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Review

Paving the way for adequate myelination: The contribution of galectin-3, transferrin and iron

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To Eduardo Soto, because he was always our scientific support and a model of friendship and generosity for all of us.

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ABSTRACT

Considering the worldwide incidence of well characterized demyelinating disorders such as Multiple Sclerosis (MS) and the increasing number of pathologies recently found to involve hypomyelinating factors such as micronutrient deficits, elucidating the molecular basis of central nervous system (CNS) demyelination, remyelination and hypomyelination becomes essential to the development of future neuroregenerative therapies. In this context, this review discusses novel findings on the contribution of galectin-3 (Gal-3), transferrin (Tf) and iron to the processes of myelination and remyelination and their potentially positive regulation of oligodendroglial precursor cell (OPC) differentiation. Studies were conducted in cuprizone (CPZ)-induced demyelination and iron deficiency (ID)-induced hypomyelination, and the participation of glial and neural stem cells (NSC) in the remyelination process was evaluated by means of both in vivo and in vitro assays on primary cell cultures.

1. Introduction

Oligodendrocytes (OLG) are glial cells in charge of myelin production in the central nervous system (CNS) and thus play a key role in demyelinating disorders, among which Multiple Sclerosis (MS) appears to exhibit the highest incidence [1–3]. Therefore, among the various trophic factors which have been identified to support OLG maturation and proliferation, e.g. fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), insulin-like growth factor 1 (IGF-1) and thyroid hormones [4–6], this review will focus on the roles of galectin-3 (Gal-3), transferrin (Tf) and iron in OLG differentiation.

OLG are known to produce most CNS Tf [7,8] and their maturation is directly related with the fact that the brain is the only organ in which Tf mRNA expression increases after birth, compelling evidence of Tf importance [9]. In this context, numerous studies published by our group since 1994 have attempted to determine whether Tf has an iron-independent trophic effect on myelin production. A critical question in assessing Tf trophic actions is whether apotransferrin (aTf, iron-free Tf) acquires iron once it is injected in vivo or from the cell culture media. Iron ubiquity and its high affinity for aTf suggest that it would rapidly bind to injected aTf even in small concentrations [10], although evidence seems to establish that it is aTf, and not iron binding to Tf, that has pro-differentiating effects [11].

On the basis of the key role of iron in OLG maturation and myelin production, and considering that hypomyelination as a consequence of iron deficits and the associated neurological sequelae persist long after these deficits have been corrected [12–14], studies in our lab targeted the possible ameliorating effects of an intracranial injection (ICI) of aTf in iron deficiency (ID) conditions, with results rendering a partial correction of myelin deficits. Subsequent assays in cultures of OLG isolated from control and ID animals revealed a smaller number of differentiated cells in ID conditions, followed by a partial recovery upon aTf treatment [15].

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Finally, it was in recent years that our group has started working on molecules apparently working as external signals in OLG differentiation, i.e. galectins (Gals), which are well known within the immune system [16], but not as widely characterized in connection with the CNS. We have identified an essential role for Gal–glycan interactions in regulating OLG differentiation leading to the control of myelin integrity and function and, in particular, we have assessed Gal-3 potential role in modulating neuroimmune processes [17].

2. Cuprizone and demyelination

The cuprizone (CPZ) model has been described as a useful tool to study demyelination and remyelination phenomena [18], with advantages such as easy reproduction and low mortality rates [19]. In mouse models, a CPZ diet has been shown to produce demyelination and OLG damage in the CNS – particularly in the corpus callosum (CC), [20] – without compromising other cell types [21–23], while its termination brings about spontaneous and almost complete remyelination in a matter of weeks [21,24,25].

Although Cammer [26] showed CPZ-induced cell damage in OLG-enriched glial cell cultures and mixed glial cell cultures from neonatal rat brains, the demyelinating effects of CPZ in vivo have proven to be very effective in mice but not in rats [20]. Rats and guinea pigs exposed to CPZ have been reported to show spongiform encephalopathy [27] but not demyelination. In turn, weaning Wistar rats fed a diet containing 0.5–2% CPZ have exhibited intramyelinic edema and OLG mitochondrial enlargement, among other abnormalities, in different areas of the cerebellum [28]. With these two exceptions, our group has pioneered the description of CPZ effects on myelin in rats [29].

Similarly to what has been described in mice [20], CPZ demyelination in rats stops upon termination of CPZ administration and is followed by spontaneous remyelination. Two weeks after toxin withdrawal, myelin yields increase substantially and myelin protein, phospholipid and galactolipid contents recover, although still remaining well below control values. A suitable model to evaluate remyelination strategies in rodents without involving the adaptive immune system, CPZ-induced demyelination by OLG degeneration in white and gray matter is accompanied by reactive gliosis [30–33], an increase in the number of resident microglia (MG) and, to a lesser extent, a rise in the number of peripheral macrophages [34]. In order to characterize the mechanism of CPZ-induced demyelination, we used rat primary oligodendroglial cell cultures and evaluated CPZ effects on cell viability, which was only significantly affected when either IFNγ or TNFα were present. In addition, in vivo experiments showed that the inhibition of microglial activation with minocycline prevented CPZ-induced demyelination. Taken together, our results demonstrate an active role for microglial cells in CPZ-induced oligodendroglial cell death and demyelination, through the production and secretion of pro-inflammatory cytokines [35].

3. Demyelinating diseases and oligodendrocyte damage. Endogenous sources of remyelination

Demyelination is defined as the process governed by the loss of myelin sheaths around neuronal axons and it is a consequence of oligodendroglial dysfunction or death, which, if sustained in time, inexorably leads to neuronal damage and neurodegeneration [36]. Among human demyelinating pathologies of the CNS, MS has a mean age of onset in young adulthood and clinical neurodegenerative manifestations in motor, sensory and cognitive disability. MS is considered a chronic autoimmune disease and is characterized by the emergence of demyelination foci in different brain areas, accompanied by a marked inflammatory response with microglial activation and astrogliosis [37]. Although the adaptive immune system plays a leading role in the acute pathogenesis of MS in most cases, primary oligodendropathy prevails in some patients [38,39]. An endogenous tissue response to CNS demyelination, remyelination tends to restore oligodendroglial population and myelin sheaths and is detected at early stages of MS. However, the process fails in the long run [40,41] and the pathological environment associated with the disease prevents successful long-term myelin recovery [42].

Much effort has been made lately in order to understand the pathophysiological complexities that unfold during MS and attention has been drawn toward developing effective strategies to stimulate myelin repair in the injured brain [43]. In this context, although animal models do not completely recapitulate human MS features, they continue to stand as useful tools for studying remyelination processes in vivo. In CPZ-induced demyelination models in rodents, several hormones such us thyroid hormones [44–47], sex hormones [48–50] or different growth factors [51–53] have been shown to stimulate myelin recovery. Despite their effectiveness in promoting remyelination, the exact mechanisms through which these factors trigger oligodendroglial generation are still unresolved.

Remyelination could proceed by two alternative mechanisms [54]: (a) a rapid response of local oligodendroglial precursor cells (OPC), which undergo differentiation/oligodendroglial maturation and (b) a process involving neural stem cell (NSC) activation and neural progenitor cell (NPC) proliferation from a subventricular zone (SVZ) niche, migration of progenitor cells toward damaged brain areas and terminal oligodendroglial differentiation [55,56].

In vitro studies are useful to dissect these particular mechanisms and allow puzzling out cell interactions and evaluating the effects of individual factors on specific cell types. OLG-enriched primary cultures have been extensively used as an in vitro model of oligodendroglial maturation. More recently, NSC and NPC biology research has become an exciting field of study within neurosciences where multipotent neural precursors can be manipulated in vitro to obtain OPC-enriched primary cultures.

4. Molecules modulating myelinogenesis, demyelination and remyelination

4.1. Galectin-3

Galectins (Gals) are a family of β-galactoside-binding lectins lacking specific individual receptors. They bind to cell surface glycoconjugates containing suitable oligosaccharides to form multivalent complexes and induce intracellular signals regulating cell survival and differentiation [57,58]. Gals also form complexes that crosslink glycosylated ligands to form dynamic lattices (Gal–glycan lattices, [59]). Gal-1 and -3 often have antagonistic roles in the immunological system: while Gal-3 plays pro-inflammatory roles, Gal-1 exerts anti-inflammatory effects [16]. Gal-3, a chimeric protein structurally composed of unusual tandem repeats of proline and glycine-rich short segments fused onto a carbohydrate-recognition domain (CRD), possesses multifaceted roles in physiological processes including the regulation of innate and adaptive immune responses [60].

Specifically in the CNS, our group was a precursor in establishing the relevance of Gal–glycan lattices in OLG physiology and identifying an essential role for Gal–glycan interactions in regulating OLG differentiation leading to the control of myelin integrity and function (Fig. 1). Our first findings showed a high expression of both Gal-1 and -3 in astrocytes (AST) and MG, and an upregulation of Gal-3 in differentiating OLG. This upregulation was accompanied by an increment in the activity of metalloproteinases

(MMP) in charge of regulating the biological activity of Gal-3 during OLG differentiation. Interestingly, recombinant Gal-3 treatment accelerated OLG differentiation in a dose- and carbohydrate-dependent manner, which is in accordance with the N-glycosylation profile observed in immature versus differentiated OLG. Strikingly, OPC cultured in media conditioned by Gal-3-expressing MG produced a higher number of differentiated OLG (myelin basic protein+ (MBP+) cells) than OPC incubated in the presence of Gal-3-deficient MG (obtained from *Lgals3*$^{-/-}$ mice). These findings received strong support from our in vivo results. The morphometric analysis of ultrastructural studies revealed a significantly lower number of myelinated axons and myelin turns (lamellae) in *Lgals3*$^{-/-}$ mice. Furthermore, the g-ratio (the ratio between the axon's diameter and the axon's diameter wrapped with myelin) was observed to be higher in *Lgals3*$^{-/-}$ mice, with myelin sheaths more loosely wrapped around axons. Accompanying these results, behavioral studies in *Lgals3*$^{-/-}$ mice rendered lower anxiety levels, which resembled those observed during early CPZ-induced demyelination. Finally, neurospheres isolated from *Lgals3*$^{-/-}$ mice showed a weaker commitment to an oligodendroglial fate than those obtained from wild type (WT) mice (Fig. 1). Additional findings by our group indicate that glial-derived Gal-3, but not Gal-1, promotes oligodendroglial differentiation. On the whole, Gal-3 contributes to myelin integrity and function, which has critical implications in the recovery of inflammatory demyelinating disorders [17].

Bibliographical evidence shows Gal-3 upregulation by inflammatory stimuli and its detrimental role in prion-infected brain tissue [61–63]. A role for Gal-3 has also been proposed in the negative regulation of lipopolysaccharide-induced inflammation [64]. On the other hand, Gal-3 mediates the activation and proliferation of MG-induced focal cerebral ischemia [65]. Moreover, when immunized with myelin OLG glycoprotein (MOG), *Lgals3*$^{-/-}$ mice exhibit a decrease in Experimental Autoimmune Encephalomyelitis (EAE) severity [66]. Due to the CPZ-mediated suppression of T cell function, the process of demyelination and remyelination in the CPZ model can be investigated without peripheral inflammation [67]. This is of fundamental importance, as brain-intrinsic effects are difficult to separate from immune-driven ones in immune-driven demyelination models such as EAE. CPZ-induced demyelination increases the number of resident MG and, to a lesser extent, peripheral macrophages [34], which have the capacity to phagocyte myelin debris, mostly through the upregulation of phagocytic receptor TREM-2b [68]. Phagocytosis of myelin debris by MG and macrophages plays an important role in the initiation of remyelination, as OPC differentiation can be inhibited by myelin [69]. Myelin phagocytosis is mediated by CR3/MAC-1 and SRAI/II, which are regulated by Gal-3-dependent activation of PI3K; as a consequence, myelin phagocytosis is deficient in *Lgals3*$^{-/-}$ MG [70].

We have used a 6-week CPZ administration model in adult *Lgals3*$^{-/-}$ mice to evaluate the involvement of Gal-3 in the demyelination process. Although our findings showed similar susceptibility to CPZ up to week 5 in both *Lgals3*$^{-/-}$ and WT mice, OPC generated in CPZ-treated *Lgals3*$^{-/-}$ mice showed diminished arborization, a possible sign of poor differentiating capacity. Strikingly, while WT mice exhibited spontaneous remyelination at week 5 –even though the demyelinating diet was still in place–, Lgals3-/- mice exhibited continuous demyelination up to week 6, accompanied by pronounced astroglial activation. Regarding cell response, an increase in Gal-3 immunoreactivity during CPZ-induced demyelination was detected in MG but not in AST. Remarkably, while CPZ-treated WT mice displayed heightened microglial activation –as evidenced by ED1 expression– and the sharp upregulation of phagocytic receptor TREM-2b, CPZ-demyelinated *Lgals3*$^{-/-}$ mice showed an increase in the number of caspase-3-activated microglial cells. Behavioral studies revealed lower innate anxiety as early as the second CPZ week in both WT and *Lgals3*$^{-/-}$ mice, although only *Lgals3*$^{-/-}$ mice experienced a reduction in locomotor activity and spatial working memory. All in all, our results indicate that Gal-3 expression in MG appears to modulate their phenotype in response to CPZ-induced demyelination [71]. These results also show that OPC generated in response to CPZ-induced demyelination in *Lgals3*$^{-/-}$ mice have decreased ability to differentiate, which could be due to the inhibitory effects exerted by impaired phagocytosis of myelin debris in *Lgals3*$^{-/-}$ MG. Moreover, this could also be explained by our previously informed findings demonstrating that conditioned media from Gal-3-expressing (but not *Lgals3*$^{-/-}$) MG successfully promote OLG differentiation (Fig. 1) [17]. Previous manuscripts have demonstrated that an interruption in OPC differentiation might be the reason for remyelination failure [36,72,73]. Therefore, *Lgals3*$^{-/-}$ mice could show a delay in remyelination due to a failure in Gal-3-induced OPC differentiation. Moreover, as *Lgals3*$^{-/-}$ mice exhibit late remyelination in an environment which is not correctly conditioned by MG, myelin generated *de novo* is aberrant and appears loosely wrapped around axons.

MG are known to release either neurotoxic or pro-recovery factors, depending on their commitment to an M1 or M2 phenotype, respectively. They have been implicated in numerous inflammatory processes in the brain [74,75] and have also been proposed to take part in the initial stages of MS. The activation and proliferation of resident MG are regulated by several factors. In particular, Gal-3 modulates microglial response to ischemic injury [65] and, as mentioned above, upregulates phagocytic receptor TREM-2b during CPZ-induced demyelination (Fig. 1) [71].

In conclusion, our results show that Gal-3 helps the myelination process by enhancing OLG differentiation and favoring oligodendroglial fate following NSC differentiation [17]. The contrasting effects of Gal-1 and Gal-3 on OLG differentiation could be explained in the context of their anti- or pro-inflammatory roles [58]. Like many cytokines and growth factors, Gals may exert a two-side effect depending on intrinsic factors including protein physicochemical properties (dimerization or oligomerization status), stability in tissue (relative activity of MMP), concentration and cell redox state, as well as extrinsic factors such as the glycosylation status of target cells [57,58]. In addition, our findings demonstrate the upregulation of Gal-3 in MG during CPZ-induced demyelination and its role in the regulation of microglial activation and phenotype, hence driving the onset of remyelination. Briefly, our studies prove that Gal-3 deficiency leads to (1) similar vulnerability to CPZ-induced demyelination but failure in triggering spontaneous remyelination, (2) a decrease in OPC differentiation, (3) aberrant microglial activation during demyelination, connected to a significant increase in the number of proliferating MG with higher levels of CD11b and caspase-3 activation but lacking ED1 expression, (4) microglial inability to induce TREM-2b expression or produce TNF-α, and (5) early behavioral alterations including lower levels of anxiety, decreased locomotor activity and impairment in spatial working memory [71]. Recent work has demonstrated an enhancement in OLG differentiation in vitro with M2 cell-conditioned media and an impairment in vivo following intra-lesional M2 cell depletion, indicating an essential role for M2 cell polarization in efficient remyelination [76]. Therefore, Gal-3, expressed in MG, could favor the onset of remyelination through the induction of an M2 phenotype or a direct effect on OLG differentiation, either extra or intracellularly. These results may thus have important implications in the development of future therapies for a variety of demyelinating diseases such as MS.

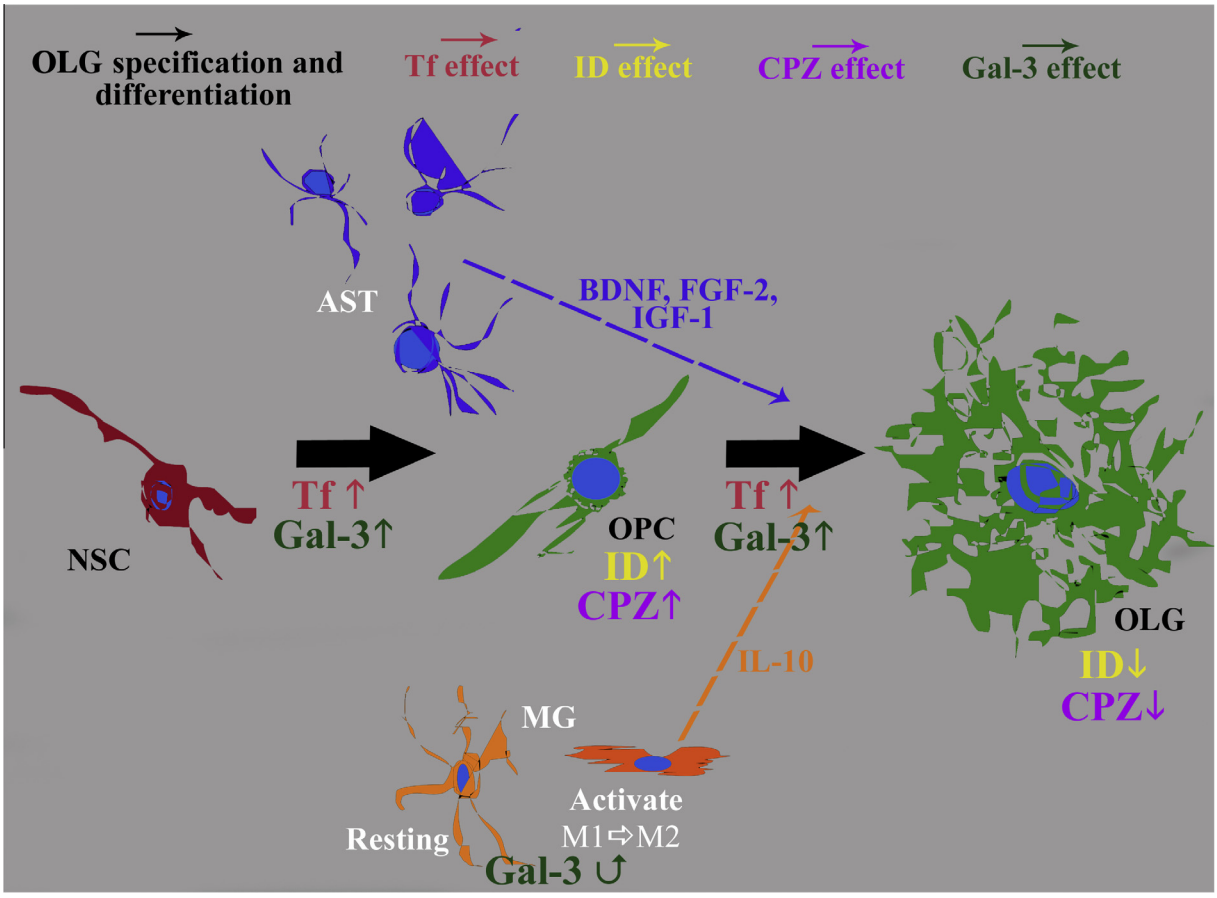

Fig. 1. Schematic illustration showing different effects: transferrin (Tf), galectin-3 (Gal-3) and iron on oligodendroglial cell (OLG) differentiation. Gal-3 participates in the conversion of M1 and M2 microglial phenotypes. Gal-3, secreted by microglia (MG), induces OLG differentiation. Apotransferrin (aTf) induces OLG lineage specification and also favors OLG differentiation. Iron deficiency (ID) increases the number of oligodendroglial precursor cells (OPC) and also diminishes OLG maturation, indicating that intrinsic OPC problems and the absence of factors secreted by astrocytes (AST) or MG affect OLG differentiation. Blue and orange arrows illustrate previously published crosstalk between AST, MG and OPC, which positively modulate OLG maturation [45,129,130].

4.2. Transferrin

Tf is the main iron carrier in the human organism and its expression is mostly restricted to the liver. However, smaller expression levels have been reported in OLG and choroid plexus epithelial cells of the CNS, and in testicular sertoli cells [7,77]. Experimental models have shown the Tf molecule to play a key role during development, with Tf-knockout mice dying shortly after birth unless treated with an exogenous Tf source [78,79]. In turn, lack of Tf in humans results in atransferrinemia (MIM #209300), a rare genetic disorder characterized by microcytic anemia and accumulation of excess iron in tissue, leading to body weakness and growth delay. In the CNS, Tf has proven to be necessary for myelin biosynthesis [8] and transgenic mice overexpressing Tf have exhibited increased myelination [80].

Studies conducted by our group since 1994 first found that an intrathecal injection of Tf in postnatal day (PND) 2–5 rats induced the expression of several myelin proteins, while an ICI of aTf in PND-3 rats increased the levels of MBP and 2′,3′-cyclic-nucleotide 3′-phosphodiesterase (CNPase) mRNA and protein, without affecting those of proteolipid protein (PLP) [11,81–83]. Subsequently, this ICI of aTf was found to increase the levels of tubulin and actin mRNA, as well as various microtubule-associated proteins (MAP) [84], although these effects occurred only when animals were injected at PND 2–5. In turn, Tf receptor expression in in vitro OPC was proven to become undetectable by Western blot when cells were allowed to differentiate into mature OLG. These data suggest the existence of a developmental time window for aTf effectiveness [11], probably related to the loss of Tf receptor expression in OLG, and are consistent with studies showing that complete Tf receptor downregulation in mature OLG at PND 20 makes them no longer responsive to exogenous Tf. In vitro studies further demonstrated that Tf addition to primary OPC cultures promotes either cell migration or maturation depending on culture conditions (Fig. 1) [85,86].

In line with these findings, Tf administration during the recovery stages of CPZ demyelination was shown to reduce the NG2-expressing cell population at the expense of an increase in the number of APC+ cells and MBP expression, thus promoting oligodendroglial generation and maturation [29]. Tf inhibits the progression of OPC cycle when using an OPC culture fraction prepared according to McCarthy and De Vellis [87]. This premature cell cycle exit subsequently leads to early cell differentiation and further favors OLG maturation [88]. In addition, similar conclusions were reached in a hypoxia/ischemia experimental model of demyelination, in terms of the positive effects of Tf on oligodendroglial regeneration and remyelination [89,90].

Moreover, given that NPC are potential targets for endogenous OLG generation both in vivo and in vitro [91–93], the effects of Tf were analyzed on rat SVZ-derived NPC cultures, with results rendering an increase in NSC proliferation [47,94]. These data are in line with findings described by Sergent-Tanguy et al. [95], who demonstrated a higher expression of Tf receptor in rat embryo NPC compared to neurons and AST cultures, and Cao et al. [96],

who proposed the Tf receptor as a putative NSC surface marker (Fig. 1).

The multipotent capacity of NSC allowed us to analyze cell commitment and OLG maturation in vitro as well. The exogenous administration of Tf to NSC cultures under differentiating conditions revealed an increase in OPC proportions and a shift toward more mature OLG [94]. Guardia Clausi et al. [89] demonstrated similar data in vivo in a model of hypoxia–ischemia where overall myelin was restored after Tf intrathecal administration. The intranasal administration of Tf was found to exert similar effects by increasing SVZ-derived OPC numbers [90] (Fig. 1).

The effects of Tf on myelination are mediated by the functional convergence of several signaling pathways associated with oligodendrogenesis and oligodendroglial maturation [4]. In terms of the downstream mediators acting after Tf binding to its receptor, our studies in primary OPC cultures have demonstrated that OLG maturation depends on Fyn kinase activation, as well as Fyn/MEK/ERK and PI3K/Akt pathways [97]. Further experiments using a dominant-negative isoform of Tau to disrupt natural Tau–Fyn interaction showed that functional Fyn is a necessary component of OLG morphological differentiation [98]. OPC have also shown to incorporate aTf through clathrin-AP2-mediated endocytosis, which indicates that, during iron delivery, Tf binds to its cognate receptors on the cell surface and enters the endosome/lysosome canonical pathway through clathrin-coated vesicles [97].

In turn, assays performed in immortalized cell lines of the oligodendroglial lineage at various differentiation stages [99] have proven that the addition of aTf increases OLG morphological differentiation and maturation. The stimulation of OLG differentiation by aTf was observed to take place through the cyclic adenosine monophosphate (cAMP)-cAMP response element-binding (CREB) protein pathway, although CREB phosphorylation was only significant in the less mature cell line (N19). These data suggest that cell maturational stages at least in part determine the signaling pathways and transcription factors through which aTf induces MBP expression. In addition, studies in these cell lines showed Tf overexpression to promote OLG morphological complexity and maturation [100] and to induce the expression of several genes related to mitochondrial function and lipid metabolism, necessary for myelin synthesis [101].

Although the precise mechanisms underlying OLG maturation in response to Tf require further description, these results contribute to elucidating the initial molecular events and the transduction signals involved in oligodendrogenesis and oligodendroglial maturation.

4.3. Iron

Iron deficiency (ID) represents one of the most prevalent nutritional deficits, affecting almost two billion people worldwide. In our studies, dietary ID during development was produced in pregnant Wistar dams by feeding them an iron-deficient diet (4 mg Fe/kg) or a control diet (40 mg Fe/kg) beginning at gestational day 5. In contrast to the abundant evidence provided by this and other studies on ID leading to anemia, less is known about the impact of moderate ID on CNS development. Recently Lee et al. [102] have established a model of non-anemic maternal ID during gestation which generates slightly anemic ID offspring. These pups exhibited reduced levels of brain iron and significant age-specific alterations in nerve conduction, but minor changes in MBP and PLP and no changes in other myelin proteins such as myelin-associated glycoprotein (MAG) and MOG. Whether acquired perinatally or during early infancy, ID has detrimental effects on brain development – including alterations in neurons and myelination – which persist regardless of and well after iron deficits have been corrected [103–107]. Furthermore, abundant evidence suggests that early life ID anemia may impact the development of neural systems implicated in neuropsychiatric disorders, in part characterized by cognitive impairments [108,109].

OLG stain for iron more robustly that any other cell type in the normal CNS [110]. Iron-enriched OLG usually appear in rows in white matter, with clearly visible cell bodies and easily detectable processes [111,112]. Among others, events involving iron uptake and homeostasis are critical to OLG maturation and their disruption can thus significantly affect myelin composition and formation.

Since the foundational work by Dallman et al. [113], ID models have targeted different aspects of deficiency severity, duration and developmental time window affected, focusing either on prenatal or postnatal time points. In particular, developmental ID impacts OLG maturation, causing long-lasting hypomyelination which continues in adulthood even after normal iron diet reinstatement [15,106,114–116].

A growing body of evidence highlights two particular and relevant approaches in ID: on the one hand, specific neuropathologies, e.g. long-term deficits in cognitive and socioemotional function [117] and schizophrenia [118–121] could be modeled by ID, as they seem to involve myelin derangement; on the other hand, specific OLG requirements and their progression to a mature and myelinating state correlate with environmental nutrient availability [116,122–124] and could thus also be studied through ID models.

From a cellular point of view, studies by our group have revealed an enlarged undifferentiated cell subpopulation in ID conditions (local OPC) capable to migrate but powerless to progress beyond this precursor stage. In other words, ID hypomyelination does not affect cell lineage specificity but generates an arrested OPC population. This larger number of OPC might be a homeostatic response in order to build a complete myelin structure, the key question being why ID local OPC remain arrested. At first glance, ID OPC appear to exhibit intrinsic alterations in terms of proliferation, migration and maturation (Fig. 1) [15,122].

In recent years, extensive research has exposed abundant AST/MG interaction with OLG, which may support, limit, preclude or drive OLG along their lineage (reviewed in Clemente et al. [125]), in line with our results obtained in CPZ demyelination. A positive crosstalk among AST processes, axons and OLG has been reported in mixed mouse spinal cord cultures [126], while dynamic changes in cell morphology and prevalence for AST differentiation around the injection site have been demonstrated in green fluorescent protein (GFP)-tagged neurospheres transplanted into the spinal cord of dysmyelinated shiverer mice. Adding up to the description of transcription factors and molecules involved in OLG maturation [127,128], our results demonstrate that ID AST exhibit an extended proliferative status, high basal expression of immature markers and a diminished response capacity. In addition, ID MG display an anomalous cytokine profile, not only basally but also under stimulus (unpublished results).

On the hypothesis that deficiencies in OLG maturation underlie ID, our group tested the effects of aTf on OLG differentiation in ID animals. The sharp reduction in myelin components observed in ID conditions at PND 24 as a sign of hypomyelination was found to be largely prevented by an ICI of aTf at PND 3. When further exploring the factors involved in these ameliorating effects, cultures of OLG isolated from ID rats exhibited fewer differentiated cells relative to controls, as evidenced by the smaller number of differentiated MBP+ cells compared to the undifferentiated PSA-NCAM+ cells [15]. Additional in vitro studies proved aTf administration to have a compensatory effect on ID OLG cultures – with an increase in the number of MBP+ cells and a decrease in the number of PSA-NCAM+ ones – and thus support in vivo findings regarding aTf effects on OLG differentiation. In this context, the ID model becomes a

powerful tool to study OLG progression toward a differentiated stage, taking into account not only OPC features and needs but also their cellular environment and thus opening doors to new targets in neuropathological approaches.

Altogether, our results indicate that aTf has ameliorating effects on demyelination and hypomyelination, while Gal-3 has proven to foster remyelination following CPZ- or LPC-induced demyelination. These findings encourage us to search for novel therapeutic strategies for the treatment of demyelinating diseases.

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