PERSPECTIVE

Role of granulocyte macrophage colony stimulating factor in regeneration of the central nervous system

Traditionally, it has been thought that the mammalian central nervous system (CNS) does not regenerate. Possibly due to the inhibitory extracellular environment post-injury as well as the limited intrinsic characteristics of adult post-mitotic neurons (Smith et al., 2015); **Figure 1**. Modulation of several molecular mechanisms promotes some degree of cell survival and axon regeneration in the adult CNS, but much remains to be elucidated. Of particular note, activating inflammatory mechanisms has been shown to either promote or limit axon regeneration *in vivo* (Filbin, 2006). The optic nerve crush injury model has been extensively used as a means of studying axon growth in the damaged CNS and has facilitated significant strides in our understanding of possible mechanisms required to enhance regeneration of the damaged adult CNS. While this model represents an initial step in guiding further research into axon regeneration, it is important to note that neurological conditions, such as stroke, are characterized by significant axonal damage and models that mimic this axon-specific damage in the brain are currently being explored.

Stroke is modelled in animals in order to gain a better understanding of the mechanisms mediating neurodegeneration, which will guide the development of novel therapeutic approaches. One of the most well characterized models of stroke is the middle cerebral artery occulsion (MCAO) model. However, there are challenges associated with reproducibility of this model in animals. The MCAO model results in significant hemipsheric brain damage, resulting in significant loss of brain tissue in human stroke cases. This significant loss of brain tissue correlates with high mortality rates in humans and in mouse models and the investigation of axonal damage mechanisms is understandably very complex. In addition, the damage induced by MCAO is particularly variable between operated animals. Conversely, the endothelin-1 (ET-1) model of stroke can be used to generate a focal and region-specific stroke in animals. Importantly, ET-1 can indeed target axonal projections, dependent upon location in the brain that the ET-1 is introduced. Using the ET-1 mouse model, we have demonstrated targeted damage of axonal projections in the corpus collasum, with associated subcortical white matter (SWM) damage, culminating in forelimb motor deficits (Theoret et al., 2016). We have also reported that granulocyte-macrophage colony-stimulating factor (GM-CSF) plays a critical role in attenuating ET-1-induced motor deficits through activation of the mTOR signalling pathway (Theoret et al., 2016). However, the ET-1 mouse model of stroke has a number of limitations in terms of its utility toward evaluating axon regeneration. Given the well established use of the optic nerve crush injury model in analysis of axon regeneration, this may be highly amenable to further evaluate the potential regenerative impact of GM-CSF.

The optic nerve has been well-characterized in terms of morphology and functionality. All the axons in the optic nerve arise from a single neuronal cell population called the retinal ganglion cells (RGCs) which can easily be experimentally manipulate. Moreover, because of its accessibility and anatomy, the eye is suitable for effective application of potential therapeutics such as growth-promoting factors like GM-CSF. Potential therapeutics can readily diffuse to the retinal ganglion cells without the need to overcome the blood-retinal barrier. The optic nerve crush model involves the use of forceps to crush and damage axonal connections in the optic nerve, this can be accompanied with administration of therapeutic agent(s), such as GM-CSF. Interestingly, and with particular relevance to the potential application of GM-CSF in brain pathologies, GM-CSF readily crosses both the blood-brain and blood-spinal barriers (McLay et al., 1997), making it an attractive therapeutic option for brain injury.

GM-CSF is a pro-inflammatory cytokine, composed of four alpha helices that bind to the alpha subunit of the GM-CSF transmembrane receptor. The alpha-subunit of the GM-CSF receptor is specific for binding GM-CSF, whereas the beta-subunit is involved in signal transduction. GM-CSF is part of the haematopoietin receptor superfamily and plays a prominent role in promoting proliferation, differentiation and maturation of hematopoietic precursors cells (Burgess and Metcalf, 1980). GM-CSF also regulates various cellular processes through the activation of intracellular signalling mechanisms such as MAPK, PI3K/Akt and JAK/STAT signalling pathways (Schallenberg et al., 2009; Legacy et al., 2013; Theoret et al., 2016). The binding of GM-CSF to the alpha subunit of the GM-CSF receptor leads to the phosphorylation of the beta subunit of the receptor by JAK2, a tyrosine kinase. GM-CSF has been shown to act as a neuronal growth factor and as a neuroprotective agent both *in vitro* and *in vivo* (Schabitz et al., 2008; Legacy et al., 2013; Hanea et al., 2016; Theoret et al., 2016). The intracellular MAPK-ERK1/2 pathway activation has been implicated in GM-CSF's role in protecting RGCs from cell death both *in vitro* and *in vivo* (Schallenberg et al., 2009). In addition, GM-CSF has been shown to stimulate neurite growth in retinal explants and cultured RGCs (Legacy et al., 2013; Hanea et al., 2016). Overall, these studies suggest that GM-CSF might be a novel therapeutic agent for neural repair following traumatic injury to the CNS.

The binding of GM-CSF to the alpha subunit of the GM-CSF receptor leads to the phosphorylation of the beta subunit of the receptor by JAK2, a tyrosine kinase. Interestingly, the expression of GM-CSF alpha-receptor is up-regulated following ischemic brain injury (Schabitz et al., 2008; Theoret et al., 2016). Furthermore, intravenous injection of GM-CSF in the MCAO rat-model resulted in significant reduction in infarct volume (Schabitz et al., 2008). Further research is needed to better understand the role of GM-CSF on axon regeneration and the intracellular signalling mechanisms that induce its proposed neuroprotective effects.

Translation of the therapeutic potential of GM-CSF from the laboratory to the clinic is slowly progressing; for instance, Jim et al. (2012) reported that GM-CSF improves cognitive function in cancer patients. Although the mechanism by which GM-CSF reduces cognitive decline is not well understood; it is hypothesized that GM-CSF treatment may result in increased angiogenesis, neurite outgrowth or neuronal survival in brain regions known

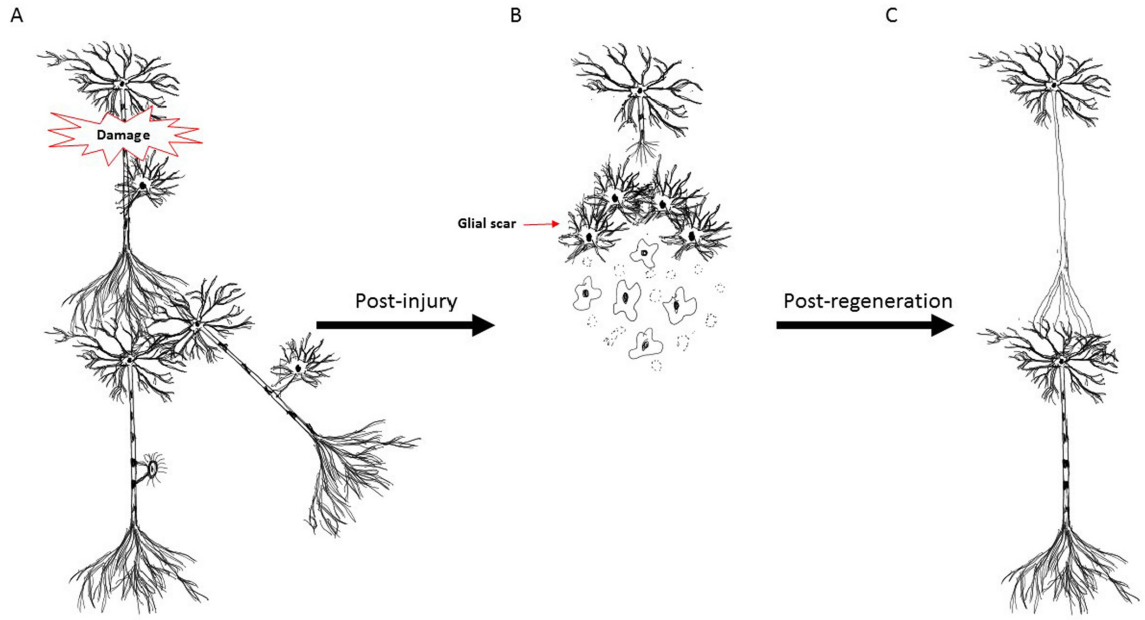

Figure 1 Schematic outlining the impact of damage and regeneration to axons in the central nervous system.
Neurons in the normal nervous system form circuits and are supported by glial cells (A). However, the central nervous system is susceptible to both physical damage and neurodegeneration, leading to neuronal death, including axonal degeneration. The environment post injury is not permissive to regeneration. The formation of the glial scar creates a physical barrier blocking axon regeneration at the injury site (B). Therapeutic agents (*e.g.*, granulocyte-macrophage colony-stimulating factor) may be able to promote axon regeneration and repair of damaged axons (C).

to be involved in cognitive function. Stroke in humans is often associated with cognitive dysfunction; this is particularly important because depending on location of a stroke, patients may experience significant cognitive decline. Further research is necessary to fully elucidate the mechanisms involved and the role of GM-CSF in post-stroke humans.

These key findings regarding the neuroprotective role of GM-CSF and its ability to enhance neurite outgrowth suggest that GM-CSF might be an ideal therapeutic agent following CNS injury. However, there seems to be many downstream intracellular effectors of GM-CSF in the CNS. There is a critical need to define the intracellular mechanisms that contribute to the effects of GM-CSF following CNS injury and to clarify whether there is cross-talk between these mechanisms in order to fully understand the role of GM-CSF in the CNS. Overall, these studies indicate that GM-CSF may be a novel therapeutic target to promote regeneration in the injured CNS and further research is necessary to clarify the impact of this compound on both axon regeneration and plasticity in the CNS.

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