to be largely astrocyte-specific.

The stark difference between wild-type and EXTL2$^{-/-}$ lesions with respect to the amount of versican present in the lesion at day 21 was an intriguing finding and necessitates asking the question of whether CSPGs in the EXTL2$^{-/-}$ lesions at day 21 are a result of protracted active synthesis, or a failure to be removed from the lesion in a timely manner. A genetic or polymerase chain reaction approach may be taken to assess whether CSPG production levels are maintained later in the progression of the lesion.

In this study we have not quantified the enzymatic activity of EXTL2. Investigating whether an increase in EXTL2 expression or its activity occur following injury may provide further insight into the regulation of CSPG synthesis. Macrophages are a major source of CSPGs (Chang et al., 2017), and loss of EXTL2 within macrophages may cause excess synthesis of CSPGs, which then may contribute to the augmentation of inflammation. Given that CSPGs, particularly versican, have a known role in the modulation of inflammation (Kim et al., 2009; Kang et al., 2017), the question of whether CSPGs deposited by microglia/macrophages locally can act in an autocrine or paracrine fashion to further promote inflammation has yet to be answered.

Unexpectedly, we did not find any deficiency in the extent of OPC repopulation of the lesion in the EXTL2$^{-/-}$ animals compared to wild-type. This is in stark contrast to several lines of evidence supporting the inhibitory nature of CSPGs to remyelination and would warrant further investigation in future studies. It is important to note that EXTL2 is not specific to versican synthesis, and that versican was the focus of this work because of its pronounced upregulation following injury in the ventral column, whereas other lectican members are not (Keough et al., 2016). NG2, a non-lectican member of the CSPG family, is highly expressed by OPCs and upregulated upon injury. There is evidence to suggest that NG2 plays a key role in the injury response of OPCs, where loss of NG2 in OPCs reduces their mitotic index and delays remyelination (Kucharova and Stallcup, 2015). Loss of EXTL2 in OPCs may similarly affect NG2 expression, thus counter-balancing the inhibitory effects of lesional versican. In addition, analysis of the rate of remyelination through a high resolution technique such as electron microscopy may reveal differences not seen by the number of oligodendrocyte-lineage cells

within the lesion, as the presence of oligodendrocytes does not necessarily indicate the production of myelin.

Although the formation of the glial scar, largely contributed to by CSPGs, is thought to be protective in limiting the area of injury, we found a significantly greater area of demyelination in the EXTL2$^{-/-}$ mice. This observation can likely be attributed to the overall more pro-inflammatory milieu generated by excess CSPG deposition, TNFα, and MMPs by EXTL2$^{-/-}$ BMDMs. This altered microenvironment may underlie both exacerbated demyelination and axonal damage. Future work should characterize whether the pro-inflammatory factors released by EXTL2$^{-/-}$ BMDMs *in vitro* are similarly elevated in the spinal cord lesion. Previous work from others have shown that the disruption of CSPG deposition in the acute phase of injury exacerbates injury and impedes remyelination and resolution of inflammation (Rolls et al., 2008). The time points we selected for this work do not necessarily reflect the acute injury phase (1-3 dpl), and future studies may focus on early time points to determine whether disruption of glial scar formation occurs in EXTL2$^{-/-}$ mice. However, a report of CSPG members in active MS lesions suggests a less clear role of CSPGs. In chronic active lesions, CSPGs are seen at the borders of the lesion, however foamy macrophages were present in the lesion core with punctate staining of CSPGs associated with the cells (Sobel and Ahmed, 2001). CSPGs, at an earlier time, may have been present uniformly throughout the lesion and are being degraded and endocytosed by macrophages from the lesion center. This, supported by our previous study in EAE (Stephenson et al., 2018), implies that CSPGs may be exacerbating inflammation rather than serving a neuroprotective role.

Existing literature on EXTL2 function remains limited, and gaps in knowledge remain on whether EXTL2 serves functions aside from its role in downregulation of CSPG synthesis. Our

cell culture studies on BMDMs suggest that loss of EXTL2 function in macrophages enhances the pro-inflammatory response with CSPG stimulation, implying a possible interaction with immune-related pathways. This possibility has yet to be explored and would be of value for future studies to examine. The reduction in MMP12 secretion by wild-type BMDMs and baseline decrease in MMP12 in the EXTL2$^{-/-}$ BMDMs was surprising. There is currently conflicting evidence on whether MMP12 is protective or detrimental in CNS inflammation (Wells et al., 2003; DaSilva and Yong, 2009), where MMP12 may be protective in experimental autoimmune encephalomyelitis but detrimental in spinal cord injury in mice, although whether these contrasting data can be attributed to differing models is unknown.

Our study investigated the role of CSPG-cell interactions and its effect on neuroinflammation, however effects independent from the direct interaction of CSPGs with cell surface receptors have not been explored. CSPGs are known to bind and sequester chemokines, cytokines, and growth factors (see Chapter 1). The effect of a chemical gradient or reservoir formed by CSPGs in the lesion likely plays a significant role in the pathogenesis of the inflammatory lesion and is possibly contributing to the exacerbation of inflammation in our EXTL2$^{-/-}$ animals. This study also did not address the closely related group of ECM, the heparan sulfate proteoglycans (HSPGs). Due to the N-acetylglucosaminyltransferase activity of EXTL2, it has been proposed that EXTL2 also plays a role in HSPG synthesis, where it is thought to be a promoter of HSPG production rather than a regulator. There is some evidence to suggest that knockdown of EXTL2 may reduce HSPG synthesis (Canals et al., 2015; Kaidonis et al., 2009). HSPGs are largely regarded as a growth-permissive ECM member. Whether HSPGs are downregulated as a result of EXTL2 knockout was not determined, and consequently, what impact reduced HSPG production may have on inflammation and repair.

Our work and others have shown that approaches to reducing CSPGs may be key in promoting regeneration in the CNS. One such approach is the use of chondroitinase ABC (ChABC), which is a bacteria-derived enzyme that cleaves glycosaminoglycan chains of CSPGs. ChABC has previously been used in SCI and white matter ischemia models, where treated animals exhibited histological and functional improvements over untreated animals (Bradbury et al., 2002; Siebert et al., 2011; Deng et al., 2015). Proteases able to cleave the core protein of CSPGs such as matrix metalloproteinases have also been explored as an option, although *in vitro* work has shown them to be neurotoxic. Pharmacological agents that reduce biosynthesis of CSPGs have been proposed as an alternative to CSPG-cleaving enzymes. For example, xylosides and fluorosamine, a fluorinated analogue of N-acetylglucosamine, are thought to inhibit the production of CSPGs, and improve remyelination (Lau et al., 2012; Keough et al., 2016). Protamine is a heparan neutralizing drug that was also found to block CSPG-mediated inhibition on OPC growth in culture (Kuboyama et al., 2017).

Overall, it can be concluded that this data supports previous reports that EXTL2 is a negative regulator of CSPG biosynthesis. We have shown that the loss of EXTL2 results in the excess production of CSPGs in response to demyelination, which consequently results in worsened inflammation. This study provides the first evidence for the role of EXTL2 in regulating the responses to CNS demyelination and identifies a novel target for future studies investigating CSPGs and their role in CNS injury.

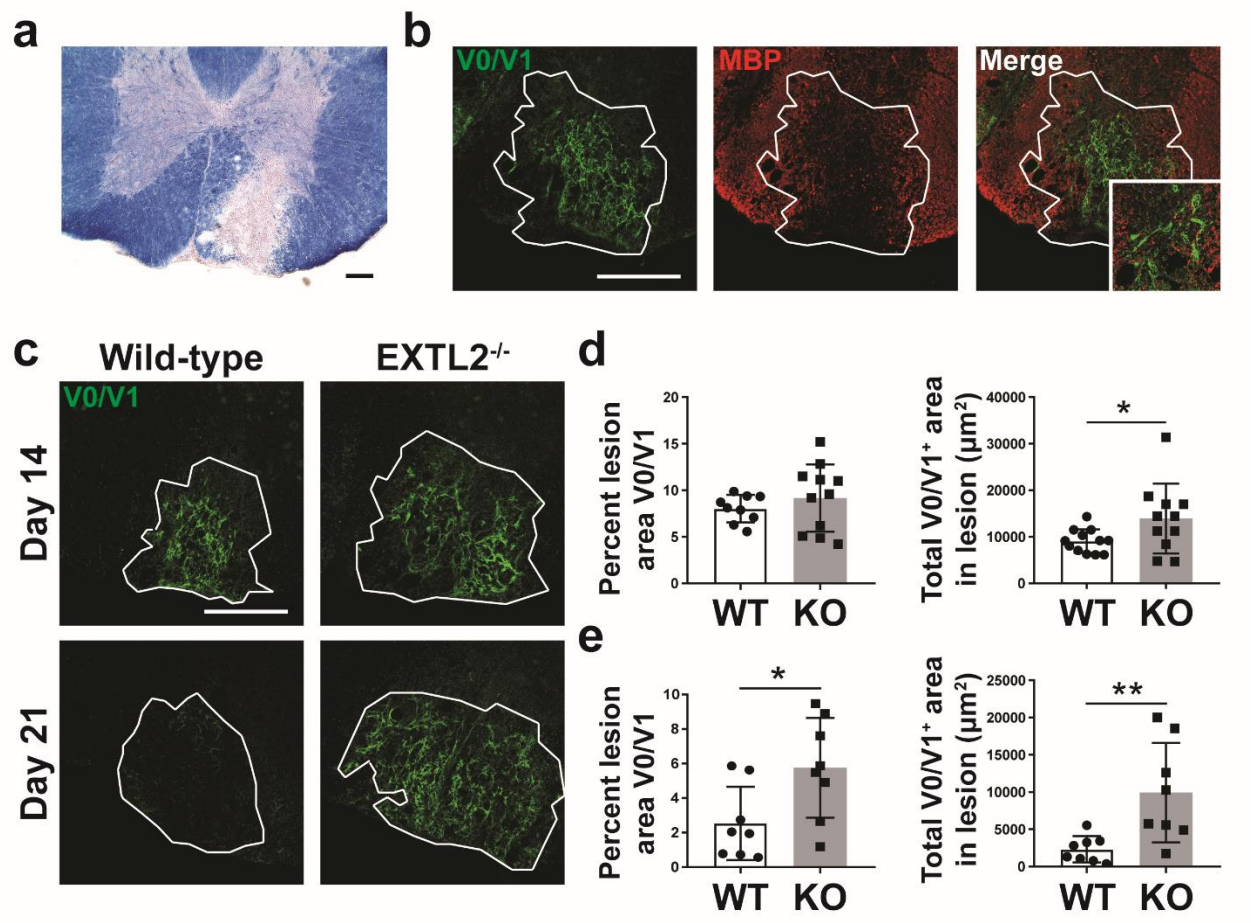

a
b
V0/V1
MBP
Merge
c
Wild-type
EXTL2-/-
V0/V1
Day 14
Day 21
d
Percent lesion area V0/V1
Total V0/V1+ area in lesion (µm2)
WT
KO
*
e
Percent lesion area V0/V1
Total V0/V1+ area in lesion (µm2)
WT
KO
*
**

Figure 2.1

Figure 2.1 **Immunolabeling of versican V0/V1 in wild-type and EXTL2$^{-/-}$ lesions.** (**a**) Representative lesion from wild-type day 14 animal stained with Eriochrome Cyanine R (scale bar = 100 μm) and (**b**) immunofluorescence staining for versican V0/V1, MBP, and merged image. Scale bar on immunofluorescent image = 200 μm. Inset shows high magnification image of versican and MBP. (**c**) Representative images of versican V0/V1 in wild-type and EXTL2$^{-/-}$ lesions at 14 and 21 dpl. Yellow outline indicates approximate lesion area. Scale bar = 200 μm. Quantification of versican area within the lesions, in terms of percent area of the whole lesion determined separately by MBP staining, and in terms of absolute area within the isolated lesion are shown in (**d**) for day 14 and (**e**) for day 21. Two-tailed, unpaired Student's t-test. * $p < 0.05$, ** $p < 0.01$

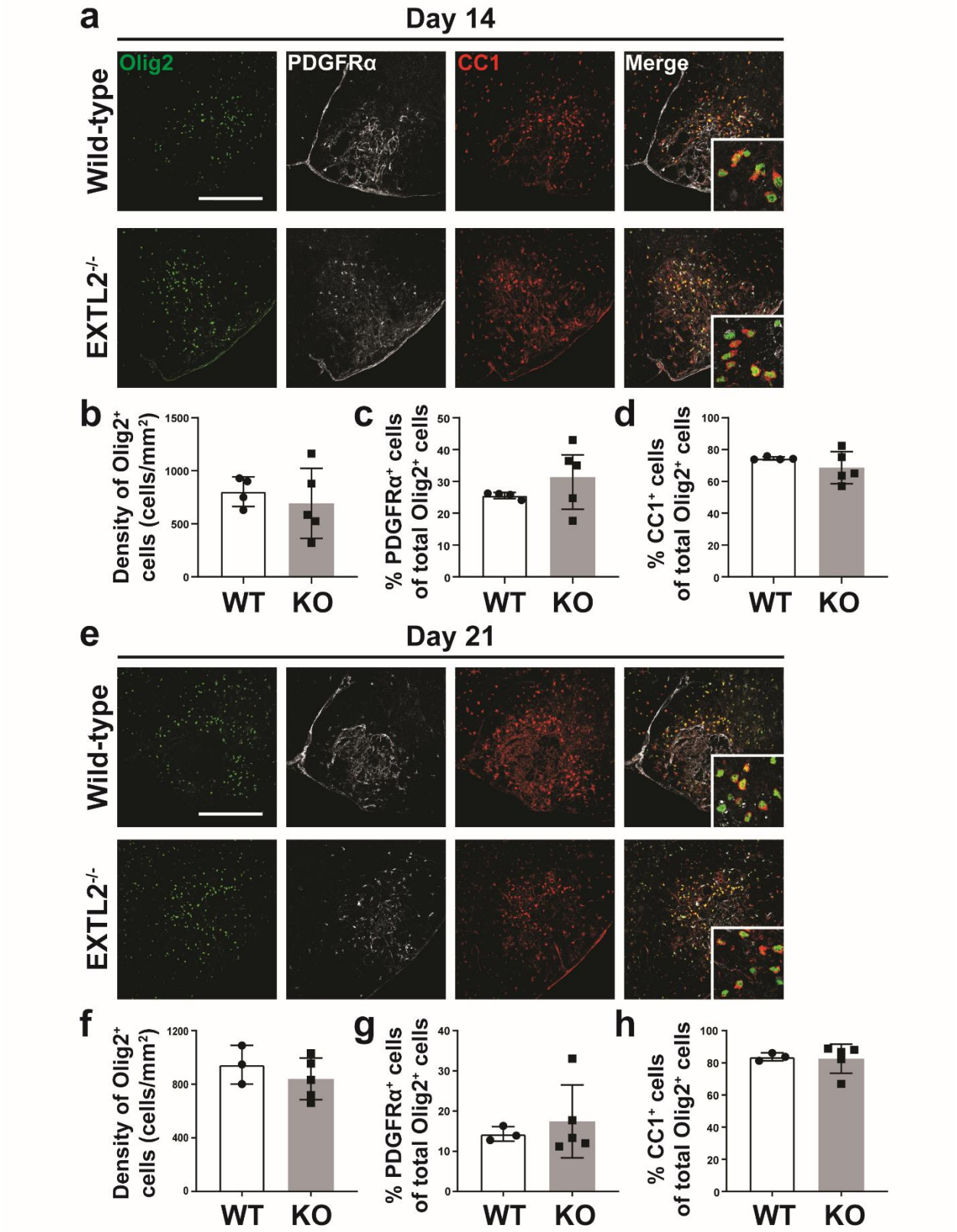

a
Day 14
Olig2
PDGFRα
CC1
Merge
Wild-type
EXTL2-/-
b
Density of Olig2+ cells (cells/mm2)
1500
1000
500
0
WT
KO
c
% PDGFRα+ cells of total Olig2+ cells
50
40
30
20
10
0
WT
KO
d
% CC1+ cells of total Olig2+ cells
100
80
60
40
20
0
WT
KO
e
Day 21
Wild-type
EXTL2-/-
f
Density of Olig2+ cells (cells/mm2)
1200
800
400
0
WT
KO
g
% PDGFRα+ cells of total Olig2+ cells
40
30
20
10
0
WT
KO
h
% CC1+ cells of total Olig2+ cells
100
80
60
40
20
0
WT
KO

Figure 2.2

Figure 2.2 **Immunostaining and quantification of oligodendrocyte lineage cells following injury.** Representative images of lesions labeled with Olig2, PDGFRα, and APC (CC1) are shown in (**a**) and (**e**), for days 14 and 21, respectively. Scale bar = 200 μm. Insets are high magnification images of individual $Olig2^+$ nuclei co-labeled with PDGFRα or APC. Density of all $Olig2^+$ nuclei are quantified for day 14 in (**b**) and day 21 in (**f**). Proportion of $Olig2^+PDGFR\alpha^+$ OPCs at both day 14 and 21, calculated as a fraction of all $Olig2^+$ cells, are shown in (**c**) and (**g**); (**c**) shows day 14 and (**g**) shows day 21 analyses. Two-tailed, unpaired Student's t-test.

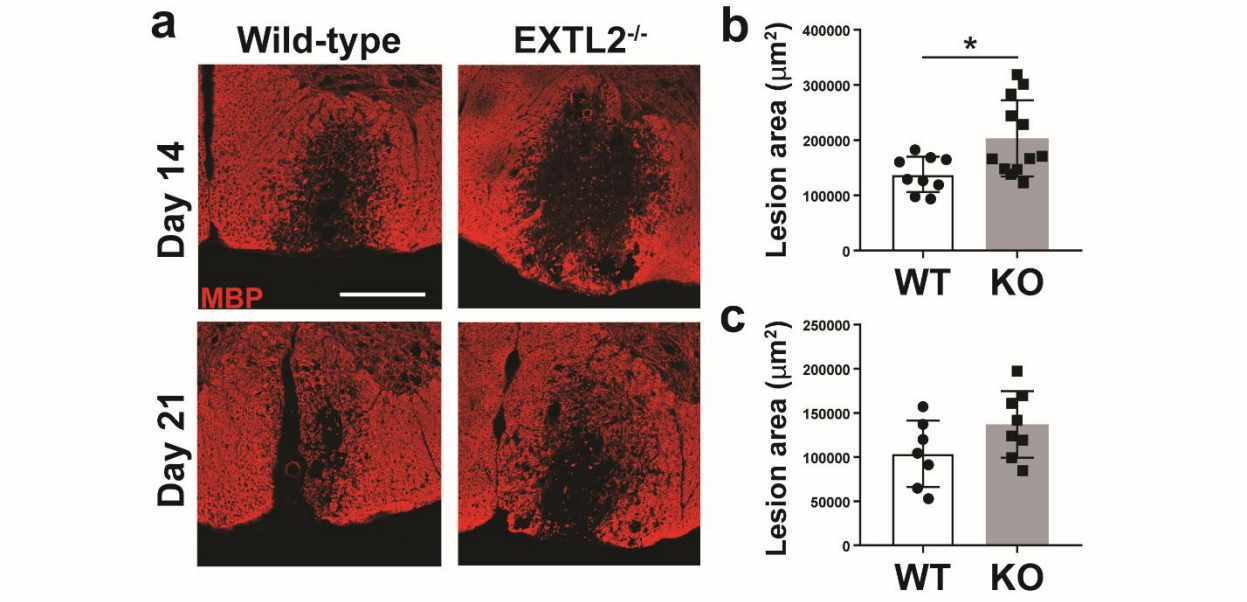

a
Wild-type
EXTL2-/-
Day 14
Day 21
MBP
b
Lesion area (μm²)
400000
300000
200000
100000
0
*
WT
KO
c
Lesion area (μm²)
250000
200000
150000
100000
50000
0
WT
KO

Figure 2.3

Figure 2.3 **Assessment of area of lesion by loss of MBP immunoreactivity.** (**a**) Representative images of MBP immunofluorescent staining in wild-type and EXTL2$^{-/-}$ mice. Scale bar = 200 μm. Lesion area determined by loss of MBP immunoreactivity was quantified for both day 14 (**b**) and day 21 (**c**). Two-tailed, unpaired Student's t-test. * $p < 0.05$.

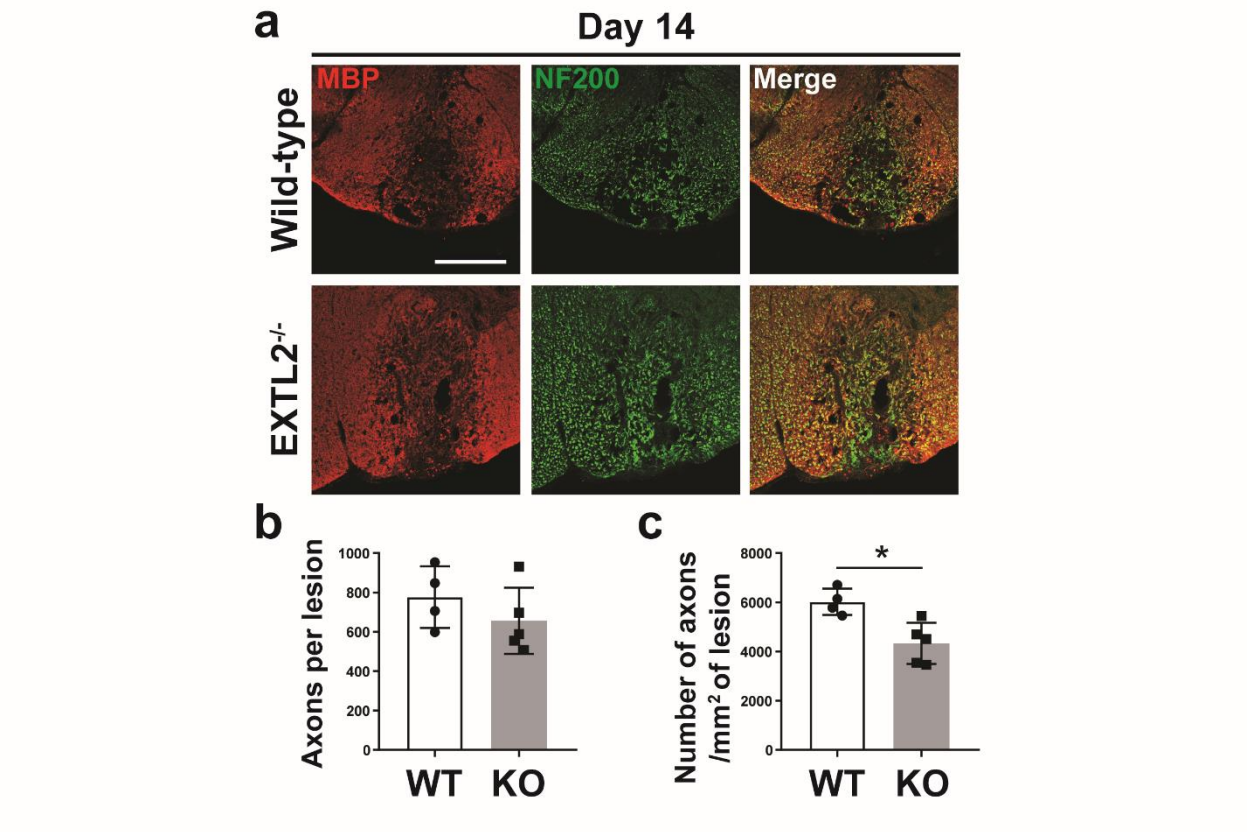

a
Day 14
MBP
NF200
Merge
Wild-type
EXTL2-/-
b
Axons per lesion
1000
800
600
400
200
0
WT
KO
c
Number of axons /mm2 of lesion
8000
6000
4000
2000
0
*
WT
KO

Figure 2.4

Figure 2.4 **Changes in axonal density in wild-type vs. EXTL2$^{-/-}$ lesions at day 14.** (**a**) Representative images from day 14 lesions labeled with MBP and NF200 to denote demyelinated area and axons, respectively. (**b**) Total number of axons per lesion. (**c**) Density of axons calculated by dividing number of axons over lesion area. Two-tailed, unpaired Student's t-test. * $p < 0.05$.

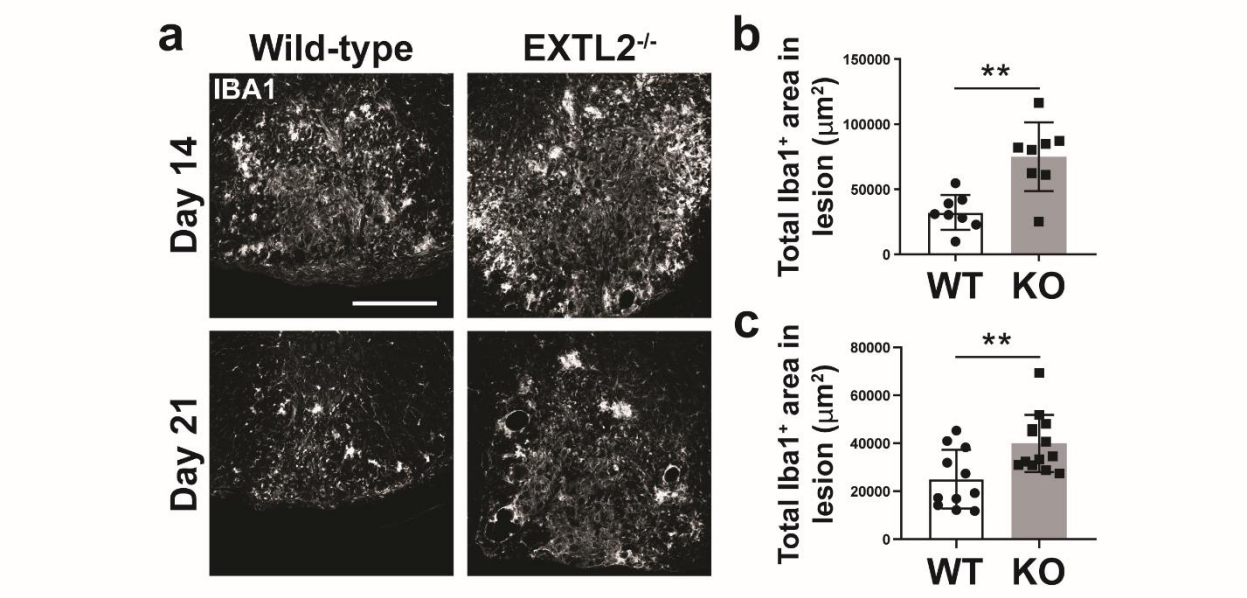

a
Wild-type
EXTL2-/-
IBA1
Day 14
Day 21
b
Total Iba1+ area in lesion (μm2)
150000
100000
50000
0
**
WT
KO
c
Total Iba1+ area in lesion (μm2)
80000
60000
40000
20000
0
**
WT
KO

Figure 2.5

Figure 2.5 **Staining and quantification of Iba1 immunoreactivity in the lesion.** Iba1 staining is shown in representative images for day 14 and day 21 in (**a**). Scale bar = 200 μm. Comparison of total $Iba1^{+}$ area within the lesion in wild-type and $EXTL2^{-/-}$ mice for day 14 (**b**) and day 21 (**c**) are shown. Two-tailed, unpaired Student's t-test. ** $p < 0.01$.

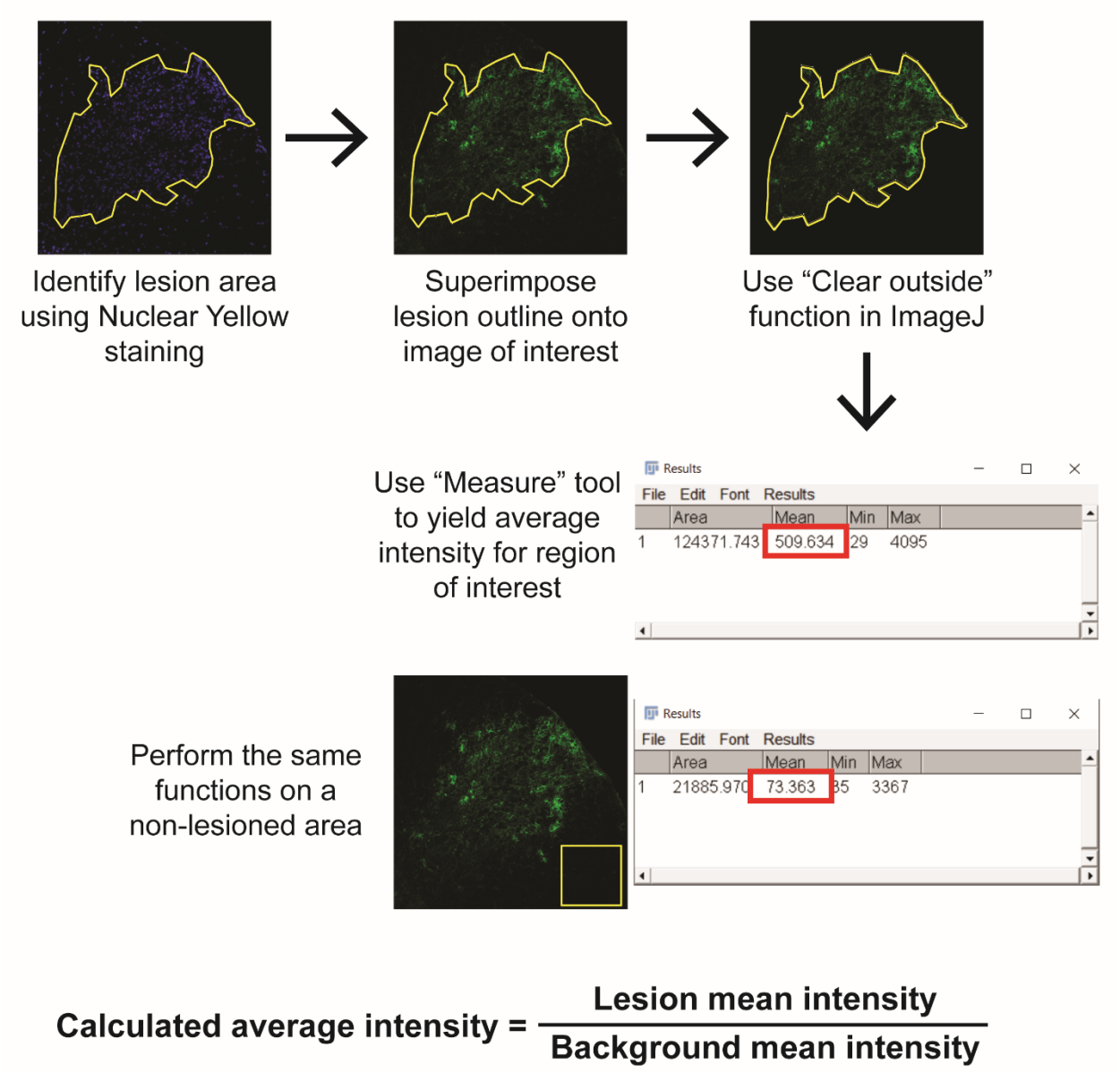

Identify lesion area using Nuclear Yellow staining
Superimpose lesion outline onto image of interest
Use “Clear outside” function in ImageJ
Use “Measure” tool to yield average intensity for region of interest
Results
File Edit Font Results
Area Mean Min Max
1 124371.743 509.634 29 4095
Perform the same functions on a non-lesioned area
Results
File Edit Font Results
Area Mean Min Max
1 21885.970 73.363 35 3367
Calculated average intensity = Lesion mean intensity / Background mean intensity

Figure 2.6

Figure 2.6 **Schematic showing workflow for calculating average intensity of staining from confocal images.** The lesion is demarcated and average intensity of label of interest is obtained using the “Measure” tool. The same is done for a non-lesioned area within the same confocal image. The average intensity of the label of interest is calculated by dividing the lesion mean intensity by the background mean intensity.

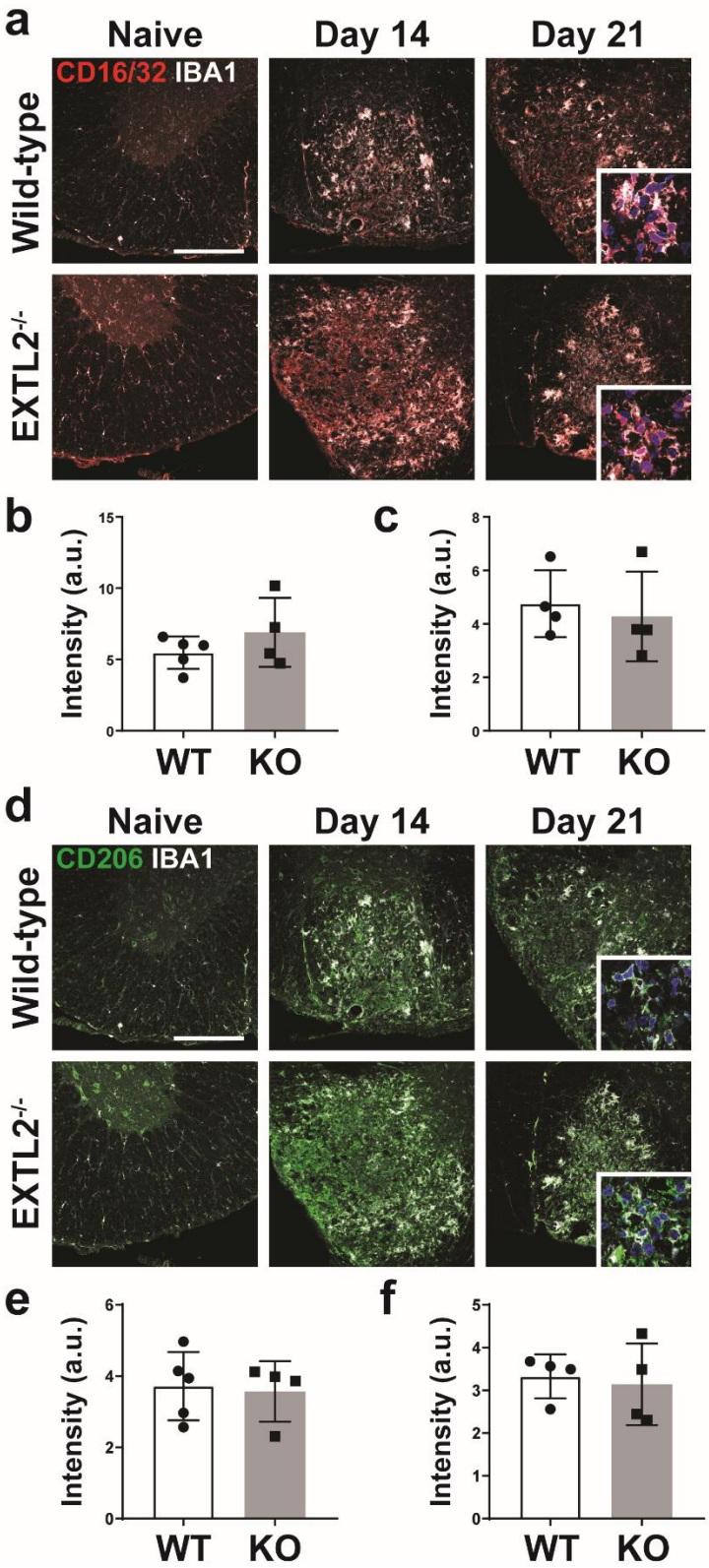

a
Naive
Day 14
Day 21
CD16/32 IBA1
Wild-type
EXTL2-/-
b
Intensity (a.u.)
WT
KO
c
Intensity (a.u.)
WT
KO
d
Naive
Day 14
Day 21
CD206 IBA1
Wild-type
EXTL2-/-
e
Intensity (a.u.)
WT
KO
f
Intensity (a.u.)
WT
KO

Figure 2.7

Figure 2.7 **Immunolabeling of microglia/macrophages and surface markers CD16/32 and CD206.** Tissue sections were probed for both CD16/32 and CD206, representative images are shown in (**a**) for CD16/32 and Iba1, and (**d**) for CD206 and Iba1. Scale bar = 200 μm. Inset images are high magnification images of nuclear yellow-stained nuclei with Iba1 (white) and CD16/32 (red) or CD206 (green). (**b**) Average lesion intensity of CD16/32 in lesions from 14 dpl. (**c**) Average lesion intensity of CD16/32 in lesions from 21 dpl. (**e**), (**f**) Average lesion intensity for CD206 in day 14 and day 21 lesions, respectively. Two-tailed, unpaired Student's t-test.

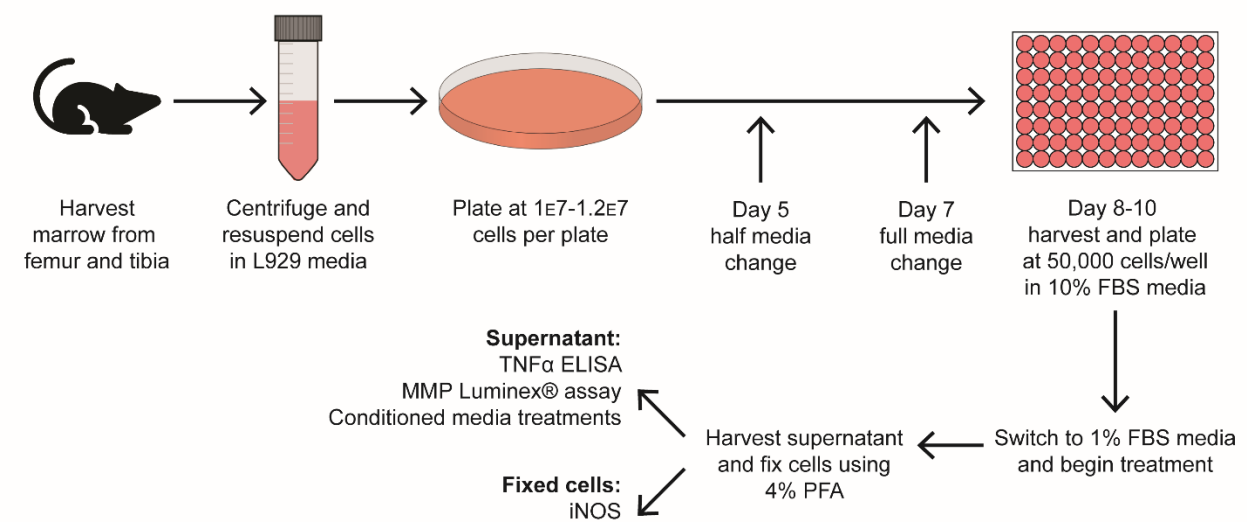

Harvest marrow from femur and tibia
Centrifuge and resuspend cells in L929 media
Plate at 1E7-1.2E7 cells per plate
Day 5 half media change
Day 7 full media change
Day 8-10 harvest and plate at 50,000 cells/well in 10% FBS media
Switch to 1% FBS media and begin treatment
Harvest supernatant and fix cells using 4% PFA
Supernatant:
TNFα ELISA
MMP Luminex® assay
Conditioned media treatments
Fixed cells:
iNOS

Figure 2.8

Figure 2.8 **Schematic of bone marrow-derived macrophage cultures.** Marrow was harvested from both femurs and tibia of female C57Bl/6 mice or EXTL2$^{-/-}$ mice aged 6-8 weeks. Cells were cultured and differentiated using L929 conditioned media for 8-10 days. Adherent cells were removed and seeded into a 96-well dark wall plate in 1% FBS-containing media. Treatment was added to wells in 1% FBS-containing media. After 24 hours, cell supernatant was removed and stored at -80^{o}C for later use and 4% paraformaldehyde was used to fix cells.

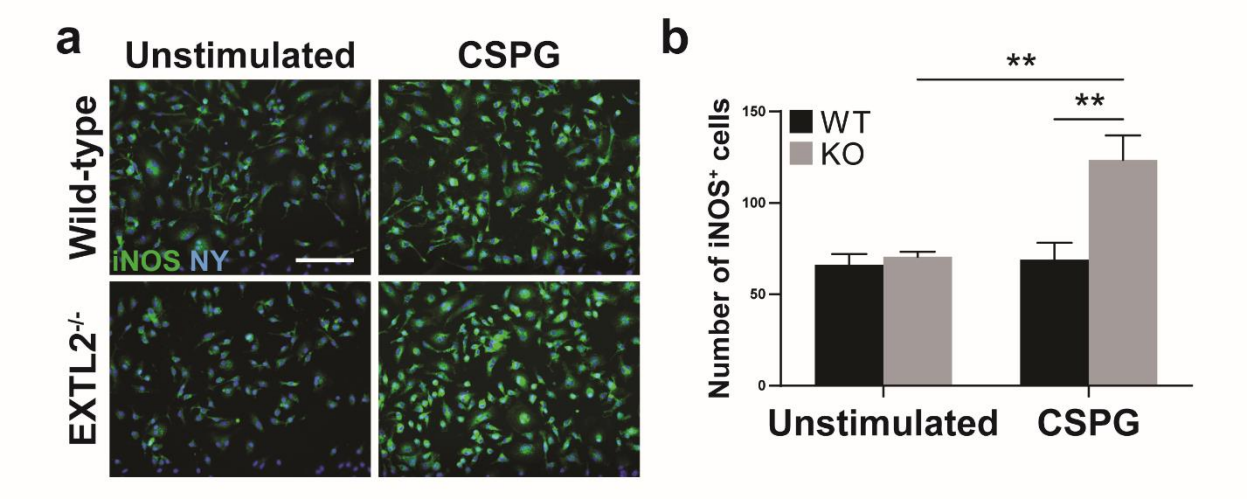

a
Unstimulated
CSPG
Wild-type
EXTL2-/-
iNOS NY
b
**
**
WT
KO
Number of iNOS+ cells
150
100
50
0
Unstimulated
CSPG

Figure 2.9

Figure 2.9 **Assessment of iNOS expression in cultured BMDMs following CSPG stimulation.** Cultured BMDMs from wild-type and EXTL2$^{-/-}$ mice were stained for iNOS and nuclear yellow (NY). Representative images for unstimulated and CSPG-stimulated cells are shown in (**a**). Scale bar = 100 µm. (**b**) comparison of number of iNOS^{+} cells between wild-type and EXTL2$^{-/-}$ cells, unstimulated and CSPG stimulation. Data shown is $n = 4$ wells per condition from 1 biological replicate. Two-way ANOVA with Sidak's multiple comparisons test. ** $p < 0.01$.

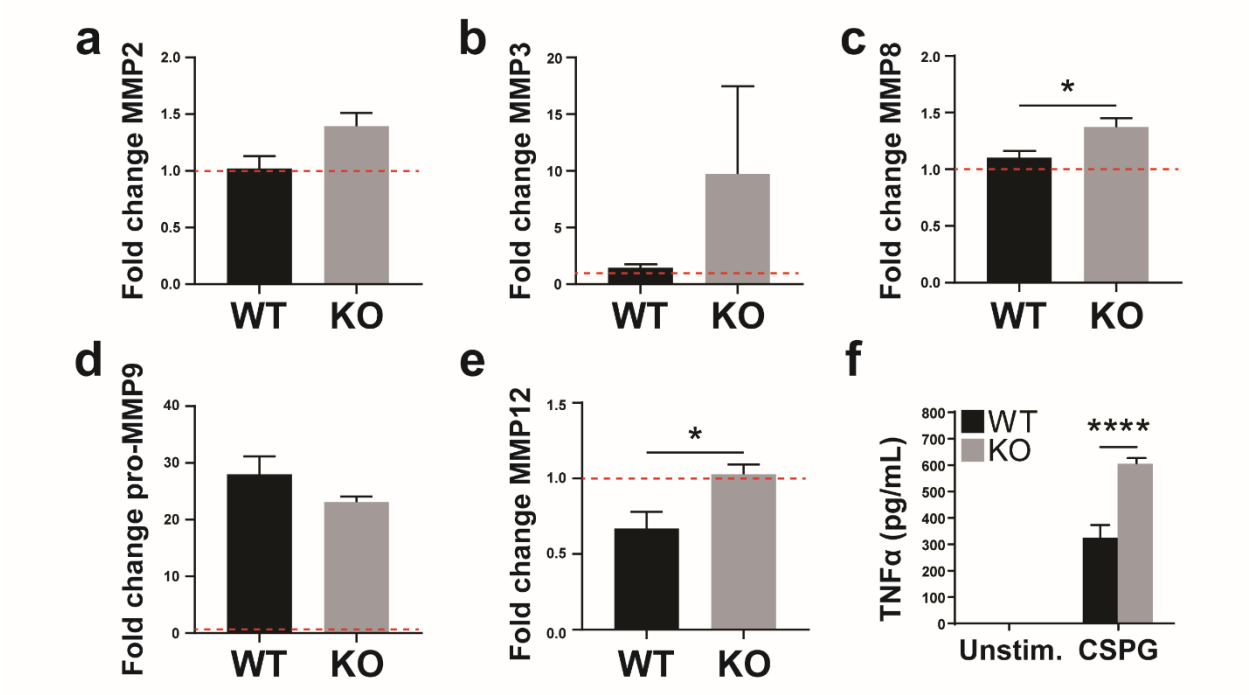

a
Fold change MMP2
2.0
1.5
1.0
0.5
0.0
WT
KO
b
Fold change MMP3
20
15
10
5
0
WT
KO
c
Fold change MMP8
*
2.0
1.5
1.0
0.5
0.0
WT
KO
d
Fold change pro-MMP9
40
30
20
10
0
WT
KO
e
Fold change MMP12
*
1.5
1.0
0.5
0.0
WT
KO
f
TNFα (pg/mL)
WT
KO
****
800
700
600
500
400
300
200
100
0
Unstim.
CSPG

Figure 2.10

Figure 2.10 **Matrix metalloproteinase 5-plex assay and ELISA analyses of conditioned media from cultured BMDMs.** Fold change of MMP2, MMP8, MMP3, pro-MMP9, and MMP12 detected in conditioned media from both wild-type and EXTL2$^{-/-}$ BMDMs, unstimulated and CSPG stimulated, are shown in (**a**), (**b**), (**c**), (**d**), (**e**), respectively. Fold change is calculated over unstimulated cells. (**f**) TNFα levels in BMDM conditioned media as determined by ELISA. $n = 4$ wells per condition from 1 biological replicate. Unpaired Student's t-test for MMP analyses. Ordinary one-way ANOVA with Tukey's multiple comparisons test for TNFα. * $p < 0.05$, **** $p < 0.0001$.

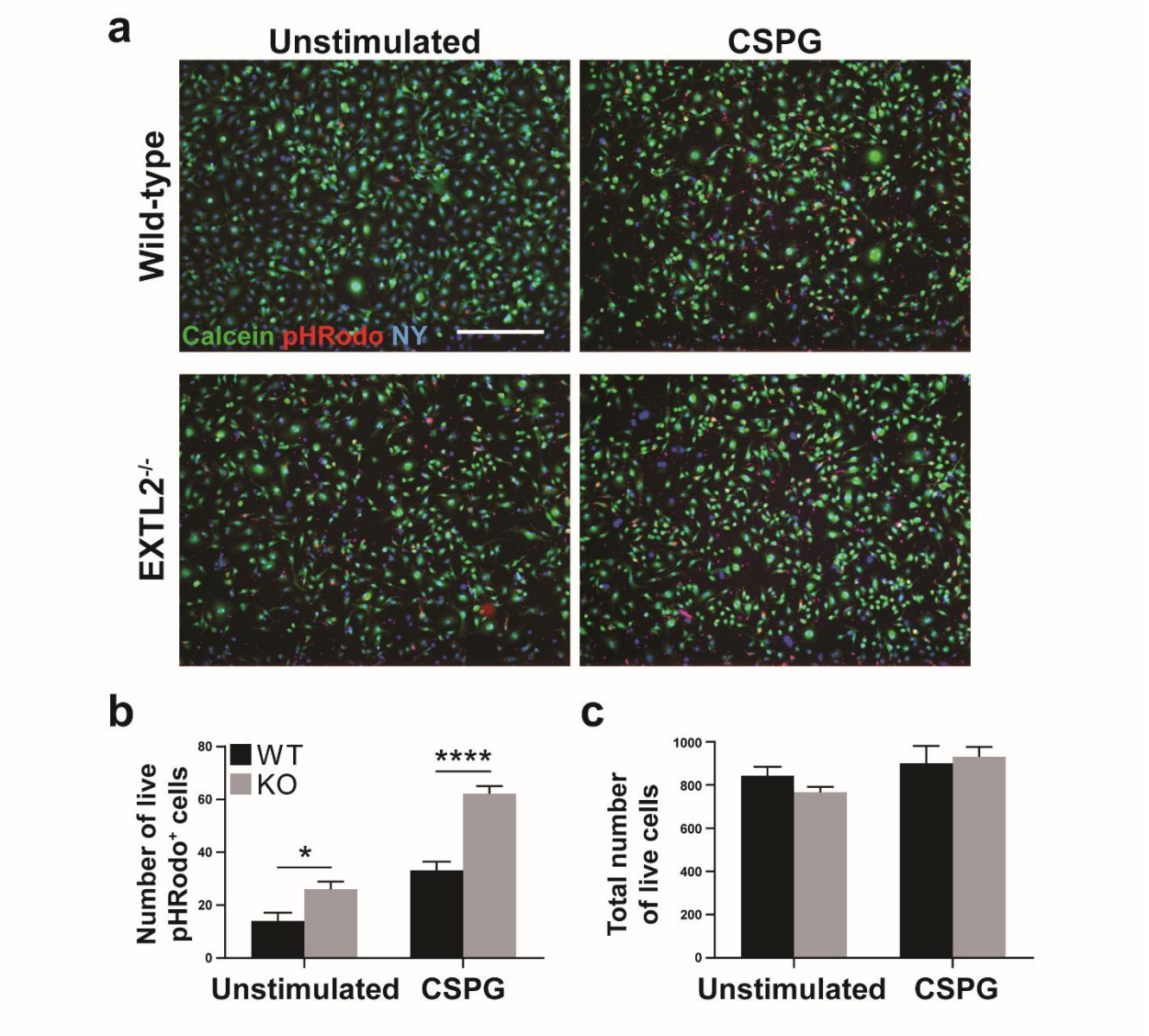

a
Unstimulated
CSPG
Wild-type
EXTL2-/-
Calcein pHRodo NY
b
Number of live pHRodo+ cells
WT
KO
*
****
Unstimulated
CSPG
c
Total number of live cells
Unstimulated
CSPG

Figure 2.11

Figure 2.11 **Phagocytosis of wild-type and EXTL2$^{-/-}$ BMDMs following 24 hour stimulation with CSPGs.** (**a**) Representative images of BMDMs labeled with Calcein AM (green), Nuclear Blue (blue), and bacteria particle-conjugated pHRodo (red). Scale bar = 200 μm. (**b**) Assessment of number of live cells (Calcein AM^{+}, Nuclear Blue^{+}) containing pHRodo in cytoplasm in either unstimulated BMDMs or BMDMs stimulated with CSPGs. (**c**) Analysis of total cell numbers counted in each field of view in each group. Nine fields of view were assessed per well. $n = 4$ wells per group from 1 biological replicate. Two-way ANOVA with Sidak's multiple comparisons test. * $p < 0.05$, **** $p < 0.0001$.

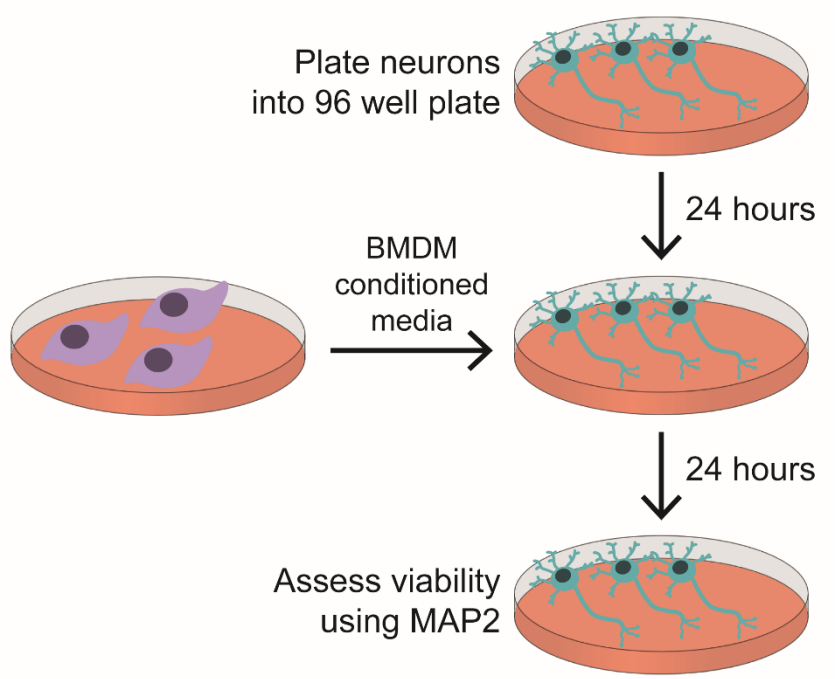


Plate neurons
into 96 well plate
24 hours
BMDM
conditioned
media
24 hours
Assess viability
using MAP2

Figure 2.12

Figure 2.12 **Schematic for the experimental paradigm for primary neurons cultured with BMDM conditioned media.** Neurons were harvested, seeded into 96-well plates, and allowed to grow for 24 hours. BMDM conditioned media was added to culture plates in 1:1 ratio with neuron growth media (see Methods). Neurons were assessed 24 hours following application of BMDM conditioned media.

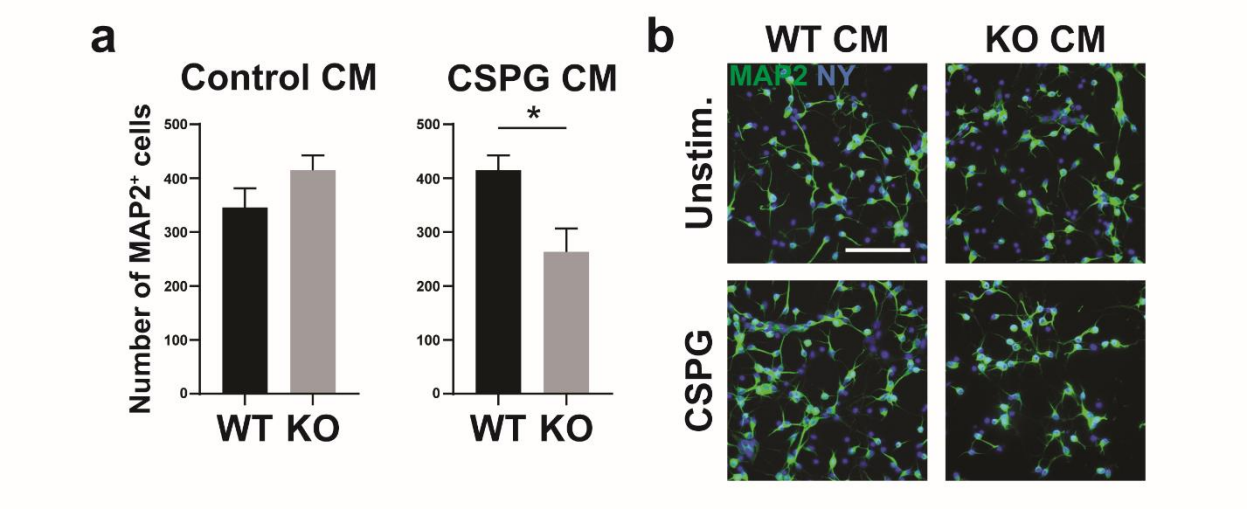

a
Control CM
CSPG CM
Number of MAP2+ cells
500
400
300
200
100
0
*
WT KO
WT KO
b
WT CM
KO CM
MAP2 NY
Unstim.
CSPG

Figure 2.13

Figure 2.13 **Effect of BMDM conditioned media on cultured neurons.** (**a**) Number of MAP2+ cells assessed for cultured neurons 24 hours following application of BMDM conditioned media. Representative images of neurons labeled with MAP2 and Nuclear Yellow shown in (**b**). Scale bar = 100 µm. $n = 4$ wells per group from 1 biological replicate. Unpaired, two-way Student's t-test. * $p < 0.05$.

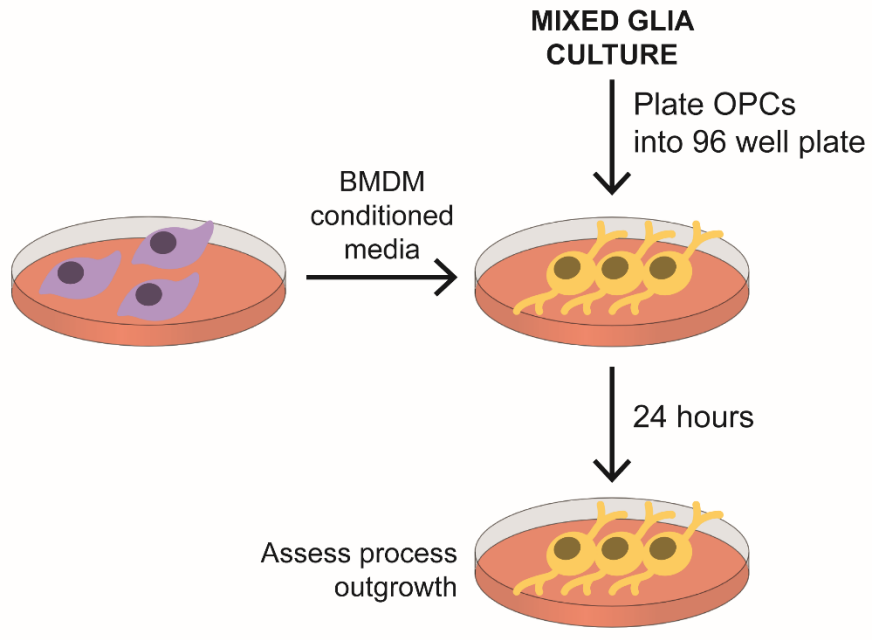

MIXED GLIA
CULTURE
Plate OPCs
into 96 well plate
BMDM
conditioned
media
24 hours
Assess process
outgrowth

Figure 2.14

Figure 2.14 **Experimental paradigm for testing effects of wild-type and EXTL2$^{-/-}$ BMDM conditioned media on OPC growth.** OPCs were harvested from mixed glia cultures and seeded into 96 well plates. BMDM conditioned media from both wild-type and EXTL2$^{-/-}$ BMDMs, unstimulated or stimulated with CSPG, was immediately added in a 1:1 ratio to OPC culture media. OPCs were allowed to grow for 24 hours before process outgrowth assessment.

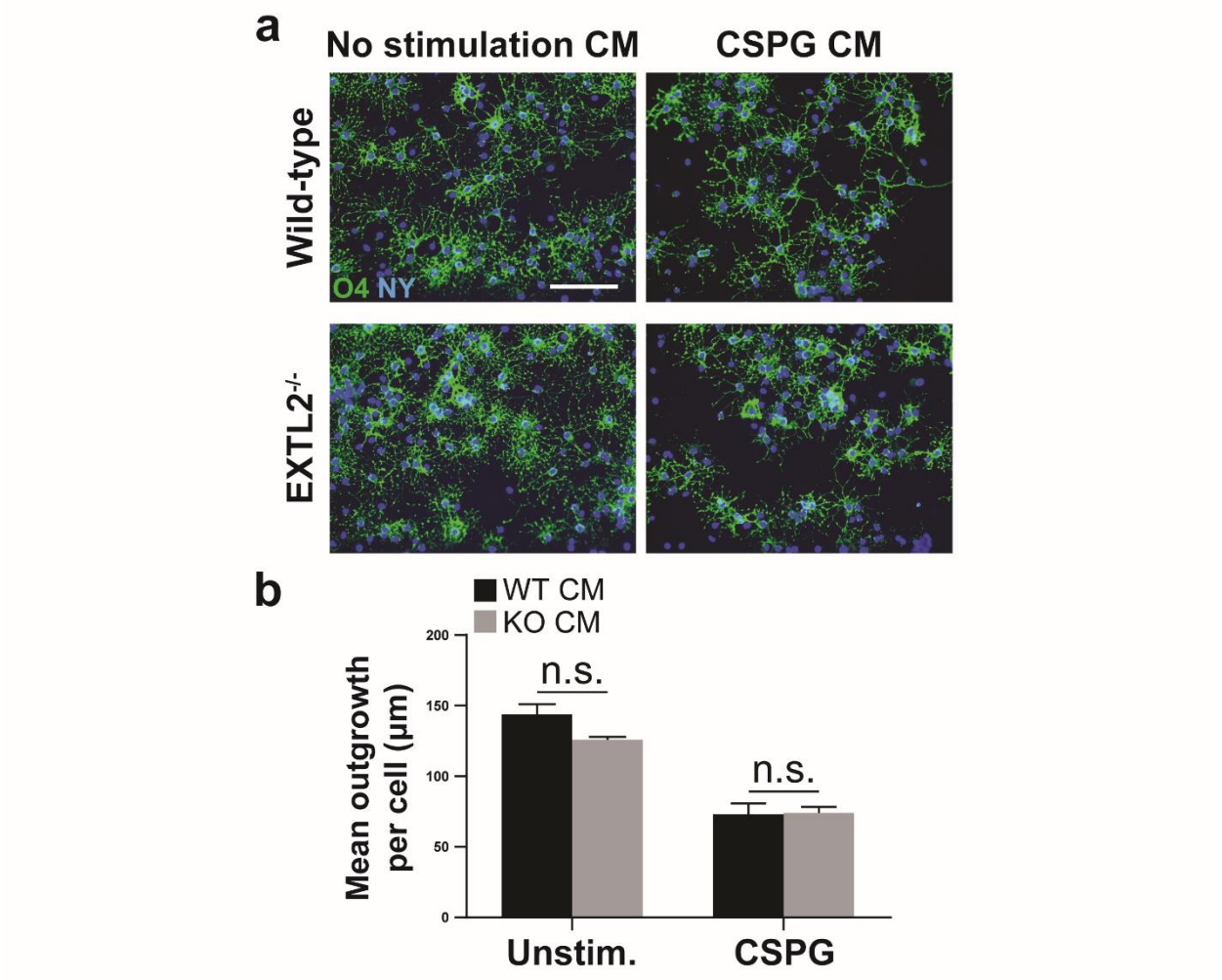

a
No stimulation CM
CSPG CM
Wild-type
EXTL2-/-
O4 NY
b
WT CM
KO CM
Mean outgrowth per cell (µm)
200
150
100
50
0
n.s.
n.s.
Unstim.
CSPG

Figure 2.15

Figure 2.15 **Analysis of OPC process outgrowth following treatment with wild-type and EXTL2$^{-/-}$ BMDM conditioned media.** (**a**) Representative images of wild-type OPCs labeled with O4 and nuclear yellow following treatment with BMDM conditioned media. Scale bar = 200 µm. (**b**) Comparison of wild-type and EXTL2$^{-/-}$ BMDM conditioned media on OPC outgrowth. *n* = 4 wells per condition from 1 biological replicate. Two-way ANOVA with Sidak's multiple comparisons test. n.s. = not significant.

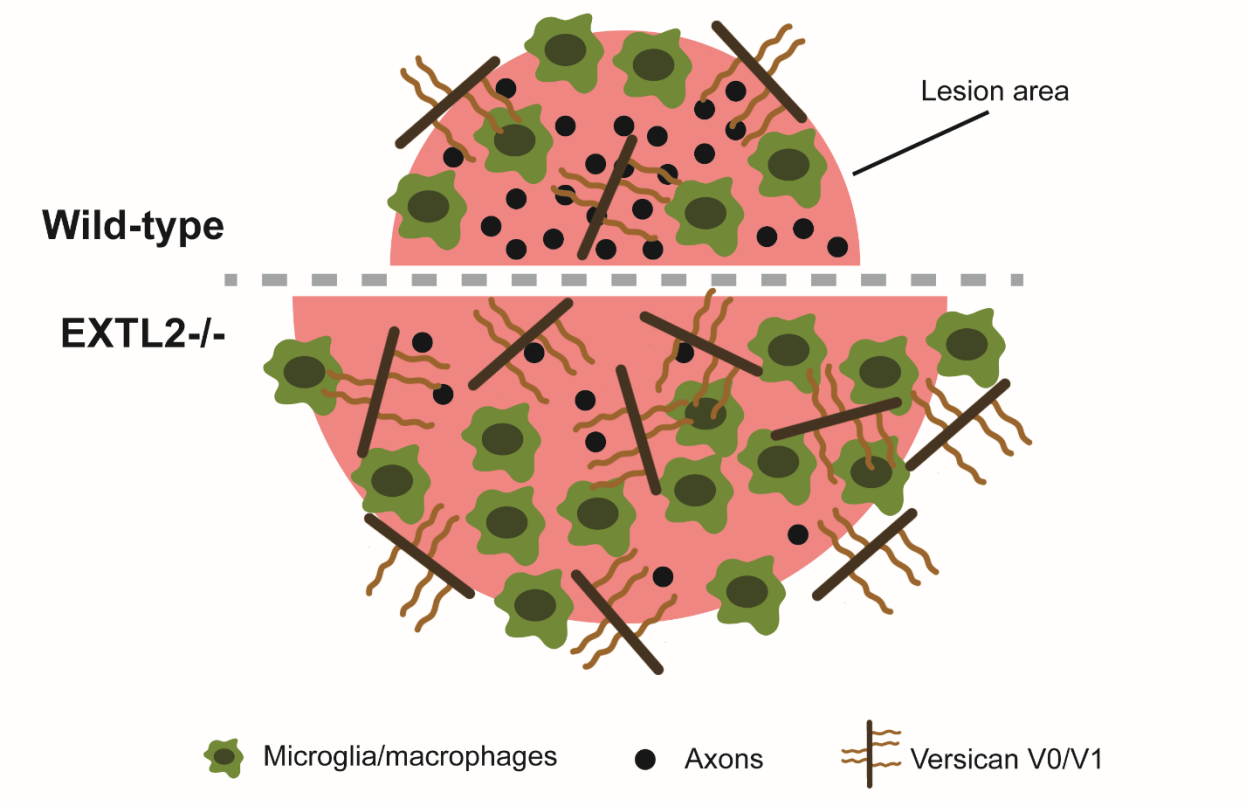

Lesion area
Wild-type
EXTL2-/-
Microglia/macrophages
Axons
Versican V0/V1

Figure 2.16

Figure 2.16 **Schematic showing the phenotypes observed in EXTL2$^{-/-}$ mice with lysolecithin-induced lesions.** EXTL2$^{-/-}$ animals have greater versican deposition into the lesion following demyelination, and this is correlated to a larger area of damage and exacerbated axon dropout within the lesion. In addition, greater amounts of Iba1 immunoreactivity was seen in EXTL2$^{-/-}$ lesions, indicative of a more inflammatory milieu compared to that of wild-type animals.

CHAPTER 3 – Conclusions and Discussion

3.1 Conclusions

Here we report that in mice lacking the EXTL2 glycosyltransferase, a lysolecithin-induced demyelination that was accompanied by higher immunoreactivity for versican V1 within the lesion did not affect oligodendrocyte repopulation compared to that occurring in wildtype mice. Instead, there was a more pronounced microglia/macrophage presence in the EXTL2$^{-/-}$ lesions and a worsened injury status as documented by more axonal loss at 14 days post-injury. In tissue culture experiments, several pro-inflammatory phenotypes including neurotoxicity were observed when EXTL2$^{-/-}$ BMDMs were stimulated with CSPGs. From these data, we conclude that an outcome of the dysregulation of CSPG production in EXTL2$^{-/-}$ mice following a demyelinating injury is the promotion of microglia/macrophage activity that contributes to axonal injury. Reducing the injury-induced production of CSPGs is likely important in attenuating inflammation and mitigating the axonal loss occurring after a demyelinating insult.

3.2 Discussion

Overall, the above chapters illustrate the importance of studying the CSPG family as modulators of both inflammation and remyelination. The work described in Chapter 2 gives further clarification on the role of CSPGs in injury, suggesting a detrimental effect in the time period beyond the acute injury phase. These results further support the notion that while their presence in the lesion immediately after an acute injury may be protective (Rolls et al., 2008), the timely clearance of CSPGs from the injury site may be necessary to facilitate more effective regeneration and resolution of inflammation.

CSPGs have been extensively studied in the context of traumatic spinal cord injury; however, the applicability of the same approaches taken to overcome the inhibitory nature of CSPGs to other white matter injuries are still largely undetermined. Chondroitinase ABC, a bacteria-derived enzyme that cleaves GAG chains from the core protein, has been tested extensively in spinal cord injury models. However, the enzyme requires focal administration, which makes it a poor option for diffuse pathologies such as MS. Furthermore, early clearance of CSPGs from the lesion, in contrast with later removal, may be detrimental, as there is evidence to suggest a protective role of CSPGs in the acute phase of injury by altering microglia function (Rolls et al., 2008) and as such, the future treatments to affect CSPGs should take the kinetics of CSPG deposition and their potentially different temporal roles, into consideration. In addition, questions remain on the roles of individual CSPG family members, the composition of their conjugated GAGs, and their enzymatic digestion products in inflammation.

CSPGs are an integral family of molecules that are key in axon guidance in development, and the maintenance of stability in the mature CNS. Perineuronal nets, of which CSPGs are a major constituent, serve important homeostatic roles in ion buffering, protection of axons from oxidative stress, and stabilization of existing synapses (Lau et al., 2013; Suttkus et al., 2014; Wang & Fawcett, 2012). In addition, CSPGs are present in most peripheral tissues (Kaplan and Holbrook, 1994; Nadanaka et al., 2013a; Purnomo et al., 2013), where they are similarly important in the maintenance of tissue health. The impact that removing non-pathological CSPGs is largely unknown; this is a future consideration for the application of drugs targeting the removal of deposited CSPGs. An ideal therapy should aim to inhibit the active synthesis of CSPGs in response to injury rather than attempt to remove CSPGs from the tissue, which may

result in unpredictable side effects. CNS injury-targeting molecules may be utilized to more specifically target the drugs to affect CSPGs to the region of interest (Mann et al., 2016).

Several ECM-degrading enzymes are also secreted in inflammation (Cossins et al., 1997; Da Silva & Yong, 2009; Larsen et al., 2003; Lemarchant et al., 2017; Nissinen & Kähäri, 2014; Wells et al., 2003), and the degradation products often also have signaling properties that were not considered in this study (Ricard-Blum and Salza, 2014). Preventing the cleaving of ECM molecules may attenuate inflammation (Peng et al., 2019), and degradation of the endothelial glycocalyx as a result of sepsis releases HSPGs into circulation, which may then enter the CNS and sequester BDNF (Hippensteel et al., 2019). There is also data to suggest that the intact CSPG molecule has signaling properties that differ from that of chondroitin sulfate GAG disaccharides, where disaccharides have been shown to be anti-inflammatory *in vitro* and *in vivo* (Rolls et al., 2006b; Cañas et al., 2010; Tan and Tabata, 2014; Melgar-Lesmes et al., 2016). This disparity in signaling effect between the intact CSPG compared to CS fragments warrants future investigation, to gain further insight into the signaling mechanisms of chondroitin sulfate fragments, and to determine whether this is a viable approach to future therapeutics. Both EAE or lysolecithin models are applicable models, where CS fragments can be applied systemically or focally to the CNS to determine whether they may serve therapeutic roles, as opposed to the inflammation-stimulating assembled CSPGs (Stephenson et al., 2018).

The model we used to study CSPGs only allowed us to focus on versican V1, as other lectican CSPG members, such as aggrecan, are not elevated within the lesion (Keough et al., 2016). As well, various forms of GAG sulfation confer differential effects; while chondroitin-4-sulfate (CS-A) exerts potent repulsive effects on cultured neurons, chondroitin-6-sulfate (CS-C) does not (Wang et al., 2008). Along the same line, treatment with blocking antibodies specific to

the chondroitin-4,6-disulfate (CS-E) in a mouse model of optic nerve crush improved axon regeneration (Brown et al., 2012). For future studies, various CS core proteins can be synthesized containing varying levels of each GAG sulfation pattern to investigate both effects of individual CSPG members and impact of sulfation patterns on function.

White and grey matter differ in the composition of ECM, both in homeostasis and in pathological conditions. Of note, aggrecan is particularly abundant in the grey matter in homeostatic conditions due to its participation in perineuronal net formation. In MS lesions traversing from white matter to cortex, significantly less versican is found in the cortical grey matter lesion area compared to white matter (Chang et al., 2012). This may underlie key differences observed in human MS pathology, where cortical lesions tend to remyelinate with much higher efficiency than white matter lesions (Strijbis et al., 2017). The impact of ECM composition on remyelination as well as inflammation is a topic that warrants more detailed characterization.

In this work we did not investigate whether peripherally-derived macrophages and CNS-resident microglia are differentially affected by the loss of EXTL2. Distinguishing macrophages from microglia is difficult to, as differentially-expressed surface markers are limited. To overcome this problem, a fate mapping approach can be taken. A TdTomato reporter mouse line is crossed with a CX3CR1-CreER line to generate tamoxifen-inducible TdTomato expression in $CX3CR1^{+}$ cells at time of injection. Due to higher turnover rate of peripheral cells, TdTomato expression is lost over time while expression is maintained in the long-lived microglia population. This allows us to reliably identify $CX3CR1^{+}Iba1^{+}TdTomato^{+}$ cells as microglia or CNS-resident macrophages. This strategy can be used to investigate whether proportions of

microglia and macrophages in the lesion are altered, and if their function is changed in demyelination.

We also did not specify which secreted factors may underlie the increased toxicity to cultured neurons. Here we have provided evidence for candidates such as MMPs or TNFα, both of which are overproduced by EXTL2$^{-/-}$ BMDMs when stimulated with CSPGs. The overproduction of such factors alludes to the possibility that EXTL2 is a suppressive mechanism that may exert effects on several inflammation-associated processes. However, an unbiased approach such as a proteomics screen may provide other candidates. Mass spectrometry can be performed on cultured EXTL2$^{-/-}$ or wild-type macrophages to study what other neurotoxic factors may be produced. Single-cell RNA sequencing experiments can also be performed on lesioned spinal cord, and the generated database can be probed for factors highly upregulated by EXTL2$^{-/-}$ microglia/macrophages compared to wild-type.

While lysolecithin-induced focal demyelination is a model that is used extensively to study demyelination, remyelination, and microglia/myeloid cell-mediated inflammation, it is a model that inadequately addresses the adaptive immune aspects (T and B cells) known to be essential in the pathogenesis and propagation of MS. Although CD4^{+} and CD8^{+} T cells are both recruited to the lysolecithin-induced lesion, their presence is transient, with very few observable T cells after 12 hours post-injection of lysolecithin (Ousman and David, 2000), making their relevance to injury progression difficult to determine. A focal experimental autoimmune encephalomyelitis (EAE) model may be a viable option to provide a more complete picture of pathology. Immunization with myelin oligodendrocyte glycoprotein with subsequent focal injection of inflammatory factors (Merkler et al., 2006) or a BBB-disrupting factor such as vascular endothelial growth factor (Sasaki et al., 2010) produces consistent focal demyelinating

lesions with significant penetration of both microglia/macrophages and $CD3^+$ T cells. Myelin oligodendrocyte glycoprotein-induced EAE does not recruit B cells to the CNS compartment, however injection of splenocytes from an EAE mouse into a syngeneic mouse induces significant B cell accumulation near the ventral lumbar spinal cord (Mannara et al., 2012).

Finally, we did not pursue a reason for why there is no deficiencies in OPC repopulation and differentiation in the $EXTL2^{-/-}$ lesions. A possible explanation is discussed in Chapter 2. This is an important question for future studies, as CSPGs have been definitively described as an inhibitor of OPC growth and remyelination.

Overall, changes to the ECM and to CSPGs is an area of study that is widely applicable to a range of both CNS and peripheral injuries. The microenvironment that cells reside in strongly dictates their phenotype and function, and this work further highlights the importance of studying and modulating the ECM to promote the efficiency of tissue healing.

REFERENCES

Agrawal SM, Lau LW, Yong VW (2008) MMPs in the central nervous system: Where the good guys go bad. Semin Cell Dev Biol 19:42–51.

Aguirre A, Dupree JL, Mangin JM, Gallo V (2007) A functional role for EGFR signaling in myelination and remyelination. Nat Neurosci 10.

Albert M, Antel JP, Brück W, Stadelmann C (2007) Extensive Cortical Remyelination in Patients with Chronic Multiple Sclerosis. Brain Pathol 17:129–138.

Alizadeh A, Dyck SM, Karimi-Abdolrezaee S (2015) Myelin damage and repair in pathologic CNS: challenges and prospects. Front Mol Neurosci 8.

Allamargot C, Pouplard-Barthelaix A, Fressinaud C (2001) A single intracerebral microinjection of platelet-derived growth factor (PDGF) accelerates the rate of remyelination in vivo. Brain Res 918:28–39.

Amano M, Nakayama M, Kaibuchi K (2010) Rho-Kinase/ROCK: A Key Regulator of the Cytoskeleton and Cell Polarity. Cytoskeleton 67:545–554.

Aparicio E, Mathieu P, Luppi MP, Gubiani MFA, Adamo AM (2013) The notch signaling pathway: Its role in focal CNS demyelination and apotransferrin-induced remyelination. J Neurochem 127:819–836.

Armstrong RC, Le TQ, Frost EE, Borke RC, Vana AC (2002) Absence of Fibroblast Growth Factor 2 Promotes Oligodendroglial Repopulation of Demyelinated White Matter. J Neurosci 22:8574–8585.

Arnett HA, Mason JL, Marino M, Suzuki K, Matsushima GK, Ting JP-Y (2001) TNFα promotes proliferation of oligodendrocyte progenitors and remyelination. Nat Neurosci 4:1116–1122.

Arnett HA, Wang Y, Matsushima GK, Suzuki K, P-Y Ting J (2003) Functional Genomic Analysis of Remyelination Reveals Importance of Inflammation in Oligodendrocyte Regeneration. J Neurosci 23:9824–9832.

Arulmoli J, Pathak MM, Mcdonnell LP, Nourse JL, Tombola F, Earthman JC, Flanagan LA (2015) Static stretch affects neural stem cell differentiation in an extracellular matrix-dependent manner. Sci Rep 5.

Asher RA, Morgenstern DA, Fidler PS, Adcock KH, Oohira A, Braistead JE, Levine JM, Margolis RU, Rogers JH, Fawcett JW (2000) Neurocan Is Upregulated in Injured Brain and in Cytokine-Treated Astrocytes. J Neurosci 20:2427–2438.

Asher RA, Morgenstern DA, Shearer MC, Adcock KH, Pesheva P, Fawcett JW (2002) Versican Is Upregulated in CNS Injury and Is a Product of Oligodendrocyte Lineage Cells. J Neurosci 22:2225–2236.

Austin JW, Gilchrist C, Fehlings MG (2012) High molecular weight hyaluronan reduces lipopolysaccharide mediated microglial activation. J Neurochem 122:344–355.

Back SA, Tuohy TMF, Chen H, Wallingford N, Craig A, Struve J, Luo NL, Banine F, Liu Y,

Chang A, Trapp BD, Bebo BF, Rao MS, Sherman LS (2005) Hyaluronan accumulates in demyelinated lesions and inhibits oligodendrocyte progenitor maturation. Nat Med 11:966–972.

Bandtlow CE, Zimmermann DR (2000) Proteoglycans in the Developing Brain: New Conceptual Insights for Old Proteins. Physiol Rev 80:1267–1290.

Bansal R, Kumar M, Murray K, Pfeiffer SE (1996) Developmental and FGF-2-Mediated Regulation of Syndecans (1– 4) and Glypican in Oligodendrocytes. Mol Cell Neurosci 7:276–288.

Baraban M, Koudelka S, Lyons DA (2017) Ca2+ activity signatures of myelin sheath formation and growth in vivo. Nat Neurosci.

Baron W, Metz B, Bansal R, Hoekstra D, De Vries H (2000) PDGF and FGF-2 Signaling in Oligodendrocyte Progenitor Cells: Regulation of Proliferation and Differentiation by Multiple Intracellular Signaling Pathways. Mol Cell Neurosci 15:314–329.

Barritt AW, Davies M, Marchand F, Hartley R, Grist J, Yip P, Mcmahon SB, Bradbury EJ (2006) Chondroitinase ABC Promotes Sprouting of Intact and Injured Spinal Systems after Spinal Cord Injury. J Neurosci 26:10856–10867.

Barros CS, Franco SJ, Mu U (2011) Extracellular Matrix: Functions in the Nervous System. Cold Spring Harb Perspect Biol:1–25.

Bechler ME, Byrne L, Ffrench-Constant C (2015) CNS Myelin Sheath Lengths Are an Intrinsic Property of Oligodendrocytes. Curr Biol 25:2411–2416.

Beggah AT, Dours-Zimmermann MT, Barras FM, Brosius A, Zimmermann DR, Zurn AAD (2005) Lesion-induced differential expression and cell association of neurocan, brevican, versican V1 and V2 in the mouse dorsal root entry zone. Neuroscience:749–762.

Benninger Y, Colognato H, Thurnherr T, Franklin RJM, Leone DP, Atanasoski S, Nave K-A, Ffrench-Constant C, Suter U, Relvas JB (2006) b1-Integrin Signaling Mediates Premyelinating Oligodendrocyte Survival But Is Not Required for CNS Myelination and Remyelination. J Neurosci 26:7665–7673.

Bernstein JJ, Getz R, Jefferson M, Kelemen M (1985) Astrocytes secrete basal lamina after hemisection of rat spinal cord. Brain Res 327:135–141.

Bignami A, Perides G, Rahemtulla F (1993) Versican, a hyaluronate-binding proteoglycan of embryonal precartilaginous mesenchyma, is mainly expressed postnatally in rat brain. J Neurosci Res 34:97–106.

Blakemore WF, Eames RA, Smith KJ, McDonald WI (1977) Remyelination in the spinal cord of the cat following intraspinal injections of lysolecithin. J Neurol Sci 33:31–43.

Blakemore WF, Murray JA (1981) Quantitative examination of internodal length of remyelinated nerve fibres in the central nervous system. J Neurol Sci 49:273–284.

Blight AR, Young W (1989) Central axons in injured cat spinal cord recover electrophysiological function following remyelination by Schwann cells. J Neurol Sci

91:15–34.

Bradbury EJ, Lawrence DFM, Popat RJ, King VR, Bennett GS, Patel PN, Fawcett JW, McMahon SB (2002) Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature 416:636–640.

Brown JM, Xia J, Zhuang B, Cho K-S, Rogers CJ, Gama CI, Rawat M, Tully SE, Uetani N, Mason DE, Tremblay ML, Peters EC, Habuchi O, Chen DF, Hsieh-Wilson LC (2012) A sulfated carbohydrate epitope inhibits axon regeneration after injury. Proc Natl Acad Sci 109:4768–4773.

Brück W (2005) Inflammatory demyelination is not central to the pathogenesis of multiple sclerosis. J Neurol 252:10–15.

Burnside ER, Bradbury EJ (2014) Review: Manipulating the extracellular matrix and its role in brain and spinal cord plasticity and repair. Neuropathol Appl Neurobiol 40:26–59.

Buss A, Pech K, Kakulas BA, Martin D, Schoenen J, Noth J, Brook GA (2009) NG2 and phosphacan are present in the astroglial scar after human traumatic spinal cord injury. BMC Neurol 9.

Butt AM, Dinsdale J (2005) Fibroblast growth factor 2 induces loss of adult oligodendrocytes and myelin in vivo. Exp Neurol 192:125–133.

Buttery PC, Ffrench-Constant C (1999) Laminin-2/Integrin Interactions Enhance Myelin Membrane Formation by Oligodendrocytes. Mol Cell Neurosci 14:199–212.

Caggiano AO, Zimber MP, Ganguly A, Blight AR, Gruskin EA (2005) Chondroitinase ABCI Improves Locomotion and Bladder Function following Contusion Injury of the Rat Spinal Cord. J Neurotrauma 22:226–239.

Canals I, Benetó N, Cozar M, Vilageliu L, Grinberg D (2015) EXTL2 and EXTL3 inhibition with siRNAs as a promising substrate reduction therapy for Sanfilippo C syndrome. Sci Rep 5.

Cañas N, Gorina R, Planas AM, Vergés J, Montell E, García AG, López MG (2010) Chondroitin sulfate inhibits lipopolysaccharide-induced inflammation in rat astrocytes by preventing nuclear factor kappa B activation. Neuroscience 167:872–879.

Carulli D, Rhodes KE, Brown DJ, Bonnert TP, Pollack SJ, Oliver K, Strata P, Fawcett JW (2006) Composition of perineuronal nets in the adult rat cerebellum and the cellular origin of their components. J Comp Neurol 494:559–577.

Chang A, Staugaitis SM, Dutta R, Batt CE, Easley KE, Chomyk AM, Yong VW, Fox RJ, Kidd GJ, Trapp BD (2012) Cortical remyelination: A new target for repair therapies in multiple sclerosis. Ann Neurol 72:918–926.

Chang MY, Kang I, Gale M, Manicone AM, Kinsella MG, Braun KR, Wigmosta T, Parks WC, Altemeier WA, Wight TN, Charles X, Frevert W (2017) Versican is produced by Trif-and type I interferon-dependent signaling in macrophages and contributes to fine control of innate immunity in lungs. Am J Physiol Cell Mol Physiol 313:1069–1086.

Cheli VT, Santiago González DA, Spreuer V, Paez PM (2015) Voltage-gated Ca++ entry promotes oligodendrocyte progenitor cell maturation and myelination in vitro. Exp Neurol 265:69–83.

Chen W, Sheng J, Guo J, Gao F, Zhao X, Dai J, Wang G, Li K (2012) Tumor necrosis factor-α enhances voltage-gated Na+ currents in primary culture of mouse cortical neurons. J Neuroinflammation 12.

Chen Y-J, Spence HJ, Cameron JM, Jess T, Ilsley JL, Winder SJ (2003) Direct interaction of β-dystroglycan with F-actin. Biochem J 375:329–337.

Clarke D, Katoh O, Gibbs R V, Griffiths SD, Gordon MY (1995) Interaction of interleukin 7 (IL-7) with glycosaminoglycans and its biological relevance. Cytokine 7:325–330.

Colognato H, Baron W, Avellana-Adalid V, Relvas JB, Evercooren AB-V, Georges-Labouesse E, ffrench-Constant C (2002) CNS integrins switch growth factor signalling to promote target-dependent survival. Nat Cell Biol 4:833–841.

Colognato H, Galvin J, Wang Z, Relucio J, Nguyen T, Harrison D, Yurchenco PD, ffrench-Constant C (2007) Identification of dystroglycan as a second laminin receptor in oligodendrocytes, with a role in myelination. Development 134:1723–1735.

Corley SM, Ladiwala U, Besson A, Yong VW (2001) Astrocytes attenuate oligodendrocyte death in vitro through an alpha-6 integrin-laminin-dependent mechanism. Glia 36:281–294.

Cossins JA, Clements JM, Ford J, Miller KM, Pigott R, Vos W, Van Der Valk P, De Groot CJA (1997) Enhanced expression of MMP-7 and MMP-9 in demyelinating multiple sclerosis lesions. Acta Neuropathol 94:590–598.

Crawford AH, Tripathi RB, Richardson WD, Franklin RJM (2016) Developmental Origin of Oligodendrocyte Lineage Cells Determines Response to Demyelination and Susceptibility to Age-Associated Functional Decline. Cell Rep 15:761–773.

Cui Q-L, Fogle E, Almazan G (2006) Muscarinic acetylcholine receptors mediate oligodendrocyte progenitor survival through Src-like tyrosine kinases and PI3K/Akt pathways. Neurochem Int 48:383–393.

Czopka T, Von Holst A, Schmidt G, Ffrench-Constant C, Faissner A (2009) Tenascin C and tenascin R similarly prevent the formation of myelin membranes in a RhoA-dependent manner, but antagonistically regulate the expression of myelin basic protein via a separate pathway. Glia 57:1790–1801.

Dai J, Bercury KK, Ahrendsen JT, Macklin WB (2015) Olig1 Function Is Required for Oligodendrocyte Differentiation in the Mouse Brain. J Neurosci 35:4386–4402.

DaSilva AG, Yong VW (2009) Matrix metalloproteinase-12 deficiency worsens relapsing-remitting experimental autoimmune encephalomyelitis in association with cytokine and chemokine dysregulation. Am J Pathol 174:898–909.

Davies JE, Tang X, Denning JW, Archibald SJ, Davies SJA (2004) Decorin suppresses neurocan, brevican, phosphacan and NG2 expression and promotes axon growth across adult rat spinal cord injuries. Eur J Neurosci 19:1226–1242.

Dawson MRL, Polito A, Levine JM, Reynolds R (2003) NG2-expressing glial progenitor cells: An abundant and widespread population of cycling cells in the adult rat CNS. Mol Cell Neurosci 24:476–488.

De Angelis F, Bernardo A, Magnaghi V, Minghetti L, Tata AM (2012) Muscarinic receptor subtypes as potential targets to modulate oligodendrocyte progenitor survival, proliferation, and differentiation. Dev Neurobiol 72:713–728.

De Biase LM, Kang SH, Baxi EG, Fukaya M, Pucak ML, Mishina M, Calabresi PA, Bergles DE (2011) NMDA Receptor Signaling in Oligodendrocyte Progenitors Is Not Required for Oligodendrogenesis and Myelination. J Neurosci 31:12650–12662.

De Yébenes EG, Ho A, Damani T, Fillit H, Blum M (1999) Regulation of the Heparan Sulfate Proteoglycan, Perlecan, by Injury and Interleukin-1α. J Neurochem 73:812–820.

Deng Y-P, Sun Y, Hu L, Li Z-H, Xu Q-M, Pei Y-L, Huang Z-H, Yang Z-G, Chen C (2015) Chondroitin sulfate proteoglycans impede myelination by oligodendrocytes after perinatal white matter injury. Exp Neurol 269:213–223.

Deshmukh VA, Tardif V, Lyssiotis CA, Green CC, Kerman B, Kim HJ, Padmanabhan K, Swoboda JG, Ahmad I, Kondo T, Gage FH, Theofilopoulos AN, Lawson BR, Schultz PG, Lairson LL (2013) A regenerative approach to the treatment of multiple sclerosis. Nature 502.

Dickendesher TL, Baldwin KT, Mironova YA, Koriyama Y, Raiker SJ, Askew KL, Wood A, Geoffroy CG, Zheng B, Liepmann CD, Katagiri Y, Benowitz LI, Geller HM, Giger RJ (2012) NgR1 and NgR3 are receptors for chondroitin sulfate proteoglycans. Nat Neurosci 15.

Dombrowski Y et al. (2017) Regulatory T cells directly promote myelin regeneration in the central nervous system. Nat Neurosci:1–10.

Duncan ID, Brower A, Kondo Y, Curlee JF, Schultz RD (2009) Extensive remyelination of the CNS leads to functional recovery. Proc Natl Acad Sci 106:6832–6836.

Dyck S, Kataria H, Alizadeh A, Santhosh KT, Lang B, Silver J, Karimi-Abdolrezaee S (2018) Perturbing chondroitin sulfate proteoglycan signaling through LAR and PTPσ receptors promotes a beneficial inflammatory response following spinal cord injury. J Neuroinflammation 15.

Dyck SM, Karimi-Abdolrezaee S (2015) Chondroitin sulfate proteoglycans: Key modulators in the developing and pathologic central nervous system. Exp Neurol 269:169–187.

Emery B (2010) Regulation of Oligodendrocyte Differentiation and Myelination. Science 330:779–782.

Engel J, Furthmayr H (1987) Electron microscopy and other physical methods for the characterization of extracellular matrix components: Laminin, fibronectin, collagen IV, collagen VI, and proteoglycans. Methods Enzymol 145:3–78.

Eyermann C, Czaplinski K, Colognato H (2012) Dystroglycan promotes filopodial formation and process branching in differentiating oligodendroglia. J Neurochem 120:928–947.

Feliu A, del Río BI, Carrillo-Salinas FJ, Hernández-Torres G, Mestre L, Puente N, Ortega-Gutiérrez S, López-Rodríguez ML, Grandes P, Mecha M, Guaza C (2017) 2-Arachidonoylglycerol Reduces Proteoglycans and Enhances Remyelination in a Progressive Model of Demyelination. J Neurosci 37:2900–2916.

Ferent J, Zimmer C, Durbec P, Ruat M, Traiffort E (2013) Sonic Hedgehog Signaling Is a Positive Oligodendrocyte Regulator during Demyelination. J Neurosci 33:1759–1772.

Fernandez-Botran R, Yan J, Justus DE (1999) Binding of interferon-γ by glycosaminoglycans: a strategy for localization and/or inhibition of its activity. Cytokine 11:313–325.

Fitzner D, Schneider A, Kippert A, Mö Bius W, Willig KI, Hell SW, Bunt G, Gaus K, Simons M (2006) Myelin basic protein-dependent plasma membrane reorganization in the formation of myelin. EMBO J 25:5037–5048.

Foote AK, Blakemore WF (2005) Inflammation stimulates remyelination in areas of chronic demyelination. Brain 128:528–539.

Fox MA, Afshari FS, Alexander JK, Colello RJ, Fuss B (2006) Growth conelike sensorimotor structures are characteristic features of postmigratory, premyelinating oligodendrocytes. Glia 53:563–566.

Frevert CW, Kinsella MG, Vathanaprida C, Goodman RB, Baskin DG, Proudfoot A, Wells TNC, Wight TN, Martin TR (2003) Binding of Interleukin-8 to Heparan Sulfate and Chondroitin Sulfate in Lung Tissue. Am J Respir Cell Mol Biol 28:464–472.

Fricker FR, Lago N, Balarajah S, Tsantoulas C, Tanna S, Zhu N, Fageiry SK, Jenkins M, Garratt AN, Birchmeier C, Bennett DLH (2011) Axonally Derived Neuregulin-1 Is Required for Remyelination and Regeneration after Nerve Injury in Adulthood. J Neurosci 31:3225–3233.

Frost EE, Buttery PC, Milner R, Ffrench-Constant C (1999) Integrins mediate a neuronal survival signal for oligodendrocytes. Curr Biol 9:1251–1254.

Frost EE, Nielsen JA, Le TQ, Armstrong RC (2003) PDGF and FGF2 regulate oligodendrocyte progenitor responses to demyelination. J Neurobiol 54:457–472.

Fuerer C, Habib SJ, Nusse R (2010) A Study on the Interactions Between Heparan Sulfate Proteoglycans and Wnt Proteins. Dev Dyn 239:184–190.

Furusho M, Dupree JL, Nave K-A, Bansal R (2012) Fibroblast growth factor receptor signaling in oligodendrocytes regulates myelin sheath thickness. J Neurosci 32:6631–6641.

Galtrey CM, Fawcett JW (2007) The role of chondroitin sulfate proteoglycans in regeneration and plasticity in the central nervous system. Brain Res Rev 54:1–18.

Galvin J, Eyermann C, Colognato H (2010) Dystroglycan modulates the ability of insulin-like growth factor-1 to promote oligodendrocyte differentiation. J Neurosci Res 88:3295–3307.

Garay L, Tüngler V, Deniselle MCG, Lima A, Roig P, De Nicola AF (2011) Progesterone attenuates demyelination and microglial reaction in the lysolecithin-injured spinal cord. Neuroscience 192:588–597.

Garbern JY, Yool DA, Moore GJ, Wilds IB, Faulk MW, Klugmann M, Nave K-A, Sistermans EA, Van Der Knaap MS, Bird TD, Shy ME, Kamholz JA, Griffiths IR (2002) Patients lacking the major CNS myelin protein, proteolipid protein 1, develop length-dependent axonal degeneration in the absence of demyelination and inflammation. Brain 125:551–561.

Garnier P, Gibbs R V, Rider CC (2003) A role for chondroitin sulphate B in the activity of interleukin 12 in stimulating γ-interferon secretion. Immunol Lett 85:53–58.

Garwood J, Garcion E, Dobbertin A, Heck N, Calco V, Ffrench-Constant C, Faissner A (2004) The extracellular matrix glycoprotein Tenascin-C is expressed by oligodendrocyte precursor cells and required for the regulation of maturation rate, survival and responsiveness to platelet-derived growth factor. Eur J Neurosci 20:2524–2540.

Gato A, Martinez ML, Tudela C, Alonso I, Moro JA, Formoso MA, Ferguson MWJ, Martínez-Alvarez C (2002) TGF-b3-Induced Chondroitin Sulphate Proteoglycan Mediates Palatal Shelf Adhesion. Dev Biol 250:393–405.

Gautier HOB, Evans KA, Volbracht K, James R, Sitnikov S, Lundgaard I, James F, Lao-Peregrin C, Reynolds R, Franklin RJM, Káradóttir RT (2015) Neuronal activity regulates remyelination via glutamate signalling to oligodendrocyte progenitors. Nat Commun 6.

Gensert JM, Goldman JE (1997) Endogenous Progenitors Remyelinate Demyelinated Axons in the Adult CNS. Neuron 19:197–203.

George EL, Georges-Labouesse E, Patel-King RS, Rayburn H, Hynes RO (1993) Defects in mesoderm, neural tube and vascular development in mouse embryos lacking fibronectin. Development 119:1079–1091.

Gibson EM, Purger D, Mount CW, Goldstein AK, Lin GL, Wood LS, Inema I, Miller SE, Bieri G, Zuchero JB, Barres BA, Woo PJ, Vogel H, Monje M (2014) Neuronal Activity Promotes Oligodendrogenesis and Adaptive Myelination in the Mammalian Brain. Science 344.

Gil JE, Woo DH, Shim JH, Kim SE, You HJ, Park SH, Paek SH, Kim SK, Kim JH (2009) Vitronectin promotes oligodendrocyte differentiation during neurogenesis of human embryonic stem cells. FEBS Lett 583:561–567.

Gonzalez GA, Hofer MP, Syed YA, Amaral AI, Rundle J, Rahman S, Zhao C, Kotter MRN (2016) Tamoxifen accelerates the repair of demyelinated lesions in the central nervous system. Sci Rep 6.

Gordon MK, Hahn RA (2010) Collagens. Cell Tissue Res 339:247–257.

Green AJ, Gelfand JM, Cree BA, Bevan C, Boscardin WJ, Mei F, Inman J, Arnow S, Devereux M, Abounasr A, Nobuta H, Zhu A, Friessen M, Gerona R, Büdingen HC Von, Henry RG, Hauser SL, Chan JR (2017) Clemastine fumarate as a remyelinating therapy for multiple sclerosis (ReBUILD): a randomised, controlled, double-blind, crossover trial. Lancet.

Gregg C, Shikar V, Larsen PH, Mak G, Chojnacki A, Yong VW, Weiss S (2007) White Matter Plasticity and Enhanced Remyelination in the Maternal CNS. J Neurosci 27:1812–1823.

Griffiths I, Klugmann M, Anderson T, Yool D, Thomson C, Schwab MH, Schneider A, Zimmermann F, McCulloch M, Nadon N, Nave K-A (1998) Axonal Swellings and

Degeneration in Mice Lacking the Major Proteolipid of Myelin. Science 280:1610–1613.

Gutiérrez J, Brandan E (2010) A novel mechanism of sequestering fibroblast growth factor 2 by glypican in lipid rafts, allowing skeletal muscle differentiation. Mol Cell Biol 30:1634–1649.

Häcker U, Hybakken K, Perrimon N (2005) Heparan sulfate proteoglycans: the sweet side of development. Nat Rev Mol Cell Biol 6:530–541.

Haist V, Ulrich R, Kalkuhl A, Deschl U, Baumgärtner W (2012) Distinct spatio-temporal extracellular matrix accumulation within demyelinated spinal cord lesions in Theiler's murine encephalomyelitis. Brain Pathol 22:188–204.

Hallmann R, Zhang X, Di Russo J, Li L, Song J, Hannocks MJ, Sorokin L (2015) The regulation of immune cell trafficking by the extracellular matrix. Curr Opin Cell Biol 36:54–61.

Hammond TR, Gadea A, Dupree JL, Kerninon C, Nait-Oumesmar B, Aguirre A, Gallo V (2014) Astrocyte-Derived Endothelin-1 Inhibits Remyelination through Notch Activation. Neuron 81:588–602.

Hara M, Kobayakawa K, Ohkawa Y, Kumamaru H, Yokota K, Saito T, Kijima K, Yoshizaki S, Harimaya K, Nakashima Y, Okada S (2017) Interaction of reactive astrocytes with type I collagen induces astrocytic scar formation through the integrin-N-cadherin pathway after spinal cord injury. Nat Med.

Hernandez M, Patzig J, Mayoral SR, Costa KD, Chan JR, Casaccia P (2016) Mechanostimulation Promotes Nuclear and Epigenetic Changes in Oligodendrocytes. J Neurosci 36:806–813.

Hippensteel JA, Anderson BJ, Orfila JE, McMurtry SA, Dietz RM, Su G, Ford JA, Oshima K, Yang Y, Zhang F, Han X, Yu Y, Liu J, Linhardt RJ, Meyer NJ, Herson PS, Schmidt EP (2019) Circulating heparan sulfate fragments mediate septic cognitive dysfunction. J Clin Invest.

Hirose J, Kawashima H, Swope Willis M, Springer TA, Hasegawa H, Yoshie O, Miyasaka M (2002) Chondroitin sulfate B exerts its inhibitory effect on secondary lymphoid tissue chemokine (SLC) by binding to the C-terminus of SLC. Biochim Biophys Acta - Gen Subj 1571:219–224.

Hirose J, Kawashima H, Yoshie O, Tashiro K, Miyasaka M (2001) Versican interacts with chemokines and modulates cellular responses. J Biol Chem 276:5228–5234.

Hsieh J, Aimone JB, Kaspar BK, Kuwabara T, Nakashima K, Gage FH (2004) IGF-I instructs multipotent adult neural progenitor cells to become oligodendrocytes. J Cell Biol 164:111–122.

Huang L, Wu Z-B, ZhuGe Q, Zheng W, Shao B, Wang B, Sun F, Jin K (2014) Glial Scar Formation Occurs in the Human Brain after Ischemic Stroke. Int J Med Sci 11:344–348.

Hubler Z et al. (2018) Accumulation of 8,9-unsaturated sterols drives oligodendrocyte formation and remyelination. Nature.

Hughes EG, Kang SH, Fukaya M, Bergles DE (2013a) Oligodendrocyte progenitors balance growth with self-repulsion to achieve homeostasis in the adult brain. Nat Neurosci 16:668–676.

Hughes EG, Kang SH, Fukaya M, Bergles DE (2013b) Oligodendrocyte progenitors balance growth with self-repulsion to achieve homeostasis in the adult brain. Nat Neurosci 16:668–676.

Hurt-Camejo E, Rosengren B, Sartipy P, Elfsberg K, Camejo G, Svensson L (1999) CD44, a cell surface chondroitin sulfate proteoglycan, mediates binding of interferon-gamma and some of its biological effects on human vascular smooth muscle cells. J Biol Chem 274:18957–18964.

Ibanez C, Shields SA, El-Etr M, Baulieu E-E, Schumacher M, Franklin RJM (2004) Systemic progesterone administration results in a partial reversal of the age-associated decline in CNS remyelination following toxin-induced demyelination in male rats. Neuropathol Appl Neurobiol:80–89.

Ikegami T, Nakamura M, Yamane J, Katoh H, Okada S, Iwanami A, Watanabe K, Ishii K, Kato F, Fujita H, Takahashi T, Okano HJ, Toyama Y, Okano H (2005) Chondroitinase ABC combined with neural stem/progenitor cell transplantation enhances graft cell migration and outgrowth of growth-associated protein-43-positive fibers after rat spinal cord injury. Eur J Neurosci 22:3036–3046.

Iozzo R V (2005) Basement membrane proteoglycans: from cellar to ceiling. Nat Rev Mol Cell Biol 6:646–656.

Irvine KA, Blakemore WF (2008) Remyelination protects axons from demyelination-associated axon degeneration. Brain 131:1464–1477.

Itoh N, Kim R, Peng M, DiFilippo E, Johnsonbaugh H, Mackenzie-Graham A, Voskuhl RR (2016) Bedside to bench to bedside research: Estrogen receptor beta ligand as a candidate neuroprotective treatment for multiple sclerosis. J Neuroimmunol 304:63–71.

Jagielska A, Lowe AL, Makhija E, Wroblewska L, Guck J, Franklin RJM, Shivashankar G V, Van Vliet KJ (2017) Mechanical Strain Promotes Oligodendrocyte Differentiation by Global Changes of Gene Expression. Front Cell Neurosci 11:1–16.

Jakovcevski I, Zecevic N (2005) Olig Transcription Factors Are Expressed in Oligodendrocyte and Neuronal Cells in Human Fetal CNS. J Neurosci 25:10064–10073.

James ND, Shea J, Muir EM, Verhaagen J, Schneider BL, Bradbury EJ (2015) Chondroitinase gene therapy improves upper limb function following cervical contusion injury. Exp Neurol 271:131–135.

Jarjour AA, Kennedy TE (2004) Oligodendrocyte Precursors on the Move: Mechanisms Directing Migration. Neurosci 10:99–105.

Jiang D, Liang J, Fan J, Yu S, Chen S, Luo Y, Prestwich GD, Mascarenhas MM, Garg HG, Quinn DA, Homer RJ, Goldstein DR, Bucala R, Lee PJ, Medzhitov R, Noble PW (2005) Regulation of lung injury and repair by Toll-like receptors and hyaluronan. Nat Med 11:1173–1179.

John GR, Shankar SL, Shafit-Zagardo B, Massimi A, Lee SC, Raine CS, Brosnan CF (2002) Multiple sclerosis: Re-expression of a developmental pathway that restricts oligodendrocyte maturation. Nat Med 8:1115–1121.

Jones LL, Margolis RU, Tuszynski MH (2003) The chondroitin sulfate proteoglycans neurocan, brevican, phosphacan, and versican are differentially regulated following spinal cord injury. Exp Neurol:399–411.

Jones LL, Yamaguchi Y, Stallcup WB, Tuszynski MH (2002) NG2 Is a Major Chondroitin Sulfate Proteoglycan Produced after Spinal Cord Injury and Is Expressed by Macrophages and Oligodendrocyte Progenitors. J Neurosci 22:2792–2803.

Jones E V., Bouvier DS (2014) Astrocyte-secreted matricellular proteins in CNS remodelling during development and disease. Neural Plast 2014.

Jurynczyk M, Jurewicz A, Bielecki B, Raine CS, Selmaj K (2005) Inhibition of Notch signaling enhances tissue repair in an animal model of multiple sclerosis. J Neuroimmunol 170:3–10.

Kadler KE, Baldock C, Bella J, Boot-Handford RP (2007) Collagens at a glance. J Cell Sci 120:1955–1958.

Kaidonis X, Liaw WC, Roberts AD, Ly M, Anson D, Byers S (2009) Gene silencing of EXTL2 and EXTL3 as a substrate deprivation therapy for heparan sulphate storing mucopolysaccharidoses. Eur J Hum Genet 18:194–199.

Kang I, Harten IA, Chang MY, Braun KR, Sheih A, Nivison MP, Johnson PY, Workman GA, Kaber G, Evanko SP, Chan CK, Merrilees MJ, Ziegler SF, Kinsella MG, Frevert CW, Wight TN (2017) Versican Deficiency Significantly Reduces Lung Inflammatory Response Induced by Polyinosine-Polycytidylic Acid Stimulation. J Biol Chem 292:51–63.

Kaplan ED, Holbrook KA (1994) Dynamic expression patterns of tenascin, proteoglycans, and cell adhesion molecules during human hair follicle morphogenesis. Dev Dyn 199:141–155.

Karimi-Abdolrezaee S, Schut D, Wang J, Fehlings MG, Zheng JC (2012) Chondroitinase and Growth Factors Enhance Activation and Oligodendrocyte Differentiation of Endogenous Neural Precursor Cells after Spinal Cord Injury. PLoS ONE 7.

Karumbaiah L, Anand S, Thazhath R, Zhong Y, Mckeon RJ, Bellamkonda R V. (2011) Targeted downregulation of N-acetylgalactosamine 4-sulfate 6-O-sulfotransferase significantly mitigates chondroitin sulfate proteoglycan-mediated inhibition. Glia 59:981–996.

Karus M, Ulc A, Ehrlich M, Czopka T, Hennen E, Fischer J, Mizhorova M, Qamar N, Brüstle O, Faissner A (2016) Regulation of oligodendrocyte precursor maintenance by chondroitin sulphate glycosaminoglycans. Glia 64:270–286.

Kassmann CM, Lappe-Siefke C, Baes M, Brügger B, Mildner A, Werner HB, Natt O, Michaelis T, Prinz M, Frahm J, Nave K-A (2007) Axonal loss and neuroinflammation caused by peroxisome-deficient oligodendrocytes. Nat Genet 39:969–976.

Kataria H, Alizadeh A, Shahriary GM, Saboktakin Rizi S, Henrie R, Santhosh KT, Thliveris JA, Karimi-Abdolrezaee S (2018) Neuregulin-1 promotes remyelination and fosters a pro-regenerative inflammatory response in focal demyelinating lesions of the spinal cord. Glia

66:538–561.

Katta K, Imran T, Busse-Wicher M, Grønning M, Czajkowski S, Kusche-Gullberg M (2015) Reduced expression of EXTL2, a member of the exostosin (EXT) family of glycosyltransferases, in human embryonic kidney 293 cells results in longer heparan sulfate chains. J Biol Chem 290:13168–13177.

Kawashima H, Atarashi K, Hirose M, Hirose J, Yamada S, Sugahara K, Miyasaka M (2002) Oversulfated chondroitin/dermatan sulfates containing GlcAbeta1/IdoAalpha1-3GalNAc(4,6-O-disulfate) interact with L- and P-selectin and chemokines. J Biol Chem 277:12921–12930.

Kawashima H, Hirose M, Hirose J, Nagakubo D, Plaas AH, Miyasaka M (2000) Binding of a large chondroitin sulfate/dermatan sulfate proteoglycan, versican, to L-selectin, P-selectin, and CD44. J Biol Chem 275:35448–35456.

Keough MB, Jensen SK, Yong VW (2015) Experimental demyelination and remyelination of murine spinal cord by focal injection of lysolecithin. JoVE:e52679.

Keough MB, Rogers JA, Zhang P, Jensen SK, Stephenson EL, Chen T, Hurlbert MG, Lau LW, Rawji KS, Plemel JR, Koch M, Ling C-C, Yong VW (2016) An inhibitor of chondroitin sulfate proteoglycan synthesis promotes central nervous system remyelination. Nat Commun 7.

Kessaris N, Fogarty M, Iannarelli P, Grist M, Wegner M, Richardson WD (2006) Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. Nat Neurosci 9:173–179.

Khaing ZZ, Milman BD, Vanscoy JE, Seidlits SK, Grill RJ, Schmidt CE (2011) High molecular weight hyaluronic acid limits astrocye activation and scar formation after spinal cord injury. J Neural Eng 8.

Khalaj AJ, Yoon J, Nakai J, Winchester Z, Moore SM, Yoo T, Martinez-Torres L, Kumar S, Itoh N, Tiwari-Woodruff SK (2013) Estrogen receptor (ER) β expression in oligodendrocytes is required for attenuation of clinical disease by an ERβ ligand. Proc Natl Acad Sci 110:19125–19130.

Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG (2009) Identification of Two Distinct Macrophage Subsets with Divergent Effects Causing either Neurotoxicity or Regeneration in the Injured Mouse Spinal Cord. J Neurosci 29:13435–13444.

Kim S, Takahashi H, Lin WW, Descargues P, Grivennikov S, Kim Y, Luo JL, Karin M (2009) Carcinoma-produced factors activate myeloid cells through TLR2 to stimulate metastasis. Nature 457:102–106.

Kipp M, Clarner T, Dang J, Copray S, Beyer C (2009) The cuprizone animal model: new insights into an old story. Acta Neuropathol 118:723–736.

Kitagawa H, Shimakawa H, Sugahara K (1999) The Tumor Suppressor EXT-like Gene EXTL2 Encodes an α1,4-N-Acetylhexosaminyltransferase That Transfers N-Acetylgalactosamine and N-Acetylglucosamine to the Common Glycosaminoglycan-Protein Linkage Region. J

Biol Chem 274:13933–13937.

Kjellén L, Lindahl U (1991) Proteoglycans: Structures and Interactions. Annu Rev Biochem 60:443–475.

Klapka N, Müller HW (2006) Collagen Matrix in Spinal Cord Injury. J Neurotrauma 234:422–435.

Kotter MRN, Setzu A, Sim FJ, Van Rooijen N, Franklin RJM (2001) Macrophage depletion impairs oligodendrocyte remyelination following lysolecithin-induced demyelination. Glia 35:204–212.

Krityakiarana W, Espinosa-Jeffrey A, Ghiani CA, Zhao PM, Topaldjikian N, Gomez-Pinilla F, Yamaguchi M, Kotchabhakdi N, De Vellis J (2010) Voluntary Exercise Increases Oligodendrogenesis in Spinal Cord. Int J Neurosci 120:280–290.

Kuboyama K, Tanga N, Suzuki R, Fujikawa A, Noda M (2017) Protamine neutralizes chondroitin sulfate proteoglycan-mediated inhibition of oligodendrocyte differentiation. PLoS ONE 12.

Kucharova K, Chang Y, Boor A, Yong VW, Stallcup WB (2011) Reduced inflammation accompanies diminished myelin damage and repair in the NG2 null mouse spinal cord. J Neuroinflammation 8.

Kucharova K, Stallcup WB (2015) NG2-proteoglycan-dependent contributions of oligodendrocyte progenitors and myeloid cells to myelin damage and repair. J Neuroinflammation 12.

Kuhlmann T, Miron VE, Cuo Q, Wegner C, Antel JP, Brück W (2008) Differentiation block of oligodendroglial progenitor cells as a cause for remyelination failure in chronic multiple sclerosis. Brain 131:1749–1758.

Kuipers HF, Rieck M, Gurevich I, Nagy N, Butte MJ, Negrin RS, Wight TN, Steinman L, Bollyky PL (2016) Hyaluronan synthesis is necessary for autoreactive T-cell trafficking, activation, and Th1 polarization. Proc Natl Acad Sci 113:1339–1344.

Kumar S, Patel R, Moore S, Crawford DK, Suwanna N, Mangiardi M, Tiwari-Woodruff SK (2013) Estrogen receptor β ligand therapy activates PI3K/Akt/mTOR signaling in oligodendrocytes and promotes remyelination in a mouse model of multiple sclerosis. Neurobiol Dis 56:131–144.

Kwok JCF, Dick G, Wang D, Fawcett JW (2011) Extracellular matrix and perineuronal nets in CNS repair. Dev Neurobiol 71:1073–1089.

Laabs TL, Carulli D, Geller HM, Fawcett JW (2005) Chondroitin sulfate proteoglycans in neural development and regeneration. Curr Opin Neurobiol 15:116–120.

Lappe-Siefke C, Goebbels S, Gravel M, Nicksch E, Lee J, Braun PE, Griffiths IR, Nave K-A (2003) Disruption of Cnp1 uncouples oligodendroglial functions in axonal support and myelination. Nat Genet 33:366–374.

Larsen PH, Wells JE, Stallcup WB, Opdenakker G, Yong VW (2003) Matrix Metalloproteinase-

9 Facilitates Remyelination in Part by Processing the Inhibitory NG2 Proteoglycan. J Neurosci 23:11127–11135.

Lassmann H, Brück W, Lucchinetti CF, Rodriguez M (1997) Remyelination in multiple sclerosis. Mult Scler 3:133–136.

Lau LW, Cua R, Keough MB, Haylock-Jacobs S, Yong VW (2013) Pathophysiology of the brain extracellular matrix: a new target for remyelination. Nat Rev Neurosci 14:722–729.

Lau LW, Keough MB, Haylock-Jacobs S, Cua R, Döring A, Sloka S, Stirling DP, Rivest S, Yong VW (2012) Chondroitin sulfate proteoglycans in demyelinated lesions impair remyelination. Ann Neurol 72:419–432.

Lee KK, de Repentigny Y, Saulnier R, Rippstein P, Macklin WB, Kothary R (2006) Dominant-Negative b1 Integrin Mice Have Region-Specific Myelin Defects Accompanied by Alterations in MAPK Activity. Glia 53:836–844.

Lee Y, Morrison BM, Li Y, Lengacher S, Farah MH, Hoffman PN, Liu Y, Tsingalia A, Jin L, Zhang P-W, Pellerin L, Magistretti PJ, Rothstein JD (2012) Oligodendroglia metabolically support axons and contribute to neurodegeneration. Nature 487:443–448.

Leiton C V, Aranmolate A, Eyermann C, Menezes MJ, Escobar-Hoyos LF, Husain S, Winder SJ, Colognato H (2015) Laminin promotes metalloproteinase-mediated dystroglycan processing to regulate oligodendrocyte progenitor cell proliferation. J Neurochem 135:522–538.

Lemarchant S, Wojciechowski S, Vivien D, Koistinaho J (2017) ADAMTS-4 in central nervous system pathologies. J Neurosci Res 95:1703–1711.

Lentferink DH, Jongsma JM, Werkman I, Baron W (2018) Grey matter OPCs are less mature and less sensitive to IFNγ than white matter OPCs: consequences for remyelination. Sci Rep 8.

Liesi P, Kaakkola S, Dahl D, Vaheri A (1984) Laminin is induced in astrocytes of adult brain by injury. EMBO J 3:683–686.

Liesi P, Kauppila T (2002) Induction of Type IV Collagen and Other Basement-Membrane-Associated Proteins after Spinal Cord Injury of the Adult Rat May Participate in Formation of the Glial Scar. Exp Neurol 173:31–45.

Lin R, Rosahl TW, Whiting PJ, Fawcett JW, Kwok JCF (2011) 6-Sulphated Chondroitins Have a Positive Influence on Axonal Regeneration. PLoS ONE 6.

Lortat-Jacob H, Garrone P, Banchereau J, Grimaud J-A (1997) Human interleukin 4 is a glycosaminoglycan-binding protein. Cytokine 9:101–105.

Lu Z, Kipnis J (2010) Thrombospondin 1--a key astrocyte-derived neurogenic factor. FASEB J 24:1925–1934.

Ludwin SK, Maitland M (1984) Long-term remyelination fails to reconstitute normal thickness of central myelin sheaths. J Neurol Sci 64:193–198.

Lundgaard I, Luzhynskaya A, Stockley JH, Wang Z, Evans KA, Swire M, Volbracht K, Gautier HOB, Franklin RJM, Ffrench-Constant C, Attwell D, Káradóttir RT (2013) Neuregulin and

BDNF Induce a Switch to NMDA Receptor-Dependent Myelination by Oligodendrocytes. PLoS Biol 11.

Luo F, Tran AP, Xin L, Sanapala C, Lang BT, Silver J, Yang Y (2018) Modulation of proteoglycan receptor PTPσ enhances MMP-2 activity to promote recovery from multiple sclerosis. Nat Commun 9:4126.

Mann AP, Scodeller P, Hussain S, Joo J, Kwon E, Braun GB, Mölder T, She Z-G, Kotamraju VR, Ranscht B, Krajewski S, Teesalu T, Bhatia S, Sailor MJ, Ruoslahti E (2016) A peptide for targeted, systemic delivery of imaging and therapeutic compounds into acute brain injuries. Nat Commun 7.

Mannara F, Valente T, Saura J, Graus F, Saiz A, Moreno B (2012) Passive Experimental Autoimmune Encephalomyelitis in C57BL/6 with MOG: Evidence of Involvement of B Cells. PLoS ONE 7:e52361.

Marella M, Ouyang J, Zombeck J, Zhao C, Huang L, Connor RJ, Phan KB, Jorge MC, Printz MA, Paladini RD, Gelb AB, Huang Z, Frost GI, Sugarman BJ, Steinman L, Wei G, Shepard HM, Maneval DC, Lapinskas PJ (2017) PH20 is not expressed in murine CNS and oligodendrocyte precursor cells. Ann Clin Transl Neurol 4:191–211.

Masaki T, Matsumura K, Saito F, Yamada H, Higuchi S, Kamakura K, Yorifuji H, Shimizu T (2003) Association of dystroglycan and laminin-2 coexpression with myelinogenesis in peripheral nerves. Med Electron Microsc 36:221–239.

Mason JL, Suzuki K, Chaplin DD, Matsushima GK (2001) Interleukin-1 Promotes Repair of the CNS. J Neurosci 21:7046–7052.

Massey JM, Hubscher CH, Wagoner MR, Decker JA, Amps J, Silver J, Onifer SM (2006) Chondroitinase ABC Digestion of the Perineuronal Net Promotes Functional Collateral Sprouting in the Cuneate Nucleus after Cervical Spinal Cord Injury. J Neurosci 26:4406–4414.

Matthews MA, St Onge MF, Faciane CL, Gelderd JB (1979) Axon sprouting into segments of rat spinal cord adjacent to the site of a previous transection. Neuropathol Appl Neurobiol 5:181–196.

Mckee CM, Penno MB, Cowman M, Burdick MD, Strieter RM, Bao C, Noble PW (1996) Hyaluronan (HA) fragments induce chemokine gene expression in alveolar macrophages: The role of HA size and CD44. J Clin Invest 98:2403–2413.

Mei F, Fancy SPJ, Shen Y-AA, Niu J, Zhao C, Presley B, Miao E, Lee S, Mayoral SR, Redmond SA, Etxeberria A, Xiao L, Franklin RJM, Green AJ, Hauser SL, Chan JR (2014) Micropillar arrays as a high-throughput screening platform for therapeutics in multiple sclerosis. Nat Med 20:954–960.

Mei F, Lehmann-Horn K, Shen Y-AA, Rankin KA, Stebbins KJ, Lorrain DS, Pekarek K, Sagan SA, Xiao L, Teuscher C, von Büdingen HC, Wess J, Josh Lawrence J, Green AJ, Fancy SPJ, Zamvil SS, Chan JR (2016a) Accelerated remyelination during inflammatory demyelination prevents axonal loss and improves functional recovery. Elife 5:1–21.

Mei F, Mayoral SR, Nobuta H, Wang F, Desponts C, Lorrain DS, Xiao L, Green JA, Rowitch

DH, Whistler J, Chan JR, Green AJ, Rowitch DH, Whistler J, Chan JR (2016b) Identification of the Kappa-Opioid Receptor as a Therapeutic Target for Oligodendrocyte Remyelination. J Neurosci 36:7925–7935.

Melgar-Lesmes P, Garcia-Polite F, Del-Rey-Puech P, Rosas E, Dreyfuss JL, Montell E, Vergés J, Edelman ER, Balcells M (2016) Treatment with chondroitin sulfate to modulate inflammation and atherogenesis in obesity. Atherosclerosis 245:82–87.

Mellion M, Edwards KR, Hupperts R, Drulović J, Montalban X, Hartung H-P, Brochet B, Calabresi PA, Rudick R, Ibrahim A, Zhang Y, Xu L, Cadavid D (2017) Efficacy Results from the Phase 2b SYNERGY Study: Treatment of Disabling Multiple Sclerosis with the Anti-LINGO-1 Monoclonal Antibody Opicinumab (S33.004). Neurology 88.

Merkler D, Ernsting T, Kerschensteiner M, Brück W, Stadelmann C (2006) A new focal EAE model of cortical demyelination: Multiple sclerosis-like lesions with rapid resolution of inflammation and extensive remyelination. Brain 129:1972–1983.

Mi S et al. (2009) Promotion of central nervous system remyelination by induced differentiation of oligodendrocyte precursor cells. Ann Neurol 65:304–315.

Mi S, Pepinsky RB, Cadavid D (2013) Blocking LINGO-1 as a Therapy to Promote CNS Repair: From Concept to the Clinic. CNS Drugs 27:493–503.

Michailov G V, Sereda MW, Brinkmann BG, Fischer TM, Haug B, Birchmeier C, Role L, Lai C, Schwab MH, Nave K-A (2004) Axonal neuregulin-1 regulates myelin sheath thickness. Science 304:700–703.

Micu I, Plemel JR, Caprariello A V, Nave K-A, Stys PK (2017) Axo-myelinic neurotransmission: a novel mode of cell signalling in the central nervous system. Nat Rev Neurosci 19:49–57.

Milner R, Crocker SJ, Hung S, Wang X, Frausto RF, del Zoppo GJ (2007) Fibronectin-and Vitronectin-Induced Microglial Activation and Matrix Metalloproteinase-9 Expression Is Mediated by Integrins a5b1 and avb5. J Immunol 178:8158–8167.

Milner R, Edwards G, Streuli C, Ffrench-Constant C (1996) A Role in Migration for the alpha-v-beta-1 Integrin Expressed on Oligodendrocyte Precursors. J Neurosci 16:7240–7252.

Minor KH, Bournat JC, Toscano N, Giger RJ, Davies SJA (2011) Decorin, erythroblastic leukaemia viral oncogene homologue B4 and signal transducer and activator of transcription 3 regulation of semaphorin 3A in central nervous system scar tissue. Brain 134:1140–1155.

Minor KH, Tang X, Kahrilas G, Archibald SJ, Davies JE, Davies SJA (2008) Decorin promotes robust axon growth on inhibitory CSPGs and myelin via a direct effect on neurons. Neurobiol Dis 32:88–95.

Miron VE, Ludwin SK, Darlington PJ, Jarjour AA, Soliven B, Kennedy TE, Antel JP (2010) Fingolimod (FTY720) Enhances Remyelination Following Demyelination of Organotypic Cerebellar Slices. Am J Pathol 176:2682–2694.

Miyamoto N, Maki T, Shindo A, Liang AC, Maeda M, Egawa N, Itoh K, Lo EK, Lok J, Ihara M,

Arai K (2015) Astrocytes Promote Oligodendrogenesis after White Matter Damage via Brain-Derived Neurotrophic Factor. J Neurosci 35:14002–14008.

Miyata S, Komatsu Y, Yoshimura Y, Taya C, Kitagawa H (2012) Persistent cortical plasticity by upregulation of chondroitin 6-sulfation. Nat Neurosci 15:414–422.

Mogha A, D'rozario M, Monk KR (2016) G Protein-Coupled Receptors in Myelinating Glia. Trends Pharmacol Sci 37:977–987.

Mundhenke C, Meyer K, Drew S, Friedl A (2002) Heparan sulfate proteoglycans as regulators of fibroblast growth factor-2 receptor binding in breast carcinomas. Am J Pathol 160:185–194.

Murtie JC, Zhou Y-X, Le TQ, Vana AC, Armstrong RC (2005) PDGF and FGF2 pathways regulate distinct oligodendrocyte lineage responses in experimental demyelination with spontaneous remyelination. Neurobiol Dis 19:171–182.

Muto J, Yamasaki K, Taylor KR, Gallo RL (2009) Engagement of CD44 by hyaluronan suppresses TLR4 signaling and the septic response to LPS. Mol Immunol 47:449–456.

Nadanaka S, Kagiyama S, Kitagawa H (2013a) Roles of EXTL2, a member of the EXT family of tumour suppressors, in liver injury and regeneration processes. Biochem J 454:133–145.

Nadanaka S, Kitagawa H (2014) EXTL2 controls liver regeneration and aortic calcification through xylose kinase-dependent regulation of glycosaminoglycan biosynthesis. Matrix Biol 35:18–24.

Nadanaka S, Zhou S, Kagiyama S, Shoji N, Sugahara K, Sugihara K, Asano M, Kitagawa H (2013b) EXTL2, a member of the EXT family of tumor suppressors, controls glycosaminoglycan biosynthesis in a xylose kinase-dependent manner. J Biol Chem 288:9321–9333.

Najm FJ et al. (2015) Drug-based modulation of endogenous stem cells promotes functional remyelination in vivo. Nature 522:216–220.

Nash B, Thomson CE, Linington C, Arthur AT, McClure JD, McBride MW, Barnett SC (2011) Functional Duality of Astrocytes in Myelination. J Neurosci 31:13028–13038.

Necas J, Bartosikova L, Brauner P, Kolar J (2008) Hyaluronic acid (hyaluronan): a review. Vet Med 53:397–411.

Nishiyama A, Lin X-H, Giese N, Heldin C-H, Stallcup WB (1996) Interaction between NG2 proteoglycan and PDGFalpha-receptor on O2A progenitor cells is required for optimal response to PDGF. J Neurosci Res 43:315–330.

Nissinen L, Kähäri V-M (2014) Matrix metalloproteinases in inflammation. Biochim Biophys Acta 1840:2571–2580.

Noble PW (2002) Hyaluronan and its catabolic products in tissue injury and repair. Matrix Biol 21:25–29.

Novak U, Kaye AH (2000) Extracellular matrix and the brain: components and function. J Clin Neurosci 7:280–290.

Nurcombe V, Ford MD, Wildschut JA, Bartlett PF (1993) Developmental regulation of neural response to FGF-1 and FGF-2 by heparan sulfate proteoglycan. Science 260:103–106.

O'Meara RW, Michalski J-P, Kothary R (2011) Integrin signaling in oligodendrocytes and its importance in CNS myelination. J Signal Transduct 2011.

O'Rourke M, Cullen CL, Auderset L, Pitman KA, Achatz D, Gasperini R, Young KM (2016) Evaluating tissue-specific recombination in a Pdgfra-CreER T2 transgenic mouse line. PLoS ONE 11:1–19.

Okada M, Miyamoto O, Shibuya S, Zhang X, Yamamoto T, Itano T (2007) Expression and role of type I collagen in a rat spinal cord contusion injury model. Neurosci Res 58:371–377.

Othman A, Frim DM, Polak P, Vujicic S, Arnason BGW, Boullerne AI (2011) Olig1 is expressed in human oligodendrocytes during maturation and regeneration. Glia 59:914–926.

Ousman SS, David S (2000) Lysophosphatidylcholine induces rapid recruitment and activation of macrophages in the adult mouse spinal cord. Glia 30:92–104.

Ozawa K, Suchanek G, Breitschopf H, Brück W, Budka H, Jellinger K, Lassmann H (1994) Patterns of oligodendroglia pathology in multiple sclerosis. Brain 117:1311–1322.

Palazuelos J, Klingener M, Raines EW, Crawford HC, Aguirre A (2015) Oligodendrocyte Regeneration and CNS Remyelination Require TACE/ADAM17. J Neurosci 35:12241–12247.

Pang Y, Zheng B, Fan L-W, Rhodes PG, Cai Z (2007) IGF-1 Protects Oligodendrocyte Progenitors Against TNFa-Induced Damage by Activation of PI3K/Akt and Interruption of the Mitochondrial Apoptotic Pathway YI. Glia 55:1416–1425.

Patrikios P, Stadelmann C, Kutzelnigg A, Rauschka H, Schmidbauer M, Laursen H, Sorensen PS, Brück W, Lucchinetti CF, Lassmann H (2006) Remyelination is extensive in a subset of multiple sclerosis patients. Brain 129:3165–3172.

Pearson CS, Mencio CP, Barber AC, Martin KR, Geller HM (2018) Identification of a critical sulfation in chondroitin that inhibits axonal regeneration. Elife 7.

Pendleton JC, Shamblott MJ, Gary DS, Belegu V, Hurtado A, Malone ML, McDonald JW (2013) Chondroitin sulfate proteoglycans inhibit oligodendrocyte myelination through PTPσ. Exp Neurol 247:113–121.

Peng X, Qiao Z, Wang Y, Li H, Xie Y, Xin M, Qiao Z, Wang Z (2019) Estrogen reverses nicotine-induced inflammation in chondrocytes via reducing the degradation of ECM. Int J Rheum Dis.

Plemel JR, Michaels NJ, Weishaupt N, Caprariello A V, Keough MB, Rogers JA, Yukseloglu A, Lim J, Patel V V., Rawji KS, Jensen SK, Teo W, Heyne B, Whitehead SN, Stys PK, Yong VW (2017) Mechanisms of lysophosphatidylcholine-induced demyelination: A primary lipid disrupting myelinopathy. Glia:1–21.

Preston M, Gong X, Su W, Matsumoto SG, Banine F, Winkler CW, Foster SC, Xing R, Struve J, Dean J, Baggenstoss B, Weigel PH, Montine TJ, Back SA, Sherman LS (2013) Digestion

products of the PH20 hyaluronidase inhibit remyelination. Ann Neurol 73:266–280.

Prineas JW, Barnard RO, Kwon EE, Sharer LR, Cho E (1993) Multiple sclerosis: Remyelination of nascent lesions. Ann Neurol 33:137–151.

Prineas JW, Connell F (1979) Remyelination In Multiple Sclerosis. Ann Neurol.

Properzi F, Carulli D, Asher RA, Muir EM, Camargo LM, Van Kuppevelt TH, Ten Dam GB, Furukawa Y, Mikami T, Sugahara K, Toida T, Geller HM, Fawcett JW (2005) Chondroitin 6-sulphate synthesis is up-regulated in injured CNS, induced by injury-related cytokines and enhanced in axon-growth inhibitory glia. Eur J Neurosci 21:378–390.

Properzi F, Fawcett JW (2004) Proteoglycans and brain repair. News Physiol Sci 19:33–38.

Properzi F, Lin R, Kwok JCF, Naidu M, Van Kuppevelt TH, Ten Dam GB, Camargo LM, Raha-Chowdhury R, Furukawa Y, Mikami T, Sugahara K, Fawcett JW (2008) Heparan sulphate proteoglycans in glia and in the normal and injured CNS: Expression of sulphotransferases and changes in sulphation. Eur J Neurosci 27:593–604.

Prydz K, Dalen KT (2000) Synthesis and sorting of proteoglycans. J Cell Sci 113:193–205.

Pu A, Stephenson EL, Yong VW (2018) The extracellular matrix: Focus on oligodendrocyte biology and targeting CSPGs for remyelination therapies. Glia 66:1809–1825.

Purnomo E, Emoto N, Nugrahaningsih DAA, Nakayama K, Yagi K, Heiden S, Nadanaka S, Kitagawa H, Hirata K-I (2013) Glycosaminoglycan overproduction in the aorta increases aortic calcification in murine chronic kidney disease. J Am Heart Assoc 2:e000405.

Pye DA, Vives RR, Turnbull JE, Hyde P, Gallagher JT (1998) Heparan sulfate oligosaccharides require 6-O-sulfation for promotion of basic fibroblast growth factor mitogenic activity. J Biol Chem 273:22936–22942.

Qin J, Sikkema AH, van der Bij K, De Jonge JC, Klappe K, Nies V, Jonker JW, Kok JW, Hoekstra D, Baron W (2017) GD1a overcomes inhibition of myelination by fibronectin via activation of protein kinase A. Implications for multiple sclerosis. J Neurosci 37:9925–9938.

Ramsden L, Rider CC (1992) Selective and differential binding of interleukin (IL)-1α, IL-1β, IL-2 and IL-6 to glycosaminoglycans. Eur J Immunol 22:3027–3031.

Redwine JM, Armstrong RC (1998) In vivo proliferation of oligodendrocyte progenitors expressing PDGFaR during early remyelination. J Neurobiol 37:413–428.

Relucio J, Menezes MJ, Miyagoe-Suzuki Y, Takeda S, Colognato H (2012) Laminin regulates postnatal oligodendrocyte production by promoting oligodendrocyte progenitor survival in the subventricular zone. Glia 60:1451–1467.

Remaud S, Ortiz FC, Perret-Jeanneret M, Aigrot M-S, Gothié J-D, Fekete C, Kvárta-Papp Z, Gereben B, Langui D, Lubetzki C, Angulo MC, Zalc B, Demeneix B (2017) Transient hypothyroidism favors oligodendrocyte generation providing functional remyelination in the adult mouse brain. Elife 6:1–20.

Rhodes KE, Fawcett JW (2004) Chondroitin sulphate proteoglycans: preventing plasticity or

protecting the CNS? J Anat 204:33–48.

Ricard-Blum S (2011) The collagen family. Cold Spring HarbPerspectBiol 3:a004978-.

Ricard-Blum S, Salza R (2014) Matricryptins and matrikines: Biologically active fragments of the extracellular matrix. Exp Dermatol 23:457–463.

Risling M, Fried K, Linda H, Carlstedt T, Cullheim S (1993) Regrowth of motor axons following spinal cord lesions: distribution of laminin and collagen in the CNS scar tissue. Brain Res Bull 30:405–414.

Rivers LE, Young KM, Rizzi M, Jamen F, Psachoulia K, Wade A, Kessaris N, Richardson WD (2008) PDGFRA/NG2 glia generate myelinating oligodendrocytes and piriform projection neurons in adult mice. Nat Neurosci 11:1392–1401.

Rogers SW, Gregori NZ, Carlson N, Gahring LC, Noble M (2001) Neuronal nicotinic acetylcholine receptor expression by O2A/oligodendrocyte progenitor cells. Glia 33:306–313.

Rolls A, Cahalon L, Bakalash S, Avidan H, Lider O, Schwartz M (2006a) A sulfated disaccharide derived from chondroitin sulfate proteoglycan protects against inflammation-associated neurodegeneration. FASEB J 20:547–549.

Rolls A, Cahalon L, Bakalash S, Avidan H, Lider O, Schwartz M (2006b) A sulfated disaccharide derived from chondroitin sulfate proteoglycan protects against inflammation-associated neurodegeneration. FASEB J 20:547–549.

Rolls A, Shechter R, London A, Segev Y, Jacob-Hirsch J, Amariglio N, Rechavi G, Schwartz M (2008) Two faces of chondroitin sulfate proteoglycan in spinal cord repair: A role in microglia/macrophage activation. PLoS Med 5:1262–1277.

Rosenberg SS, Kelland EE, Tokar E, De La Torre AR, Chan JR (2008) The geometric and spatial constraints of the microenvironment induce oligodendrocyte differentiation. Proc Natl Acad Sci 105:14662–14667.

Rutka JT, Apodaca G, Stern R, Rosenblum M (1988) The extracellular matrix of the central and peripheral nervous systems: structure and function. J Neurosurg 69:155–170.

Sahel A, Ortiz FC, Kerninon C, Maldonado PP, Angulo MC, Nait-Oumesmar B (2015) Alteration of synaptic connectivity of oligodendrocyte precursor cells following demyelination. Front Cell Neurosci | wwwfrontiersinorg 9.

Sanchez MA, Armstrong RC (2017) Postnatal Sonic hedgehog (Shh) responsive cells give rise to oligodendrocyte lineage cells during myelination and in adulthood contribute to remyelination. Exp Neurol.

Sarrazin S, Lamanna WC, Esko JD (2011) Heparan Sulfate Proteoglycans. Cold Spring Harb Perspect Biol 3.

Sasaki M, Lankford KL, Brown RJ, Ruddle NH, Kocsis JD (2010) Focal experimental autoimmune encephalomyelitis in the lewis rat induced by immunization with myelin oligodendrocyte glycoprotein and intraspinal injection of vascular endothelial growth factor.

Glia 58:1523–1531.

Schachtrup C, Ryu JK, Helmrick MJ, Vagena E, Galanakis DK, Degen JL, Margolis RU, Akassoglou K (2010) Fibrinogen Triggers Astrocyte Scar Formation by Promoting the Availability of Active TGF-□ after Vascular Damage. J Neurosci 30:5843–4854.

Scheibner KA, Lutz MA, Boodoo S, Fenton MJ, Powell JD, Horton MR (2006) Hyaluronan Fragments Act as an Endogenous Danger Signal by Engaging TLR2. J Immunol 177:1272–1281.

Schönherr E, Järveläinen HT, Sandell LJ, Wight TN (1991) Effects of platelet-derived growth factor and transforming growth factor-beta 1 on the synthesis of a large versican-like chondroitin sulfate proteoglycan by arterial smooth muscle cells. J Biol Chem 266:17640–17647.

Scott-Drew S, ffrench-Constant C (1997) Expression and function of thrombospondin-1 in myelinating glial cells of the central nervous system. J Neurosci Res 50:202–214.

Selvaraju R, Bernasconi L, Losberger C, Graber P, Kadi L, Avellana-Adalid V, Picard-Riera N, Baron A, Evercooren AB-V, Cirillo R, Kosco-Vilbois M, Feger G, Papoian R, Boschert U (2004) Osteopontin is upregulated during in vivo demyelination and remyelination and enhances myelin formation in vitro. Mol Cell Neurosci 25:707–721.

Shee WL, Ong WY, Lim TM (1998) Distribution of perlecan in mouse hippocampus following intracerebroventricular kainate injections. Brain Res 799:292–300.

Sherman DL, Brophy PJ (2005) Mechanisms of axon ensheathment and myelin growth. Nat Rev Neurosci 6:683–690.

Siebert JR, Osterhout DJ (2011) The inhibitory effects of chondroitin sulfate proteoglycans on oligodendrocytes. J Neurochem 119:176–188.

Siebert JR, Steencken AC, Osterhout DJ (2014) Chondroitin sulfate proteoglycans in the nervous system. Biomed Res Int 2014.

Siebert JR, Stelzner DJ, Osterhout DJ (2011) Chondroitinase treatment following spinal contusion injury increases migration of oligodendrocyte progenitor cells. Exp Neurol 231:19–29.

Šišková Z, Baron W, De Vries H, Hoekstra D (2006) Fibronectin impedes myelin sheet-directed flow in oligodendrocytes: A role for a beta 1 integrin-mediated PKC signaling pathway in vesicular trafficking. Mol Cell Neurosci 33:150–159.

Šišková Z, Yong VW, Nomden A, Van Strien ME, Hoekstra D, Baron W (2009) Fibronectin attenuates process outgrowth in oligodendrocytes by mislocalizing MMP-9 activity. Mol Cell Neurosci 42:234–242.

Sloane JA, Batt CE, Ma Y, Harris ZM, Trapp BD, Vartanian T, Sidman RL, Israel B (2010) Hyaluronan blocks oligodendrocyte progenitor maturation and remyelination through TLR2. Proc Natl Acad Sci 107:11555–11560.

Smith EM, Mitsi M, Nugent MA, Symes K (2009) PDGF-A interactions with fibronectin reveal

a critical role for heparan sulfate in directed cell migration during Xenopus gastrulation. Proc Natl Acad Sci 106:21683–21688.

Snaidero N, Möbius W, Czopka T, Hekking LHP, Mathisen C, Verkleij D, Goebbels S, Edgar J, Merkler D, Lyons DA, Nave K-A, Simons M (2014) Myelin Membrane Wrapping of CNS Axons by PI(3,4,5)P3-Dependent Polarized Growth at the Inner Tongue. Cell 156:277–290.

Sobel RA, Ahmed AS (2001) White matter extracellular matrix chondroitin sulfate/dermatan sulfate proteoglycans in multiple sclerosis. J Neuropathol Exp Neurol 60:1198–1207.

Soderblom C, Luo X, Blumenthal E, Bray E, Lyapichev K, Ramos J, Krishnan V, Lai-Hsu C, Park KK, Tsoulfas P, Lee JK (2013) Perivascular Fibroblasts Form the Fibrotic Scar after Contusive Spinal Cord Injury. J Neurosci 33:13882–13887.

Sozmen EG, Rosenzweig S, Llorente IL, Ditullio DJ, Machnicki M, Vinters H V, Havton LA, Giger RJ, Hinman JD, Carmichael ST (2016) Nogo receptor blockade overcomes remyelination failure after white matter stroke and stimulates functional recovery in aged mice. Proc Natl Acad Sci 113:E8453–E8462.

Spicer AP, Joo A, Bowling RA (2003) A Hyaluronan Binding Link Protein Gene Family Whose Members Are Physically Linked Adjacent to Chrondroitin Sulfate Proteoglycan Core Protein Genes. J Biol Chem 278:21083–21091.

Spivak-Kroizman T, Lemmon MA, Dikic I, Ladbury JE, Pinchasi D, Huang J, Jaye M, Crumley G, Schlessinger J, Lax I (1994) Heparin-Induced Oligomerization of FGF Molecule Is Responsible for FGF Receptor Dimerizatio Activation, and Cell Proliferation. Cell 79:15–1024.

Srivastava T, Sherman LS, Back SA (2018) A TLR/AKT/FoxO3 immune tolerance–like pathway disrupts the repair capacity of oligodendrocyte progenitors. J Clin Invest.

Starkey ML, Bartus K, Barritt AW, Bradbury EJ (2012) Chondroitinase ABC promotes compensatory sprouting of the intact corticospinal tract and recovery of forelimb function following unilateral pyramidotomy in adult mice. Eur J Neurosci 36:3665–3678.

Stassart RM, Fledrich R, Velanac V, Brinkmann BG, Schwab MH, Meijer D, Sereda MW, Nave K-A (2013) A role for Schwann cell–derived neuregulin-1 in remyelination. Nat Neurosci 16:48–54.

Stephenson EL, Mishra MK, Moussienko D, Laflamme N, Rivest S, Ling C-C, Yong VW (2018) Chondroitin sulfate proteoglycans as novel drivers of leucocyte infiltration in multiple sclerosis. Brain.

Stoffels JMJ, De Jonge JC, Stancic M, Nomden A, Van Strien ME, Ma D, Šišková Z, Maier O, Ffrench-Constant C, Franklin RJM, Hoekstra D, Zhao C, Baron W (2013a) Fibronectin aggregation in multiple sclerosis lesions impairs remyelination. Brain 136:116–131.

Stoffels JMJ, Hoekstra D, Franklin RJM, Baron W, Zhao C (2015) The EIIIA domain from astrocyte-derived fibronectin mediates proliferation of oligodendrocyte progenitor cells following CNS demyelination. Glia 63:242–256.

Stoffels JMJ, Zhao C, Baron W (2013b) Fibronectin in tissue regeneration: timely disassembly

of the scaffold is necessary to complete the build. Cell Mol Life Sci 70:4243–4253.

Strijbis EMM, Kooi E-J, Van Der Valk P, Geurts JJG (2017) Cortical Remyelination Is Heterogeneous in Multiple Sclerosis. J Neuropathol Exp Neurol 76:390–401.

Stringer SE, Mayer-Proschel M, Kalyani A, Rao MS, Gallagher JT (1999) Heparin Is a Unique Marker of Progenitors in the Glial Cell Lineage. J Biol Chem 274:25455–25460.

Struve J, Maher PC, Li YQ, Kinney S, Fehlings MG, Kuntz IV C, Sherman LS (2005) Disruption of the hyaluronan-based extracellular matrix in spinal cord promotes astrocyte proliferation. Glia 52:16–24.

Sugahara K, Mikami T, Uyama T, Mizuguchi S, Nomura K, Kitagawa H (2003) Recent advances in the structural biology of chondroitin sulfate and dermatan sulfate. Curr Opin Struct Biol 13:612–620.

Sun J-J, Ren Q-G, Xu L, Zhang Z-J (2015) LINGO-1 antibody ameliorates myelin impairment and spatial memory deficits in experimental autoimmune encephalomyelitis mice. Sci Rep 5.

Sundberg M, Skottman H, Suuronen R, Narkilahti S (2010) Production and isolation of NG2 + oligodendrocyte precursors from human embryonic stem cells in defined serum-free medium. Stem Cell Res 5:91–103.

Suttkus A, Rohr SO, Weigel S, Glöckner P, Arendt T, Morawski M (2014) Aggrecan, link protein and tenascin-R are essential components of the perineuronal net to protect neurons against iron-induced oxidative stress. Cell Death Dis 5:e1119.

Takagi J, Yang Y, Liu J, Wang J, Springer TA (2003) Complex between nidogen and laminin fragments reveals a paradigmatic β-propeller interface. Nature 424:969–974.

Takano R, Hisahara S, Namikawa K, Kiyama H, Okano H, Miura M (2000) Nerve growth factor protects oligodendrocytes from tumor necrosis factor-alpha-induced injury through Akt-mediated signaling mechanisms. J Biol Chem 275:16360–16365.

Tan G-K, Tabata Y (2014) Chondroitin-6-sulfate attenuates inflammatory responses in murine macrophages via suppression of NF-κB nuclear translocation. Acta Biomater 10:2684–2692.

Tauchi R, Imagama S, Natori T, Ohgomori T, Muramoto A, Shinjo R, Matsuyama Y, Ishiguro N, Kadomatsu K (2012) The endogenous proteoglycan-degrading enzyme ADAMTS-4 promotes functional recovery after spinal cord injury. J Neuroinflammation 9.

Termeer C, Benedix F, Sleeman J, Fieber C, Voith U, Ahrens T, Miyake K, Freudenberg M, Galanos C, Simon JC (2002) Oligosaccharides of Hyaluronan Activate Dendritic Cells via Toll-like Receptor 4. J Exp Med 195:99–111.

Testa D, Prochiantz A, Di Nardo AA (2018) Perineuronal nets in brain physiology and disease. Semin Cell Dev Biol.

Tomlinson L, Huang PH, Colognato H (2018) Prefrontal Cortex NG2 Glia Undergo a Developmental Switch in Their Responsiveness to Exercise. Dev Neurobiol.

Tomlinson L, Leiton C V, Colognato H (2016) Behavioral experiences as drivers of oligodendrocyte lineage dynamics and myelin plasticity. Neuropharmacology 110:548–562.

Tran JQ, Rana J, Barkhof F, Melamed I, Gevorkyan H, Wattjes MP, de Jong R, Brosofsky K, Ray S, Xu L, Zhao J, Parr E, Cadavid D (2014) Randomized phase I trials of the safety/tolerability of anti-LINGO-1 monoclonal antibody BIIB033. Neurol Neuroimmunol Neuroinflammation 1.

Tripathi RB, Clarke LE, Burzomato V, Kessaris N, Anderson PN, Attwell D, Richardson WD (2011) Dorsally and Ventrally Derived Oligodendrocytes Have Similar Electrical Properties but Myelinate Preferred Tracts. J Neurosci 31:6809–6819.

Tripathi RB, Rivers LE, Young KM, Jamen F, Richardson WD (2010) NG2 Glia Generate New Oligodendrocytes But Few Astrocytes in a Murine Experimental Autoimmune Encephalomyelitis Model of Demyelinating Disease. J Neurosci 30:16383–16390.

Urbanski MM, Kingsbury L, Moussouros D, Kassim I, Mehjabeen S, Paknejad N, Melendez-Vasquez C V (2016) Myelinating glia differentiation is regulated by extracellular matrix elasticity. Sci Rep 6.

Van't Veer A, Du Y, Fischer TZ, Boetig DR, Wood MR, Dreyfus CF (2009) Brain-derived neurotrophic factor effects on oligodendrocyte progenitors of the basal forebrain are mediated through trkB and the MAP kinase pathway. J Neurosci Res 87:69–78.

Viehover A, Miller RH, Park S-K, Fischbach G, Vartanian T (2001) Neuregulin: An Oligodendrocyte Growth Factor Absent in Active Multiple Sclerosis Lesions. Dev Neurosci 23:377–386.

Viganò F, Möbius W, Götz M, Dimou L (2013) Transplantation reveals regional differences in oligodendrocyte differentiation in the adult brain. Nat Neurosci 16:1370–1372.

VonDran MW, Singh H, Honeywell JZ, Dreyfus CF (2011) Levels of BDNF impact oligodendrocyte lineage cells following a cuprizone lesion. J Neurosci 31:14182–14190.

Wake H, Lee PR, Fields RD (2011) Control of Local Protein Synthesis and Initial Events in Myelination by Action Potentials. Science 333:1647–1651.

Wang D, Fawcett JW (2012) The perineuronal net and the control of CNS plasticity. Cell Tissue Res 349:147–160.

Wang H, Katagiri Y, McCann TE, Unsworth E, Goldsmith P, Yu Z-X, Tan F, Santiago L, Mills EM, Wang Y, Symes AJ, Geller HM (2008a) Chondroitin-4-sulfation negatively regulates axonal guidance and growth. J Cell Sci 121:3083–3091.

Wang Y, Imitola J, Rasmussen S, O'Connor KC, Khoury SJ (2008b) Paradoxical dysregulation of the neural stem cell pathway sonic hedgehog-Gli1 in autoimmune encephalomyelitis and multiple sclerosis. Ann Neurol 64:417–427.

Warford JR, Lamport A-C, Clements DR, Malone A, Kennedy BE, Kim Y, Gujar SA, Hoskin DW, Easton AS (2018) Surfen, a proteoglycan binding agent, reduces inflammation but inhibits remyelination in murine models of Multiple Sclerosis. Acta Neuropathol Commun 6.

Weerth SH, Rus H, Shin ML, Raine CS (2003) Complement C5 in Experimental Autoimmune Encephalomyelitis (EAE) Facilitates Remyelination and Prevents Gliosis. Am J Pathol 163:1069–1080.

Weil M-T, Möbius W, Winkler A, Ruhwedel T, Wrzos C, Romanelli E, Bennett JL, Enz L, Goebels N, Nave K-A, Kerschensteiner M, Schaeren-Wiemers N, Stadelmann C, Simons M (2016) Loss of Myelin Basic Protein Function Triggers Myelin Breakdown in Models of Demyelinating Diseases. Cell Rep 16:314–322.

Welliver RR, Polanco JJ, Seidman RA, Sinha AK, O'Bara MA, Khaku ZM, Santiago González DA, Nishiyama A, Wess J, Feltri ML, Paez PM, Sim FJ (2018) Muscarinic Receptor M3R Signaling Prevents Efficient Remyelination by Human and Mouse Oligodendrocyte Progenitor Cells. J Neurosci 38:6921–6932.

Wells JE, Rice TK, Nuttall RK, Edwards DR, Zekki H, Rivest S, Yong VW (2003) An adverse role for matrix metalloproteinase 12 after spinal cord injury in mice. J Neurosci 23:10107–10115.

Winkler CW, Foster SC, Itakura A, Matsumoto SG, Asari AA, McCarty OJT, Sherman LS (2013) Hyaluronan oligosaccharides perturb lymphocyte slow rolling on brain vascular endothelial cells: Implications for inflammatory demyelinating disease. Matrix Biol 32:160–168.

Winkler S, Stahl RC, Carey DJ, Bansal R (2002) Syndecan-3 and perlecan are differentially expressed by progenitors and mature oligodendrocytes and accumulate in the extracellular matrix. J Neurosci Res 69:477–487.

Wolswijk G (1998) Chronic Stage Multiple Sclerosis Lesions Contain a Relatively Quiescent Population of Oligodendrocyte Precursor Cells. J Neurosci 18:601–609.

Woodruff RH, Franklin RJM (1999) Demyelination and remyelination of the caudal cerebellar peduncle of adult rats following stereotaxic injections of lysolecithin, ethidium bromide, and complement/anti-galactocerebroside: A comparative study. Glia 25:216–228.

Woodruff RH, Fruttiger M, Richardson WD, Franklin RJM (2004) Platelet-derived growth factor regulates oligodendrocyte progenitor numbers in adult CNS and their response following CNS demyelination. Mol Cell Neurosci 25:252–262.

Yamaguchi Y, Mann DM, Ruoslahti E (1990) Negative regulation of transforming growth factor-beta by the proteoglycan decorin. Nature 346:281–284.

Yamashita T, Miyamoto Y, Bando Y, Ono T, Kobayashi S, Doi A, Araki T, Kato Y, Shirakawa T, Suzuki Y, Yamauchi J, Yoshida S, Sato N (2017) Differentiation of oligodendrocyte progenitor cells from dissociated monolayer and feeder- free cultured pluripotent stem cells. PLoS ONE 12.

Yao L, Phan F, Li Y (2013) Collagen microsphere serving as a cell carrier supports oligodendrocyte progenitor cell growth and differentiation for neurite myelination in vitro. Stem Cell Res Ther 4.

Yick L-W, Wu W, So K-F, Yip HK, Kwok-Yan Shum D (2000) Chondroitinase ABC promotes axonal regeneration of Clarke's neurons after spinal cord injury. Neuroreport 11:1063–

1067.

Yue T, Xian K, Hurlock E, Xin M, Kernie SG, Parada LF, Lu QR (2006) A Critical Role for Dorsal Progenitors in Cortical Myelination. J Neurosci 26:1275–1280.

Zhang W, Sun F, Niu H, Wang Q, Duan J (2015) Mechanistic insights into cellular immunity of chondroitin sulfate A and its zwitterionic N-deacetylated derivatives. Carbohydr Polym 123:331–338.

Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, Phatnani HP, Guarnieri XP, Caneda C, Ruderisch N, Deng S, Liddelow SA, Zhang C, Daneman R, Maniatis T, Barres BA, Jia X, Wu Q (2014) An RNA-Sequencing Transcriptome and Splicing Database of Glia, Neurons, and Vascular Cells of the Cerebral Cortex. J Neurosci 34:11929–11947.

Zhang Y, Zhang H, Wang L, Jiang W, Xu H, Xiao L, Bi X, Wang J, Zhu S, Zhang R, He J, Tan Q, Zhang D, Kong J, Li X-M (2012) Quetiapine enhances oligodendrocyte regeneration and myelin repair after cuprizone-induced demyelination. Schizophr Res 138:8–17.

Zhang Z, Coomans C, David G (2001) Membrane heparan sulfate proteoglycan-supported FGF2-FGFR1 signaling: evidence in support of the "cooperative end structures" model. J Biol Chem 276:41921–41929.

Zhao C, Fancy SPJ, Franklin RJM, Ffrench-Constant C (2009) Up-regulation of oligodendrocyte precursor cell ??V integrin and its extracellular ligands during central nervous system remyelination. J Neurosci Res 87:3447–3455.

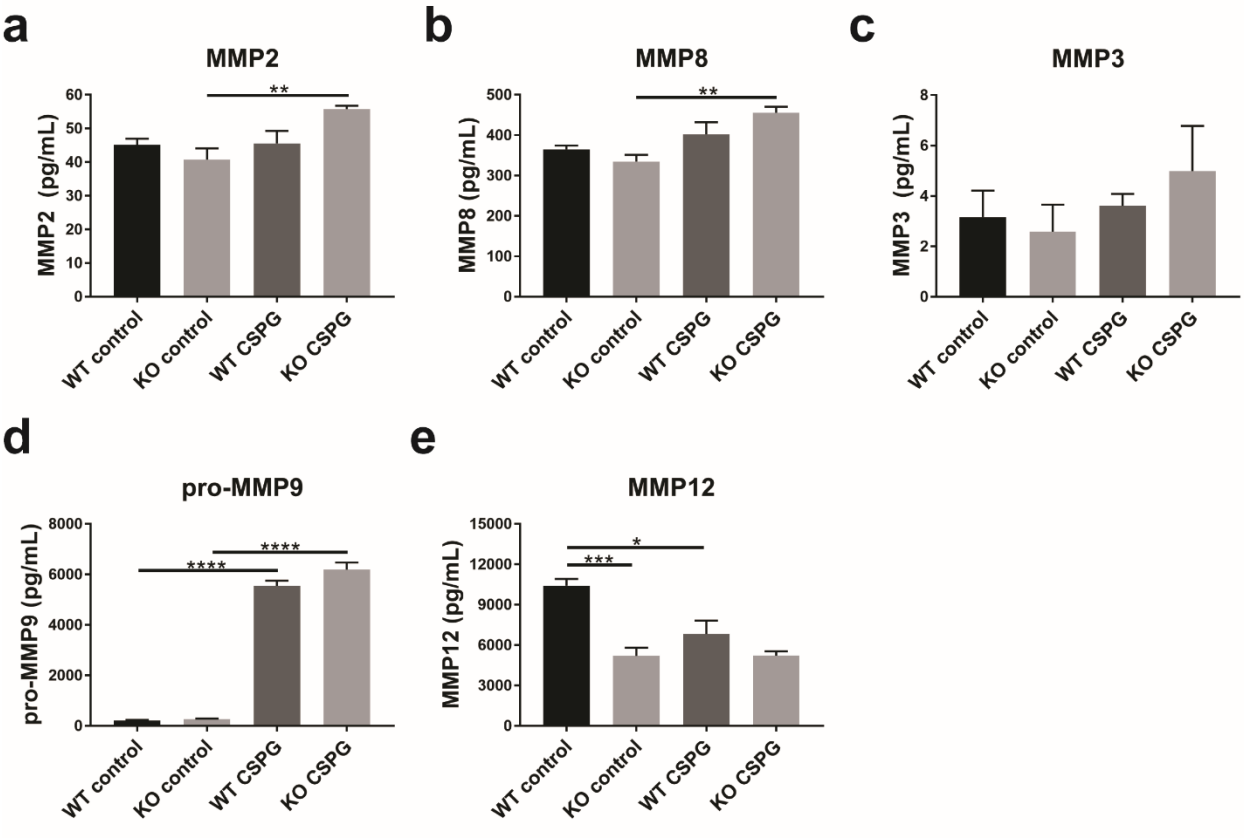
Zhornitsky S, Yong VW, Koch MW, Mackie A, Potvin S, Patten SB, Metz LM (2013) Quetiapine Fumarate for the Treatment of Multiple Sclerosis: Focus on Myelin Repair. CNS Neurosci Ther 19:737–744.

Zhu K, Sun J, Kang Z, Zou Z, Wu X, Wang Y, Wu G, Harris RA, Wang J (2019) Repurposing of omeprazole for oligodendrocyte differentiation and remyelination. Brain Res 1710:33–42.

Zhu Q, Zhao X, Zheng K, Li H, Huang H, Zhang Z, Mastracci T, Wegner M, Chen Y, Sussel L, Qiu M (2014) Genetic evidence that Nkx2.2 and Pdgfra are major determinants of the timing of oligodendrocyte differentiation in the developing CNS. Development 141:548–555.

Zuchero JB, Fu M-M, Sloan SA, Ibrahim A, Olson A, Zaremba A, Dugas JC, Weinbar S, Caprariello A V, Kantor C, Leonoudakis D, Lariosa-Willingham K, Kronenberg G, Gertz K, Soderling SH, Miller RH, Barres BA (2015) CNS Myelin Wrapping Is Driven by Actin Disassembly. Dev Cell 34:152–167.

Appendix A



Appendix A. **Raw values of MMP measurements from BMDM conditioned medium.** Comparison of absolute quantities of MMP2 (**a**), 8 (**b**), 3 (**c**), 12 (**e**), and pro-MMP9 (**d**) from conditioned medium shown as pg/mL from wild-type (WT) and EXTL2$^{-/-}$ (KO) BMDMs under basal control conditions and with CSPG stimulation. Data shown is from $n = 4$ wells derived from 1 biological replicate. Two-way ANOVA with Tukey's post hoc test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Appendix B

The copyright form for my published review, titled “The extracellular matrix: Focus on oligodendrocyte biology and targeting CSPGs for remyelination therapies” is included below.

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