Journal Club

Editor's Note: These short reviews of recent *JNeurosci* articles, written exclusively by students or postdoctoral fellows, summarize the important findings of the paper and provide additional insight and commentary. If the authors of the highlighted article have written a response to the Journal Club, the response can be found by viewing the Journal Club at www.jneurosci.org. For more information on the format, review process, and purpose of Journal Club articles, please see http://jneurosci.org/content/preparing-manuscript#journalclub.

A Therapeutic Link between Astrogliosis and Remyelination in a Mouse Model of Multiple Sclerosis

Craig Pearson

National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland 20824, and Department of Clinical Neurosciences, University of Cambridge, Cambridge CB2 1TN, United Kingdom

Review of Feliú et al.

Injuries to the mammalian CNS often provoke astrogliosis, a process in which reactive astrocytes surround the site of injury to restrict tissue damage (Voskuhl et al., 2009; Hamby and Sofroniew, 2010). Formation of the glial scar has historically been viewed as a short-term solution that creates long-term problems: while astrogliosis effectively limits the spread of inflammation and infection, it also creates a physical and chemical barrier to the regeneration and remyelination of axons (Cregg et al., 2014). Many therapies for CNS injuries have thus sought to remove this barrier by blocking astrogliosis and restricting or neutralizing the glial scar. However, in certain contexts, blocking reactive astrocyte formation has been found to impede functional recovery (Anderson et al., 2016), suggesting that some astrocytes may exert neuroprotective effects (Faulkner, 2004). Therefore, rather than prohibit astrogliosis entirely, alternative therapies have sought to specifically target molecules produced by reactive astrocytes that inhibit axon regeneration and remyelination.

Received Oct. 2, 2017; revised Nov. 6, 2017; accepted Nov. 7, 2017.

I thank Dr. Alessia Tassoni and Irene Falk for comments on the manuscript.

Correspondence should be addressed to Craig Pearson, National Institutes of Health, Building 50, Room 4142, Bethesda, MD 20817. E-mail: craig.pearson@nih.gov.

DOI:10.1523/JNEUROSCI.2844-17.2017

 0270-6474/18/380029-03$15.00/0

Among these molecules, substantial attention has been paid to chondroitin sulfate proteoglycans (CSPGs), which are extracellular matrix proteins that inhibit neuronal growth *in vivo* and *in vitro* and have been linked to remyelination failure (Galtrey and Fawcett, 2007). Therapies that selectively remove the inhibitory glycosaminoglycan (GAG) chains of CSPGs have been vital to studies of axon regeneration and remyelination in many models of CNS injury and disease (Soleman et al., 2013). Given the complexity of extracellular responses to acute and chronic damage, it is crucial to develop a clear understanding of how therapeutic interventions modify the inhibitory microenvironment to ensure their success.

One disease that might benefit from treatments targeting CSPGs associated with astrogliosis is multiple sclerosis (MS), an inflammatory disease characterized by multiple focal regions of demyelination and axonal damage, known as plaques, in the brain and spinal cord (Compston and Coles, 2008). At the time of diagnosis, most patients exhibit a relapsing–relapsing remitting disease course, in which periods of disability are followed by periods of recovery; however, many patients eventually enter a secondary progressive phase in which disabilities accumulate without recovery (Compston and Coles, 2008). Whereas demyelinated axons in MS plaques may spontaneously remyelinate in the relapsing–remitting phase, the failure of remyelination in the progressive phase leads to extensive neurodegeneration, resulting in severe dysfunction and disability (Franklin, 2002). Curiously, these demyelinated plaques are not typically depleted of myelin-forming oligodendrocytes (Chang et al., 2002).

The inability of oligodendrocytes in MS lesions to successfully remyelinate damaged axons suggests that some aspect of the lesion environment, such as astrogliosis, inhibits the remyelination process. Astrocytes in the white matter of MS lesions associate with CSPGs, and remyelination tends to be more robust in areas with fewer reactive astrocytes and lower levels of CSPGs, in conjunction with higher numbers of oligodendrocyte precursor cells (OPCs; Chang et al., 2012). Furthermore, CSPGs inhibit OPC growth and differentiation *in vitro*, and enzymatic digestion of their GAG chains reverses this inhibition (Siebert and Osterhout, 2011; Lau et al., 2012; Pendleton et al., 2013). Moreover, *in vivo* therapies that block CSPG synthesis (Lau et al., 2012; Keough et al., 2016), digest GAG chains (Bartus et al., 2014), or disrupt CSPG receptors (Lang et al., 2015) have all been shown to improve axon regeneration and remyelination, providing convincing evidence that CSPGs antagonize the remyelination process. Together, these findings point to CSPGs as an important target for MS therapies.

In an article published in *The Journal of Neuroscience*, Feliú et al., (2017) describe a pharmacological treatment that reduces CSPG deposition in demyelinating lesions of the spinal cord and leads to improved motor function in a mouse model of MS, illustrating an important new link between CSPGs and remyelination (Feliú et al., 2017). To study changes in the extracellular environment of demyelinated lesions, the researchers examined mice inoculated with Theiler's murine encephalomyelitis virus (TMEV), which stimulates a progressive model of MS in which the chronic phase is accompanied by astrogliosis (Feliú et al., 2015). The spinal cords of TMEV-infected mice were analyzed at 85 d postinjury (dpi), during the chronic phase of the disease, and 105 dpi, a well established time point for assessing early remyelination (Warrington et al., 2000). Astrogliosis was evaluated by immunostaining for GFAP and vimentin. CSPG levels were measured using immunostaining with an antibody (CS-56) specific to the GAG chains of CSPGs, as well as with RT-PCR and Western blot. By 85 dpi, a robust increase in reactive astrocytes was observed in conjunction with CSPG levels and increased expression of CSPG-related mRNA and proteins in demyelinated spinal cord lesions. CSPGs were closely associated with reactive astrocytes. Given the evidence that astrocytes produce CSPGs following CNS injury (McKeon et al., 1999), this observation suggests that the CSPGs in TMEV mice were likely produced by reactive astrocytes. Encouragingly, treating mice with xyloside, which inhibits CSPG synthesis, reduced markers of astrogliosis and CSPGs, and also rescued motor function as measured by spontaneous motor activity (Feliú et al., 2017).

Having confirmed that TMEV produces demyelinated lesions characterized by reactive astrocytes and elevated CSPG deposition, Feliú et al. (2017) sought to determine whether the endogenous cannabinoid 2-arachidonoylglycerol (2-AG) affects astrogliosis. 2-AG plays a central role in promoting oligodendrocyte proliferation and differentiation (Gomez et al., 2011; Gomez et al., 2015). Previous work showed that a single injection of 2-AG reduced white matter damage after spinal cord injury in rats (Arevalo-Martin et al., 2010) and that sustained delivery over 14 d delayed disease onset in mice with experimental autoimmune encephalomyelitis, another commonly used model of MS (Lourbopoulos et al., 2011). However, the effects of 2-AG on astrogliosis had not yet been explored. To produce elevated 2-AG levels *in vivo*, Feliú et al. (2017) pharmacologically inhibited the hydrolysis of 2-AG by monoacylglycerol lipase (MAGL) using a reversible MAGL inhibitor, UCM03025. After 10 d of treatment, the MAGL inhibitor yielded significant functional improvements in spontaneous motor activity and reduced immunostaining for markers of reactive astrocytes and CSPGs (Feliú et al., 2017). This indicates that the previously demonstrated therapeutic effects of 2-AG administration may be due in part to its reduction of astrocyte-derived CSPGs and that these beneficial effects can be achieved by pharmacologically inhibiting 2-AG hydrolysis.

In addition to reducing inhibitory components of the glial scar, inhibiting 2-AG hydrolysis increased the number of OPCs and mature oligodendrocytes in the demyelinated lesions of TMEV mice at 85 dpi. Western blot analysis indicated that levels of myelin basic protein were restored to sham levels in UCM03025-treated mice. This suggests that suppressing MAGL and elevating 2-AG enabled OPCs to migrate to the lesion and differentiate into mature oligodendrocytes, thereby restoring the endogenous mechanism of remyelination. This observation was extended by assessing motor activity at 105 dpi without further UCM03025 treatment. CSPG immunostaining and protein levels remained lower in treated mice than in controls, even at this later time point. Furthermore, lesions examined by transmission electron microscopy at 105 dpi revealed newly formed myelin sheaths and an increase in myelinated axons in the spinal cords of mice treated with the MAGL inhibitor, supporting the conclusion that remyelination was indeed taking place. Interestingly, UCM03025 administration led to reduced immunostaining for a marker of microglial activation and lowered levels of mRNA for the immune mediator IL-1β, indicating that 2-AG may also play an immunomodulatory role.

Understanding the mechanisms that prevent remyelination in MS is vital to developing effective therapies, particularly for patients in the progressive phase of the disease. The discovery that enhancing endogenous 2-AG levels also reduces CSPG accumulation in demyelinated lesions is a significant step toward establishing a new therapeutic target. Our knowledge of the role of the glial scar in tissue remodeling after CNS injury has become increasingly nuanced, with evidence that astrogliosis is not uniformly inhibitory to repair. Astrocytic scar formation was found to support axon regeneration after spinal cord injury in mice (Anderson et al., 2016), and it was recently discovered that reactive astrocytes can be classified into at least two subtypes, only one of which inhibits the repair of neuronal pathways (Liddelow et al., 2017). This dichotomy extends to the molecules produced by reactive astrocytes: emerging evidence shows that even CSPGs themselves, long considered an unequivocal barrier to axonal growth, can undertake both permissive and inhibitory functions depending on their structural features. For instance, it has been demonstrated that the different sulfation patterns of CSPG GAG chains influence how neurons respond to these molecules, with cultured neurons avoiding 4-sulfated GAGs yet growing readily over 6-sulfated GAGs (Wang et al., 2008). Shifts in the ratio of 4-sulfated GAGs to 6-sulfated GAGs in the brain were recently linked to age-related declines in plasticity in mice, suggesting that selective targeting of inhibitory GAG chain motifs may make for more effective therapies (Foscarin et al., 2017).

More research is required to untangle the complex interactions between neurons and their environment after injury or disease. It would be valuable, for instance, to study whether CSPG GAG chain sulfation changes after inhibiting 2-AG hydrolysis in TMEV mice or whether selectively targeting inhibitory sulfate groups without directly reducing CSPG levels might improve functional outcomes in a similar manner. The relationship between CSPGs, OPCs, and inflammatory cells also deserves further investigation. Feliú et al., (2017) found that inhibiting 2-AG hydrolysis reduces microglial activation and IL-1β mRNA levels, suggesting that this treatment also modulates the inflammatory response. Isolating the contributions of these various effects may prove challenging due to their interdependence. As the mechanisms underlying axon regeneration and remyelination become better described, it is increasingly vital to elucidate how the extracellular matrix contributes to these injury response processes and to develop therapeutic interventions that selectively and effectively promote neuronal repair. The findings reported by Feliú et al., (2017) expand our understanding of how the glial scar contributes to the failure of remyelination in chronic disease and suggest promising future avenues of investigation and potential treatments for demyelinating conditions like MS.

References

Anderson MA, Burda JE, Ren Y, Ao Y, O'Shea TM, Kawaguchi R, Coppola G, Khakh BS, Deming TJ, Sofroniew MV (2016) Astrocyte scar formation aids central nervous system axon regeneration. Nature 532:195–200. CrossRef Medline

Arevalo-Martin A, Garcia-Ovejero D, Molina-Holgado E (2010) The endocannabinoid 2-arachidonoylglycerol reduces lesion expansion and white matter damage after spinal cord injury. Neurobiol Dis 38:304–312. CrossRef Medline

Bartus K, James ND, Didangelos A, Bosch KD, Verhaagen J, Yáñez-Muñoz RJ, Rogers JH, Schneider BL, Muir EM, Bradbury EJ (2014) Large-scale chondroitin sulfate proteoglycan digestion with chondroitinase gene therapy leads to reduced pathology and modulates macrophage phenotype following spinal cord contusion injury. J Neurosci 34:4822–4836. CrossRef Medline

Chang A, Tourtellotte WW, Rudick R, Trapp BD (2002) Premyelinating oligodendrocytes in chronic lesions of multiple sclerosis. N Engl J Med 346:165–173. CrossRef Medline

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. CrossRef Medline

Compston A, Coles A (2008) Multiple sclerosis. Lancet 372:1502–1517. CrossRef Medline

Cregg JM, DePaul MA, Filous AR, Lang BT, Tran A, Silver J (2014) Functional regeneration beyond the glial scar. Exp Neurol 253:197–207. CrossRef Medline

Faulkner JR, Herrmann J, Woo MJ, Tansey KE, Doan NB, Sofroniew MV (2004) Reactive astrocytes protect tissue and preserve function after spinal cord injury. J Neurosci 24:2143–2155. CrossRef Medline

Feliú A, Moreno-Martet M, Mecha M, Carrillo-Salinas FJ, de Lago E, Fernández-Ruiz J, Guaza C (2015) A Sativex-like combination of phytocannabinoids as a disease-modifying therapy in a viral model of multiple sclerosis. Br J Pharmacol 172:3579–3595. CrossRef Medline

Feliú A, Bonilla Del Río I, 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 37:8385–8398. CrossRef Medline

Foscarin S, Raha-Chowdhury R, Fawcett JW, Kwok JCF (2017) Brain ageing changes proteoglycan sulfation, rendering perineuronal nets more inhibitory. Aging (Albany NY) 9:1607–1622. CrossRef Medline

Franklin RJ (2002) Why does remyelination fail in multiple sclerosis? Nat Rev Neurosci 3:705–714. CrossRef Medline

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. CrossRef Medline

Gomez O, Sanchez-Rodriguez A, Le M, Sanchez-Caro C, Molina-Holgado F, Molina-Holgado E (2011) Cannabinoid receptor agonists modulate oligodendrocyte differentiation by activating PI3K/Akt and the mammalian target of rapamycin (mTOR) pathways. Br J Pharmacol 163:1520–1532. CrossRef Medline

Gomez O, Sanchez-Rodriguez MA, Ortega-Gutierrez S, Vazquez-Villa H, Guaza C, Molina-Holgado F, Molina-Holgado E (2015) A basal tone of 2-arachidonoylglycerol contributes to early oligodendrocyte progenitor proliferation by activating phosphatidylinositol 3-kinase (PI3K)/AKT and the mammalian target of rapamycin (MTOR) pathways. J Neuroimmune Pharmacol 10:309–317. CrossRef Medline

Hamby ME, Sofroniew MV (2010) Reactive astrocytes as therapeutic targets for CNS disorders. Neurotherapeutics 7:494–506. CrossRef Medline

Keough MB, Rogers JA, Zhang P, Jensen SK, Stephenson EL, Chen T, Hurlbert MG, Lau LW, Rawji KS, Plemel JR, Koch M, Ling CC, Yong VW (2016) An inhibitor of chondroitin sulfate proteoglycan synthesis promotes central nervous system remyelination. Nat Commun 7:11312. CrossRef Medline

Lang BT, Cregg JM, DePaul MA, Tran AP, Xu K, Dyck SM, Madalena KM, Brown BP, Weng YL, Li S, Karimi-Abdolrezaee S, Busch SA, Shen Y, Silver J (2015) Modulation of the proteoglycan receptor PTPσ promotes recovery after spinal cord injury. Nature 518:404–408. CrossRef Medline

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. CrossRef Medline

Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, Bennett ML, Münch AE, Chung WS, Peterson TC, Wilton DK, Frouin A, Napier BA, Panicker N, Kumar M, Buckwalter MS, Rowitch DH, Dawson VL, Dawson TM, Stevens B, et al (2017) Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541:481–487. CrossRef Medline

Lourbopoulos A, Grigoriadis N, Lagoudaki R, Touloumi O, Polyzoidou E, Mavromatis I, Tascos N, Breuer A, Ovadia H, Karussis D, Shohami E, Mechoulam R, Simeonidou C (2011) Administration of 2-arachidonoylglycerol ameliorates both acute and chronic experimental autoimmune encephalomyelitis. Brain Res 1390:126–141. CrossRef Medline

McKeon RJ, Jurynec MJ, Buck CR (1999) The chondroitin sulfate proteoglycans neurocan and phosphacan are expressed by reactive astrocytes in the chronic CNS glial scar. J Neurosci 19:10778–10788. Medline

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. CrossRef Medline

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

Soleman S, Filippov MA, Dityatev A, Fawcett JW (2013) Targeting the neural extracellular matrix in neurological disorders. Neuroscience 253:194–213. CrossRef Medline

Voskuhl RR, Peterson RS, Song B, Ao Y, Morales LB, Tiwari-Woodruff S, Sofroniew MV (2009) Reactive astrocytes form scar-like perivascular barriers to leukocytes during adaptive immune inflammation of the CNS. J Neurosci 29:11511–11522. CrossRef Medline

Wang H, Katagiri Y, McCann TE, Unsworth E, Goldsmith P, Yu ZX, Tan F, Santiago L, Mills EM, Wang Y, Aviva J, Symes AJ, Geller HM (2008) Chondroitin-4-sulfationnegativelyregulates axonal guidance and growth. J Cell Sci 121:3083–3091. CrossRef Medline

Warrington AE, Asakura K, Bieber AJ, Ciric B, Van Keulen V, Kaveri SV, Kyle RA, Pease LR, Rodriguez M (2000) Human monoclonal antibodies reactive to oligodendrocytes promote remyelination in a model of multiple sclerosis. Proc Natl Acad Sci U S A 97:6820–6825. CrossRef Medline