

● PERSPECTIVE

Current opinion on a role of the astrocytes in neuroprotection

Central nervous system (CNS) injuries remain a leading cause of functional disabilities worldwide, often resulting in permanent neurological impairments, due to the inability to repair and regenerate damaged connections. A major contributing factor to this loss of regenerative capacity is the formation of glial scar tissue (Cregg et al., 2014), traditionally regarded as a potent mechanical and molecular barrier to repair. The glial scar comprises primarily reactive astrocytes, a subset of NG2 glia, inflammatory cells and extracellular matrix (ECM) glycoproteins – mainly chondroitin sulfate proteoglycans (CSPG; Cregg et al., 2014). The transition of astrocytes into their reactive state, as a consequence of pathological insults to the CNS, including trauma, inflammation and strokes, is characterized by changes in their morphology, gene/protein expression and role. These features are regulated by a host of intrinsic and extrinsic factors that modulate astrocyte reactivity, behaviour, proliferation and ECM secretions, ultimately culminating in the formation of the glial scar (Sofroniew, 2009). While astrogliosis and glial scarring have been well studied, especially in the context of repair inhibition, new evidence has begun to shed light on the previously unspecified neuroprotective role of reactive astrocytes after injury. Therefore, this perspective will discuss the most recent and significant research that has had altered the once held view of the astrocyte being solely inhibitory to repair following CNS injury.

Consistent with the terminologies defined by Anderson and colleagues (Anderson et al., 2016), we refer to astrogliosis as the general all-inclusive description of the diverse responses of reactive astrocytes to CNS injury. The use of the terms ‘glial scarring’ and ‘glial scar’ refers to the formation of and the consequent border formed by astrocytes between healthy and necrotic tissue after CNS injury, respectively.

Towards a new understanding of reactive astrocytes in neuroprotection: Due to the inhibitory nature of the glial scar, studies focussed on ablating scar formation to promote functional regeneration after CNS injuries (Cregg et al., 2014) have resulted in some conflicting outcomes. While some have reported successful improvements in functional recovery (Spanevello et al., 2013), others have demonstrated no significant improvements compared to controls (Anderson et al., 2016). A contributing factor to these conflicting results has been the long-standing view that astrogliosis is synonymous with glial scarring and is, therefore, categorically detrimental to regeneration. While it is well known that reactive astrocytes can exacerbate secondary neuronal degeneration (Liddel et al., 2017), previous evidence suggests that they also play a crucial neuroprotective role in the early stages after CNS injury, e.g., limiting neuronal apoptosis, maintaining homeostatic balance and providing trophic support to surviving neurones (Sofroniew, 2009; Anderson et al., 2014; Cregg et al., 2014). This is further supported by more recent ground-breaking studies, discussed below, demonstrating that reactive astrocytes possess environment and age-dependent plasticity that can induce phenotypic changes and confer neuroprotection.

Notably, a landmark study by the Sofroniew laboratory (Anderson et al., 2016) demonstrated that the prevention of scar formation or chronic glial scar ablation after spinal cord injury (SCI) significantly impaired functional recovery and regenerative processes. Indeed, contrary to established dogma, the presence of a glial scar was required for axonal regeneration in the presence of exogenous growth factors (Anderson et al., 2016). This study provided clear evidence that astrogliosis, and to some extent glial scarring, is necessary to achieve functional recovery. This hypothesis is supported by evidence that reactive astrocytes exhibit remarkable diversity in their morphology, gene expression and signalling mechanisms (Anderson et al., 2014). This diversity correlates to high levels of functional heterogeneity that is likely dependent on a host of intrinsic and extrinsic regulatory factors (Sofroniew, 2009; Hara et

al., 2017), cellular interactions (Liddel et al., 2017), types of injury (Zamanian et al., 2012), proximity to the injury (Shannon et al., 2007) and the age at which injury occurs (Teo et al., 2018).

For example, in a series of experiments, two distinct reactive astrocyte subpopulations were identified by the Barres laboratory through gene expression analyses (Zamanian et al., 2012; Liddel et al., 2017). Specifically, neurotoxic ‘A1 astrocytes’, induced by activated microglia following traumatic injury; and, neuroprotective ‘A2 astrocytes’, induced following ischemic injury. While both subtypes shared a cluster of important reactive genes, A2 astrocytes, in particular, possessed a molecular profile that is indicative of neuroprotection (Zamanian et al., 2012). In contrast, neurotoxic A1 astrocytes lacked phagocytic activity and actively induced neuronal apoptosis through a secreted soluble neurotoxin (Liddel et al., 2017). Gene expression analyses have also allowed for the characterisation of a subset of reactive astrocytes that undergo a secondary transformation into ‘scar-forming’ astrocytes (Hara et al., 2017). This study demonstrated that interactions with type I collagen, through an integrin/N-cadherin pathway, transform reactive astrocytes into scar-forming astrocytes. This transformation was associated with an increase in extracellular matrix gene expression, consistent with scar formation (Hara et al., 2017). Abrogation of this secondary transformation through integrin blockade resulted in improved axonal regeneration and functional outcomes, without affecting the beneficial roles of the existing reactive astrocyte population. Importantly, the transformation of reactive astrocytes into scar-forming astrocytes was not unidirectional but is instead reversible (Hara et al., 2017). These findings demonstrate a level of environment-dependent plasticity not previously observed with reactive astrocytes, and leads to the question: is astrocyte reactivity in itself a reversible process? Regardless, this study affirms that environmental factors heavily influence the identity and function of reactive astrocytes following CNS injuries, which is underpinned by unique gene expression profiles.

Recent reports have also demonstrated evidence of direct neuroprotective functions by reactive astrocytes after CNS injury. The expression of growth associated protein-43 (GAP43), traditionally associated with growth cones and synaptic plasticity, was recently reported on a subset of reactive astrocytes (Hung et al., 2016). This study provided clear evidence that GAP43 suppresses microglial activation and attenuates production of proinflammatory cytokines (interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α)), likely through inhibition of the Toll-like receptor 4/nuclear factor kappa B (NF- κ B) mediated pathway. In addition, GAP43 knock-down exacerbates secondary neuronal degeneration following CNS injury, likely due to increased excitotoxicity, due to the consequent downregulation of excitatory amino acid transporter 2 (EAAT2)/glial glutamate transporter 1 (GLT1) transcription on GAP43 depleted reactive astrocytes (Hung et al., 2016). This study demonstrated that GAP43 expression on a subset of reactive astrocytes affords direct neuroprotection following CNS injury through regulation of inflammatory responses, attenuation of proinflammatory cytokine release and increased protection against neuronal excitotoxicity. Furthermore, the transformation of a subset of reactive astrocytes within the ischemic penumbra into specialised phagocytic astrocytes has been confirmed following transient ischemic injury (Morizawa et al., 2017). In this study, it was reported that phagocytic astrocytes, induced by the ATP-binding cassette transporter A1 (ABCA1) pathway, emerge over a more chronic period compared with the acuter microglial activation. Interestingly, astrocytic phagocytosis, in this context, not only functioned to facilitate clearance of cell debris, likely limiting secondary injury, but also contributed to synaptic remodelling in the penumbra post-injury (Morizawa et al., 2017).

While most studies have focussed on the mature CNS, we have recently demonstrated that the age at which CNS injury occurs governs the intrinsic and extrinsic regulation of astrogliosis, significantly impacting glial scar severity and functional outcomes (Teo et al., 2018). Using a model of perinatal and adult focal ischemic injury in the nonhuman primate, we demonstrated that astrogliosis in early life occurred independently of several key intrinsic and

extrinsic regulators of adult astrogliosis; specifically signal transducer and activator of transcription 3 (STAT3), lipocalin2, and collagen 1 (Teo et al., 2018). While the lack of these regulators did not abrogate infant scar formation, the severity of glial scarring was significantly reduced following early-life injury, compared to an injury sustained during adulthood. These difference in regulatory mechanisms is also likely tied to the temporal profile of astrocyte reactivity after injury as reactive astrocyte proliferation occurred at a more acute time point in the infant compared to the adult. Most importantly, the chronic scar observed in infants is more permissible to improved neuronal sparing and functional recovery long-term (Teo et al., 2018). It remains unclear if the reduced severity of infant glial scarring is a function of ongoing developmental processes, age-related phenotypic differences in reactive astrocytes or a combination of both. However, our study provides corroborating evidence that early-life astrogliosis and scarring is more permissive to neuronal survival. Further elucidation of the molecular characteristics that govern infant astrogliosis could enable future manipulation of astrocyte reactivity and scar formation in adults as a therapeutic target after CNS injury.

Considerations for therapeutic developments: Before further exploration into the therapeutic potential of reactive astrocyte induced neuroprotection, several issues will need to be addressed. Firstly, the transient nature of the reactive astrocyte-induced neuroprotection after injury (Hung et al., 2016; Morizawa et al., 2017). The most likely cause of the emergence, and potentially the subsequent depletion, of neuroprotective reactive astrocytes after injury is due to the injury-dependent microenvironment at the lesion site. A further interrogation into the specific factors that govern the emergence and functions of these subpopulations will be needed to determine if the neuroprotection afforded by these factors can be triggered, enhanced and extended through exogenous means. Secondly, it remains to be seen if the neuroprotection observed in these studies can be recapitulated in (i) multiple models of CNS injuries and (ii) other clinically relevant model species. This is due, in part, to the poor translational outcomes of pre-clinical studies to clinical applications that have brought to light inter- and intra-species differences in the cellular response to CNS injuries. For example, the pathophysiological time-course of cellular and molecular events are protracted in humans and nonhuman primates compared to rodent models of CNS injuries (Teo et al., 2018). Therefore, it is important that the mechanisms that underpin these neuroprotective roles be evaluated and understood from a variety of species and injury models to ensure the scientific rigour of these observations.

Conclusion: Astrogliosis and glial scarring is a requisite part of the CNS response to injury. However, it is becoming evident that the impact of astrogliosis and glial scarring on neural repair and functional recovery is highly dependent on the diverse subtypes and roles of reactive astrocyte present after injury. The studies discussed here highlight a profound shift in the perception of astrogliosis and glial scarring from a categorically detrimental process to one that is highly complex in functions, heterogeneity and plasticity. Thus, while glial scarring is dependent on astrogliosis, both are separate processes governed by distinct astrocyte sub-populations. More importantly, these newly-described properties of reactive astrocytes present ideal therapeutic targets for harnessing endogenous repair mechanisms to improve functional recovery after CNS injuries.

Over the past couple of years, research from leading groups has been instrumental in our understanding of the beneficial role of the astrocyte following CNS injury. When once it was deemed an inhibitor of neural repair and that abrogating astrogliosis was essential to any potential recovery, the tide is changing. The studies outlined in this Perspective demonstrate that the astrocyte should be considered a potential 'friend', with the caveat being that there is significant heterogeneity in astrocyte behaviour. Harnessing the good side of the astrocyte following CNS injury should be an area of concerted focus. Particular attention needs to be paid to the specific molecular pathways responsible for a more suitable environment for neural repair to occur.

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Open peer review reports:

Reviewer 1: Margot Mayer-Proschel, University of Rochester, USA.

Comments to authors: The authors selected an interesting topic, which is well worth a perspective. The information provided is for the most part interesting and compelling.

Reviewer 2: Joe E. Springer, University of Kentucky, USA.

Comments to authors: This brief review on A1/A2 astrocyte phenotypes in CNS injury is well written, informative, and very timely. This reviewer's only comment is that it might be helpful to include a more specific statement linking microglia associated pro-inflammatory cytokines to activation of reactive astrocytes and GAP-43 signaling (induced by TLR4/NF- κ B/STAT3 as described in Hung et al. (2016)), and possibly the age-related differences reported by Teo et al. (via STAT3).

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