PERSPECTIVE

Novel approaches for the development of peripheral nerve regenerative therapies

Schwann cells are the myelinating glial cells of the peripheral nervous system (PNS). By establishing lipid-rich myelin sheaths around large-caliber axons, they ensure that electrical signal transmission is accelerated–a process referred to as saltatory signal propagation. Apart from this prominent physiological function, these cells also exert important pathophysiological roles in PNS injuries or diseases. In contrast to the central nervous system (CNS), the adult PNS retains a remarkably high degree of intrinsic regeneration. As a consequence, transected axons and damaged myelin sheaths can be repaired and nerve functionality can be restored. This spontaneous regenerative capacity depends on (inter) actions of macrophages, neurons, and Schwann cells. Although highly specialized and tightly interacting with axons, Schwann cells can revert upon nerve injury or disease to an immature and repair-mediating phenotype (Arthur-Farraj et al., 2012). Dedifferentiated Schwann cells participate in myelin clearance and attract macrophages for further clearance, enabling Wallerian degeneration of distal nerve stumps to proceed. They were also shown to positively influence injured axons and to stimulate regrowth of their tips toward their target cells in the periphery. Finally, re-established axons can be wrapped up again by redifferentiating Schwann cells, thereby generating new isolating myelin sheaths. Thus, spontaneous peripheral nerve regeneration can be mainly attributed to Schwann cells and their particular and specific responses to trauma and disease. This is remarkable cell behavior and implies that these cells have a large capacity to switch and adjust their transcriptional programs, most likely by means of epigenetic controls (Jacob et al., 2011; Heinen et al., 2012). Moreover, multiple interactions with cells and components of the immune system were recently revealed (Tzekova et al., 2014).

Despite this well-developed intrinsic repair function, the overall capacity of peripheral nerves to heal and functionally restore remains limited, particularly in pathological conditions such as inherited, toxic, inflammatory, and diabetic neuropathies, as well as after deep traumatic lesions. The underlying reasons for this impairment remain to be fully elucidated, but it is likely that regenerative Schwann cell functions are defective, either due to immunological processes or due to kinetic aspects, *i.e.*, nerve restoration cannot cope with recurring degeneration. Given that neuropathies of different etiologies are quite common, it is remarkable that no therapeutic approaches exist to promote, stabilize, accelerate, or even trigger peripheral nerve repair. This unmet therapeutic need related to axonal and myelin regeneration therefore requires increased attention to develop novel treatment strategies. In addition, because nerve regeneration, which includes remyelination of spared axons, myelination of regenerated axons, and axonal regeneration, is time-consuming, long-lasting processes need to be supported.

Two recent preclinical investigations from our laboratory addressed to what degree well-described pharmacological treatments could affect Schwann cells and their potential to adopt a repair-mediating phenotype, and thus serve as future medications for patients with PNS injuries or diseases.

In the first study, we investigated glial responses upon exposure to Fingolimod/FTY720P (Heinen et al., 2015) (**Figure 1**). Fingolimod is a sphingosine-1-phosphate (S1P) receptor agonist and is an approved treatment for relapse-remitting multiple sclerosis (Gilenya) (Ingwersen et al., 2012). Currently, it is undergoing a clinical trial for the treatment of chronic inflammatory demyelinating polyneuropathies (CIDP; ClinicalTrials.gov Identifier: NCT01625182). Fingolimod modulates S1P signaling and prevents immune cells from exiting lymphoid tissues (Ingwersen et al., 2012). Previous studies have indicated that Schwann cells express all five S1P receptors affecting glial migration and cytoskeletal dynamics and interfering with myelination *in vitro* [see Heinen et al. (2015) for further references]. Moreover, in experimental autoimmune neuritis (EAN), the rodent model of PNS Guillain-Barré syndrome (GBS), FTY720P application led to substantial amelioration of the disease course (Zhang et al., 2008), most likely due to its immunomodulatory action. Possible direct FTY720P-related neuroregenerative effects have not yet been investigated. In our study we stimulated primary neonatal and adult rat Schwann cells with Fingolimod/FTY 720P and investigated its impact on the regeneration-promoting phenotype. We found that this treatment resulted in the activation of a number of dedifferentiation markers, including the transcription factor cJun, which was recently described to reprogram Schwann cells to act as repair-mediating cells (Arthur-Farraj et al., 2012). While it interfered with the expression of mature markers and myelin, Fingolimod also negatively affected intracellular Akt signaling, which is known to be critically involved in Schwann cell maturation (Heinen et al., 2015). Besides this shift toward a dedifferentiated cellular state, FTY720P-treated Schwann cells also increased growth factor expression, which in turn rendered these cells more potent in enhancing neurite outgrowth–even on inhibitory substrates, as evidenced by dorsal root ganglion neuron stimulation by conditioned media of FTY720P-treated Schwann cells. Therefore, these findings provide strong evidence that S1P receptor stimulation supports the generation of a repair-promoting cellular phenotype, suggesting that Fingolimod/Gilenya should be further investigated for PNS regenerative treatments. Currently, it is not clear which of the five S1P receptors initially described on Schwann cells [Heinen et al. (2015) and references therein] are responsible for Fingolimod's promotion of cellular dedifferentiation. A more detailed description of involved receptor subtypes and further signaling cascades is currently being undertaken, along with a translation towards clinically relevant *in vivo* models.

In the second study we examined whether immunoglobulins can affect glial cell homeostasis, differentiation, or Schwann cell-dependent nerve regenerative processes (Tzekova et al., 2015) (**Figure 1**). Intravenous immunoglobulins

(IVIG) mainly consist of polyclonal human immunoglobulin G (IgG). They are generally used for the treatment of immune deficiencies, but they are also given to patients suffering from polyneuropathies. IVIG most likely act on several immunomodulatory mechanisms, and positive effects on disease severity and recovery have been reported for EAN (Tzekova et al., 2015). Their underlying mode of action is not well understood, but it certainly includes Fc-dependent and F(ab')2-dependent mechanisms blocking cellular receptors, neutralizing cytokines, complement and autoantibodies, as well as modulation of activating and inhibitory FcγR expression on immune cells (Lunemann et al., 2015). Of note, recent data indicate that endogenous antibodies also participate in myelin clearance and axon regeneration after peripheral nerve injury (Vargas et al., 2010), suggesting an intrinsic contribution of immune/neural (cell) interactions to spontaneous nerve repair processes.

We discovered that IVIG specifically bind to Schwann cells, including interactions *via* the high-affinity 1a Fc receptor (Fcgr1a/CD64) expressed on their surface. On stimulation with IVIG, non-differentiating Schwann cells reduced proliferation rates, accelerated growth of cellular protrusions, and transiently increased myelin gene expression as well as myelination-related signaling pathways. Myelin expression of differentiation-competent Schwann cells was enhanced in the long-term and *in vitro* myelination was improved. Importantly, myelin responses could not be detected when IgG1 control antibodies were applied. Moreover, we were able to demonstrate that IVIG stimulate interleukin-18 production by Schwann cells and that this cytokine instructs them to promote axonal growth from sensory neurons *ex vivo*. We therefore concluded that polyvalent immunoglobulin preparations can positively influence the Schwann cell differentiation process and that it enhances their regenerative potential. Currently, it is not known how IVIG-dependent signals act on Schwann cells or how they can stimulate maturation pathways and gene expression. Based on our findings, it is conceivable that, similar to immune cells, Fc-dependent and F(ab')2-dependent mechanisms account for this process.

Given that Schwann cells are central components of the peripheral nerve repair process, and given that this activity critically depends on their plastic differentiation potential, our findings using two approved medications for the treatment of (among others) inflammatory demyelinating diseases of the CNS are of particular interest. While the establishment of a dedifferentiation process can be enforced *via* S1P receptor activation, the subsequent redifferentiation stage was clearly promoted in the presence of and upon interaction with IVIG. Although these are preclinical findings mainly drawn from observations *ex vivo*, they strongly indicate that Schwann cell differentiation can indeed be modulated specifically and that these treatments could be used to overcome regeneration restrictions imposed by multiple pathological conditions of the PNS. Several polyvalent immunoglobulin preparations are approved and on the market, and besides Gilenya/Fingolimod several novel S1P receptor agonists have been developed – some of them are already evaluated in clinical trials (for example, Siponimod; ClinicalTrials.gov Identifier: NCT01665144). Of note, as discussed in our articles (Heinen et al., 2015) (Tzekova et al., 2015), similar treatments have also been shown to affect oligodendroglial precursor cells (OPCs), which are immature glial cells of the CNS that can eventually give rise to new oligodendrocytes. Specific OPC responses *ex vivo* or in experimental demyelination conditions were reported upon FTY720P stimulation, and immunoglobulin M (IgM) was discovered as a potent OPC differentiation inducer that led to a clinical trial on hIgM22 (ClinicalTrials.gov Identifier: NCT01803867).

Moreover, recent findings revealed that Herceptin, a monoclonal antibody directed against human epidermal growth factor receptor 2 (erbB2), promotes axonal outgrowth after peripheral nerve transection (Placheta et al., 2014) (**Figure 1**). The underlying mechanism awaits future analyses because Herceptin's actions could not be attributed to altered neuregulin/erbB2 signaling. Because this antibody was not found to be effective in our experiments and served (together with Avastin and Synagis) as a control, this might be of interest considering potential combinatory treatments (*e.g.*, IVIG). In addition to testing immunoglobulins and S1P receptor agonists in suitable *in vivo* paradigms, optimal windows of opportunity need to be established and parallel overlapping or counteracting effects on the immune system need to be explored.

The search for active ingredients in polyvalent immunoglobulin preparations (Fc receptor or Schwann cell antigen–directed) as well as studies of S1P receptor activation by means of more specific ligands might further pave the way for novel repair therapies for patients with different peripheral nerve conditions. The studies presented here also demonstrate that Schwann cells exert a high degree of immunocompetence and that multiple signaling interfaces between immune and Schwann cells exist, and these could be explored for pharmacological modulation.

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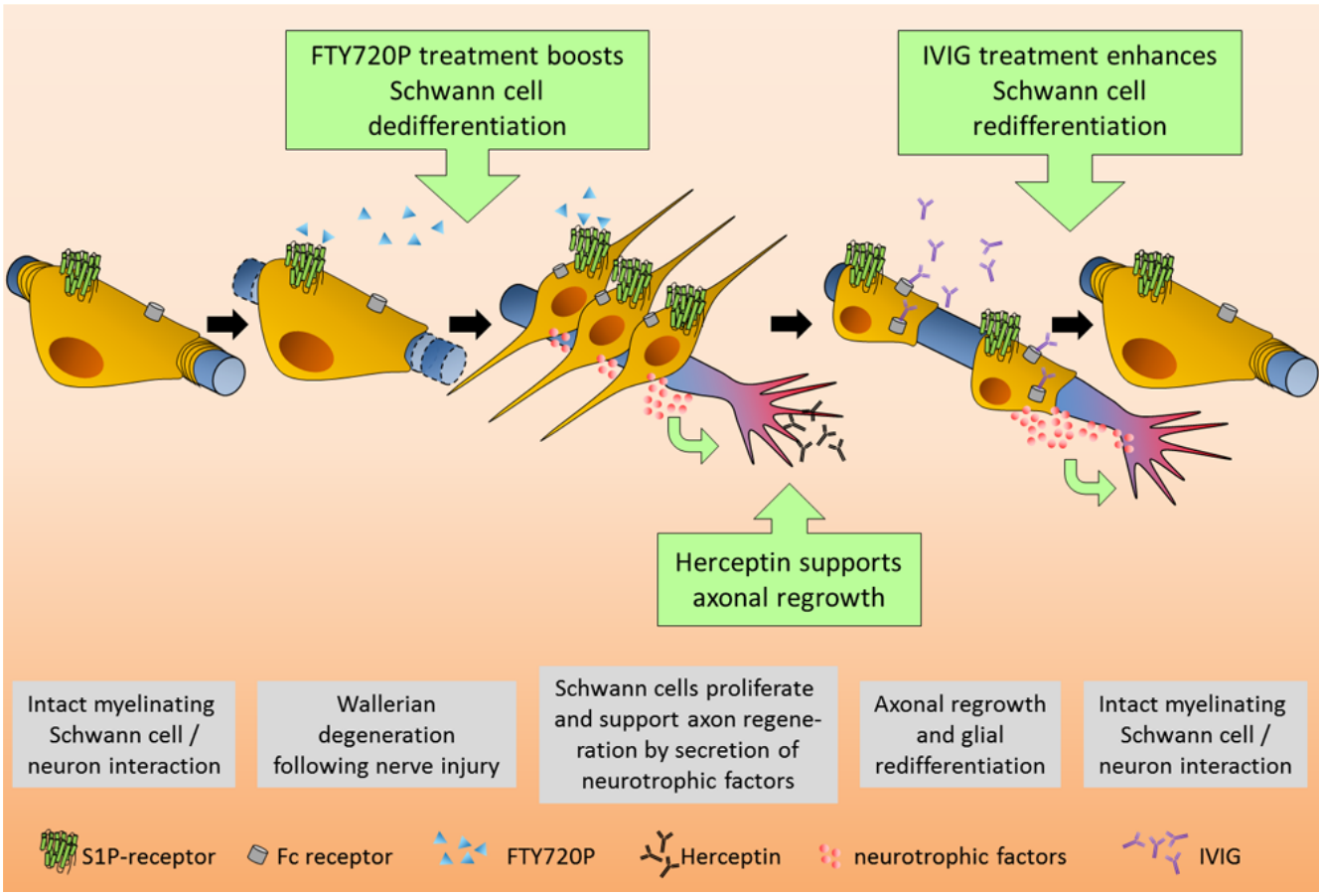

Figure 1 Schwann cell responses and degeneration- and regeneration-related subprocesses following peripheral nerve injury.
Schwann cell dedifferentiation resulting in the generation of a repair-mediating phenotype can be promoted/accelerated by means of Fingolimod stimulation. This is accompanied by growth factor production and promotion of axonal outgrowth effects by these glial cells. Intravenous immunoglobulins (IVIG) treatment was found to enhance Schwann cell redifferentiation and the generation of myelin sheaths. The monoclonal antibody Herceptin can specifically support axonal growth in injured nerves.

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