Thrombin in peripheral nerves: friend or foe?

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Differently from the central nervous system (CNS), the peripheral nervous system (PNS) exhibits a high regenerative capacity. This ability is related to the remarkable plasticity of Schwann cells (SCs) which after nerve injury convert to a repair-promoting phenotype to a large extent. Nerve injury is accompanied by a rapid rise of thrombin levels (Bushi et al., 2016; Gera et al., 2016). Thrombin is the key effector protease of the coagulation cascade which elicits hormone-like actions by the activation of G-protein coupled receptors known as protease-activated receptors (PARs). Inflammation and coagulation are two complex and interconnected pathways whose mutual interactions have been only partially elucidated.

Our recent work demonstrates that thrombin is able to favor or inhibit SC pro-regenerative role in different experimental settings (Pompili et al., 2017, 2020). In particular, low levels of thrombin induce SCs to release in culture factors which promote neurite extension (Pompili et al., 2017). Conversely, high levels of thrombin inhibit the pro-regenerative capacities of SCs and cause a precocious demyelination of the paranodes in *ex vivo* nerve explants (Pompili et al., 2020). Our studies were conducted *in vitro* and in *ex vivo* nerve explants and it would be improper to directly translate these findings *in vivo* where the local level reached by thrombin after nerve injury is difficult to be assessed. Nevertheless, other studies reported the capacity of thrombin at picomolar concentrations to exert cytoprotective signal while switching to a proinflammatory profile when its concentration is raised (Willis Fox and Preston, 2020). Although significant progress has been made recently, the molecular basis of this concentration-dependent feature of thrombin remains to be fully elucidated.

SCs in peripheral nerve injury: During development, myelinating SCs wrap large caliber axons in a myelin sheath while axons of small caliber are enveloped by non-myelinating SCs. Upon injury, damaged axons respond by generating a distress signal detected by SCs, which undergo extensive reprogramming. SCs gradually lose contact with the distal stump axon and convert into a repair phenotype which in the end promotes and guides axonal repair. In the injured site, repair SCs participate in the disintegration and removal of damaged axons during the process of Wallerian degeneration and assist myelin debris clearance to create a regrowth favourable environment. Myelin clearance is achieved by the digestion of myelin fragments and the recruitment and activation of invading macrophages. Afterwards, SCs secrete trophic factors to support survival of damaged neurons and promote axon regrowth. Repair SCs extend long parallel processes and align in tracts called bands of Büngner to guide the regrowing axon back to innervate its former target. Finally, SCs proliferate, upregulate pro-myelinating genes, re-differentiate into myelinating SCs and remyelinate the regenerated axon. This repair machinery requires a dynamic and orchestrated regulation of SC plasticity and reprogramming following axon injury (Boerboom et al., 2017).

Unfortunately, although peripheral nerves display an impressive regenerative capacity as compared to the CNS, recovery for patients suffering from traumatic injuries and others peripheral neuropathies is often incomplete. This is mainly a result of the slow regeneration rate and of the absence of a long-lasting repair-supportive environment. Moreover, the PNS repair ability decreases over time. SCs slowly lose their plasticity in an age-dependent way and the PNS environment becomes unsupportive to regeneration. Therefore, a greater understanding of the mechanisms driving SC plasticity is of utmost interest. Extensive research has been devoted to highlight the molecular mechanisms involved in SC reprogramming to provide mechanistic insights and novel therapeutic strategies to treat peripheral nerve injuries. Recent work demonstrates the involvement of morphogenetic transformations, epigenetic mechanisms and highlight many transcription factors and signaling pathways critical in this process. However, their induction and the temporal/quantitative activation as well as their mutual interactions have not been completely elucidated (Nocera and Jacob, 2020).

Thrombin and PARs in peripheral nerves: Regeneration of peripheral nerves after damage appears to correlate inversely with thrombin levels. Numerous studies support the involvement of coagulation factors, and specifically of the thrombin pathway, in SC-mediated regeneration and axonal function. It is known that the lesion of a peripheral nerve is accompanied by a local dramatic increase in thrombin activity respect to the uninjured control (Bushi et al., 2016; Gera et al., 2016) and that both thrombin and the extrinsic pathway FX/FXa are locally generated in sciatic nerve (Gera et al., 2018). In the PNS, low concentrations of thrombin were found to enhance the regeneration of mouse peripheral nerve after its crushing, while high concentrations had deleterious effects (Gofrit and Shavit-Stein, 2019). Furthermore, Lino et al. (2007) demonstrated that SC-derived serpin protease nexin-1, an endogenous thrombin inhibitor, is required for normal reinnervation after sciatic nerve crush, possibly to protect structures from prolonged and extensive proteolytic attacks. Thrombin is the key effector protease of the coagulation cascade mediating hemostasis, thrombosis, and inflammatory responses to vascular injury predominantly through the PAR1. PAR1 is the prototypic member of a family of four G-protein-coupled receptors that respond to extracellular proteases via a unique proteolysis-dependent activation mechanism (Willis Fox and Preston, 2020). The thrombin receptor PAR1 is strategically located in both the PNS and CNS, further supporting its possible role in various neurological disease processes. In the PNS, PAR1 is found at the node of Ranvier on specific glial structure known as the SC microvilli and its activation with high doses of thrombin and PAR1 agonists causes a conduction block in motor fibers (Shavit et al., 2008). On the other hand, low levels of thrombin generate activated protein C which, when coupled with its receptor, endothelial protein C receptor, can activate PAR1 (Gera et al., 2016). This activation has a neuro-regenerative effect (Festoff and Citron, 2019). In this connection, our data show that PAR1 stimulation with low doses of thrombin and PAR1 agonist peptides increases neurotrophic and neuroprotective properties of cultured SCs (Pompili et al., 2017, 2020). Indeed, the activation of PAR1 by low levels of specific agonist peptides in SC cultures enhances their ability to release molecules already known to promote nerve regeneration such as decorin, macrophage migration inhibitory factor and matrix metalloproteinase-2 (Pompili et al., 2017). Conversely, cultured SCs treated with high levels of thrombin show a reduced capacity of promoting PC12 neurite extension respect to control (Pompili et al., 2020).

These divergent effects of low and high levels of thrombin on SCs are also observed in sciatic nerve *ex vivo* explants. In this model system although the nerve is transected the three-dimensional relationship between SCs and the axon is mainly maintained. No evident modification in the morphology of SCs and of the nodes are observed in this model using low levels of thrombin. Conversely, the increase in the concentration of thrombin (or PAR1 agonist peptide) determines a profound rearrangement of SCs with the disappearance of the Cajal bands. Actually, our data show that nerve fibers treated with high levels of thrombin present evident signs of calcium-mediated demyelination at the paranodes (**Figure 1**). Although these data can be reproduced by PAR1 agonist peptides and inhibited by a

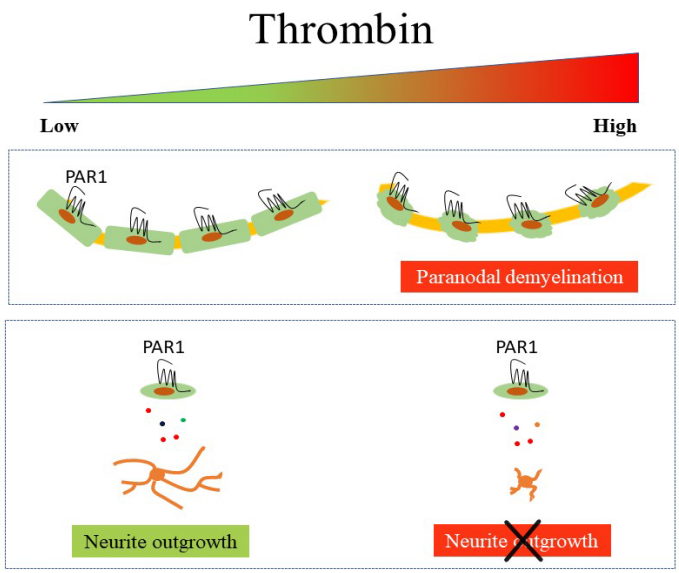

Figure 1 | **Schematic representation of the postulated dual role for thrombin in peripheral nerves.** Schwann cells express the thrombin receptor PAR1 on their plasma membrane. Low levels of thrombin support the pro-regenerative capacities of Schwann cells facilitating neurite outgrowth in neuronal cell cultures (lower panel). Conversely, the rise of thrombin levels which accompanied injuries of the peripheral nerves reduces the neurotrophic properties of Schwann cells in culture (lower panel) and determines paranodal demyelination in *ex vivo* nerve explants (upper panel). PAR1: Protease-activated receptor 1.

PAR1 inhibitor (SCH79797) since agonist and antagonists are used at high concentrations the possibility of an off-target effect should be considered. In adequate concentration thrombin can activate other PAR family members such as PAR2 and PAR4 (Kremers et al., 2018). Moreover, the formation of PAR1 homo and heterodimers with other PARs is known to lead to a distinctive signaling output upon activation (Kremers et al., 2018). The presence and role of the other PARs in peripheral nerves have not been reported yet.

Conclusion: Together, all these data contribute towards considering thrombin, which appears deregulated at sites of PNS injury, a possible novel therapeutic target. Potentially a pharmacological reduction of its excessive activity at the level of nerve lesions could circumvent the demyelinating properties of this protease and restore the SC neurotrophic activities. Interestingly, in a murine model for Guillaine-Barre syndrome the slowing of nerve conduction velocity and the destruction of the architecture of the node of Ranvier are accompanied by elevation of thrombin levels in sciatic nerve together with a reduction of nodal PAR1 expression. Thrombin inhibitors normalizes both the conduction velocity and the nodal architecture (Shavit-Stein et al., 2019).

Vorapaxar is a PAR1 antagonist already approved for the treatment of patients with myocardial infarction. Other promising molecules targeting PAR1 signaling are pepducins and parmodulins (Flaumenhaft and De Ceunynck, 2017).

Since there are currently no therapies to effectively protect myelin or to promote nerve regeneration after lesion of the PNS, the inhibition of the thrombin pathway could be a possible future treatment to prevent demyelination and promote remyelination in cases of injury or disease of peripheral nerves.

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