Stressed axons craving for glial sugar: links to regeneration?

Elisabetta Babetto, Bogdan Beirowski*

Extract
The contrary but interrelated processes of axon degeneration and regeneration are the *yin* and *yang* of many neurodegenerative conditions. Here we discuss recent evidence for metabolic cross-talk between glia and injured axons regulating these processes. We especially focus on potential bioenergetic mechanisms as to how axon-flanking glia may promote regeneration.

Introduction: Axons are amongst, if not the most, vulnerable compartments of neuronal circuits. Axon degeneration (AxD) followed by attempts of axon repair are closely linked pivotal elements in many neurodegenerative conditions. However, their cellular and molecular underpinnings and the relationship between them remain only poorly defined. AxD and subsequent regeneration can be modeled through the induction of Wallerian degeneration (WD). WD is a process triggered by experimental axon injury and comprises a set of molecular and cellular events in different cell types by which degenerating axons and myelin are cleared away to set the stage for axon regeneration.

In the central nervous system, WD is slow and axonal regrowth generally fails. In contrast, WD in the peripheral nervous system progresses faster, yielding a permissive terrain with greater potential for axon regeneration and functional restoration. Additionally, unlike central neurons, peripheral neurons inherently possess the ability to self-repair and activate intrinsic growth programs after injury. For these reasons as well as the experimental manipulability and the genetic similarity to humans, the rapid dismantling of injured axons with subsequent vigorous growth of new axon segments is widely studied *in vivo* by transecting peripheral nerves in rodents. In this paradigm, the axon segments distal to a site of nerve injury undergo rapid structural fragmentation (i.e., initiating WD) (**Figure 1A** and **Figure 2**, top). By contrast, the axon segments proximal to the injury site connected to the neuronal cell bodies typically survive (no WD), and then start to regenerate through the distal nerve stump. In favorable cases, this regeneration results in reinnervation of the target and functional recovery. Schwann cells (SCs), the predominant glia of the peripheral nervous system, have emerged as a key factor for the ability of peripheral nerve axons to regenerate. First, with respect to early phases of WD, SCs quickly sense axon injury (Wong et al., 2017) and possess the unique capacity to promote axonal dismantling by the formation of contractile actomyosin constrictions that rapidly separate dead axon stumps into small fragments (Catenaccio et al., 2017; Vaquie et al., 2019). Actin polymerization in SCs also facilitates the fragmentation of myelin sheaths associated with the disconnected axonal corpses in the distal nerve stump (Jung et al., 2011). Together, the axon and myelin fragmentation are important prerequisites for debris removal and subsequent axon regeneration. Concerning later events of WD, an additional and perhaps more substantial prerequisite for successful axon regeneration is the activation of a glial program that converts denervated SCs into specialized 'repair cells'. These cells provide strong regenerative support in the distal nerve stump through a spectrum of functions orchestrated by the transcription factor c-Jun (Arthur-Farraj et al., 2012). As such, c-Jun upregulation in denervated SCs suppresses myelin genes, activates glial autophagy for debris digestion (Gomez-Sanchez et al., 2015), remodels the SC structure for the formation of regeneration tracks, and results in the upregulation of a network of trophic factors released by SCs that support axon growth. Consequently, the deletion of c-Jun in denervated SCs abolishes the repair phenotype, resulting in marked degenerative delays in the distal nerve segment, and profound nerve regenerative failure.

Schwann cells antagonize axon breakdown through their glycolytic activity: We recently uncovered a novel role of SCs during early stages of WD that precedes and appears to be functionally distinct from the above pro-regenerative roles (Babetto et al., 2020). In essence, we showed that SCs in the distal nerve stump counteract the death of experimentally injured axons through the release of glycolytic substrates and neurometabolic coupling (**Figure 1A**). This mechanism builds on the earlier discovery indicating that injury-induced AxD is regulated by a conserved neuronal program of subcellular self-destruction that leads to a fatal energetic crisis of axons (Yang et al., 2015). In this context, and for the first time demonstrating a non-cell autonomous regulation of the axonal self-destruction program, we found that SCs rapidly upregulate their glycolytic metabolism and supply glycolytic end-products to antagonize the energetic depletion of injured axons. The glycolytic shift in SCs is driven by the mammalian target of rapamycin complex 1 (mTORC1) pathway and the downstream transcription factors Hif1α and c-Myc. Together, Hif1α and c-Myc promote the expression of a wide spectrum of glycolytic enzymes as well as glial glucose and monocarboxylate transporters in SCs (MCT1/4). The glycolytic switch then leads to the increased production and release of lactate from SCs flanking injured axons. This substrate is taken up by injured axons via axonal monocarboxylate transporters (MCT2), and is then used in axonal mitochondria for energy production (**Figure 1A**). The ATP supply counteracts the energetic crisis and extends the life span of injured axons. Accordingly, if the glycolytic switch on injury is experimentally deactivated in SCs, axon disintegration in the distal nerve stump proceeds faster (**Figure 1B**).

This function clearly differs from the more established pro-regenerative roles of SCs regulated by the c-Jun repair program. To reconcile these apparently antagonistic functions, we propose that the glycolytic injury response of SCs is directed at energizing and mending compromised axons. This purpose is significant if we consider that in many chronic neurodegenerative diseases axons face only mild and temporary injuries. This is often associated with progressive axonal energetic decline, but does not invariably lead to axon death. This situation preempts the need for axonal regeneration if reenergized axons can escape demise and recover. In contrast, if the axon damage is more severe and evokes the irreversible commitment to axon death, the activation of the c-Jun repair program in SCs ensures the swift preparation for an environment permissive for the regeneration of new axons. We hypothesize that both SC functions are at play and fulfill critical neuroprotective functions in subacute and chronic disease settings. Importantly, future studies will have to elucidate potential molecular commonalities between these anti-degenerative and pro-regenerative programs. For example, it is conceivable

that the glycolytic upsurge in SCs is also controlled by the induction of c-Jun, similar to the activation of glial autophagy (Gomez-Sanchez et al., 2015). In support for a molecular overlap, a recent study suggests that mTORC1 promotes the elevation of c-Jun in SCs following nerve injury (Norrmen et al., 2018). Therefore, mTORC1 signaling may be the central driver for the activation of the dichotomic SC injury responses, and c-Jun with downstream autophagy upregulation could potentially also account for the glycolytic switch. The fact that the glycolytic boost, mTORC1, and c-Jun upregulation all begin in SCs very early after nerve injury in the injured distal nerve stump is consistent with such model.

Do Schwann cell glycolysis and metabolic coupling promote axon regeneration? Interestingly, a recent commentary suggests the speculative idea that the newly identified SC support mechanism to energetically stabilize injured axons could promote the regeneration of new axons and also neuronal survival (Trimarco and Taveggia, 2020). This could occur by the same token through energetic support of axon growth, or alternatively through signaling functions of monocarboxylates released from SCs. A prerequisite for such mechanisms would be that SCs sustain the glycolytic upregulation and substrate release up to the late stages of WD in which axonal regeneration begins. Whether or not this really occurs in SCs is currently unknown. Intriguingly, a recent study alludes to a signaling role of monocarboxylates by demonstrating that lactate released from hyperglycolytic *Drosophila* glia supports axon regeneration through modulation of neuronal GABA-B receptors and downstream cAMP signaling (Li et al., 2020). Apart from such non-cell-autonomous crosstalk mechanisms, a sustained glycolytic upregulation in SCs enhancing the glial biosynthetic capacity through parallel activation of the pentose phosphate pathway (a glycolytic side pathway) could also support the myelination of regrown axons. Indeed, axonal re-myelination by SCs is an anabolic challenge and an important final step toward successful functional nerve repair.

From a bioenergetic vantage point, we envision at least three mutually not exclusive possibilities as to how SC-mediated support of axon regeneration could function. Firstly, because axon regeneration is a highly energy-demanding process, the transfer of monocarboxylates from denervated SCs into proximal regenerating axon stumps could support axonal mitochondrial respiration and ATP production requisite for axon elongation (**Figure 2a**). Indeed, these energetic features combined with the striking mitochondrial redistribution into growing proximal axon stumps, have been identified as key determinants for successful axon regeneration (Cartoni et al., 2016; Han et al., 2016; Zhou et al., 2016). Secondly, the glial shuttling of energetic substrates into proximal axon stumps is expected to support neuronal survival and fitness through the ATP-dependent retrograde transport of neurotrophin-containing signaling endosomes. Moreover, this mechanism could also support the retrograde axonal transport of injury signals that activate

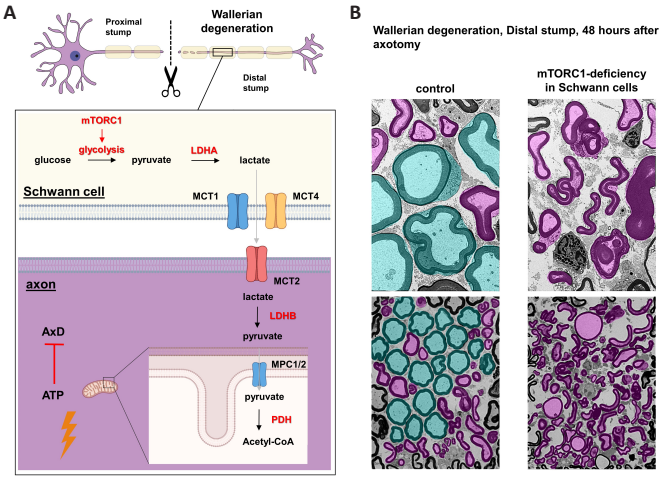

Figure 1 | **Support of injured axons by SCs in the distal disconnected nerve stump through metabolic coupling.**
(A) Model for the regulation of lactate production and release, driven by mTORC1 and glycolytic upregulation in injury-activated SCs to support the survival of disconnected axons. The glycolysis-derived pyruvate is converted to lactate by LDHA and released from SCs via MCT1/4 monocarboxylate transporters. The lactate is then shuttled into axons through MCT2, converted to pyruvate by LDHB, imported via MPC1/2 into the mitochondrial matrix, converted to Acetyl-CoA by the pyruvate dehydrogenase complex (PDH), and finally metabolized to generate ATP, which delays AxD. (B) Electron microscopy (top) and semithin light microscopy (bottom) of cross sections from mouse distal sciatic nerve stumps undergoing WD (48 hours after sciatic nerve transection). Note the majority of intact axons (turquoise) in the control nerve stump versus exclusively degenerated axons (magenta) in the distal nerve stump from mutant mice with SCs lacking mTORC1 expression. Unpublished data. AxD: Axon degeneration; LDHA/B: lactate dehydrogenase A/B; MCT1/2/4: monocarboxyate transporter 1/2/4; MPC1/2: mitochondria pyruvate carriers 1/2; mTORC1: mammalian target of rapamycin complex 1; PDH: Pyruvate Dehydrogenase; SCs: Schwann cells; WD: Wallerian degeneration.

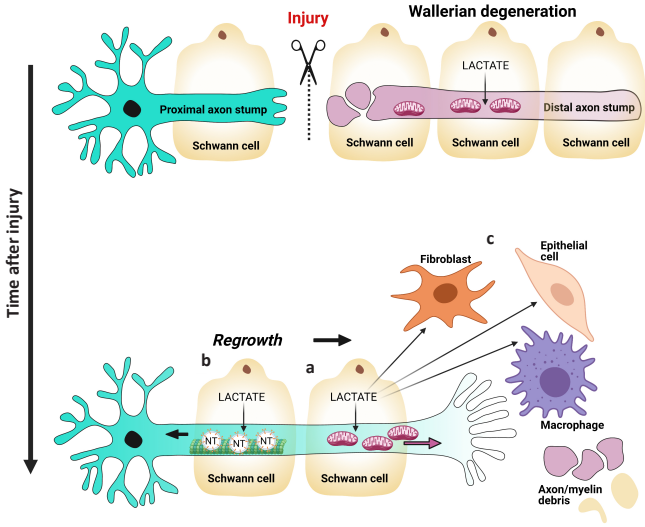

Figure 2 | **Models for the support of axon regeneration by Schwann cell lactate.**
Schematic depicting putative mechanisms as to how lactate released by denervated Schwann cells may promote axon regeneration: (a) through the support of axonal mitochondrial ATP production requisite for axon elongation; (b) through the support of energy-dependent retrograde transport of NT-containing signaling endosomes and injury signals that activate the expression of RAGs; (c) through the support of cell types other than neurons that play an important role in the directed regeneration of proximal axon stumps. NT: Neurotrophin; RAGs: regeneration associated genes.

the expression of regeneration associated genes (RAGs) in the neuronal cell body (**Figure 2b**). In this context it would be valuable in the future to investigate if the glycolytic upregulation occurs also in SCs located proximal to the nerve lesion site as this could place SCs closer to the neuronal cell bodies and hence in a better position to support these aspects. Lastly, it is conceivable that the release of the glycolytic intermediates from SCs supports the function of other cell types such as macrophages, mesenchymal, and endothelial cells that have been shown to play a central role in the directed regeneration of proximal axon stumps (Cattin et al., 2015) (**Figure 2c**). In fact, these cell types can be critically influenced by their metabolic microenvironment, which includes extracellular lactate levels, to regulate regenerative functions (Zhang et al., 2020). All in all, these models are consistent with the finding of delayed sciatic nerve axon regeneration in MCT1 heterozygous null mice (Morrison et al., 2015).

Conclusions: Our recent work opens the opportunity for a new perspective on the role of SCs for axon degeneration and potentially also regeneration mechanisms implicating bioenergetic axon-glia crosstalk. We and others have shown that the axonal dismantling process previously believed to be exclusively executed by the neuron can be also non-cell autonomously regulated by SCs. The glycolytic pathway controlled by mTORC1 in SCs occupies a central position in the regulation of the resistance of injured axons to degeneration. Future studies are warranted to elucidate if the glial glycolytic switch and metabolic coupling pathway could be exploited to safeguard damaged axons from breakdown in models of chronic neurodegenerative conditions such as peripheral neuropathies or Amyotrophic lateral sclerosis. These studies may also need to concentrate on the potential role of the release of glial energetic substrates to promote axonal regeneration and neuronal survival. Finally, it will be important to examine if the knowledge gained can be applied to protect central axons and increase the efficiency of regeneration in the central nervous system.

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Elisabetta Babetto, Bogdan Beirowski*
Hunter James Kelly Research Institute, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY, USA (Babetto E, Beirowski B)
Department of Biochemistry, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY, USA (Beirowski B)
Department of Pharmacology and Toxicology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY, USA (Babetto E)
***Correspondence to:** Bogdan Beirowski, MD, PhD, bogdanbe@buffalo.edu.
https://orcid.org/0000-0002-1241-1777 (Bogdan Beirowski)
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