Regional diversity in oligodendrocyte progenitor cells
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DOI:
10.33612/diss.165785295

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2021

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Chapter 6

Summary and future perspectives

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Summary

Myelin is a stack of multiple lipid-bilayers that is wrapped around axons, functionally isolating the axon, thereby facilitating saltatory conduction and providing metabolic support. In the chronic demyelinating disease multiple sclerosis (MS), both myelin and myelinating oligodendrocytes (OLGs) are lost in a local manner, leading to focal demyelination, inflammation and neurodegeneration, culminating in neurological disability. New myelin can be produced by surviving or newly differentiated OLGs during a regenerative process called remyelination, which is essential for neuronal survival and functional recovery and preventing irreversible neuronal damage. Remyelination by newly differentiated OLGs is more efficient and is a multi-step process that involves the migration of activated oligodendrocyte progenitor cells (OPCs) to the demyelinated lesions, their proliferation at the site of injury, and the differentiation into mature myelinating OLGs. Although efficient in early stages of the disease, remyelination becomes less efficient upon aging and MS disease duration. Current treatments for MS are limited to immunosuppressive or immunomodulating disease-modifying treatments and do not prevent neurodegeneration. Enhancing endogenous remyelination might prove to be beneficial to halt neurodegeneration and therefore disease progression in MS.

Remyelination is more efficient in grey matter (GM) than in white matter (WM) areas of the brain. This remarkable regional difference is observed in MS lesions, and in an experimental rodent model for de- and remyelination. Regional differences in remyelination efficiency may not only be attributed to differences in the permissiveness of the local micro-environment but also to diversity in cells. Indeed, NPCs from different brain regions have been segregated based on their proliferation and differentiation rate, injury response, and electrical properties. Moreover, NPCs from WM (wmOPCs) differentiate equally well upon transplantation into either GM or WM, while NPCs from GM (gmOPCs) remain more immature irrespective of the environment, indicating cell-intrinsic differences. Regional differences in NPCs will undoubtedly affect regional responses to injury and recovery, contributing to pathology. Therefore, the aim of the work presented in this thesis was to investigate intrinsic differences between gmOPCs and wmOPCs, and whether these differences putatively affect in vitro NPC behavior and their response to signaling factors that are present in MS lesions. The obtained knowledge will contribute to a better understanding of regional NPC diversity and remyelination, and may open therapeutic avenues aimed at stimulating region-specific endogenous remyelination in demyelinating diseases, including MS.

Chapter 1 provides a comprehensive overview on current literature concerning diversity in macroglia, i.e., OLGs, NPCs and remyelination-modulating astrocytes, from GM and WM in the developing and the adult brain, and how these differences may contribute to regional differences in remyelination efficiency in experimental models and in MS pathology. Although postnatal NPCs display relatively minor transcriptomic differences, OPCs acquire clear morphological and functional properties in GM and WM. In an experimental, inflammatory demyelination model for MS, OPCs become transcriptionally heterogeneous upon remyelinating injury, and in an experimental model for successful remyelination, OPCs revert first to a more immature stage. On the other hand, the transcriptome of OPCs is hardly altered in MS. Mature oligodendrocytes (OLGs) display more transcriptional heterogeneity and can be segregated into six distinct populations. In MS, the abundance of two of these populations is reduced, while in a rodent model for immune-mediated demyelination oligodendrocyte lineage cells start expressing immune-related proteins. Astrocytes, known orchestrators of remyelination, also display transcriptomic, morphological and functional diversity in GM and WM. However, in the healthy brain astrocytes are less heterogeneous than mature OLGs based on their transcriptome. In fact, rather than intrinsic heterogeneity, astrocytes display a high functional plasticity conforming to the needs of their local micro-environment. This becomes especially apparent upon demyelinating injury, where the response to and presence of molecular factors differ between GM and WM. Hence, macroglia display diversity between GM and WM, both in the adult brain and upon injury, which likely contributes to the differences in remyelination robustness in GM and WM.

In chapter 2 potential differences in parameters that are important for successful remyelination, including proliferation, migration, differentiation and myelin membrane formation, between primary neonatal OPCs from GM (gmOPCs) and
WM (wmOPCs) were investigated. Our findings revealed that, in vitro, gmOPCs and wmOPCs were distinct in oligodendrocyte lineage maturity, morphology and in their responses to environmental signals, including pro-inflammatory cytokines present in MS lesions. GmOPCs elaborated a less complex branched network of processes (Fig. 1-1) and proliferated more when exposed to mitogens compared to wmOPCs (Fig. 1-2). By contrast, wmOPCs differentiated faster than gmOPCs (Fig. 1-3) and had higher transcript levels of oligodendrocyte lineage maturity genes. In addition, wmOPCs were more affected by IFNγ-mediated inhibition of proliferation, differentiation and detrimental effects on process arborization, which is potentiated by TNFα (Fig. 1-4). As OPCs revert to a more immature state upon demyelination\(^5\), the less mature gmOPCs may have an advantage in remyelination, being more responsive to signals involved in the initiation of the remyelination process and less susceptible to pro-inflammatory cytokines. The more mature status of wmOPCs may be beneficial to developmental myelination, which proceeds early in WM\(^537–540\), and in myelin remodeling in the adult brain\(^101\).

Locally secreted molecular signals direct OPC proliferation and differentiation. As gmOPCs and wmOPCs differ in response to these signals, chapter 3 addressed whether the primary cilium, a cell-signaling hub transiently carried on OPCs\(^59\), may function in a region-dependent manner. Using primary neonatal OPCs from GM and WM we revealed that in the neonatal cortex more oligodendrocyte lineage cells carry a primary cilium than in the corpus callosum (Fig 1-5). Upon toxin-induced demyelination the number of primary cilium-displaying oligodendrocyte lineage cells was increased in the cortex, while remaining similar in the corpus callosum. Furthermore, this organelle was displayed more and earlier on gmOPCs compared to wmOPCs in vitro (Fig. 1-5). Disruption of the formation of the primary cilium by a knockdown of IFT88 resulted in reduced mitogen-induced proliferation of gmOPCs and Wnt3a-mediated inhibition of differentiation of immature gmOLGs (Fig. 1-6), but not their WM counterparts. Hence, the primary cilium may be indispensable for the regulation of proliferation and differentiation of gmOPCs, but not wmOPCs, indicating regional diversity in OPCs in their reaction to extracellular signals. The elucidation of ligands and signaling pathways localized at the primary cilium affecting gmOPC behavior in normal and pathological conditions may contribute to our understanding of regional differences in remyelination, and the failure thereof.

Mature OLGs may be segregated into regionally diverse groups\(^28,34,176\), and anecdotal evidence indicates putative regional differences in GM and WM myelin\(^195,196\). Furthermore, the myelin composition in MS differs from myelin obtained from healthy subjects\(^541\). Upon demyelination, myelin debris has to be removed from...
the local environment for successful remyelination to occur, as myelin debris inhibits OPC differentiation. Peripheral infiltrating macrophages and CNS-resident microglia remove myelin debris and, in addition, orchestrate remyelination upon ingestion of myelin. Therefore, in chapter 4, regional differences in myelin composition of GM and WM and its effect on OPCs, bone marrow-derived macrophages (BMDMs) and microglia were investigated. We revealed that the proportions of myelin proteins and lipids of GM and WM myelin differ, both in rat and in MS (Fig. 1-7). When OPCs were cultured on a myelin coating from rat GM and WM myelin, OPC proliferation and differentiation was inhibited. The observed effect was more pronounced for WM OPC proliferation than GM OPC proliferation (Fig. 1-8). In contrast, a coating of MS myelin only inhibited GM OPC differentiation. Exposure to rat GM and WM myelin similarly altered the activation status of BMDMs and microglia that were pre-polarized into an anti-inflammatory phenotype, although expression of the pro-inflammatory marker Nos2 is reduced upon rat myelin exposure in alternatively activated BMDM, and increased in alternatively activated microglia (Fig. 2-1). Exposure to MS WM myelin, but not MS GM myelin, interfered with mRNA expression of alternatively activated markers in BMDMs. Furthermore, both rat GM and WM myelin exposure increased mRNA expression of Lgals3 and Lif in BMDMs and microglia respectively (Fig. 2-2), while MS myelin exposure hardly altered transcript levels of pro-OPC differentiation factors. Although these factors are known to promote OPC differentiation, culturing OPCs in medium conditioned by BMDMs and microglia that were exposed to rat and MS GM and WM myelin hardly affected gmOPC and wmOPC differentiation. However, BMDM-conditioned and microglia-conditioned medium increased gmOPC, but not wmOPC differentiation (Fig. 1-9). Overall, although a myelin coating had a clear effect on OPC behavior, GM and WM myelin had similar effects on OPC, BMDM and microglia behavior. Therefore, the observed difference in remyelination capacity of GM and WM is probably only slightly due to differences in myelin composition. The distinct response of gmOPCs and wmOPCs to rat myelin and BMDM and microglia-conditioned medium, and the differing effect of rat myelin exposure on BMDM and microglia indicate that locally present cells in demyelinated GM and WM are probably more important determinants for the regional difference in remyelination capacity.

Whether the observed intrinsic differences in OPCs from GM and WM is reflected in their transcriptome, and how this is affected by age, was studied in chapter 5. The antibody A2B5 has been used as an OPC marker and to isolate CNS progenitor cells capable of remyelination. After ex vivo isolation of progenitor cells, including OPCs, using the A2B5 antibody, we found that the transcriptome of A2B5-positive cells from cortex (cxA2B5) and corpus callosum (ccA2B5) substantially differs at postnatal day (P)7, but this difference is diminished in the adult brain at P250. Gene ontology (GO) annotation from differentially expressed genes (DEGs) indicates that A2B5-positive cells from different regions and age are involved in different cell processes. When comparing A2B5 from P7, cxA2B5 had more transcripts of genes involved in synapse organization, while ccA2B5 more abundantly expressed genes involved in cilium organization and assembly (Fig. 3). However, gene expression of cell type specific markers and deconvolution, indicated that A2B5-positive cells at P7 and P250 consisted of different proportions of cell types, making it difficult to distinguish alterations with age. More specifically, A2B5-positive cells also consisted of endothelial cells at P7 and microglia at P250 (Fig. 3). Overall, we find that antibody A2B5 immunoreactivity is likely not restricted to OPCs, and the cell types bound by antibody A2B5 likely differ at P7 and P250. However, based on gene expression, the cell types bound by A2B5 do not differ between cx and cc within P7 or P250, indicating that A2B5-positive cells are transcriptomically, and possibly functionally, different in

Figure 2. Graphical abstract of the key findings in chapter 4. Nos2 expression is reduced in alternatively-activated BMDMs and increased in alternatively activated microglia upon rat GM and WM myelin exposure, while expression of Lgals3 is increased in BMDMs, and Lif in microglia upon rat myelin exposure.
cx and cc at either age. Future studies investigating the identity of A2B5-positive cells from GM and WM at P7 and P250, including scRNA-seq and co-labeling with cell type specific markers, may reveal whether A2B5-positive cells are progenitors or that different CNS cell types display the A2B5 antigen on their surface. Given their remyelinating capacities, functional differences of A2B5-positive between region and age will contribute to our understanding of remyelination.

Taken together, the findings described in this thesis contribute to a deeper understanding in why remyelination is more robust in GM than in WM. Our in vitro findings indicate that gmOPCs are likely better equipped for remyelination; gmOPCs were more immature and more receptive to micro-environmental signals -including those derived from macrophages and microglia- that are beneficial for remyelination. The finding that a higher percentage of gmOPCs than wmOPCs display a primary cilium may contribute to their receptiveness to extracellular signals. Furthermore, gmOPCs are less responsive to rat myelin and less susceptible to pro-inflammatory cytokines that are present in MS lesions. In addition, upon demyelination, WM myelin is more detrimental to wmOPC proliferation than GM myelin to gmOPC proliferation. Also, the use of antibody A2B5 to isolate CNS cells indicated regional transcriptomic heterogeneity of these cells in GM and WM and alterations in cell type proportions with age. Most experiments in this thesis were conducted with neonatal OPCs and myelin derived from rodent brain, therefore future studies focusing on adult OPCs and A2B5-positive cells will elucidate how OPC and A2B5-positive cell diversity in GM and WM is affected by aging in their respective environment. Notably, rodent OLG generation is not very different from human, rodent OPCs having similar cell division times and age-related changes in OLG production rates. Furthermore, both in rodent and in MS, remyelination is more efficient in GM than WM. However, the findings described in this thesis should be translated to human or humanized models, including use of primary human OPCs from GM and WM tissue and (patient-derived) induced pluripotent stem cell (iPS)-derived cells. In addition, the findings described in this thesis emphasize that OPC diversity -both in region and age of origin- should be taken into account when conducting experiments focusing on remyelination. This is also valid when investigating potential therapeutic agents to promote remyelination in MS, as their effect may be different in GM and WM.
Concluding remarks and future perspectives

The findings in this thesis provided insight in OPC diversity in GM and WM of the brain, its relevance for (re)myelination-focused experiments in vivo and in vitro, and will be helpful in elucidating novel therapeutic pathways enhancing endogenous remyelination either in GM or WM MS lesions. Such a regional-specific approach in search for a remyelination promoting therapeutic agent is currently lacking. In fact, most in vitro studies use neonatal gmOPCs, while WM areas are often screened in experimental models. Ultimately, this may lead to conflicting findings. In the following paragraphs the implication of OPC diversity for remyelination efficiency and failure of remyelination in MS lesions in GM and WM is discussed, as well as future perspectives of a remyelination enhancing treatment for GM and WM MS lesions.

Role of OPC diversity in regional remyelination efficiency: intrinsic differences and plasticity

Our findings provide compelling evidence that OPCs in GM and WM are diverse, which likely contributes to the more robust and faster remyelination in GM than WM. Neonatal gmOPCs prolife\(\text{erated more and showed delayed maturation}^\text{compared to wmOPCs, while morphologically displaying a less complex morphology, all of which are characteristic for being less mature than wmOPCs (chapter 2). As OPCs in the adult CNS adopt a more immature state upon demyelination}^\text{75}, the more immature status of gmOPCs may be beneficial for their remyelinating capacities as they may initiate remyelination faster. Furthermore, differentiating oligodendrocyte lineage cells transiently carry a primary cilium, a cell signaling hub. Primary cilia were carried earlier on oligodendrocyte lineage cells from GM (chapter 3), and a higher percentage of oligodendrocyte lineage cells from GM than WM carried a primary cilium upon demyelination, which probably makes them more reactive to micro-environmental, remyelination-orchestrating, signals. Indeed, gmOPCs were more responsive to recruitment factors compared to wmOPCs; gmOPCs proliferated more upon exposure to mitogens and OPC migration was inhibited more in wmOPCs than gmOPCs upon exposure to astrocyte-conditioned medium (chapter 2). In that regard it is surprising that in chapter 5 we found that at P7, genes involved in primary cilium formation are more abundantly expressed in A2B5-positive cells from the cc than cx. It has to be remarked however that the A2B5-positive population does not solely contain OPCs. Since the primary cilium is a cell signaling hub involved in multiple processes requiring extracellular signals, it would be relevant to further study its role in OPC behavior, i.e., OPC migration and tiling. Known chemotactic and chemorepulsive factors to guide OPC migration and tiling, achieved by self-avoidance, that are linked to the primary cilium are for example TGFβ, PDGF-A, SHH and FGF. Although OPCs are often observed in MS lesions, they fail to remyelinate. In vitro, OPC differentiation often does not occur until a threshold in OPC density is achieved. In vivo, highly motile OPCs find a niche using local self-avoidance cues, i.e. tiling, thereby achieving an intrinsic cellular density. This process might be impaired in MS lesions, i.e., OPCs may not recognize that the threshold OPC density is achieved, resulting in impaired OPC differentiation and, hence, remyelination. This may be of special importance in GM remyelination, as more oligodendrocyte lineage cells in GM started displaying a primary cilium upon demyelination. The pro-inflammatory cytokine IFNγ, present in MS lesions, was more detrimental to wmOPCs than gmOPCs; in our experimental set-up we exposed OPCs transiently to IFNγ, which resulted in decreased OPC proliferation and differentiation (chapter 2), particularly in wmOPCs. Therefore, transient exposure to cytokines can have long lasting effects on OPC differentiation. It will be worthwhile for future studies to investigate the consequences of a transient exposure to cytokines on pre-myelinating OLGs present in MS lesions. Additionally, the effect of anti-inflammatory cytokines or other factors that are present in MS lesions, such as extracellular matrix molecules, than those used in this study are yet to be determined. As a transient pro-inflammatory cytokine exposure affected the proliferation, differentiation, and arborization of OPCs -especially in wmOPCs-, it may also affect the production of local myelin. It will prove valuable to compare the composition of developmental myelin to that of myelin formed during remyelination, i.e., when OPCs have been exposed to cytokines, and how myelin composition is changed upon aging. The newly described cuprizone-induced de- and remyelination paradigm in aged mice may be a good model to perform this. In addition, this model could be used to isolate OPCs, i.e., by use of antibody A2B5 or anti-PDGFRα antibodies, on which scRNA-seq could be performed. This study would obtain valuable insights in local transcriptomic responses in OPCs upon demyelination and remyelination in young and aged mice. In parallel, to assess intrinsic differences in OPCs and the effect of the

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microenvironment in different regions in the brain on remyelination capacity, homono- and heterotopic transplantsions of gmOPCs and wmOPCs into demyelinated GM and WM areas could be performed in this model.

Although the proportions of myelin proteins and lipids differs in GM and WM in both rat and MS, in our hands this only marginally resulted in differing effects of myelin exposure on OPCs, BMDMs and microglia (chapter 1,4). However, we exposed cells to equal protein amounts of GM and WM myelin. Since myelin is more abundant in WM compared to GM, and the detrimental effect of myelin was more pronounced in wmOPCs, this may result in the WM myelin being more detrimental to remyelination in the WM than the GM lesion environment. Future studies should further explore differences in GM and WM myelin by lipidomic and proteomic analysis, including a comparison between MS and age-matched healthy subjects, especially since the effect of MS myelin on OPCs, BMDMs and microglia was less pronounced than rat myelin. It is possible however that MS myelin does not provide the correct signals to these cells, thereby frustrating the tightly regulated remyelination process. Furthermore, as microglia are the foremost cell type phagocytosing myelin[30-33], are more abundant than macrophages in the rim of active WM lesions[34], and microglia also display regional heterogeneity[35], it is recommended to study the effect of GM and WM myelin phagocytosis on GM and WM microglia, since in our study whole-brain microglia were used and microglia from GM and WM are transcriptomically diverse[36].

Altogether, gmOPCs and the GM lesion environment may be more permissive for remyelination than wmOPCs and the WM lesion environment. Of note, proteins but not lipids associated with myelin debris, including Ephrin3B, affect Fyn, PKC and RhoA-mediated signaling, preventing OPC differentiation[37,38]. Pharmacological intervention in binding to the receptor and these downstream signal transduction pathways in gmOPCs and wmOPCs may pose possible pharmacological targets aimed at enhancing endogenous remyelination. In addition, enhancing myelin phagocytosis, especially in WM MS lesions, may prove a valuable target. Since OPCs are present in most WM MS lesions but fail to differentiate[39,40] owing to multiple reasons depending on the lesion stage[41], stimulating OPC differentiation via known[24] and yet to be determined factors in a lesion-specific approach may prove to be successful in enhancing remyelination.

Role of OPC diversity in regional remyelination failure in MS: therapeutic avenues

The research in this work was performed with rat-derived OPCs, and it has to be determined whether the findings can be translated to MS. In favor of evolutionary conserved differences between cells in GM and WM, remyelination is more robust in GM in both MS lesions[26,27] and a rodent model for remyelination[24-25]. Furthermore, OPC generation is highly similar between rodent and human based on cell division and production rate[25]. However, recent findings indicate that remyelination in remyelinated MS lesions is predominantly performed by adult OLGs that remain in demyelinated lesions[26,27], instead of newly-formed OLGs derived from OPCs like in rodent models for remyelination[24]. Of note, these studies focused on WM MS lesions and did not include GM MS lesions. Furthermore, it has been postulated that OPC-mediated remyelination may be impaired in MS, resulting in adaptive remyelination by demyelination-surviving OLGs[30], which is supported by novel studies describing myelination in the adult human CNS is performed by differentiating OPCs[30-32], reviewed in[33]. As the work presented here indicates regional differences in OPCs, including their response to MS lesion relevant environmental factors, between GM and WM (chapter 1-5), one might speculate that, in contrast to in WM MS lesions, in GM MS lesions OPCs may contribute to remyelination. Further carbon-dating studies exploring which cells remyelinate GM MS lesions may yield new insights into the observed difference in GM and WM remyelination. A recent paper identifying OLG subpopulations in the WM of the healthy and MS brain found two mature OLG subpopulations to be less abundant in MS[34]. Whether these OLG populations are also lacking in GM MS lesions, or whether in the GM mature OLG populations differ and/or exist per se as was first indicated by Pio del Rio-Hortega[35-37], is currently unclear and remains to be addressed in future studies.

Accumulating evidence indicates that MS is not a single disease, but rather a demyelinating syndrome which can manifest upon various molecular triggers[38]. With a diversity in etiology, a regenerative, i.e., remyelination-enhancing, treatment upon the manifestation of this demyelinating syndrome will prove highly valuable. The findings described here add to our understanding of the process of remyelination in different regions, which is paramount for the development of such a treatment. Thus, gmOPCs and wmOPCs intrinsically differ, which probably contributes to the reported difference in remyelination efficiency of GM and WM. GmOPCs were
less mature than wmOPCs and might be more susceptible to extracellular signals coordinating remyelination by more abundant display of the primary cilium (chapter 2,3). As the primary cilium is involved in gmOPC differentiation (chapter 3), and sufficient numbers of OPCs are present in GM MS lesions, while ultimately failing to differentiate\textsuperscript{14,46} stimulating gmOPC differentiation, i.e., by modulating primary cilium display and the timing thereof, or by manipulation of the subsequent downstream intracellular signaling cascade, might prove beneficial for restoring endogenous GM remyelination in MS lesions. Therefore, primary cilia display in GM MS lesions warrants further investigation. Since the primary cilium was not involved in wmOPC proliferation and differentiation in vitro, this approach will likely not stimulate WM remyelination. Considering wmOPCs were more sensitive to the detrimental effect of rat myelin, a myelin phagocytosis enhancing therapy would be beneficial for enhancing remyelination in WM MS lesions (see below).

Like other endogenous repair processes, remyelination efficiency decreases with age\textsuperscript{14,46}. Human and rodent A2B5-positive cells have remyelinating capacities when transplanted in a de- or dysmyelinated environment\textsuperscript{57–59,490–495}. These remyelinating cells had transcriptomic differences between cx and cc at P7 and P250, while even more transcriptomic differences were found between A2B5-positive cells from P7 and P250. Unexpectedly, deconvolution estimated that A2B5-positive cells also consisted of endothelial cells at P7 and microglia at P250. However, estimated cell proportions were similar within a given age, indicating regional heterogeneity (chapter 5). Hence, based on our transcriptomic data, remyelinating A2B5-positive cells in the CNS likely have specialized functions that differ in GM and WM, and in time. However, it cannot be excluded that these differences are a reflection of alterations in A2B5 epitope expression of different CNS cell types. Although ccA2B5 were transcriptomically better equipped for cell signaling, our data show that gmOPCs were more responsive to some environmental signals in vitro (chapter 2,3), while wmOPCs were more responsive to myelin and proinflammatory cytokines (chapter 1,4). Our findings indicate that a rat WM myelin coating impedes wmOPC proliferation more than a rat GM myelin coating gmOPC proliferation, although MS myelin did not have the same effect (chapter 4). Given that normal human myelin has been reported to prominently inhibit OPC differentiation\textsuperscript{492}, this may be due to MS myelin being chemically different from myelin from healthy subjects, lacking the potential to evoke an appropriate effect in MS myelin-exposed cells. It has been reported that while the abundance of MBP is decreased in normal appearing white matter compared control white matter, PLP abundance remains stable\textsuperscript{594,595}. Furthermore, the activity of CNP is reported to be lower in MS myelin compared to normal myelin, indicating that either the enzymatic function is impaired or CNP levels are altered in MS\textsuperscript{595}. Also, differences in lipid composition between WM myelin from healthy subjects and MS patients have been reported, although conflicting. Previous studies report a lower abundance\textsuperscript{597–599} or unchanged levels\textsuperscript{599–601} of certain phospholipids, higher/lower or normal abundance of cerebrosidesulfatide\textsuperscript{597–598}, or differences in fatty acid content and unsaturation degree\textsuperscript{598–599,600–602} or not\textsuperscript{599–600,603}. However, a relative increase of protein to lipids in WM MS myelin is observed\textsuperscript{604}. Whether MS myelin from WM modulates BMDMs and microglia activation and behavior differently than normal WM myelin, or wmOPCs are less susceptible to MS WM myelin to transiently block OPC differentiation, accelerating myelin removal from the demyelinated WM lesions might prove highly beneficial for remyelination to occur. Indeed, multiple drugs that enhance myelin debris removal are currently under investigation. The RXR pathway agonist bexarotene is trialed in MS patients. While this drug enhances remyelination, and has a known positive effect on myelin debris clearance by alleviating aging effects on monocytes and macrophages, it is unclear whether this is causally related\textsuperscript{606–608}. Additional novel candidates enhancing myelin debris removal include the blockage of soluble TNF\textsuperscript{571}, the P2X4R agonist ivermectin\textsuperscript{609}, recombinant human rHlgM22\textsuperscript{610} and TREM2 activation\textsuperscript{677}. Furthermore, intraperitoneal M-CSF administration concomitant with a cuprizone-diet induces increased myelin debris uptake in the corpus callosum upon cuprizone-induced demyelination\textsuperscript{677–679}.

Taken together, the development of distinct remyelination-enhancing therapies for GM and WM lesions may prove beneficial for MS. While remyelination in GM MS lesions is more efficient compared to WM MS lesions, also in GM MS lesions remyelination ultimately fails. Stimulating gmOPC differentiation, possibly via agonistic or antagonistic signaling over the primary cilium, may be suitable for enhancing GM remyelination. Enhancing WM remyelination would possibly benefit more from the accelerated removal of myelin debris removal from the demyelinated lesion.