

● EDITORIAL COMMENTARY

The complex contribution of the astrocyte scar

It is tempting to assign positive or negative roles to components of neurotrauma pathology, in an effort to generate an ordered picture and design therapeutic strategies accordingly. However nature is seldom so obliging. This principle is elegantly illustrated in a recent publication from Anderson, Sofroniew and colleagues (Anderson et al., 2016) which describes use of a variety of complementary approaches to demonstrate that the astrocyte scar can be beneficial for central nervous system axonal regeneration. Conventional wisdom states that astrocytes are a barrier to axon regrowth, with reports correlating failed axon regeneration with the presence of mature astrocytes and astrocytic scars. Described mechanisms include activation of the physiological stop pathways (Liuzzi and Lasek, 1987) and production of inhibitory chondroitin sulphate proteoglycans (CSPGs) (Silver and Miller, 2004). However it is now becoming apparent that the source of inhibitory molecules may not necessarily be astrocytes.

Anderson et al. (2016) began their exploration by utilizing transgenic mouse models to inhibit key elements of the astrocyte scar acutely following spinal cord injury. Selective killing of proliferating scar-forming astrocytes or genetic knockdown of critical STAT3 signalling prevented formation of the astrocytic scar, associated with increased axonal dieback in axons of the descending corticospinal tract and the ascending sensory tract (AST). Axons of the descending serotonergic (5HT) tract were largely unaffected. No spontaneous regeneration was seen despite the absence of astrocytes. Ablation of chronic astrocytic scars using genetically targeted diphtheria toxin resulted in similar outcomes. While numbers of animals per group were somewhat low at $n = 5-6$ in this study, the images and accompanying quantification render convincing, the key finding that astrocytes are not the sole inhibitor of axon regeneration following spinal cord injury.

CSPGs are regarded as the key inhibitory component of the astrocytic scar (Silver and Miller, 2004). In genetically modified mice with no astrocyte scar following SCI, CSPGs were still prominently present, associ-

ated with glial fibrillary acidic protein (GFAP)-negative cells. Genome-wide RNA sequencing of astrocyte- and non-astrocyte-specific ribosome-associated RNA (ramRNA) revealed that individual CSPG ramRNAs were expressed by both astrocytes and non-astrocyte cells in SCI lesions. The commonly used growth inhibitory CSPG aggrecan was not expressed by scar forming astrocytes at either the ramRNA or immunohistochemically detected protein levels; other CSPG isoforms were expressed by both cell classifications. Furthermore, scar-forming astrocytes and non-astrocyte cells within the SCI lesions were reported as upregulating multiple axon-growth-permissive matrix molecules, including axon growth-supporting CSPGs such as NG2 and neuroglycan C, as well as laminins. It is therefore clear that the presence of astrocytes is not required for upregulation of axon growth-inhibitory CSPGs and that other cells are likely contributing to axon growth inhibition.

An axon regenerative approach was then employed, using pre-conditioning lesions to the sciatic nerve, in combination with the exogenous growth factors BDNF and NT3 delivered following spinal cord injury *via* synthetic hydrogel depots. Regeneration of AST axons was clearly demonstrated in animals that had received both the conditioning lesion and growth factors, despite the presence of astrocytes. Indeed, regenerating axons grew through and beyond dense astrocytic scars. Similar treatment of spinal cord injury in genetically modified mice without astrocytes or an astrocytic scar resulted in an attenuated response, indicating that astrocytic scar formation aided, rather than inhibited AST axon regeneration after SCI. The study demonstrates that axon regeneration is possible despite the presence of axon-inhibitory molecules. While the astrocytic scar is not required for inhibition of axon regeneration, Anderson et al. (2016) stopped short of identifying an alternative culprit. Other cell types that generate axon-inhibitory CSPGs include oligodendrocytes, oligodendrocyte precursor cells and NG2+ cells (Silver et al., 2015). In addition, myelin molecules such as MAG, MOG and NogoA are known inhibitors of axon regeneration (Schwab and Thoenen, 1985; Huang et al., 1999), and likely contribute to effects observed in the current study.

An interesting finding of the study from Anderson

et al. (2016) is the absence of significant regeneration without both a conditioning lesion and exogenous growth factors. Neither a conditioning lesion delivered to the peripheral nervous system at the sciatic nerve, nor growth factors alone, were effective at promoting robust regeneration. Pre-conditioning injuries in the peripheral nervous system of mammals (Neumann and Woolf, 1999), and axotomy of species such as goldfish and frogs, has been reported to result in substantial axon regeneration in the spinal cord. Similarly, administration of growth factors such as NT-3 in hydrogels has led to reported increases in axon regrowth and enhanced functional outcomes (Piantino et al., 2006). The lack of an effect of these individual interventions in the current study may reflect use of mice rather than rats, differences in regions of assessment, hydrogel constituents and/or timing of assessments. Nevertheless, the lack of robust repetition of previously reported positive outcomes is a further caution towards over-interpretation of positive pre-clinical findings in the field of axonal regeneration.

Much effort has been devoted to limiting the effects of the astrocyte scar on axon regrowth in an effort to enhance regeneration. However it is becoming increasingly apparent that cellular contributors to pathology exist along a spectrum from damaging to protective. It has recently been postulated that different types of injury produce different types of reactive astrocyte, with some types being inhibitory and others not (Liddelov and Barres, 2016). As such, therapeutic strategies may have to be tailored to specific astrocytic subtypes in order not to do more harm than good. Furthermore, an individual cell may exhibit markers of both damaging and protective phenotypes. For example, following traumatic brain injury, microglia/macrophages cannot be precisely defined as a polarized “M1-only” or “M2-only” phenotype, but display a mixed phenotype due to the complex signaling events surrounding them (Morganti et al., 2016). Perhaps a similar situation is contributing to the current controversy regarding astrocyte involvement in pathology, with astrocytes expressing a mixed phenotype dependent upon the nature of the insult present within that cell’s microenvironment. It may thus be necessary to focus therapeutic efforts to limiting anti-regenerative moieties rather

than the cells that produce them.

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