On the cause of multiple sclerosis
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2016

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Download date: 11-06-2021
Chapter 6

Summary and Perspectives
SUMMARY

In the central nervous system (CNS), oligodendrocytes (OLGs) form myelin sheaths that are necessary for enwrapping axons, a prerequisite for the efficient propagation of neuron-mediated saltatory conduction of impulses. Damage to OLGs or myelin, which simultaneously may affect the integrity of axons, has deleterious consequences, as exemplified by the chronic demyelinating disease multiple sclerosis (MS). Specifically, the disease is characterized by inflammation, demyelination, failure of remyelination, and axonal loss. As a consequence these patients usually suffer from typical features such as progressive disability of mechanical functions and cognitive impairment. A detailed understanding of extra- and intracellular molecular mechanisms that underlie the process of myelination and its regulation, such as the biosynthesis and transport of specific myelin membrane components to the myelin sheath, including signaling that may trigger these events, will be imperative in developing an effective therapy for demyelinating diseases, which is conspicuously lacking so far.

Myelination entails the biogenesis of a membrane system that extends from the OLG plasma membrane, and eventually recognizes and enwraps axons. Important in this context is the emerging knowledge that OLGs should be considered as polarized cells, exposing two distinct surface membrane domains, i.e., the cell body plasma membrane and the myelin membrane. It is therefore anticipated that valuable mechanistic insight into the polarized nature of OLGs in general, and myelin biogenesis in particular, might be derived from previous studies, focused on similarly polarized epithelial cells. In Chapter 1 the extent to which membrane polarity of OLGs in terms of trafficking and sorting mirrors that of epithelial cells is highlighted. Current knowledge of the trafficking of several myelin specific proteins, including the major myelin proteins proteolipid protein (PLP) and myelin basic protein (MBP), and the role of myelin-enriched galactolipids as sorting platforms, is discussed, as well as the role of additional molecular entities in sorting and trafficking, such as myelin and lymphocyte protein (MAL) and SNAREs, and their polarity-specific features. In particular, based on the functioning of a common recycling endosomal system in epithelial cells, which is instrumental in the regulation and maintenance of their polarity, we propose the potential involvement of a similar system in OLGs. Among others this is inferred from studies on the transectyotic trafficking of PLP from the cell body plasma membrane to the myelin membrane, a type of trafficking already known from polarized trafficking and membrane maintenance in epithelial cells.

In Chapter 2, we demonstrate that the t-SNAREs syntaxin 3 and 4 display a polarity-specific distribution in (polarized) OLGs. Consistent with a typical polarized distribution in epithelial cells, syntaxin 3 localizes to the ‘apical-like’ cell body plasma membrane, while syntaxin 4 localizes near the ‘basolateral-like’ myelin membranes. Consistently, during OLG maturation, syntaxin 4 was upregulated, whereas syntaxin 3 levels remained constant. Lentiviral downregulation of syntaxin 4 resulted in a complete eradication of MBP protein expression. Remarkably, downregulation of sheet-localized syntaxin 4 has little if any effect on the vesicular delivery of other myelin-directed proteins, including PLP, integrin α6, and
the viral model protein VSV G. Not unexpectedly, our findings indicate that the lack of MBP expression upon syntaxin 4 downregulation is not related to defects in granule assembly or transport of MBP mRNA per se. Rather, most interestingly, the data strongly favors the hypothesis that downregulation of the syntaxin 4 machinery leads to a transcriptional suppression of MBP mRNA. Taken the role of syntaxin 4 in docking of transport vesicles, a possible mechanism would be the necessity of syntaxin 4 to deliver a (still unknown) component to the myelin membrane, that is crucial in initiating MBP mRNA transcription. In support of this, we have shown that adding conditioned medium can rescue the effect of syntaxin 4 downregulation. So most excitingly, our data indicate the potential involvement of a syntaxin 4 dependent mechanism that likely plays a role in triggering an autocrine signaling pathway that initiated MBP transcription.

In an earlier study we demonstrated that transcytotic transport of PLP, next to MBP the other major myelin resident protein, is a key element in the mechanism of myelin assembly. PLP is transported to the myelin membrane in an indirect manner via the cell body plasma membrane, which relies on syntaxin 3 and proceeds via a transcytotic mechanism, mediated by sulfatide. This process is characterized by a dynamic, protein conformation-driven partitioning of PLP into distinct membrane domains, thereby governing biosynthetic and transcytotic transport steps of the protein, respectively. This transcytotic transport pathway may thus allow for a regulated timing of PLP’s appearance in myelin membranes, thereby preventing premature compaction. To investigate the role of proteins, capable of regulating polarized trafficking in epithelial cells, we investigated in Chapter 3 the effect of MAL, a well-known regulator of polarized transport in epithelial cells and expressed in OLGs after PLP, on PLP trafficking in OLGs. Our data suggest that MAL, rather than interfering with vesicular trafficking of de novo synthesized PLP to the (apical) cell body membrane, controls the lateral segregation of PLP into distinct membrane microdomains once myelin is formed, presumably by modulating in a competitive manner PLP’s interaction with the sphingolipid sulfatide. We propose that at such conditions, PLP may acquire direct access from the plasma membrane to the sheath via lateral diffusion, possibly avoiding in this manner increasingly frustrated vesicular transport into the sheath when myelin compaction is finalized.

In the CNS, the extracellular matrix protein laminin-2, present on developing axons, is important in regulating MBP protein expression, and hence, OLG maturation. For example, laminin-2 is involved in mediating interactions between integrins and growth factors, initially localized in separate membrane microdomains, so-called lipid rafts, from where MBP translation is initiated. Galactosylceramide (GalC) and sulfatide are important constituents of such microdomains, while sulfatide may also serve as a receptor for laminin-2. We examined the relevance of these galactolipids in laminin-2-dependent OLG maturation, as reflected by MBP expression, in Chapter 4. Interruption of laminin-2-sulfatide interactions impeded the expression of MBP and the formation of myelin membranes. Our data, based upon immunoprecipitation among other techniques, support a mechanism in which laminin-2 engagement facilitates lateral interactions between integrin α6β1 and sulfatide in membrane microdomains. This interaction likely provides a platform within the OLG plasma
membrane that enables interactions between PDGFαR and integrin α6. Therefore, given that activation of integrin α6β1 initiates the translation of MBP, sulfatide likely acts as a response modifier of integrin signalling, leading to MBP expression. Consequently, any mechanism that disrupts laminin-2-sulfatide interactions might contribute to remyelination failure. Since aggregates of fibronectin are capable of impeding differentiation of OPCs in demyelinated MS lesions, the effect of fibronectin-galactolipid interactions on MBP expression was studied as well in Chapter 4. Interestingly, anti-sulfatide antibodies, but not anti-GalC antibodies promoted the formation of MBP-positive myelin membrane formation on fibronectin. Of particular interest for MS pathology is our observation that inhibition of morphological OLG differentiation by fibronectin dominated over the promoting potential of laminin-2. In fact, our data indicate that fibronectin prevents sulfatide segregation and proper integration into membrane microdomains, thereby frustrating MBP expression on laminin-2. Hence, these findings may explain the quiescence of OPCs in MS lesions, and rationalize the potential of anti-sulfatide antibodies to trigger remyelination.

The level of pro-inflammatory cytokine TNFα is elevated in MS lesions and contributes to MS pathology. Therefore, we examined in Chapter 5 the specific influence of TNFα on OLG myelin maintenance, as studied in primary mature rat OLGs and myelinated cultures. Remarkably, in TNFα-treated myelinated cultures, the length of the MBP-positive myelin segments, i.e., internodes, was reduced as compared to untreated cultures. In TNFα-treated mature OLGs, MBP protein, but not its mRNA, was retracted from the myelin membranes towards the cell body and primary processes. This difference in localization was reversible upon removal of TNFα, blocked in the presence of an anti-TNFFR1 antibody, and was not apparent with other pro-inflammatory cytokines (IFNγ, IL1β), thus highlighting the specificity of TNFα. Our findings further revealed that a TNFα-induced reversible disorganization of the actin cytoskeleton resulted in a redistribution of MBP and GalC from actin-dependent to actin-independent membrane microdomains. This may indicate that continuous exposure to TNFα in MS lesions not only influences remyelination by targeting proliferation, differentiation and survival of OPCs, as previously reported, but also alters the internodal length of existing myelin by altering the actin cytoskeleton, causing a subsequent lateral relocalization of MBP and GalC. Moreover, we anticipate that the in vivo significance of these data may relate to a necessary flexibility of existing myelin segments, when per demyelinated segment at least two newly formed but shorter myelin segments require integration within the demyelinated area of the axon. In order to do so, the existing myelin segments have to decompact, which can be accomplished by disruption of the actin cytoskeleton and retraction of MBP to non-compacted areas.
PERSPECTIVES

The work presented in this thesis underlines the polarized nature of OLGs, and the importance of correct trafficking of myelin components, including its regulation by internal and external cues, for myelination to occur. Future research should link the (dys)regulation of polarized trafficking to (re)myelination defects in demyelinating diseases, such as MS. For example, of interest in this regard would be the identification of the molecular factor(s) that is (are) secreted via the syntaxin 4-mediated SNARE machinery, and which initiates MBP, but not PLP mRNA transcription. In fact, since MBP expression is imperative for myelination to occur, identification of this specific factor may provide a means to promote myelination in MS lesions. It is tempting to suggest that the absence of MBP, as a result of syntaxin 4 downregulation, might perturb the ability of OPCs to properly polarize. This notion would be in line with similar observations reported for MDCK cells, where disruption of the basolateral targeting signal of syntaxin 4 leads to the inability of MDCK cells to establish a polarized morphology. Accordingly, a polarized morphology might thus be necessary to trigger MBP transcription. Given that the myelin-enriched lipids GalC and its derivative, sulfatide, are expressed prior to the expression of MBP, we anticipate a role for these lipids in MBP protein expression. Indeed, preliminary qPCR analysis show that MBP mRNA levels are significantly increased in the OLG-derived progenitor cell line, OLN-93, engineered to only express GalC (unpublished observations). Moreover, GalC transport to the plasma membrane is prevented upon syntaxin 4 downregulation, indicating that the galactolipid might be a crucial component of the membrane and a key player in establishing membrane polarity.

Consistent with the polarized nature of OLGs, we have previously shown that transcytotic transport of the other major myelin resident protein, PLP, mediated by syntaxin 3 and sulfatide, is a key element in the mechanism of myelin assembly. This transcytotic transport pathway may allow for a regulated timing of PLP’s appearance in myelin membranes, thereby preventing premature and ectopic compaction. Intriguingly, when, in the frame of the work presented in this thesis, the localization of syntaxin 3 was examined in cells grown on laminin-2, i.e., when myelin membrane formation is more extensive than in cells cultured on the inert substrate poly-L-lysine (PLL), it became apparent that syntaxin 3 relocalized preferentially towards the myelin membrane, like syntaxin 4. This may suggest that as a function of time and upon myelin maturation, syntaxin 3 mediated transport of PLP to the plasma membrane may be altered. Indeed, our data in Chapter 3 revealed that the MAL proteolipid, which is expressed after myelin membrane synthesis, redirects PLP transport from a transcytotic route to a ‘direct’, vesicle-independent transport route, by exerting control on the membrane microdomain localization of PLP upon its biosynthesis. Such a direct transport route, implying PLP transport by lateral diffusion between the apical-like plasma membrane and the maturing basolateral myelin membrane, is more efficient during myelin maintenance and protein turnover, since it does not require vesicular transport, as in apical to basolateral transcytosis. Hence, MAL interferes with membrane polarity, and in this context
it is of interest that a regulatory link appears to exist between MAL and GalC expression, further emphasizing the role of galactolipids in the expression and trafficking of the structural major myelin components MBP and PLP. Interestingly, Genome Wide Association Studies have identified galactosylceramidase, involved in the lysosomal degradation of GalC, as an MS susceptibility gene, implying that perturbed GalC levels may result in remyelination defects in MS. Further investigations, addressing issues as to whether MAL is expressed earlier on laminin-2 than on PLL, and whether its expression may interfere with the localization of syntaxin 3 and 4, would be of interest.

Parameters that drive the polarization of OLGs in vitro are still unknown. Several (in vitro) studies have shown that distinct extracellular cues can modulate cell polarity development. Evidently, a critical step in OLG polarization during developmental myelination is its initial interaction with an axon, which further paves the way for segregation of the cell body plasma membrane and myelin membrane (extending from the tip of a primary process). For the correct timing of subsequent axonal myelination a careful balance between axonal-derived activators, such as laminin-2 and L1, and clearance of inhibitory axonal signals of OLG differentiation, such as galectin-4, PSA-NCAM and Jagged, is essential. Likely these axon-derived signals are also involved in the establishment and/or maturation of OLG polarity. In this thesis, we showed that MBP expression and OLG maturation on laminin-2 is dependent on sulfatide. Of note, syntaxin 4 and MBP mRNA are present in sulfatide-devoid OLGs that are cultured on laminin-2 (unpublished observations). Since sulfatide expression precedes the establishment of axonal contact-dependent signals, its interaction with axonal laminin-2 might thus act as a focal point in OLG-axon adhesion that controls the asynchronous, localized differentiation and polarization of OLGs. Consequently, any event that interferes with laminin 2-sulfatide interaction, as is likely the case in MS, might contribute to remyelination failure. Indeed, the microenvironment encountered by OLGs in an MS lesion differs from that during development or upon demyelination in control (healthy) white matter, thereby constituting a basis for OLG polarity impairment, and hence remyelination failure. The persistent presence of inflammatory mediators, such as TNFα, and the extracellular matrix (ECM) substrate fibronectin in MS lesions, impedes remyelination. TNFα-exposure of mature OLGs results in retraction of MBP from the myelin membranes towards the cell body and primary processes, an induced reversible disorganization of the actin cytoskeleton, and a redistribution of MBP and GalC from actin-dependent to actin-independent membrane microdomains (Chapter 5). In preliminary experiments, in which OPCs were treated with TNFα, myelin-like membrane formation was inhibited, which may reflect a defect in OLG polarity development. Of particular interest for clarifying MS pathology is our observation that inhibition of morphological OLG differentiation by fibronectin dominated over the promoting potential of laminin-2 (Chapter 4). Likely fibronectin interferes with OLG polarity establishment, as fibronectin inhibits myelin membrane-directed vesicular trafficking and prevents sulfatide segregation to membrane microdomains (Chapter 4), emphasizing the importance of MS-related changes in myelin directed transport. Therefore, these findings may explain the quiescence and absence of polarity development in OPCs in MS lesions, and the potential of anti-sulfatide antibodies to trigger remyelination. However, anti-sulfatide
antibodies inhibit myelin membrane formation in cultures grown on physiological (‘healthy’) laminin-2 (Chapter 3), thus displaying the undesirable potential to induce demyelination in healthy white matter. Therefore, the development of modified anti-sulfatide antibodies, capable of inducing ECM-independent remyelination is required.

Taken together, in generating a myelin membrane, OLGs acquire a polarized phenotype, whereby the myelin membrane in cultured OLGs is served by a ‘basolateral-like’ and the cell body plasma membrane by an ‘apical-like’ transport mechanism, reflected by the distinct localization of syntaxin 3 and syntaxin 4, and the regulation by galactolipids. This specific polarized distribution seems less apparent in more mature myelinating cells, in which alternative transport pathways of myelin components may occur, possibly by lateral diffusion when myelin compaction progresses, as shown by PLP transport upon MAL expression. In this context further examination of the polarized nature of OLGs and the similarity with mechanisms reported in polarized epithelial cells, including the existence and potential role of a common recycling endosomal system, is of major interest. This also holds for the need of acquiring a better understanding of the composition of membrane microdomains containing GalC and/or sulfatide, and the formation of signalling platforms to initiate myelination and the proper trafficking of myelin components to the myelin compartment. This knowledge will also be imperative for devising therapeutic means to induce OPC polarity to overcome remyelination failure in MS lesions.