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Myelin Biogenesis And Oligodendrocyte Development: Parsing Out The Roles Of Glycosphingolipids

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Abstract

The myelin sheath is an extension of the oligodendrocyte (OL) plasma membrane enriched in lipids which ensheaths the axons of the central and peripheral nervous system. Here we review the involvement of glycosphingolipid in myelin/OL functions; including the regulation of OL differentiation, lipid raft-mediated trafficking and signaling, and neuron-glia interactions.

Keywords

oligodendrocytes; myelin; glycosphingolipids

Myelin is a lipid rich membrane produced by oligodendrocytes (OLs) in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (PNS). The composition of myelin is approximately 70% lipid and 30% protein, which is in stark contrast to other membranes, which are typically 30–50% lipid (67). The low capacitance, high resistance membrane envelops and electrically insulates the axon allowing for the rapid and efficient propagation of impulses. In addition to its role as an insulator, myelin provides trophic support to the axon and maintains its long-term integrity. Thus, myelin function extends beyond providing insulation; it facilitates tri-directional communication between the neuron, the myelinating cell, and the environment (66). Various classes of lipids are present in OL and myelin membranes, including cholesterol, phospholipids, and glycosphingolipids (GSLs). Cholesterol and GSL are the major lipid components of myelin, making up approximately 27% and 31% of total myelin lipids, respectively. GSLs are a class of sphingolipids that have a sphingoid base, a straight chain amino alcohol with 18–20 carbon atoms which normally carrying a saturated or unsaturated fatty acid chain. Depending upon the attachment of a mono- or oligosaccharide head group to the sphingoid base, different subclasses of GSL such as cerebroside, sulfatides, and gangliosides are formed. This review will focus mainly on the contributions of two specific glycosphingolipids enriched in the myelin membrane: galactocerebroside (GalC, galactosylceramide, GalCer) and its sulfated derivative, sulfatide, along with a brief discussion on gangliosides, the sialic acid containing GSLs. Studies reviewed here briefly focus on the role of GSLs in the regulation of OL differentiation, signaling in GSL-cholesterol-enriched microdomains (“lipid rafts”), neuron-glia interactions, and the creation of specialized domains along the axon. For detailed reviews on these topics, we refer the reader to our book containing chapters on myelin lipid (34,99).

GLYCOSPHINGOLIPID BIOSYNTHESIS

Glycosphingolipid biosynthesis has been described in detail elsewhere (99). Briefly, GSL synthesis begins in the ER with the condensation of L-serine and palmitoyl-CoA to form 3-ketosphinganine. Subsequent reactions produce ceramide, the precursor for galactolipids, gangliosides, and sphingomyelin (Fig. 1). The enzyme UDP-galactose:ceramide galactosyltransferase (CGT) acts on ceramide to synthesize GalC with the addition of a galactose residue in the ER and transports it to the outer leaflet of the plasma membrane lipid bilayer. A subpopulation of GalC is transported to the Golgi where a cerebroside sulfotransferase (CST) adds a sulfate to the 3'-OH group of galactose producing sulfatide, which is also targeted to the outer leaflet of the plasma membrane. In order to produce gangliosides, ceramide is sequentially converted by glucosyltransferase (GlcT) to glucocerebroside (GlcCer) and then by galactosyltransferase (GalT1) to lactosylceramide (LacCer), the substrate for the biosynthesis of most gangliosides. All gangliosides, excluding GM4, are generated by the stepwise action of sialyltransferases (SATI, II, III) that add sugar and sialic acid residues to LacCer in the Golgi to yield GM3, GD3, and GT3, which are the backbones for complex gangliosides (not shown). GM4 has a distinct biosynthetic pathway, as it is produced from GalC, and the reaction is catalyzed by a sialyltransferase (SAT). Like GalC and sulfatide, gangliosides are transported to the outer leaflet of the plasma membrane.

ROLE OF GSL IN THE REGULATION OF OLIGODENDROCYTE DIFFERENTIATION

Oligodendrocytes are derived from neuroectodermal cells within the subventricular zone and proceed through a developmental lineage in which surface antigens, several of which are GSLs, are expressed on the membrane at distinct time points. Based on the sequential expression of these lipid and protein antigens, the different developmental stages of the OL lineage have been well characterized (74) (Fig. 2). Early OL progenitors are bipolar, migratory, proliferative cells recognized by monoclonal antibody (mAb) A2B5, against surface gangliosides, GD3, GT3, and O-acetylated GT3, which are subsequently downregulated as the progenitors differentiate into an immature OL. Early progenitors mature into Late progenitors (Pro-oligodendroblasts, Pro-OL) which elaborate more processes. In addition to A2B5 immunoreactivity, they express POA, an unidentified sulfated glycolipid recognized by the monoclonal antibody O4 (5,6,7,38,96). The next stage in development is the transient "Pre-GalC" stage where proliferation ceases and cells are on the verge of entering terminal differentiation marked by immunoreactivity against R-mAb (a monoclonal antibody that recognizes GalC and sulfatide) (2,4,6,79). Finally, the OLs enter terminal differentiation, developing a more complex morphology and begin to abundantly synthesize GalC (recognized by O1 mAb in addition to R-mAb) and sulfatide (O4 at this stage recognizes sulfatide in addition to POA). Myelin proteins, such as 2'-3'-cyclic nucleotide 3' phosphohydrolase (CNP), myelin-associated glycoprotein (MAG), myelin basic protein (MBP), myelin/oligodendrocyte glycoprotein (MOG), and proteolipid protein (PLP) are also sequentially expressed along with progressive morphological maturation of cells. Mature OLs form 'myelin-like' membranes *in vitro* and myelin sheaths that enwrap axons *in vivo*.

Several earlier studies suggest that GSL are involved in OL terminal differentiation. For example, when OLs are treated with R-mAb, which recognizes both GalC and sulfatide, terminal differentiation of late OL progenitors to GalC⁺ OLs is inhibited (Fig. 2), suggesting that these GSLs can act as sensors and transmitters of environmental information (4). Specifically, antibodies against sulfatide, but not GalC, produced the observed arrest in terminal differentiation (7). Thus, a model was proposed that anti-sulfatide antibodies mimic a natural ligand, which activates intracellular signaling pathways preventing entry into terminal differentiation. Upon removal of the antibody, differentiation proceeds normally (7),

suggesting that sulfatide is a negative regulator of OL differentiation. Later studies substantiated the *in vitro* findings when it was shown that increased numbers of terminally differentiated OLs were produced both *in vitro* and *in vivo* in the forebrain of mice null for either both GalC and sulfatide (CGT-null animals) (7) or sulfatide alone (CST-null animals) (41). Since enhanced differentiation was observed in both of these mutants, it suggests that loss of sulfatide was responsible for this effect. Increased numbers of OLs were also observed in the spinal cord of CGT (60) and CST (92) null mice.

The mechanism of glycosphingolipid-mediated regulation of OL biology remains to be fully understood. The pioneering work of Dyer and Benjamins showed that exposure of mature OLs to anti-GalC or anti-sulfatide results in redistribution of GalC over internal domains of MBP, the disruption of microtubules and microfilaments within the myelin-like sheets, and the influx of extracellular Ca^{2+} (30–32). Similar loss of myelin-like membranes and down-regulation of myelin genes was also observed with R-mAb (3), which together suggest that lipid-mediated signaling is involved in maintaining the myelin-like membranes of OLs *in vitro*. This association with MBP may be functional, for upon antibody treatment, MBP becomes dephosphorylated and detergent insoluble through its association with the cytoskeleton. Consistent with these results, exposure of myelinated axons to O1 or O4 IgM antibodies leads to distinctive dysmyelination *in vivo* (83) that is characterized by a widening of the myelin intra-period lines.

ROLE OF GSL IN SIGNALING AND INTRACELLULAR SORTING OF PROTEINS VIA LIPID RAFTS

“Lipid rafts” are highly dynamic cholesterol and glycosphingolipid-enriched micro domains in the plasma membrane that are considered to be platforms of protein sorting and signal transductions (18,42,75,93). These microdomains have been previously shown to permit the specific inclusion or exclusion of molecules into particular compartments for intracellular trafficking and signaling. The enrichment of GSLs and cholesterol in the myelin sheath creates an environment compatible with the existence of lipid rafts. These can be isolated biochemically as low density, detergent-insoluble membrane fractions [also known as detergent-insoluble glycosphingolipid/cholesterol-enriched micro domains (DIGs), glycolipid-enriched domains (GEMs), or detergent-resistant or insoluble membranes (DRMs, DIMs)] (18,42,93). The functions of lipid rafts in intracellular trafficking and signaling, including in OLs/myelin, has been extensively described elsewhere (11,37,39,93,94). Here we review a few select lipid-raft associated proteins of myelin.

Myelin and lymphocyte protein (MAL), upregulated in mature OLs (49), is associated with lipid rafts in OLs and Schwann cells (33,35,36,51) and is involved in the apical sorting machinery of polarized cells (20,59). It is speculated that MAL may be involved in intracellular targeting of neurofascin-155 (NF155) (86), a key protein required for paranode formation which is also associated with lipid rafts (87). Several other myelin proteins are known to associate with rafts, including CNP (50), MOG (50), PLP/DM20 (95), peripheral myelin protein 22, protein zero, plasmalogen (40) and Fibroblast growth factor receptor-2 (19,39). It should be noted, however, that the study of membrane rafts is complex since the presence of a particular protein in detergent insoluble domains is not a definitive indication of its role in trafficking or signaling within the raft micro domain.

The evidence for lipid raft involvement in signal transduction stems from the presence of several signaling molecules, such as receptor and non-receptor tyrosine kinases, G proteins, GPI-anchored proteins, adaptor proteins, and other molecules critical for signal transduction in lipid rafts (46). Proteins can either be constitutively present in rafts or sequestered into or excluded from these microdomains at critical periods. For example, Platelet derived growth

factor receptor alpha (PDGF-R α) is recruited into rafts as OLs shift from a proliferative phenotype and enter a differentiation program, shifting the role of PDGF from a mitogen to a survival factor (9). Extracellular matrix induced colocalization of integrins with PDGF or neuregulin within OL rafts enables precise signaling critical for OL function (8,9). Fibroblast growth factor receptor-2 also partitions between raft and non-raft microdomains of OLs and myelin (19). Its signaling in rafts is coupled with the PI3K/Akt pathway but not the Ras/Mek/Erk pathway. It is known that Fyn kinase, a signaling molecule present at the initiation of myelination, is also raft associated (52). In addition, the adhesion molecules NCAM and F3 are contained in rafts and may be important for the adhesive interactions required for myelin biogenesis and maintenance (52).

Using antibody cross-linking techniques, we have demonstrated that the repartitioning of molecules into detergent insoluble fractions corresponds to altered OL physiology. Specifically, cross-linking of both MOG and MAG results in the repartitioning of these proteins into the detergent-insoluble fraction, accompanied by altered phosphorylation of specific proteins and cytoskeletal changes (56–58).

ROLE OF GSL IN NEURON-GLIAL INTERACTIONS

Myelin biogenesis requires extension of OL processes, recognition and adherence to the axon, stabilization of these contacts, initiation of the spiral wrapping of the myelin membrane, termination of wrapping after a certain number of turns, and formation of compact and non-compact myelin with specialized domains of axon-glial contact (101). There is a tremendous amount of information that is still unknown about these processes. Elucidating the role of GSL in these interactions is an emerging field of investigation (23,78,87).

Myelinated axons have four distinct structural and functional domains: node (N), paranode (PN), juxtaparanode (JPN), and internode (INT) (10,71,72,77,85,88,91) (Fig 3). The myelinated regions, called the internodes, are interrupted by non-myelinated regions, called the nodes of Ranvier, which is the site of voltage-gated Na⁺ channel clustering. Paranode, the region next to the node, forms adhesion junctions with the paranodal cytoplasmic loops of myelin. This forms a barrier that limits access to the internodal periaxonal space between axon and myelin. This barrier separates the voltage-gated Na⁺ channels of the node from the voltage-gated K⁺ channels of the juxtaparanode, a region next to the paranode. Isolation and maintenance of the separation between these channels by the paranode is important for saltatory conduction of impulses down the axon.

The central role of GalC and sulfatide in the stabilization and maintenance of axoglial adhesion at the paranode in the CNS was revealed through the analysis of CGT (25,27,28) and CST (43,61) deficient animals which showed paranodal defects, with a more severe phenotype observed in CGT-null mutants. Specifically, in the mutant mice the paranodal loops of myelin were everted away from the axon, due to a loss of septate-like junctions (transverse bands), which normally attach the paranodal loop of myelin to the axon (16,25,29,43,61,62). As a result, the ion channels did not remain confined to their particular domain and redistributed along the axon (27,45,80,82). This resulted in aberrant propagation of impulses along the axon leading to functional impairment including mild tremor, hindlimb weakness, and altered gait in the CGT and CST null mice (14,22,25,43). The mechanism of how the loss of these GSL may lead to the observed phenotype in these animals is poorly understood. It is possible that since sulfatide binds many proteins, including extracellular matrix and cell adhesion protein such as laminin, amphoterin and tenascin-R (64,73,81), the loss of adhesion at the paranode could involve a direct loss of interaction of sulfatide with an adhesive axonal protein. Alternatively, an indirect mechanism has been proposed for the role of GSLs. In this model, an adhesion molecule such as NF-155 clusters into GSL-rich lipid rafts at the paranodal loops

of myelin; this results in co-clustering of its neuronal binding partners, caspr/contactin and the stabilization of axo-glial junctions at the paranode; disruption of lipid rafts due to loss of GalC and sulfatide results in the loss of this co-clustering and thus axon-glial contact at the paranode (87). Consistent with this, NF-155 and caspr/contactin are no longer clustered at the paranode in the CGT- and CST-null mice (27,44,45,76,87). Interestingly, reduced association of NF155 with rafts and disruption of paranodal junctions was also observed in active multiple sclerosis lesions and in animal models of demyelination (54,55). GalC and sulfatide also play a role in axon – Schwann cell interactions in the PNS. For example, the withdrawal of myelin sheath from the axon has been observed in postnatal CGT-null mice (26), and disordered Schwann cell microvilli that do not properly contact the axon have been observed in CST-null mice (44).

Although in the rodent direct evidence for a role of GalC and sulfatide in compaction of myelin at the internodes remains to be demonstrated, phylogenetic studies on invertebrates suggest that GalC and sulfatide can potentially be involved in the formation of the compact, multilamellar sheath. For example, earthworms which lack GalC, sulfatide, and sphingomyelin have loosely wrapped, noncompacted myelin (68,69); shrimp that have GluCer at levels comparable to GalC, but no GalC or sulfatide per se, have an uncompacted concentric arrangement of myelin layers. Structurally, it has been suggested that GalC can form tightly packed bilayers by utilizing intermolecular hydrogen bonds, and the *trans* interactions between GalC and sulfatide play a role in the attachment of the extracellular faces of the myelin membrane (12,13). Although the role of GalC and sulfatide in the initial compaction of myelin is questionable, their role in the maintenance of compact myelin is evident since CGT-null and CST-null mice develop myelin defects with age, including vacuolation, splitting, and redundant myelin sheets (21,22,26,61). It should be noted that CGT-null mice lack not only GalC and sulfatide but also monogalactosyl diacylglycerol, N-Acetylneuraminyl-galactosylceramide (GM4), and seminolipid, while GluC synthesis is upregulated (14,15,22,25). Similarly, CST-null mice are not only deficient in sulfatide, but also in seminolipid (43). Thus, these lipids could in theory also contribute to the overall phenotype of these mutants.

ROLE OF GANGLIOSIDES IN OL/MYELIN BIOLOGY

It has been suggested that gangliosides bind to several growth factor tyrosine kinase receptors, which regulate their activity (63). For example, GM3 binds to the epidermal growth factor receptor and inhibits its tyrosine kinase activity; GM1 binds and activates TrkA (nerve growth factor receptor); GM1 and GM3 inhibit FGF receptor activity (17), and several gangliosides inhibit the dimerization of PDGFR (102). Additionally, the carboxy terminus of FGF-2 interacts with several gangliosides (84). It therefore seems plausible that specific gangliosides in the local environment can potentially modulate the receptor tyrosine kinase activity of growth factor receptors such as PDGF and FGF receptors, which are known to influence proliferation and differentiation of OL progenitors (1,74). Further, the addition of GM3 but not other GM or GD ganglioside species enhanced differentiation of OLs in culture suggesting a role of gangliosides in OL biology (107). Nevertheless, experiments utilizing the addition of exogenous gangliosides should be interpreted with caution since ganglioside metabolism is complex, and it is difficult to distinguish between direct and indirect effects of a ganglioside species that are responsible for a particular effect.

Certain sialated gangliosides, notably GD1a and GT1b are expressed on the axonal membrane and the sialic acid binding protein, MAG on the periaxonal surface of the myelin membrane (24,48,100). These molecules are thus perfectly positioned to interact with each other on opposing surfaces for facilitating axon-glial interaction and promoting myelin stability (89, 103,104,106). Consistent with this, mice lacking MAG show abnormality in the formation of

the periaxonal cytoplasmic collar suggest that MAG-ganglioside interaction is important for the organization and maintenance of periaxonal space and cytoplasmic collar (53,65).

Ganglioside function in normal myelination has been further elucidated using mutants deficient in a combination of gangliosides. Some mutations lead to a severe phenotype, while other mutations produce animals with normal myelination. For example, in initial studies mice with a disrupted GM2/GD2 synthase gene that lacked complex gangliosides exhibited only subtle defects in their nervous systems (98), but later studies revealed pronounced pathology in these mice, including axonal degeneration, an increase in unmyelinated fibers and redundant myelin loops, disruption of paranodal junction, and mislocalization and dysfunction of ion channels (70,90,97). In contrast, mutants that lack -b and -c series gangliosides (GD3 synthase-null) do not show demyelination in the brain (47). The most severe phenotype was observed in the transgenic mice in which there was a total absence of all ganglio-gangliosides (*Siat9* and *Galgt1* double knock). These mice showed profound axonal degeneration, CNS white matter vacuolization, and perturbed paranodal stability evident by the presence of paranodal loops that face away from the axon (105). These studies together support a role of gangliosides in axon-glial interaction.

In conclusion, GSL are at the forefront of OL/myelin biology contributing to the formation and stability of a competent myelin sheath by specifically influencing OL differentiation, protein and lipid trafficking, signaling, neuron-glial interactions, and the creation of specialized domains.

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Fig. 1. Biosynthesis of Glycosphingolipids

Ceramide is converted to galactocerebroside (GalC) by the enzyme UDP-galactose:ceramide galactosyltransferase (CGT) and then to sulfatide by cerebroside sulfotransferase (CST). Ceramide is also sequentially converted by glucosyltransferase (GlcT) to glucocerebroside (GlcCer) and then by galactosyltransferase (GalT1) to lactosylceramide (LacCer). Gangliosides, excluding GM4, are generated from LacCer by the stepwise action of sialyltransferases (SAT I, II, III) to yield GM3, GD3, and GT3, the backbones for complex gangliosides (not shown). GM4 is produced from GalC, by a sialyltransferase (SAT). Adapted from Taylor et al., 2004.

Fig. 2. Diagrammatic representation of oligodendrocyte differentiation pathway

Each stage of oligodendrocyte maturation is defined by the expression of specific lipid and protein markers, change in morphology, migratory and proliferative capacity of cells. The most commonly used lipid markers and names of antibodies are shown. Lineage progression can be reversibly blocked in culture by treatment with R-mAb antibody. OL: oligodendrocyte; POA: pro-oligodendroblast antigen; GalC: galactocerebroside; SUL: sulfatide; GD3: ganglioside. A2B5, O4, O1, R-mAb: names of antibodies. Adapted from Pfeiffer et al., 1993; Taylor et al., 2004.

Fig. 3. Disruption of Axon-Glia interaction in the CGT- and CST-null mice

Oligodendrocyte processes wrap around axons to form the myelin sheath. Myelinated regions of the axons (internodes), are interrupted by non-myelinated regions (Nodes of Ranvier). Astrocytic processes cuff the nodes. Myelinated axons have four distinct domains: node (N), paranode (PN), juxtaparanode (JPN), and internode (INT). In the CGT- or CST-null mice the paranodal loops of myelin are everted away from the axon, due to a loss of junctions (transverse bands) and the disruption of co-clustering of myelin protein, NF-155 with its axonal partners Caspr/contactin as in the wild type mice. Adapted from Salzer 2003.