NEURAL REGENERATION RESEARCH
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Not only myelination: the immune-inflammatory functions of oligodendrocytes

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Oligodendrocytes (OLs) are highly specialized cells of the central nervous system (CNS). Their primary and most investigated role is to form myelin, a multilamellar fatty membrane that enwraps axons ensuring their insulation and the saltatory conduction of nerve impulses. The formation of myelin is a complex process during which the OL precursor cells (OPCs, also known as NG2-glia) become mature OLs through a highly regulated program of differentiation. In addition, OPCs persist in the adult grey and white matter parenchyma, representing approximately 6% of the total number of CNS cells. Beyond their role in myelin generation and turn-over, it is now clear that OPCs have the capability to control tissue homeostasis and to sense and react to inflammation which characterizes many neurological diseases.

OPCs control CNS homeostatic state: Under physiological conditions, OPCs are essential for the maintenance of microglia in a quiescent state (**Figure 1**, top panel), and their depletion has been shown to downregulate microglia homeostatic signature (Zhang et al., 2019; Liu and Aguzzi, 2020). Gain- and loss-of-function studies further demonstrated that OPC regulation of microglial homeostasis is mediated by the release of transforming growth factor-beta 2 (TGF-β2). In turn, TGF-β2 up-regulated the expression of CX3CR1, a microglial receptor activated by fractalkine that promotes a neuroprotective phenotype of microglia (Zhang et al., 2019). In a different study, NG2-glia ablation was shown to induce hippocampal neuronal cell death, paralleled by an increase in microglia activation and in the mRNA levels of pro-inflammatory interleukin (IL)-1β, IL-6 and tumor necrosis factor (Tnf). These detrimental effects were accompanied by reduced levels of the neuroprotective hepatocyte growth factor (Hgf). Interestingly, administration of mouse recombinant HGF in OPC-ablated rats significantly preserved hippocampal neurons and attenuated microglia activation (Nakano et al., 2017).

In a similar fashion, both genetic ablation of NG2-glia and reduced OPC density caused by chronic social stress exposure were found to impair the physiological functions of astrocytes, including glutamate reuptake from the synaptic cleft. The resulting disruption of glutamatergic transmission contributed to the development of a depressive-like behaviour, underlining the importance of OPCs for preserving normal brain activity. In this context, OPCs were shown to interact with astrocytes by releasing fibroblast growth factor 2 (FGF2), as selective knockdown of FGF2 in NG2-expressing cells was sufficient to induce a depressive-like phenotype (Birey et al., 2015).

Hence, results from OPC depletion studies suggest a new physiological role for OPCs in maintaining the homeostatic and protective functions exerted by microglia and astrocytes, thus preserving neuronal integrity.

OLs orchestrate the immune-inflammatory response in neuropathological conditions: CNS-resident astrocytes and microglia, together with circulating immune cells, are classically considered the main actors orchestrating the inflammatory response. Nevertheless, it is now evident that OLs have immunomodulatory properties, through which they play an active contribution to the immune-inflammatory response in neurological diseases. This is relevant not only for neuroinflammatory conditions, such as multiple sclerosis (MS), but also for other neurological disorders, including Parkinson's disease, Alzheimer's disease, stroke, epilepsy, and amyotrophic lateral sclerosis, in which inflammation strongly contributes to neurodegeneration.

In experimental models of CNS injury, deficiency of OPCs has been shown to exacerbate neuroinflammation and neurodegeneration. In mice with ablated NG2-glia, microglia acquired aberrant activation, contributing to a more aggressive lipopolysaccharide-induced neuroinflammatory response. Moreover, in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced mouse model of Parkinson's disease, OPC-depleted mice displayed a significant increase in the number of reactive microglia in the substantia nigra. This led to higher loss of tyrosine hydroxylase-positive dopaminergic neurons as compared to control animals with preserved OPC population (Zhang et al., 2019).

To interact with neighbouring immune cells, OLs express a wide range of cytokines, chemokines, and several receptors for immune-related molecules. In OPCs isolated from cuprizone-fed mice with chronic demyelination, the gene expression of the two inflammatory mediators IL-1β and Ccl2 was found to be significantly upregulated compared to cells derived from control mice. Accordingly, an increase of CCL2 protein levels in OPCs was also revealed in active lesions of MS patients (Moyon et al., 2015). These data suggest that demyelination-activated OPCs express chemoattractant inflammatory mediators which can enhance, in an autocrine fashion, their own motility and tissue regenerative capacities (Moyon et al., 2015).

Recent studies highlighted a critical role of oligodendroglial TNF receptor 2 (TNFR2), the receptor of the membrane-bound form of TNF (tmTNF), in modulating the immune-inflammatory response following demyelination. In the experimental autoimmune encephalomyelitis (EAE) model of MS, mice selectively lacking the oligodendroglial TNFR2 displayed earlier microglial activation and immune cell infiltration from the periphery. This led to increased demyelination, widespread axonal loss, and impaired remyelination compared to wild-type controls (Madsen et al., 2020). Interestingly, gene expression profiling of OLs sorted from oligodendroglial TNFR2 knockout mice after EAE showed significant upregulation of numerous inflammatory mediators when compared to naïve mice, while the same was not observed in wild-type controls (Madsen et al., 2020). Thus, TNFR2 activation on OLs might be beneficial to dampen the production of inflammatory signals, limiting excessive neuroinflammation and consequently slowing down demyelination (**Figure 1**, bottom panel). Importantly, *in vitro* experiments also suggested that, even though both OPCs and immature OLs have inflammatory capacity, TNFR2 is involved in the modulation of this process more in OPCs than in OLs (Madsen et al., 2020). On this basis, a bulk RNA sequencing (RNAseq) analysis has been performed *in vitro* on primary OPCs, isolated from wild-type and TNFR2-ablated mice, and exposed to either pro-inflammatory cytokines or vehicle for 3 hours (Desu et al., 2021). Comparison between control and stimulated OPCs from wild-type mice revealed that most of the downregulated genes were associated with differentiation, while upregulated genes were correlated with inflammatory responses. Of note, stimulated cells from TNFR2 knockout mice were found to further upregulate genes associated with metabolism, immunity,

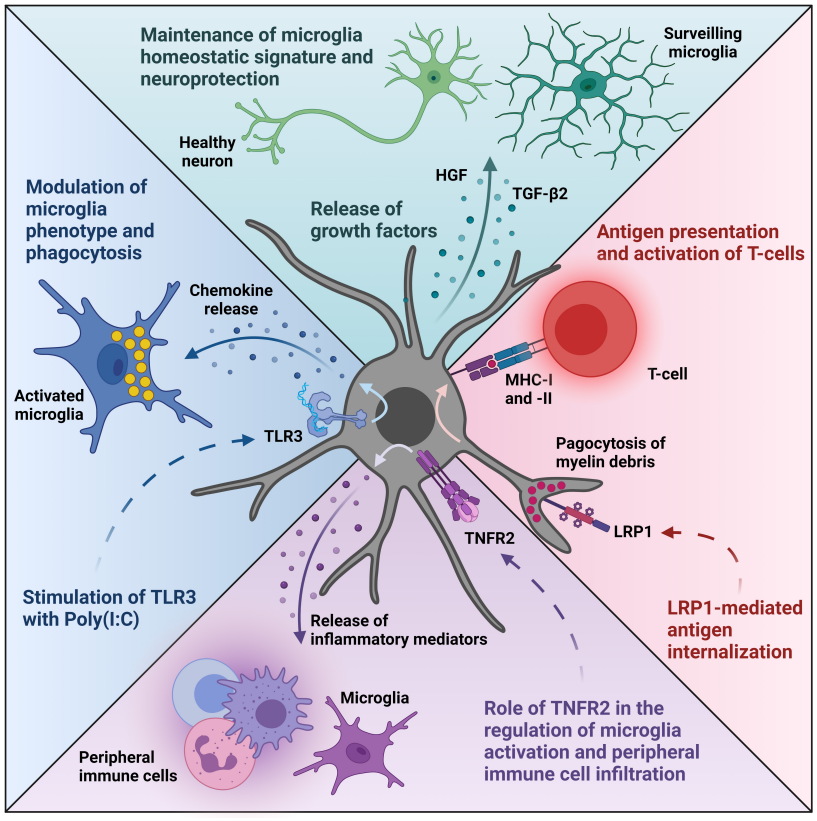

Figure 1 | **The immune-inflammatory functions of oligodendrocytes.**
In physiological conditions, the release of growth factors (e.g. hepatocyte growth factor [HGF], transforming growth factor-beta 2 [TGF-β2]) by oligodendrocyte precursor cells (OPCs) has been shown to be essential for the maintenance of microglia in a surveillant state, protecting neurons from degeneration (Nakano et al., 2017; Zhang et al., 2019) (top panel). Upon central nervous system (CNS) injury, oligodendrocytes (OLs) express a wide range of inflammatory mediators and several receptors for immune-related molecules, which enable them to sense inflammation and to react. Oligodendroglial tumor necrosis factor receptor 2 (TNFR2) has been shown to regulate the expression of inflammatory molecules, controlling excessive microglia activation and immune cell infiltration from the periphery (Madsen et al., 2020; Desu et al., 2021) (bottom panel). Stimulation of toll-like receptor 3 (TLR3) influences the immunomodulatory properties of OLs, inducing the production of soluble factors able to modulate microglia phenotype and phagocytic capacity (Boccazzi et al., 2021) (left panel). OPCs also express the machinery for antigen processing and presentation, therefore being able to enhance the survival, proliferation, and cytokine production in T-cells, perpetuating a detrimental immune response (Falcao et al., 2018; Kirby et al., 2019) (right panel). Globally, these results support an emerging role of OL lineage cells in maintaining immune homeostasis and in modulating inflammatory reaction during CNS diseases.

and inflammation compared to wild-type stimulated cells. Thus, TNFR2 ablation exacerbated the immunomodulatory and inflammatory function of OPCs following inflammatory stimulation, reducing their capacity to proliferate and differentiate. (Desu et al., 2021). These data pointed at a role of TNFR2 in limiting the pro-inflammatory phenotype of OPCs. In addition, bioinformatic analysis suggested that almost all chemokines released by OPCs could interact with the corresponding receptors expressed by naïve microglia. These interactions were determined to have higher statistical significance for ligands expressed by stimulated TNFR2-deficient OPCs compared to wild-type stimulated cells, which can be indicative of a stronger interaction between microglia and OPCs in the absence of TNFR2. Overall, these in silico data indicated the likelihood that OPC-produced chemokines may act on microglia, enhancing their activation state and migration (Desu et al., 2021).

Recently, differences between OPCs and immature OLs in the sensitivity to neuroinflammation have been also highlighted *in vivo*, using a mouse model mimicking inflammation-mediated white matter injury of preterm born infants by intraperitoneal injection of IL-1β (Boccazzi et al., 2021). In this experimental paradigm, $O4^+$ immature OLs showed a greater upregulation of toll-like receptor 3 (Tlr3), IL-1β, interferon (Ifn)-β, Ccl2 and Cxcl10 as compared to $PDGFR\alpha^+$ OPCs. Further analysis in purified primary OPC cultures demonstrated that, upon *in vitro* TLR3 stimulation with Poly(I:C), $PDGFR\alpha^+$ OPCs significantly increased the release of CCL2, CCL3, CCL5 and CCL11 in their culture medium. These molecules potentially represent important cues for attracting immune cells, like microglia, near the area of demyelination. Interestingly, we also demonstrated that, depending on their maturation state, TLR3-activated OLs play a critical role in modulating microglia phenotype and function (**Figure 1**, left panel). Conditioned medium derived from primary OPCs, treated with Poly(I:C) during proliferation, increased the expression of the immunomodulatory markers Socs3 and Il1rn in primary microglial cultures. On the contrary, microglial cells exposed to conditioned medium from Poly(I:C)-treated differentiating OPCs increased the expression of the pro-inflammatory marker Nos2 and acquired a typical pro-inflammatory phenotype. Further, conditioned medium derived from both proliferating and differentiating cells stimulated with Poly(I:C) increased the phagocytic activity of microglia (Boccazzi et al., 2021). Additional studies are needed to understand how this diverse OL differentiation stage-specific capacity of shaping microglia response might impact on myelin lesion formation and repair in neuroinflammatory diseases.

It is worth mentioning that several chemokines, cytokines, and surface adhesion molecules produced by inflammatory-stimulated OPCs can also potentially interact with the respective receptors expressed by astrocytes. This evidence suggests an additional role of OPCs in controlling astrocyte functional phenotype at sites of myelin injury, in turn affecting remyelination kinetics. However, specific studies investigating how OPCs may influence astrocytes activation and function are still lacking, encouraging further research in this direction.

Another fascinating mode of action by which OPCs could modulate the immune response is by interacting with the neurovascular unit to regulate the infiltration of immune cells from the peripheral circulation. Indeed, following EAE, juxtavascular and perivascular OPCs have been observed to increase their accumulation near blood vessels. In particular, OPCs were mostly associated with microvessels characterized by altered tight junction staining patterns and barrier leakage. Thus, it can be speculated that, under inflammatory conditions, vascular OPCs may amplify blood-borne immune cell recruitment by releasing chemoattractant molecules and by altering the permeability of the blood brain barrier (Girolamo et al., 2019).

OLs trigger T-cell activation via antigen processing and presentation: Recent single-cell RNAseq analysis of OL linage cells in EAE (Falcao et al., 2018) and in MS tissue (Jakel et al., 2019) revealed that OLs can acquire a disease-specific state characterized by the expression of immune-related genes. These data emphasize the new vision of OLs as immunologically active cells in MS pathogenesis. In particular, confirming what already observed in the early '90,

transcriptomic analysis revealed that OL lineage cells respond to demyelination also by expressing genes involved in antigen processing and presentation, including major histocompatibility complex class I and II (MHC-I and -II; **Figure 1**, right panel). *In vitro* experiments showed that the direct contact with T-cells induces OPCs to express MHC-I and -II and in turn cross-present antigens to $CD8^+$ and $CD4^+$ T-cells. A similar response was also found to be triggered by the exposure to IFN-γ, alone or in combination with other Th1 cytokines (Falcao et al., 2018; Jakel et al., 2019; Desu et al., 2021). Hence, this mechanism increases T-cell activity due to enhanced survival, proliferation, and cytokine production, establishing a negative feedback loop that ultimately promotes OL degeneration. These findings suggest that OPCs may be co-opted by the immune system to perpetuate the autoimmune response in MS (Falcao et al., 2018; Kirby et al., 2019).

Finally, a subset of OPCs was shown to be capable of internalizing fluorescent microspheres and myelin debris through phagocytosis. IFN-γ treatment did not alter OPC phagocytic activity (Falcao et al., 2018); however, it is not possible to exclude that a different inflammatory trigger could further stimulate this function. Regarding the mechanism regulating OPC phagocytosis, low-density lipoprotein receptor-related protein 1 (LRP1) gene silencing in OLs blocked myelin vesicles uptake, highly suggesting the involvement of this protein in antigen internalization. Accordingly, conditional knockout mice with OPC-restricted LRP1-deficiency were characterized by decreased expression of the antigen cross-presentation machinery, including MHC-I (Fernandez-Castaneda et al., 2020). Notably, OPC-specific LRP1-ablated mice displayed an improved clinical score and a delayed disease onset of EAE as compared to control littermates. Moreover, the knockout of LRP1 in OPCs enhanced remyelination in the cuprizone model. These protective effects were accompanied by decreased infiltration of T-cells and myeloid cells in the spinal cord of animals lacking LRP1 expression in OPCs at EAE peak compared to control mice (Fernandez-Castaneda et al., 2020).

In conclusion, the results presented above disclose a new and exciting point of view in the field of OL research, putting these cells in the spotlight of CNS immune-inflammatory processes. Based on the early involvement of OLs in several diseases, spanning from neurodevelopmental (e.g., schizophrenia and autism) to neurodegenerative disorders, the identification of the molecular mechanisms underpinning the immunomodulatory functions of OLs may provide novel therapeutic targets for controlling neuroinflammation and promoting CNS repair.

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Date of submission: July 20, 2021
Date of decision: September 27, 2021
Date of acceptance: December 24, 2021
Date of web publication: April 29, 2022

https://doi.org/10.4103/1673-5374.342678
How to cite this article: *Boccazzi M, Raffaele S, Fumagalli M (2022) Not only myelination: the immune-inflammatory functions of oligodendrocytes. Neural Regen Res 17(12): 2661-2663.*

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C-Editors: Zhao M, Liu WJ, Li JY; T-Editor: Jia Y