PERSPECTIVE

Neuronal gene transcription modulates demyelination and remyelination in a mouse model of multiple sclerosis

The role of the neuronal compartment in multiple sclerosis: Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease affecting the central nervous system (CNS). It is usually characterized by initial relapses and remissions with subsequent progressive neurological deterioration. MS is the most common acquired neurological disorder affecting young adult persons. Considering the early age of onset of the disease and the massive physical and cognitive restrictions patients face as disease progresses, the identification of the cause and the development of therapeutic strategies is of immense importance.

The pathological hallmark of MS is the occurrence of multifocal demyelinated lesions in the brain and spinal cord of patients. At early stages of the disease, active lesions are associated with inflammation, de- and remyelination. As disease progresses, remyelination fails and axons devoid of myelin eventually degenerate. Clinical symptoms are mainly caused by the ongoing neurodegeneration of demyelinated axons in the grey and white matter tracts (Ellwardt and Zipp, 2014). Hence, current therapeutic approaches do not only concentrate on hindering demyelination, but also on promoting oligodendrocyte precursor cell (OPC) maturation and remyelination.

Inflammatory processes play a key role in disease pathogenesis, thus, MS is commonly considered to be caused by an autoimmune response. For example, breakdown of the blood-brain barrier, autoreactive T cells, monocyte infiltration and autoantibodies are commonly found in patients suffering from MS (Yadav et al., 2015). Beside the components of the peripheral immune system, activation of brain resident inflammatory cells (*i.e.*, astrocytes and microglia) is a classical feature of MS and influences disease progression considerably (Claycomb et al., 2013).

Astrocytes are the most abundant cell type in the CNS with functions ranging from building the blood-brain barrier to establishing a scaffold for migrating neurons during brain development. One of the most intriguing functions of astrocytes is the regulation of the adaptive and innate immune system in the CNS. Activated astrocytes in lesions of MS patients can influence the activation and infiltration of T cells into demyelinated lesions for example by controlling the permeability of the blood-brain barrier, by presenting antigens or by secreting pro-inflammatory chemokines. Additionally, an essential function of astrocytes is the activation of microglia (Claycomb et al., 2013; Skripuletz et al., 2013).

Myelin and cell debris within MS lesions are mainly cleared away by microglia, the primary phagocytic cells in the CNS. Microglia activity can be regulated by astrocytes either by direct cell-cell contacts, or through chemokine secretion. Depending on the mode of activation, microglia can develop into pro-inflammatory M1 cells promoting further inflammation and eventually leading to neurotoxicity. On the other hand, microglia can polarize to anti-inflammatory M2 cells, which have neuroprotective functions and promote maturation of OPCs. Interestingly, M2 microglia are only present in active MS lesions and at the border of chronic lesions, where remyelination is still ongoing (Ellwardt and Zipp, 2014). This possibly indicates an essential role of M2 microglia in promoting remyelination. Taken together, the exact regulation of astrocyte and microglia activation seems to be critical for keeping the balance between neuroprotection and neurotoxicity in pathological situations like MS.

In addition to the demyelination and neuroinflammation, axonal degeneration is a typical characteristic of MS lesions. Neuronal pathology like axonal swellings, Wallerian degeneration and axonal transections have been repeatedly described in MS patients and MS mouse models and are commonly accepted to be secondary to axonal demyelination and inflammation (Trapp and Nave, 2008). Recent research has increasingly focused on the role of the neuronal compartment in MS development and progression. Interestingly, studies in MS patients with relapsing remitting MS (RR-MS) receiving immunomodulatory drugs have shown that while suppression of the immune system alleviates clinical symptoms during relapses, it does not slow down disease progression (Shirani et al., 2012). Additionally, experiments with mice lacking the oligodendroglial protein 2′,3′-cyclic nucleotide phosphodiesterase (CNPase) have shown axonal pathology without myelin impairment (Lappe-Siefke et al., 2003). Those findings indicate that the etiology of neurodegeneration cannot solely be explained by demyelination or neuroinflammation. In sum, recent research has identified the neuronal compartment and axon-glia interactions as key players in MS development. Hence, it is considerable that demyelination and neuroinflammation are (at least in some cases) secondary to neuronal damage.

Paracrine regulation demyelination and remyelination by neuronal serum response factor (SRF) gene transcription: In our laboratory, we have focused on the role of the transcription factor SRF, which is highly expressed in neurons. SRF regulates the transcription of immediate early genes (IEGs), cytoskeletal genes and genes related to actin dynamics (Knöll and Nordheim,

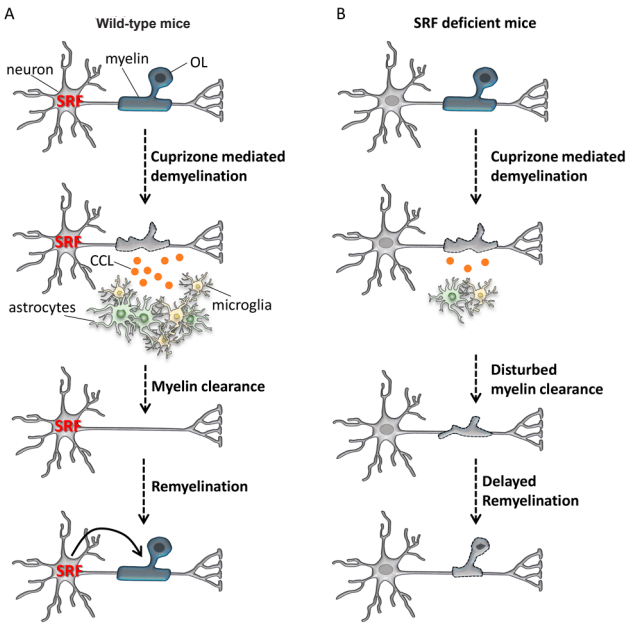

Figure 1 Model for the regulation of inflammation, demyelination and remyelination by serum response factor (SRF).
(A) In wild-type mice, cuprizone treatment leads to myelin destruction. This results in an SRF mediated secretion of chemokines by neurons. Secreted chemokines activate astrocytes and microglia, which in turn clear myelin debris away. The proper debris clearance allows for an efficient remyelination. (B) Depletion of SRF in neurons leads to decreased production of CCLs. Due to the low CCL levels, astrocytes and microglia are activated to a much lesser extent. As a result, myelin debris cannot be cleared and hinder the remyelination process. OL: Oligodendrocyte; SRF: serum response factor; CCL: CC chemokine ligand.

2009). Thereby, it can control diverse neuronal processes in the developing brain, including neuronal migration, axon guidance and regeneration and growth cone dynamics. Besides those neuron specific processes, we analyzed the role of SRF in myelin development in mice. We demonstrated that mice lacking SRF in forebrain neurons only, fail to generate myelin upon birth. Further, we identified a mechanism by which neurons regulate the maturation of pre-myelinating oligodendrocytes in a paracrine fashion (Stritt et al., 2009).

In our recent study, we aimed at analyzing the role of SRF in demyelination and remyelination in young adult mice with an already established myelinated neuronal circuit. Our data show that the deletion of *Srf* in neurons of adult mice can influence demyelination and inflammation in the cuprizone mouse MS model of toxic demyelination. In fact, whereas wild-type mice had a robust demyelination upon cuprizone treatment, the lack of SRF in forebrain neurons resulted in higher amounts of non-removed damaged myelin. Since oligodendroglia (OLs) of *Srf* deficient mice where similarly depleted by cuprizone as OLs of wildtype mice, we concluded that *Srf* mutant mice had problems efficiently clearing away the remaining myelin debris (**Figure 1**; Anastasiadou et al., 2015). Moreover, we demonstrated that SRF depletion in neurons results in disturbed induction of brain resident inflammatory cells (*i.e.*, microglia and astrocytes). Previous reports have already shown that astrocytes and microglia play an essential role in removing cell and myelin debris. Therefore, this defective induction of inflammation is a compelling explanation for the impaired myelin debris clearance observed in *Srf* mutant mice. In our mouse model, the deletion of *Srf* was restricted to neurons, indicating the involvement of a paracrine mechanism. Hence, our results support the importance of the crosstalk between OLs, neurons, astrocytes and microglia for the regulation of demyelination and inflammation in pathological conditions. Interestingly, in contrast to neuronal SRF depletion, the deletion of *Srf* only in OLs did not influence demyelination or inflammation upon cuprizone treatment. This indicates that mainly neurons (but not OLs) use a SRF mediated paracrine pathway for the induction of brain resident immune cells.

Besides demyelination and inflammation, a major issue in MS pathology is the compromised remyelination. Hitherto, it is not known why OPCs eventually stop differentiating, resulting in remyelination failure. Considering that SRF can regulate OPC differentiation during development (Stritt et al., 2009), we investigated whether SRF also influences OL function during remyelination. In wild-type mice, we detected rapid generation and maturation of OPCs during remyelination. In line, the expression of myelin genes, which had been strongly reduced during demyelination, showed an overshooting reaction reaching even higher levels than untreated control mice. In contrast, generation and maturation of OPCs during remyelination were delayed in mice lacking SRF in forebrain neurons. Additionally, the expression of genes encoding for myelin proteins was reduced in those mice (Anastasiadou et al., 2015). Hence, we demonstrated that neuronal SRF can influence OL function during remyelination in a paracrine fashion.

In order to shed some light on the interaction of the involved cell types, we searched for mediators of the neuron-glia crosstalk. In this search, we considered molecules that fulfill two criteria: i) they can be secreted by neurons and ii) they are related to inflammation. By gene expression analysis, we identified a group of chemokines, the CC chemokine ligands (CCLs), which are highly induced during demyelination. Particularly CCL2, CCL3 and CCL5 show reduced expression in SRF deficient mice (Anastasiadou et al., 2015). CCLs have previously been reported to activate and attract astrocytes and microglia and are also known to be secreted by neurons. Therefore, we suggest that SRF activity in neurons can modify the expression of CCLs during demyelination, thereby influencing the communication of neurons with astrocytes and microglia (**Figure 1**). Nevertheless, the contribution of different CCLs to the neuron-glia crosstalk still has to be elucidated *e.g.*, by the use of CCL2, CCL3 or CCL5 deficient mice.

Taken together, our results underline the importance of a well-orchestrated communication between neurons and different types of glia cells for the regulation of essential processes in pathological demyelination and in remyelination. Our observations suggest that neurons are actively involved in activation/recruiting of brain resident inflammatory cells, thereby regulating myelin debris clearance. Additionally, our data emphasize the delicate balance of inflammation leading either to neurodegeneration or neuroprotection. Current therapeutic strategies for MS aim at suppressing CNS inflammation and enhancing neuroprotection. Therefore, molecular and cellular insights obtained in rodent MS models, highlighting the importance of the neuronal compartment for disease progression, might prove useful for the understanding of mechanisms involved in the onset of human MS disease. Finally, the investigation of neuronal signaling mechanisms, such as SRF mediated gene transcription, identified in MS mouse models, might provide new valuable molecular targets relevant to human MS therapy.

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