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Deregulated Sphingolipid Metabolism and Membrane Organization in Neurodegenerative Disorders

Marco Piccinini · Federica Scandroglio · Simona Prioni · Barbara Buccinnà · Nicoletta Loberto · Massimo Aureli · Vanna Chigorno · Elisa Lupino · Giovanni DeMarco · Annarosa Lomartire · Maria Teresa Rinaudo · Sandro Sonnino · Alessandro Prinetti

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Abstract Sphingolipids are polar membrane lipids present as minor components in eukaryotic cell membranes. Sphingolipids are highly enriched in nervous cells, where they exert important biological functions. They deeply affect the structural and geometrical properties and the lateral order of cellular membranes, modulate the function of several membrane-associated proteins, and give rise to important intra- and extracellular lipid mediators. Sphingolipid metabolism is regulated along the differentiation and development of the nervous system, and the expression of a peculiar spatially and temporarily regulated sphingolipid pattern is essential for the maintenance of the functional integrity of the nervous system: sphingolipids in the nervous system participate to several signaling pathways controlling neuronal survival, migration, and differentiation, responsiveness to trophic factors, synaptic stability and synaptic transmission, and neuron–glia interactions, including the formation and stability of central and peripheral myelin. In several neurodegenerative diseases, sphingolipid metabolism is deeply deregulated, leading to the expression of abnormal sphingolipid patterns and altered membrane organization that participate to several events related to the pathogenesis of these diseases. The most impressive consequence of this deregulation is represented by anomalous sphingolipid–protein interactions that are at least, in part, responsible for the misfolding events that cause the fibrillogenic and amyloidogenic processing of disease-specific protein isoforms, such as amyloid β peptide in Alzheimer's disease, huntingtin in Huntington's disease, α-synuclein in Parkinson's disease, and prions in transmissible encephalopathies. Targeting sphingolipid metabolism represents today an underexploited but realistic opportunity to design novel therapeutic strategies for the intervention in these diseases.

F. Scandroglio · S. Prioni · N. Loberto · M. Aureli · V. Chigorno · S. Sonnino · A. Prinetti
Center of Excellence on Neurodegenerative Diseases, Department of Medical Chemistry, Biochemistry and Biotechnology, University of Milan, 20090 Segrate, Italy

M. Piccinini · B. Buccinnà · E. Lupino · G. DeMarco · A. Lomartire · M. T. Rinaudo
Section of Biochemistry, Department of Medicine and Experimental Oncology, University of Turin, Turin, Italy

A. Prinetti (✉)
Dipartimento di Chimica, Biochimica e Biotecnologie per la Medicina, Università degli Studi di Milano, Via Fratelli Cervi 93, 20090 Segrate, Italy
e-mail: alessandro.prinetti@unimi.it

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Abbreviations
Ganglioside and glycosphingolipid nomenclature is in accordance with the IUPAC-IUBMB recommendations [1]

AD	Alzheimer's disease
CJD	Creutzfeldt–Jakob disease
CNS	Central nervous system
GalCer	Galactosylceramide
GD	Gaucher disease
GlcCer	Glucosylceramide
GPL	Glycerophospholipids
GSL	Glycosphingolipids

HD	Hungtington's disease
MAG	Myelin-associated glycoprotein
NPD	Niemann–Pick disease
PD	Parkinson's disease
PNS	Peripheral nervous system
PrP	Prion protein
SL	Sphingolipids
SM	Sphingomyelin

Introduction

Structure and Functions of Sphingolipids

Cell membrane lipids, at least in vertebrates, are represented by glycerophospholipids (GPL), sphingolipids (SL), and cholesterol. Polar, amphipatic lipids, such as GPL and SL, participate as major structural lipids to the formation of the basic matrix of all cellular membranes in eukaryotes due to their aggregative properties (the tendency of their hydrophobic portions to associate together excluding water molecules and that of their hydrophilic portions to interact with the extra- and intracellular aqueous environments). GPL are by far the major structural lipids in cellular membranes and phosphatidylcholine that accounts in most cases for more than 50% of all cell membrane phospholipids is the main bilayer-forming lipid. SL are minor components of cell membranes, and many complex glycosphingolipids (GSL), including gangliosides, are not bilayer-forming lipids (in water solution, they tend to form micellar aggregates due to the large size of their polar headgroups). However, they can be inserted in the glycerolipid bilayer through their hydrophobic ceramide moiety. It should be noted that, even if minor components respect to the bulk of a cell membrane, their local concentration can be relatively high: SL are mainly associated with the external leaflet of the plasma membrane, and in some cells and tissues, such as the myelin sheath and neurons, they are particularly abundant (e.g., in cultured cerebellar neurons, they represent about 5% of total amphipatic lipids).

The hydrophobic moiety of SL, ceramide (Fig. 1), is a long-chain amino alcohol, [2] (2*S*, 3*R*, 4*E)* 2-amino-1, 3-dihydroxy-octadec-4-ene, trivially known as sphingosine,

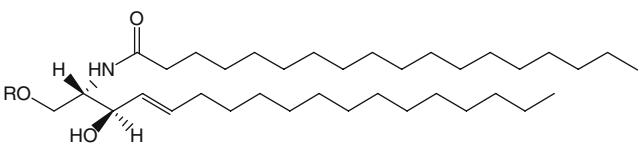

Fig. 1 Ceramide, the hydrophobic backbone of sphingolipids. Ceramide can be heterogeneous in its fatty acid and sphingoid base composition. *R* symbolizes the hydrophilic headgroup of sphingolipids, phosphocholine in the case of SM or a saccharide in the case of GSL

linked via an amide bond with a fatty acyl chain that can be very heterogeneous (as in the case of GPL) with regard to the chain length and the presence of unsaturations.

The hydrophilic head group of SL is phosphocholine in the case of sphingomyelin (SM; the only known phosphosphingolipid in mammals, where it is ubiquitously expressed in tissues and cells, but abundant within the nervous system both in neurons and myelinizing oligodendrocytes) or an oligosaccharide chain in the case of GSL (Fig. 2). The oligosaccharide chain of GSL can be very simple (as is the case for galactosylceramide, GalCer, one of the main myelin lipids) or it can reach a very high degree of complexity (as in polysialylated gangliosides, abundant in differentiated neurons). In addition to neutral GSL, in eukaryotes, two families of acid GSL are also present, represented by (1) sulfatides, containing an *O*-linked sulfate group on a glucose or galactose residue, among which 3-*O*-sulfogalactosylceramide is highly enriched in myelin sheath, representing up to 6% of myelin lipids); and (2) gangliosides, characterized by the presence of sialic acids, sugars containing a carboxyl group. Sialic acid [3] is the name that collectively indicates the derivatives of 5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid or neuraminic acid. In human, the most abundant sialic acid is the 5-*N*-acetyl derivative, but about 10% of the total ganglioside sialic acid is represented by the 9-*O*-acetyl-*N*-acetylneuraminic acid [4], and polysialogangliosides containing this sugar structure have been characterized in mice brains [5, 6]. GSL are ubiquitous components of mammal cell membranes, but are particularly abundant in the nervous system, and within the nervous system, gangliosides are present at high levels in neurons. Keeping in mind that SL are concentrated at the subcellular level in the plasma membrane, where they reside asymmetrically in the extracellular leaflet, and that they are not randomly distributed, but rather concentrated in restricted membrane areas [7, 8] due to their spontaneous segregation respect to GPL, it can be predicted that their local concentration in specific "lipid membrane domains" is very high. Remarkably, membrane segregation of SL seems to be higher in neurons than in any other cell type so far investigated.

The presence of (glyco)sphingolipids deeply affects the structural properties of a cellular membrane. GSL included in PC bilayers imply a curvature stress to the membrane that is probably relevant in the stabilization of the architecture of polarized cell membrane areas (such as the pre- and postsynaptic areas in neurons) and for membrane geometry dynamics in processes such as vesiculation and budding. In addition, SL, in particular GSL, greatly contributes to the creation of lateral order in biological membranes [7, 8]. SL tend to segregate in biological membranes with the formation of sphingolipid-enriched areas that are more ordered than the surrounding membrane

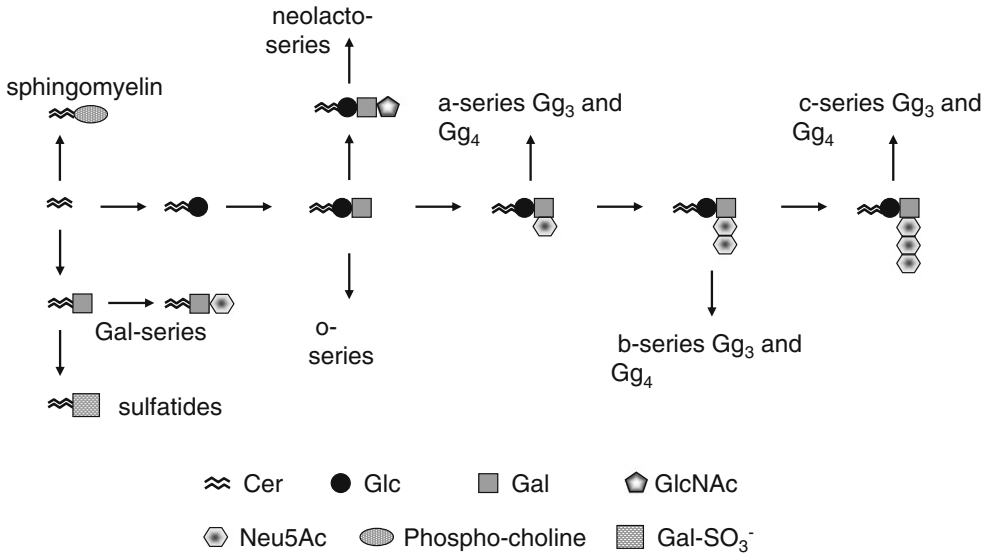

Fig. 2 The structure of the main sphingolipid classes

environments, being in this regard similar to a liquid-ordered or a metastable gel phase. This behavior is driven by the unique biophysical and geometrical properties of SL among polar lipids:

1. Due to the common hydrophobic ceramide backbone, characterized by the presence of an amide linkage and of a hydroxyl group, all SL can act as donors and acceptors for the formation of hydrogen bonds [7, 8], thus participating in the formation of a hydrogen bond network at the water/lipid interface that strongly stabilizes the lateral segregation of these lipids within the membrane bilayer.
2. GSL are hallmarked by the presence of a bulky oligosaccharide hydrophilic headgroup (the volume occupied by an "average" sugar GSL headgroup is much larger than that occupied by phosphocholine, the bulkiest headgroup present in phospholipids). Phase separation with clustering of GSL in a phospholipid bilayer is thus favored by the minimization of the interfacial free energy required to accommodate the amphipathic molecule in the bilayer. As mentioned above, this energetically favored event imposes a positive curvature stress to the membrane [9–26].
3. GSL clustering can be facilitated and stabilized by the formation of carbohydrate–water interactions, i.e., hydrogen bonds involving the GSL sugar headgroups and water molecules associated with the oligosaccharide chains [27]. It has been estimated that each GSL oligosaccharide chain is surrounded by 40–70 water molecules [17, 28], and strong interactions between water and the oligosaccharide chain of GM1 ganglioside have been observed by NMR studies [14], suggesting that water bridges between saccharides play an important role in organizing a net of hydrogen bonds able to stabilize GSL clustering.
4. Some SL classes, such as SM and gangliosides (at least in the nervous system), contain high levels of saturated acyl chains (such as palmitic and stearic acid). The presence of saturated acyl chains (that can be tightly packed with a high degree of order in the hydrophobic core of a bilayer) is another factor that favors the phase separation of a rigid, liquid-ordered phase. As example, in the case of GM1 ganglioside, it has been shown that its distribution in the fluid phase of a phospholipid bilayer [29] is directly correlated with the degree of unsaturation.

The lateral order imposed by SL segregation in cellular membranes has important consequences on the function of membrane-associated proteins, thus affecting several relevant biological events. It has been proposed that the association of a protein with a SL-enriched membrane area with reduced fluidity with respect the surrounding bilayer might represent a way to restrict the lateral motility of the protein. This could favor more stable interactions with other proteins segregated in the same domains or prevent interactions with other proteins preferentially localized in fluid membrane regions.

On the other hand, the complex oligosaccharide chains of GSL, oriented toward the extracellular environment at the plasma membrane level, seem to be made for specific interactions, and several examples of interactions between GSL and other molecules belonging to the same membrane (*cis* interactions) or to the extracellular environment (including soluble molecules, such as microbial toxins, extracellular matrix components, and molecules inserted in the plasma membrane of neighboring cells; trans-interactions) have been described. Apart from the association with sphingolipid-enriched plasma membrane domains (lipid rafts), the ability of GSL and gangliosides, in particular, to

laterally interact with and to modulate the activity of membrane-associated proteins, such as receptor tyrosine kinases, has been widely documented (reviewed in [30–40]), especially in the nervous system. Obviously, the clustering of a certain protein within SL-enriched membrane domains would favor its interactions with lipid components of the rafts, and the high enrichment in lipid rafts of several receptor and non-receptor protein kinases and other signaling proteins suggested novel models for the interpretation of ganglioside-mediated signal transduction. In some cases, SL–protein interactions imply a specific, medium-affinity interaction between the GSL oligosaccharide chain and some part of the protein that could be represented by amino acid residues belonging to the extracellular loops of the protein, sugar residues in the glycans of a glycosylated protein, or the hydrophilic portion of a glycosylphosphatidylinositol (GPI) anchor in the case of GPI-anchored proteins. On the other hand, the association of a protein with a rigid membrane area could induce conformational changes in the polypeptide chain affecting its functional activity, independently of the formation of specific high-affinity lateral interactions with other raft components.

Lastly, as for GPL, catabolic fragments derived from plasma membrane SL by the action of hydrolytic enzymes can represent or be converted to simple lipid mediators (ceramide, sphingosine, and sphingosine 1-phosphate) that are capable of modulating cell proliferation, differentiation, motility, or apoptotic cell death by affecting specific signaling cascades. In this sight, the hydrolysis of SM by different sphingomyelinases with the production of bioactive ceramide has been described by many authors. More recently, a few papers reported the possibility that GSL hydrolysis might also represent a mechanism for signaling ceramide production [41].

Metabolism and Intracellular Traffic of Sphingolipids

Both the biosynthesis and the degradation of plasma membrane SL take place in intracellular districts. Therefore, the regulation of plasma membrane SL composition in a certain cell or tissue is the result of (a) the activities of biosynthetic and catabolic enzymes that are developmentally regulated in a tissue-specific fashion; (b) a bidirectional flow of molecules from and to the plasma membrane that mainly occurs via vesicular traffic, even if non-vesicular transport via SL-binding proteins plays an important role in specific steps [42–44]. The early steps in the de novo biosynthetic pathway of SL occur at the cytosolic face of the endoplasmic reticulum, where the enzyme activities responsible for the reaction sequence leading to the formation of ceramide are localized (Fig. 3). At least six different genes encoding for (dihydro)ceramide synthases with unique tissue distribution and preference for different acyl CoA as substrates have been so far identified [45]. The fate of the neosynthesized ceramide, as common precursor of SM and GSL, is determined by the existence of different specific delivery mechanisms to the sites where the following steps of the synthesis of complex SL take place. Ceramide reaches the luminal side of the trans-Golgi apparatus, the main site for its conversion to SM by sphingomyelin synthase 1 [46] by at least two different mechanisms, vesicular transport and non-vesicular transport mediated by the ceramide transfer protein CERT [47] that shows a preference for ceramides with C16–C20 fatty acids. GalCer, the precursor of galacto-GSL series (Table 1), is formed at the luminal side of the ER [48], while ceramide used for the synthesis of all other GSL is transferred to the Golgi apparatus by vesicular transport where it is stepwise glycosylated by membrane-bound glycosyltransferases responsible for the sequential addition of sugar residues to the growing oligosaccharide chain (Fig. 3). Glucosylceramide (GlcCer), the common glycosylated precursor of ganglio-, globo-, isoglobo-, lacto-, and neolacto- series GSL (Table 1) is formed by a ceramide glucosyltransferase activity localized at the cytosolic side of the Golgi membrane. The exact site of GlcCer synthesis in the Golgi apparatus is still debated (different regions of the Golgi or even specialized ER subregions, such as the mitochondria-associated ER subcompartment) and the movement of GlcCer along the Golgi likely involves different pathways, with evidence for the importance of non-vesicular mechanisms mediated by the GlcCer transfer protein FAPP2 [49]. Eventually, neosynthesized GalCer and GlcCer can be delivered to the luminal side of the Golgi apparatus, where all the transferases (galactosyltransferases, sialyltransferases, GalNAc transferases, and GalCer sulfotransferase), responsible for the synthesis of more complex GSL by the sequential addition of sugar residues to the growing oligosaccharide chain are localized (Fig. 3); alternatively, they can directly reach the plasma membrane [50]. Neosynthesized GSL move through the Golgi apparatus to the plasma membrane following the mainstream exocytotic vesicular traffic.

Relatively little is known about the regulation of SL biosynthesis that has been regarded for a long time as the main mechanism responsible for the formation of a cell-specific GSL pattern. It is generally assumed that GSL synthesis is mainly regulated at the transcriptional level through the control of the expression levels of glycosyltransferases or transporter proteins. This notion has been supported by the observation that changes in cellular GSL patterns, such as those occurring in the nervous system during neuronal development and oncogenic transformation, are paralleled by changes in the expression of the corresponding glycosyltransferases. However, the highly compartmentalized nature of SL metabolism suggests that differential intracellular flows of different GSL can influence the final GSL composition of

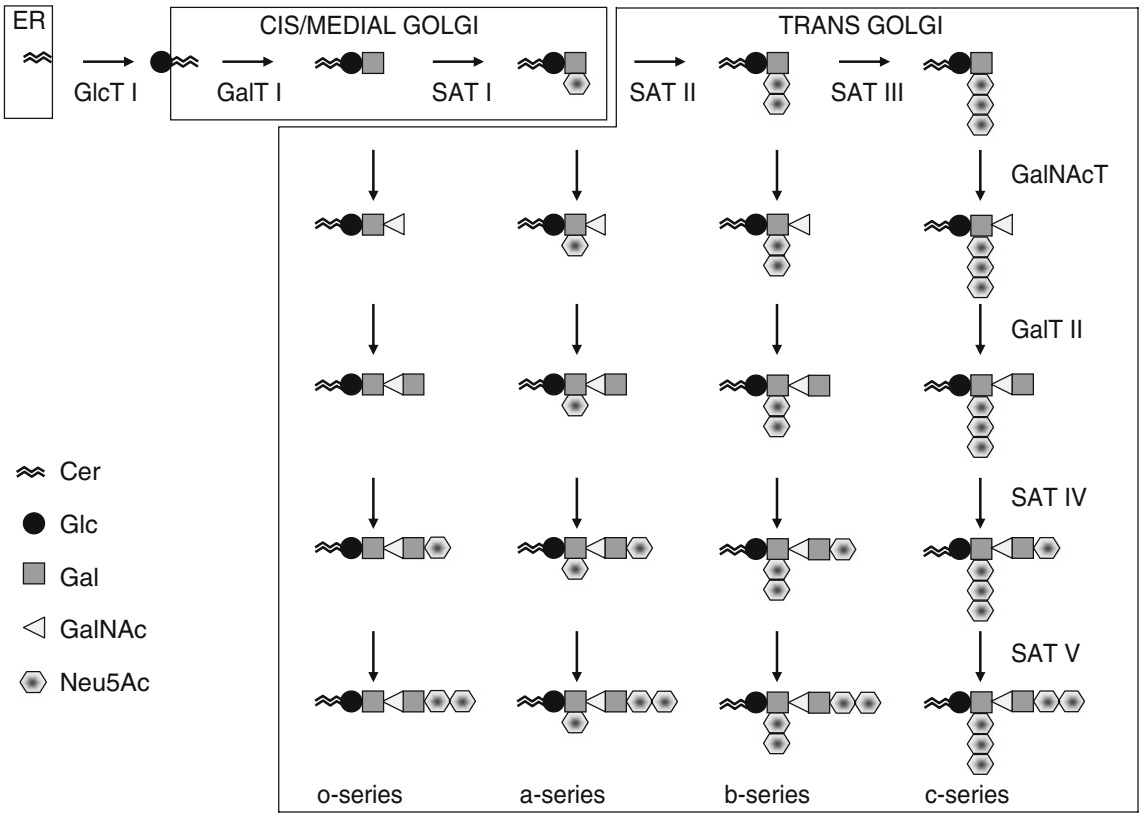

Fig. 3 Schematic representation of the biosynthetic pathway for ganglio-series gangliosides. The synthesis of ceramide, the common biosynthetic precursor of all SL, occurs at the cytosolic face of the endoplasmic reticulum. Neosynthesized ceramide is then delivered by specific mechanisms to the sites where the synthesis of complex SL takes place. Ceramide used for the synthesis of GlcCer and all GlcCer-based GSL is transferred to the Golgi by vesicular transport. GlcCer is formed by a ceramide glucosyltransferase activity localized at the cytosolic side of the Golgi membrane and eventually is delivered (likely by a non-vesicular mechanisms mediated by the GlcCer transfer protein FAPP2) to the luminal side of the Golgi, where all the glycosyltransferases responsible for the synthesis of more complex GSL by the sequential addition of sugar residues to the growing oligosaccharide chain are located

the plasma membrane, independently of the expression levels of relevant glycosyltransferases.

The degradation of plasma membrane GSL takes place in the lysosomes that are reached by the endocytic vesicular flow through the early and late endosomal compartment. Along their route to the lysosomes, GSL originally resident at the plasma membrane can be diverted to intracellular sites (presumably the Golgi apparatus) where they undergo direct glycosylation with the formation of more complex products, able in turn to reach again the plasma membrane. Moreover, simple sphingoid molecules such as ceramide and sphingosine generated in lysosome can escape further degradation and be recycled for the re-synthesis of signaling SL or complex plasma membrane SL. At least, in some tissues and cell types (for example, in neurons) [51, 52], the recycling of SL catabolic products for biosynthetic purposes seems to be quantitatively very relevant, thus representing a further potential mechanism for the regulation of SL turnover at the level of intracellular traffic. However, very little is known about the mechanisms of escape from the lysosome and the transfer of these intermediates to the Golgi or other cellular districts.

On the other hand, the plasma membrane is not just the cellular district where complex SL are concentrated to exert their relevant biological function, but rather, it is also an active site for SL metabolic remodeling. The production of bioactive ceramide has been regarded for a long time as mainly due to SM hydrolysis by sphingomyelinases [53], resident in the plasma membrane or translocated to it from intracellular sites upon stimulus [54, 55]. More recently, it has been shown that a sphingomyelin synthase enzyme activity (SMS2), encoded by a different gene with respect to that coding for the enzyme distributed in the Golgi apparatus, is also present at the plasma membrane [56]. Thus, two different enzyme activities are present allowing the reciprocal regulation of ceramide and SM levels within the plasma membrane in response to changes in cellular physiology, without the need of any sorting of the substrates to intracellular sites of metabolism. Plasma membrane-associated ceramidases and sphingosine kinases

Table 1 Structures of main nervous system glycosphingolipids

Glucose series, GlcCer	
GlcCer	ß-Glc-(1-1)-Cer
Galactose series, GalCer	
GalCer	ß-Gal-(1-1)-Cer
SM4s	$^{-}O_3S$-3-β-Gal-(1-1)-Cer
GM4	α-Neu5Ac-(2-3)- ß-Gal-(1-1)-Cer
Lactose series, LacCer	
LacCer	ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
GM3	α-Neu5Ac-(2-3)-ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
GD3	α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
Neolacto-tetraose series, nLC_4Cer	
3′-LM1	α-Neu5Ac-(2-3)-ß-Gal-(1-4)-ß-GlcNAc-(1-3)- ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
Ganglio-triose series, Gg_3Cer	
asialoGM2	ß-GalNAc-(1-4)-ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
GM2	ß-GalNAc-(1-4)-[α-Neu5Ac-(2-3)-]ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
Ganglio-tetraose series, Gg_4Cer	
AsialoGM1	ß-Gal-(1-3)-ß-GalNAc-(1-4)-ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
GM1	ß-Gal-(1-3)-ß-GalNAc-(1-4)-[α-Neu5Ac-(2-3)-]ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
GD1a	α-Neu5Ac-(2-3)-ß-Gal-(1-3)-ß-GalNAc-(1-4)-[α-Neu5Ac-(2-3)-]ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
GD1b	ß-Gal-(1-3)-ß-GalNAc-(1-4)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-]ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
GD1b-lactone	ß-Gal-(1-3)-ß-GalNAc-(1-4)-[α-Neu5Ac-(2-8,1-9)-α-Neu5Ac-(2-3)-]ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
GT1a	α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-ß-Gal-(1-3)-ß-GalNAc-(1-4)-[α-Neu5Ac-(2-3)-]ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
GT1b	α-Neu5Ac-(2-3)-ß-Gal-(1-3)-ß-GalNAc-(1-4)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-]ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
O-Acetyl-GT1b	α-Neu5Ac-(2-3)-ß-Gal-(1-3)-ß-GalNAc-(1-4)-[α-Neu5,9Ac_2-(2-8)-α-Neu5Ac-(2-3)-]ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
GT1c	ß-Gal-(1-3)-ß-GalNAc-(1-4)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-]ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
GQ1b	α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-ß-Gal-(1-3)-ß-GalNAc-(1-4)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-]ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
O-Acetyl-GQ1b	α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-ß-Gal-(1-3)-ß-GalNAc-(1-4)-[α-Neu5,9Ac_2-(2-8)-α-Neu5Ac-(2-3)-]ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
GQ1c	α-Neu5Ac-(2-3)-ß-Gal-(1-3)-ß-GalNAc-(1-4)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-]ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
GP1c	α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-ß-Gal-(1-3)-ß-GalNAc-(1-4)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-]ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
Ganglio-pentaose series, Gg_5Cer	
Fuc-GM1	α-Fuc-(1-2)-ß-Gal-(1-3)-ß-GalNAc-(1-4)-[α-Neu5Ac-(2-3)-]ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
GalNAc-GM1	ß-GalNAc-(1-4)-ß-Gal-(1-3)-ß-GalNAc-(1-4)-)-[α-Neu5Ac-(2-3)-]ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
Fuc-GD1b	α-Fuc-(1-2)-ß-Gal-(1-3)-ß-GalNAc-(1-4)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-]ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
GalNAc-GD1a	ß-GalNAc-(1-4)-[α-Neu5Ac-(2-3)-]ß-Gal-(1-3)-ß-GalNAc-(1-4)-[α-Neu5Ac-(2-3)-]ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
Alfa series	
Chol-1α-a	α-Neu5Ac-(2-3)-ß-Gal-(1-3)-[α-Neu5Ac-(2-6)-]ß-GalNAc-(1-4)-[α-Neu5Ac-(2-3)-]ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
Chol-1β	ß-Gal-(1-3)-[α-Neu5Ac-(2-6)]-ß-GalNAc-(1-4)- [α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-]ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
GT1α	α-Neu5Ac-(2-3)-ß-Gal-(1-3)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-6)-]ß-GalNAc-(1-4)-ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
GQ1α	α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-ß-Gal-(1-3)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-6)-]ß-GalNAc-(1-4)-ß-Gal-(1-4)-ß-Glc-(1-1)-Cer
Chol-1α-b	α-Neu5Ac-(2-3)-ß-Gal-(1-3)-[α-Neu5Ac-(2-6)-]ß-GalNAc-(1-4)-[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-]ß-Gal-(1-4)-ß-Glc-(1-1)-Cer

have been described, putatively responsible for the generation of sphingosine and/or sphingosine-1-phosphate at the cell surface [57–59].

More than 20 years ago, the observations that both a sialidase [60–63] and a sialyltransferase [64] are active in synaptosomal membranes led to the hypothesis that a physiologically relevant sialylation–desialylation cycle for gangliosides can be operative at the plasma membrane level. Some information is also available about the in situ sialylation of gangliosides at the cell surface. The existence

of a synaptosomal membrane sialyltransferase in brain has been confirmed by metabolic studies in chicken embryos [65] and rat brain [66, 67], and it has been shown that dexamethasone treatment markedly increased GM3 synthesis, possibly due to increased enzyme activity of GM3 synthase at the plasma membrane [68]. Thus, GSL sialylation might occur outside the Golgi compartment and could be relevant in modulating plasma membrane GSL patterns. The existence of a plasma membrane-associated sialidase distinct from the lysosomal enzyme in nervous cells was suggested by several studies. Among others, it has been shown that cultured rat cerebellar granule and human neuroblastoma cells possess the capability to desialylate exogenously added gangliosides under experimental conditions blocking endocytosis and lysosomal activity [69–71], a process blocked by a cell-impermeable sialidase inhibitor [72]. A membrane-bound sialidase was purified from human brain gray matter [69, 70] and bovine brain [73], and eventually the cDNA sequence of a specific membrane-linked sialidase, subsequently termed Neu3, distinct from other known sialidases, has been cloned in human [74], bovine [75], and mouse [76]. Remarkably, the ability of Neu3 to modulate the cell surface glycolipid composition was not restricted to *cis* interactions. In fact, mouse Neu3 overexpressed in COS-7 cells was able to hydrolyze ganglioside substrate belonging to the surface of neighboring cells [77], representing the first and so far only known example of transcellular SL metabolism. More recently, the presence of other glycolipid hydrolases in the plasma membrane has been demonstrated in cultured fibroblasts [78, 79]. Both lysosomal GSL-metabolizing enzymes delivered at the cell surface during the repair of the plasma membrane [80] by a retrograde flow of lysosomal components and specific membrane-associated glycosylhydrolase isoforms seem to account for these activities.

Finally, (glyco)sphingolipids can be released from the cell surface to the extracellular environment as monomers or aggregates, such as shedding vesicles [81–84], and shed gangliosides could be taken up by neighboring cells [85]. Thus, intercellular exchange of SL could represent a further mechanism for the regulation of cell lipid composition.

Role of Sphingolipids in the Development and Function of Nervous System

GSL are vital for multicellular organisms. GSL-deficient cells, such as the GM-95 mutant melanoma cell line, lacking ceramide glucosyltranferase activity [86] and embryonic stem cells from ceramide glucosyltranferase knockout mice [87] are able to survive, grow, and undergo in vitro differentiation as those from wild-type animals. However, ceramide glucosyltranferase knockout mice are embryonically lethal and showed no cellular differentiation beyond the primitive germ layers [88].

The crucial role of GSL in the development and maintenance of the proper functions of the nervous system has been demonstrated by an impressive and multifaceted body of evidence (schematically summarized in Fig. 4).

1. GSL patterns undergo deep qualitative and quantitative modifications during the development of the nervous system: in chicken [89], rodent [90], and human brain [91], the total gangliosides contents increased several-fold from the embryonic stages to the postnatal life. These increases were accompanied by a dramatic shift from simple gangliosides (GM3 and GD3) to more complex species (GM1, GD1a, GD1b, GT1b). A similar increase in the quantity and in the complexity of gangliosides has also been observed during differentiation in cultured neurons of different origin and in mouse neural precursor cells [89, 90, 92–97]. In humans, the phase of rapid ganglioside increase started from the sixth month of gestation and reached the maximum value at about 5 years of age [91]. Along the adult life, a progressive loss of gangliosides with aging has been reported in human and mouse brain. The trends of variations are very complex and different for different brain areas, glycolipid species, and age ranges; however, no sex-related differences were observed [91, 98–102]. The most pronounced ganglioside changes associated with aging (substantially similar in whole brain, brain white and gray matter, parietal and frontal cortex, and cerebellum) were an increase in the simpler gangliosides (GM3 and GD3), a reduction of the complex gangliosides of the a-pathway (GD1a and GT1a), and an increase in GD1b [99, 101, 102]. The expression of galactolipids, such as GalCer and sulfatide, two GSL highly enriched in central and peripheral myelin, is also dramatically regulated during the development of the nervous system. During mid-embryonic stages of mouse brain development, GlcCer, but not GalCer or sulfatide, is expressed [90]. Their synthesis starts in the embryonic development when oligodendrocytes enter terminal differentiation and is upregulated during the postnatal extension of the myelin sheaths [103]. In human cerebral gray matter, the concentration of myelin lipids starts to decrease after 20 years of life [101, 102].
2. Experimental manipulations allowing modification of the concentration or pattern of GSL in the plasma membrane profoundly affect the behavior of neural cells. The addition of exogenous gangliosides exerts neuritogenic, neurotrophic, and neuroprotective effects in cultured neurons and neural cell lines and in animal models of neural lesions [104–108]. In particular, GM1 ganglioside is able to potentiate the neuritogenic effect

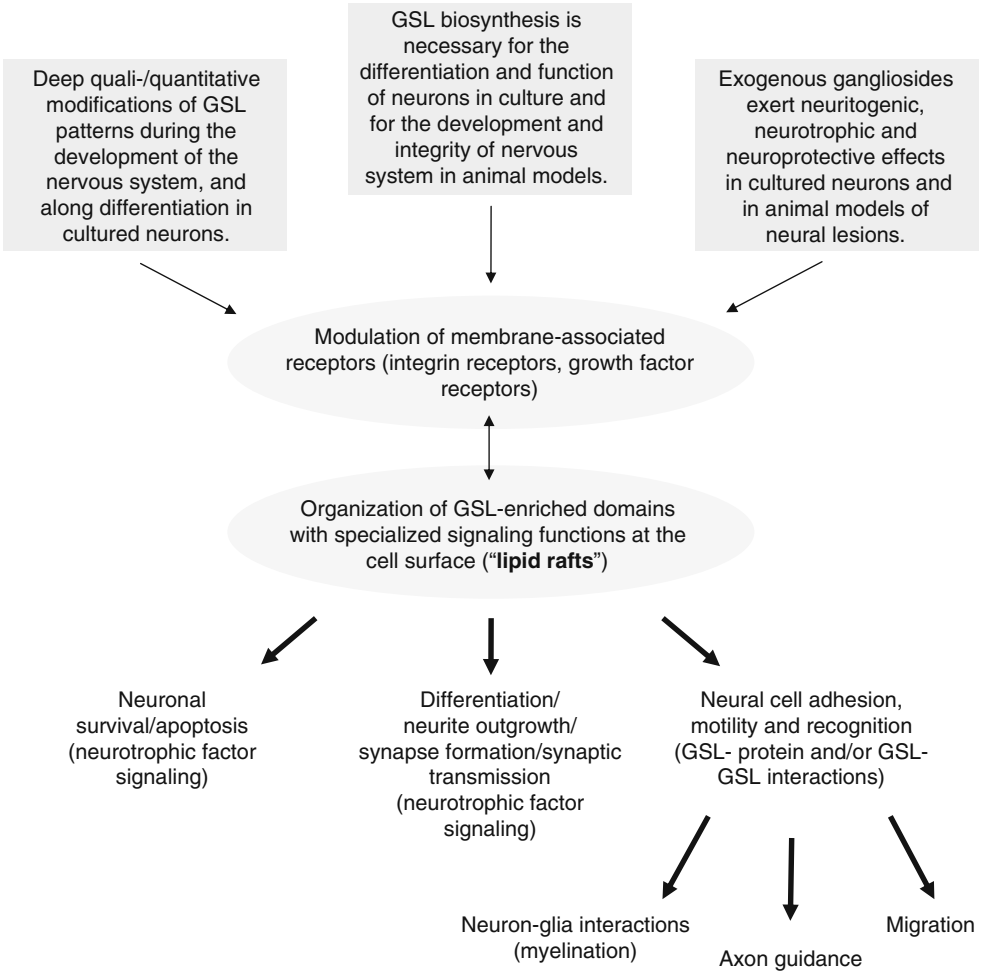

Fig. 4 The multifaceted role of GSL in the development and in the maintenance of the nervous system functions

of nerve growth factor (NGF) in PC12 cells, i.e., it is able to induce neuronal differentiation in the presence of an NGF concentration that is ineffective by itself [109–111]. Increased surface expression of GM1 by treatment cells with bacterial sialidase potentiated PGE1-induced neurite formation [112, 113]. Furthermore, administration of exogenous GM1 and GM3 induced c-Src activation and neuritogenesis in neuroblastoma cells [114]. Treatment with pharmacological inhibitors of ceramide synthase or ceramide glucosyltranferase, or selective depletion of cell surface SL, achieved by treating living cells with bacterial sphingomyelinases [115, 116] or with endoglycoceramidase (able to remove the oligosaccharide chain from cell surface GSL) [117] caused SL depletion and disorganization of SL-enriched domains [118–123], thus affecting domain-mediated biological functions, including survival in neurotumoral cell lines and oligodendrocytes, axonal transport and sorting [124–127], and finally TAG-1 signaling in cerebellar neurons [117].

3. Many pieces of evidence indicated that SL biosynthesis is necessary for nervous system development. Blockade of (glyco)sphingolipid biosynthesis by pharmacological inhibition of GlcCer synthase or ceramide synthase reduced axonal elongation and branching in cultured hippocampal and neocortical neurons [128–130], synapses formation and activity [131], and NGF-induced neurite outgrowth in human neuroblastoma and PC12 cells [132, 133]. Neural cell-specific deletion of GlcCer synthase in mice led to severe neurological defects in the early postnatal life and death within 3 weeks [134], demonstrating the importance of GSL for the maturation of the nervous system. On the other hand, pharmacologically induced stimulation of GSL biosynthesis stimulated neurite outgrowth, formation of functional synapses, and synaptic activity in cultured cortical neurons [130, 131], and induced expression of GD3 synthase was able to switch neuroblastoma cells to a differentiated phenotype [135]. NGF- and forskolin-induced neuronal differentiation in PC12 cells was accompanied by the up-regulation of several glycosyltransferase activities (GalGb3-, GM3-, GD1a-, and GM2 synthases) [136], and bFGF-stimulated axonal growth in cultured hippocampal neurons resulted in the activation of ceramide glucosyltranferase [137]. Glycosyltranferase expression and activity

showed important changes in the developing mouse brain. In particular, the regulation of the two glycosyltransferases at the branching point in the biosynthetic pathway of gangliosides (sialyltransferase II, ST-II, or GD3 synthase, and GalNAc transferase, GalNAcT, or GM2/GD2 synthase) seems to account for the differential expression of gangliosides during brain development. SAT-II activity, but not its expression levels, decreased, and GalNAcT activity increased during development [90, 138, 139]. On the other hand, increased GalCer and sulfatide levels during oligodendrocyte development and myelination are mainly driven by the concomitant increased expression of GalT-III (GalCer synthase) [90]. Remarkably, the expression of several lysosomal glycosidases (Neu1, Neu3, glucosylceramidase, galactosylceramidase, lysosomal acid β-galactosidase (β-Gal), β-*N*-acetylhexosaminidase α- and β-subunits) and of some co-factors involved in the catabolic pathway of SL remained unvaried in the developing mouse brain, suggesting that this pathway is not significantly responsible for the GSL compositional changes associated with the development of the nervous system [90]. However, it has been recently suggested that the activity of the plasma membrane-associated ganglioside sialidase Neu3 might have a role in modifying the cell surface ganglioside composition, causing a decrease of GM3 and shift from polysialylated ganglioside species to GM1, with deep consequences on very important cellular events, including neuronal differentiation. In neuroblastoma cell lines, Neu3 expression increased during pharmacologically induced neuronal differentiation [140], and Neu3 gene transfection induced neurite outgrowth [140] and enhanced the effect of differentiating agents on the extension or branching of neurites [76]. Conversely, inhibition of plasma membrane sialidase activity resulted in the loss of neuronal differentiation markers [69, 70, 141]. In cultured hippocampal neurons, Neu3 activity regulated the local GM1 concentration, determining the neurite's axonal fate by a local increase in TrkA activity [142] and affecting axonal regeneration after axotomy [143].

The multiple roles of GSL in regulating cellular function essential for the development and the homeostasis of the nervous system can be explained by their ability to modulate the activity of plasma membrane via direct SL–protein or indirect (mediated by lipid rafts) lateral interactions (*cis* interactions), as discussed above [144–147] (Fig. 4). SL, together with many classes of proteins involved in mechanisms of signal transduction that are relevant for neural cell biology, such as (1) receptor tyrosine kinases (including neurotrophin receptors Trk A, Trk B, Trk C, c-Ret, ErbB, the ephrin receptor Eph), GPI-anchored receptors (the GDNF family receptor GFRα), G protein-coupled receptors (including cannabinoid receptors and neurotransmitter receptors such as α1-, β1-, β2-adrenergic, adenosine A1, γ-aminobutyric acid GABAb, muscarinic M2, glutamate metabotropic mGLUR, serotonin 5HT2), (2) non-receptor tyrosine kinases of the Src family, (3) adapter and regulatory molecules of tyrosine kinase signaling, (4) heterotrimeric and small GTP-binding proteins, (5) protein kinase C isoenzymes, (6) cell adhesion molecules, including integrins, Notch1, NCAMs, TAG-1, Thy-1, F3/contactin, (7) ion channels, proteins involved in neurotransmitter release, postsynaptic density complex proteins [92, 93, 147–155] segregate in lipid rafts present in cultured neural cells (neurons, oligodendrocytes, astrocytes, and neurotumoral cell lines), as well as in different brain regions, myelin, and synaptic plasma membranes. This particular clustering affects neurotrophic factor signaling [147, 148, 151, 152], cell adhesion and migration [147, 156, 157], axon guidance, synaptic transmission [147, 158], neuron–glia interactions [159, 160], and myelin genesis [161].

Glycosphingolipids and Myelin

An interesting example of the multifaceted roles of GSL in the nervous system is represented by their involvement in the formation and maintenance of myelin. In particular, two different kinds of trans-interactions involving GSL seem to importantly contribute to the wrapping and stabilization of the multilayered myelin sheath and to functional myelin–axon communication.

As mentioned above, the galactolipids GalCer and sulfatide are the major GSL in myelin, and their synthesis is maximal in rat at the time of maximal myelination and in cultured oligodendrocytes during the formation of membrane sheaths [162, 163]. Studies on galactolipid-knockout mice revealed their importance in the creation of a compactly wrapped myelin that is essential for a fast rate of nerve conduction and in the stabilization of the paranodal loops [164–166]. These roles are at least, in part, explained by the ability of GalCer and sulfatide to act as trans-ligands for each other by carbohydrate–carbohydrate interactions (reviewed in [161, 167]). GalCer-sulfatide interaction in oligodendrocyte membranes regulate the co-clustering and distribution of several myelin proteins, deeply affecting the organization of myelin lipid rafts that are crucial for myelin formation, maintenance, and function [168] and participate in myelin–axonal communication.

On the other hand, long-term axon–myelin stability is due to the trans-interaction between the axonal gangliosides GD1a and GT1b and the myelin-associated glycoprotein (MAG) [169, 170].

MAG is a neural cell adhesion molecule belonging to a subgroup of the immunoglobulin superfamily, termed

sialoadhesins, which is selectively generated by oligodendrocytes in the central nervous system and Schwann cells in the peripheral nervous system. MAG represents ~1% of the central nervous system (CNS) and ~0.1% of the peripheral nervous system (PNS) myelin proteins [171]; it is found on the periaxonal surface of oligodendrocytes (CNS) and Schwann cells (PNS) as well as in the Schmidt–Lanterman incisures and the paranodal loops of PNS [172]. MAG is a type 1 integral membrane protein composed of five extracellular Ig-like domains followed by a single transmembrane domain and a cytoplasmic C-terminal domain [173, 174]. Two distinct MAG isoforms are known, the large MAG (L-MAG, 626 aa) and the small MAG (S-MAG, 582 aa), which originate by alternative splicing of the primary transcript. The two isoforms are identical in their extracellular and transmembrane domains, but differ in their cytoplasmic domain, which is shorter in S-MAG. L-MAG is the predominant variant in human CNS, whereas the two variants coexist in rodents; in PNS in contrast, S-MAG is the most abundant isoform in humans and rodents [175, 176]. About 30% by the molecular mass of MAG, estimated on the basis of electrophoretic mobility on polyacrylamide gels around 100 kDa (L-MAG) and 95 kDa (S-MAG), consists of carbohydrates organized to form oligosaccharide chains linked to the extracellular domain where eight glycosylation sites have been detected. The *N*-linked oligosaccharide chains are of the complex type and contain the HNK-1 epitope characterized by the sequence SO_4-3GlcAβ1→3Galβ1→4GlcNAc [177, 178]. The MAG extracellular domain bears a significant structural similarity to the two sialic acid-binding adhesion molecules CD22 (a member of the immunoglobulin superfamily expressed by B lymphocytes) and sialoadhesin (a macrophage receptor), both included in the above-mentioned sialoadhesin subgroup. MAG preferentially binds to *O*-linked glycans bearing the terminal sequence NeuAcα2→3Galβ1→3GalNAc. For this reason, MAG is also classified as a Siglec (sialic acid-binding immunoglobulin-like lectin), a subgroup of the Ig superfamily integral membrane proteins with an extracellular domain consisting of an amino-terminal V-set Ig-like domain followed by a variable number of C2-set Ig-like domains [179, 180]. The two above-mentioned brain gangliosides localized on the axonal membrane, GD1a and GT1b, that bear the terminal sequence NeuAcα2→3Galβ1→3GalNAc have been shown in vitro and in vivo to act as physiological MAG ligands [181–183]. Arginine 118, in the first Ig-like domain of MAG is believed to be the major determinant for this interaction [184].

The intracellular domains of the two MAG isoforms appear to mediate different functions. The L-MAG cytoplasmic domain contains a tyrosine residue (Tyr 620) that constitutes a phosphorylation site described to interact with Fyn, one of the non-receptor tyrosine kinases of the Src family, as well as with the phospholipase Cγ and the calcium-binding protein S100β, thus pointing to a functional role for L-MAG in signal transduction [185–188]. The CNS myelin of the L-MAG mutant mice, in which the physiological full-length L-MAG is substituted with a truncated form lacking the cytoplasmic domain, displays most of the pathological abnormalities reported for the total MAG knockout mice (see below). However, in contrast to total MAG knockout mice, PNS axons and myelin of older L-MAG mutant animals do not degenerate, thus indicating that S-MAG is sufficient to maintain PNS integrity [189]. In this respect, the cytoplasmic domain of S-MAG has been reported to bind to tubulin and microtubules, thus providing a dynamic link between the axonal surface and myelinating cell cytoskeleton [190].

Usually, MAG is not found in Triton X-100 resistant lipid domains [191]. However, antibody-mediated cross-linking of MAG on the surface of cultured-differentiated oligodendrocytes resulted in the redistribution of MAG into Triton X-100-insoluble complexes. This event was associated with the internalization of MAG/anti-MAG complexes, increased phosphorylation of Fyn, dephosphorylation of serine and threonine residues on specific proteins, such as lactate dehydrogenase and the β-subunit of the trimeric G protein complex Gβ1-2, cleavage of α-fodrin, a non-erythroid alfa spectrin involved in the organization and stability of the cytoskeleton and transient depolymerization of actin microfilaments [188, 192]. These modifications have been proposed to be part of a signaling cascade relying either on the reorganization of protein domains on the plasma membrane of oligodendrocytes or the MAG function as a mediator of axon–glia communication, which might have implications for the mutual regulation of the formation and stability of axons and myelin.

MAG expression begins early in the process of myelination [193] and continues at relatively high level in mature animals [173]. Evidence exists that, in MAG null mice, the formation of compact myelin in the CNS is significantly delayed in young and adult stages [194, 195]. Furthermore, in the CNS of these animals, the ultrastructure of compact myelin was unaffected although an abnormal periaxonal cytoplasmic collar was consistently observed [195, 196] associated with alterations of distal oligodendrocyte processes [197]. Although in the PNS of young MAG null mice the myelin formation was unaffected, in aging animals' myelin and axon, degeneration was a feature, so implicating MAG in the stability of both myelin and axons [198]. More recent reports [169, 199], in which MAG null mice extensively back-crossed to C57BL/6 background were used, revealed in CNS and PNS of aged animals a quantitatively and qualitatively similar axonal degeneration and a decrease in axonal caliber and neurofilament spacing [173]. The phenotype of mice lacking the gene Galgt1 required for the

synthesis of complex gangliosides including GD1a and GT1b was strikingly similar to that of MAG null mice [169]; in this regard, the two strains exhibited quantitatively and qualitatively similar alterations in CNS and PNS. These data, besides strengthening the view that MAG and complex gangliosides are major determinants of axon–myelin stability in CNS and PNS, give support to the hypothesis that the interaction between MAG on myelin and gangliosides on the axonal membrane plays a critical role in the long-term axon–myelin stability [170].

Inhibitory molecules expressed in CNS myelin are largely responsible for the failure of axonal regeneration after injury to the brain or spinal cord [200]. MAG has been identified as one of the several myelin-associated inhibitors of axonal regeneration [201–203]. The demonstration that recombinant MAG and antibody cross-linking of cell surface GT1b on hippocampal neurons [204] and GD1b and GT1b on cerebellar granule neurons [160] inhibited axon outgrowth, suggested a potential role for gangliosides as MAG receptors in axon outgrowth inhibition. Furthermore, the demonstration that MAG, together with other myelin-associated inhibitors of axonal regeneration (Nogo-A and OMgp), binds to Nogo-R1 (NgR1), a GPI-anchored protein expressed in many types of neurons in CNS [205–207], suggests a potential role of NgR1 as a MAG receptor in axon outgrowth inhibition. Further studies have demonstrated that additional molecules are required for the intracellular transduction of signals originated from NgR1 to the RhoA- and RhoA-associated kinase pathway. Two classes of transmembrane co-receptors have been so far shown to associate with NgR1, such as p75 and TROY, both belonging to the tumor necrosis factor receptor family, and LINGO1, a functional component of the Nogo receptor signaling complex, thus originating a multisubunit complex constituted by NgR1-p75/TROY-LINGO1 [203]. The negative impact exerted on axonal regeneration via NgR by the ability of MAG to bind sialic acid residues has been a matter of an intense debate. Initial reports indicated that MAG inhibition was acid sialic-independent [206, 207]. However, recent studies demonstrated that the binding of MAG with NgR1 or NgR2 is sensitive to sialidase action [208, 209]. Interestingly, NgR1 and NgR2 are almost exclusively found within Triton X-100 insoluble lipid microdomains [208]. With that in mind, several MAG receptor components, including p75 and GT1b, are localized on lipid rafts [204]; it was proposed that gangliosides promote a stable clustering of the MAG-NgR1-p75-LINGO receptor–ligand complex [210]. Based on recent results, the hypothesis has been raised that multiple and perhaps cell type-specific receptors for MAG-determined inhibition of axonal outgrowth exist [211, 212].

Deregulated Sphingolipid Metabolism and Membrane Organization in Nervous System Pathology

On the basis of the considerations reported in the previous paragraphs, it can be easily predicted that alterations in SL metabolism and/or changes in the SL-driven membrane organization can lead to important nervous system dysfunctions. Not surprisingly, several pieces of evidence indeed indicate that SL are important not only in physiological but also in pathological conditions in the nervous system and that: (1) GSL metabolism is altered with important consequences in many neurological diseases, including Alzheimer's and Huntington's diseases (Table 2); (2) altered organization of SL-enriched membrane domains is linked with the pathogenesis of spontaneous and transmissible neurodegenerative diseases (Table 3). A number of molecules causally connected to such diseases are associated with these domains. The most prominent examples are represented by the amyloid precursor protein (APP) in Alzheimer's disease (AD) by α-synuclein in Parkinson's disease (PD) and by the prion protein in transmissible spongiform encephalopathies. The generation of the aberrant forms of these proteins which are responsible for the onset of the disease seem to be localized in lipid rafts and/or dependent on the structure of these membrane domains [213, 214].

Sphingolipid Storage Diseases

A wide group of inherited lysosomal storage disorders caused by defects in SL metabolism (sphingolipidoses; reviewed in [215–217]) are characterized by severe neurological involvement. Lysosomal storage disorders are caused by the reduced or absent activity of lysosomal proteins, which results in the intralysosomal accumulation of undegraded metabolites. For sphingolipidoses, the defective gene encodes for either a hydrolase involved in SL catabolism or an activator protein required for the proper activity of a SL hydrolase. Most sphingolipidoses are characterized by prominent neurological involvement. In particular, the infantile forms are the most severe (death usually occurs in the early years of life) and are characterized by an acute brain involvement. The enzymatic, genetic, and molecular bases underlying the metabolic deficiency have been extensively studied and basically elucidated for most of these diseases. However, even if it is undoubtedly clear that the intralysosomal accumulation of unmetabolized substrates is the primary cause of the disease, the molecular mechanisms leading from this event to the pathology are still obscure, and very likely, the primary defect does affect multiple secondary biochemical and cellular mechanisms that could be indeed the main cause of tissue damage and death in sphingolipidosis. Since SL metabolism and traffic is a complex network of interdependent events, and

Table 2 Alterations in (glyco)sphingolipid metabolism in neurological diseases or diseases with neurological impairment

	Primary biochemical defect	Alteration in SL metabolism/membrane organization	
Lysosomal sphingolipid storage diseases	Defective or lacking activity of a lysosomal SL catabolic enzyme or activator protein	Accumulation of undegraded SL substrate	[215–217]
		Secondary alterations in SL metabolism (GM3 and GM2 accumulation)	[223, 224]
		Altered lipid rafts organization	[221, 224]
Alzheimer's disease	Misfolding and aggregation of variant amyloid β-protein	Reduced ganglioside concentration in several brain areas and altered ratios of a-series to b-series gangliosides	[101, 102, 226–230]
		Reduced sulfatide content	[231, 232]
		Elevated levels of simpler gangliosides (GM3 and GM2)	[99, 229]
		Accelerated lysosomal ganglioside degradation	[233]
		Altered lipid rafts organization (higher levels of raft-associated GM1 and GM2)	[234]
Huntington's disease	Huntingtin mutation, misfolding and aggregation of mutated protein	Altered white matter SM fatty acid composition	[276, 277]
		Reduced ganglioside concentration in erythrocytes, striatum and caudate	[278–280]
		Abnormal expression of glycosyltranserase genes	[280]
		Increased GD3 levels	[280]
Parkinson's disease	Loss of dopaminergic neurons, misfolding and aggregation of α-synuclein	Glucocerebrosidase deficiency sensitizes to PD	[284]
Prion diseases	Prion infection, misfolding and aggregation of prion protein	Reduced ganglioside content with a shift from complex to simpler species (GM3, GD3, GD2)	[302–307]
		Appearance of novel alkali-labile species	[307]
		Alterations in the long-chain base composition of gangliosides	[303, 304]
		Altered lipid rafts organization	[297, 298]
Autosomal recessive infantile-onset symptomatic epilepsy syndrome	Loss of function mutation of GM3 synthase	Lack of GM3 and of GM3-derived GSL, increase of LacCer, o-series gangliosides and globo- and neolacto-series GSL	[308]
Severe malignant autosomal recessive osteopetrosis	Osteopetrosis associated transmembrane protein-1 (OSTM1) mutation	Accumulation of GM3 and GM2	[313]
		No changes in lysosomal glycohydrolases	[313]

the recycle of catabolic fragments originated in the lysosome for biosynthetic purposes is quantitatively relevant, it can be expected that the blockade of proper SL catabolism at the lysosomal level leads to the jamming of the overall flow of metabolites, with consequences on the SL composition in all cellular districts, including the plasma membrane. The resulting SL-enriched membrane domains with non-physiological composition might be responsible for altered signaling events involved in the onset of the cellular damage and of tissue pathology. This hypothesis has been recently confirmed by several observations: (1) in a cell model of Gaucher disease (GD), impaired lysosomal catabolism of GlcCer led to the accumulation of GlcCer at the plasma membrane level in lipid rafts, possibly explaining the altered lipid and protein sorting observed in this pathological condition [218]; moreover, it has been reported that GD is associated with insulin resistance [219]. Since insulin receptor function is regulated by its interaction in lipid rafts with GSL [220] and in particular, GM3 ganglioside, this suggests that the altered lipid raft organization in Gaucher cells might be responsible for altered responsiveness to insulin; (2) psychosine (galactosylsphingosine) is one of the galactoslylsphingolipids that accumulates in the brain of Krabbe disease (human globoid cell leukodystrophy) patients due to the deficient activity of β-galactosylceramidase. Psychosine accumulates in lipid rafts from brain and sciatic nerve from twitcher mice (the animal model for the infantile variant of the disease) and from human Krabbe patients, leading to an altered distribution of lipid raft proteins and to inhibition of protein kinase C [221]; (3) in brains from ASMKO mice, an animal model for Niemann–Pick disease (NPD) type A (due to deficient activity of the lysosomal acid sphingomyelinase) [222], in addition to the expected SM accumulation, we observed an unexpected remodeling of the fatty acid composition of the accumulated SM and a significant increase in ganglioside content, mainly due to the accumulation of monosialogangliosides GM3 and

Table 3 Molecules causally connected to spontaneous and transmissible neurodegenerative diseases associated with lipid rafts

Disease	Protein	Reference
Alzheimer's disease	Amyloid precursor protein	[225, 239–241, 243, 244]
Transmissible spongiform encephalopathies	Prion protein	[294–298]
Parkinson's disease	α-Synuclein	[287, 330]

GM2, leading to a non-conventional lipid raft organization [223, 224].

Alzheimer's Disease

Disregulated brain ganglioside metabolism has been reported in brain of AD patients and in transgenic mice models of the disease (reviewed in [225]). The patterns of ganglioside alterations in AD are very complex and differ according to age of onset and type of mutation, suggesting that different GSL-regulated events contribute to the onset of different AD forms. However, a consistent finding was a reduced ganglioside concentration (associated with altered ratios of a-series to b-series gangliosides) in the majority of brain regions of AD and dementia of the Alzheimer type-affected patients [101, 102, 226–230] with respect to age-matched healthy controls. A reduced sulfatide content in AD post-mortem brain samples has also been reported [231, 232]. Remarkably, as mentioned above, age-associated ganglioside loss has been reported in humans during physiological senescence. In addition, elevated levels of simpler gangliosides (GM3 and GM2) have been reported in the cerebral cortex of AD patients [229] and from APP^{SL} mice, expressing the Swedish and London mutations of human APP [99]. Since it has been shown that GM1 degradation is enhanced in cultured fibroblasts from AD patients with respect to control cells, leading to increased production of GM3 and GM2 [233], it can be assumed that accelerated ganglioside degradation at the lysosomal level contribute to the changes in GSL patterns observed in AD. Remarkably, no or only minor changes in ganglioside composition have been reported in cerebellum, a region usually lacking Aβ plaques and regarded as non-vulnerable to the disease [99]. On the other hand, lipid rafts from the frontal and temporal cortices of AD patients contain a higher concentration of gangliosides GM1 and GM2 respect to age-matched control brains [234]. Alterations in ganglioside metabolism associated with AD are probably reflected by the presence of anti-GM1 antibodies in AD patients (as well in patients with other forms of dementia, but not in non-demented patients with other neurodegenerative diseases) with respect to age-matched controls [235].

Even if altered ganglioside metabolism seems to be a signature of AD and contribute to multiple aspects of the disease, as discussed more in detail below, a recent study revealed multiple abnormalities targeting the gene expression of several enzymes that control SL metabolism in dementia and AD patient brains [236]. These changes were detectable at the earliest clinically recognizable stages of dementia and AD and became evident at the later stages of the disease. In addition to the down-regulation of enzymes involved in GSL synthesis (that is consistent with the above-reported ganglioside depletion observed in AD), the enzymes controlling ceramide de novo synthesis were upregulated, in particular, in the frontal and temporal cortices, suggesting that a widespread alteration in SL metabolism, leading to an unbalance between the generation of protective and pro-apoptotic SL mediators, is involved in AD-associated neurodegeneration across cortical regions.

Altered ganglioside expression and membrane organization could contribute to the amyloidogenic process in AD at least in three different ways: (1) by modulating the functions of APP as signaling molecule and the proteolytic processing of APP in an amyloidogenic direction; and (2) by favoring the conversion of soluble Aβ to the insoluble form.

APP is a transmembrane protein that can undergo different proteolytic pathways. APP can be cleaved by α-secretase yielding soluble APP. Alternatively, APP is processed with the production of the Aβ amyloid peptide, which accumulates in the brain lesions (senile plaques) that are commonly thought to cause AD [237]. The physiological function of APP remains poorly understood; however, several studies suggest that APP can transduce signals across the membrane [238]. APP is enriched within lipid rafts [239–241] where it interacts with the subunit of G_o proteins ($G_{\alpha o}$). APP stimulation by a specific antibody inhibits the basal $G_{\alpha o}$ GTPase activity [239]. Since an APP form, carrying a missense mutation (V642I) associated with familiar AD constitutively activates $G_{\alpha o}$ [242], the regulation of $G_{\alpha o}$ by APP within lipid rafts is likely to be relevant for the physiopathological function of APP itself.

Lipid rafts from cultured cells and mammalian brains contain not only APP, but also APP-derived proteolytic fragments, including Aβ, and several proteolytic enzymes involved in APP processing [225, 243]. They are enriched in cholesterol (whose role in controlling APP processing and in the pathogenesis of AD, even if still strongly debated, is probably very important [244]) and, of course, in SL. Disturbance of lipid raft organization resulted in the reduction of APP association with the domains and inhibited the generation of Aβ amyloid peptide [241]. Some evidence indicates that non-amyloidogenic α-secretase processing of APP occurs within lipid rafts. In non-neuronal cell lines, caveolin-1, a principal component of caveolae-like lipid membrane domains was reported to be physically

associated with APP, and α-secretase-mediated processing of APP was dependent on the expression levels of caveolin-1 itself [240]. Exogenous addition of GM1 ganglioside to SH-SY5Y neuroblastoma cells decreased the secretion of soluble APPα and stimulated the production of Aβ [245]. In the same cell line (and in others as well), GSL depletion obtained by pharmacological inhibition of GlcCer synthase resulted in a reduced secretion of APP and Aβ peptides, an effect reversed by the addition of exogenous brain gangliosides [59]. In SL-deficient cell lines, cellular levels and maturation of APPβ were reduced [246], while the secretion of soluble APPα was greatly increased [247], and again, these effects were counteracted by restoring normal cellular SL levels. Exogenous ceramide and treatments able to raise cellular ceramide levels enhanced the production of Aβ by affecting the β- but not the γ-cleavage of APP [248]. On the other hand, lipid rafts from mouse brain are enriched in active β- and γ-secretases and seem to be the main cellular site where the amyloidogenic processing of APP leading to the production of Aβ amyloid occurs [249–251]. All these data strongly suggest that altered SL metabolism, leading to anomalous lipid raft organization, affects APP signaling function and APP amyloidogenic vs. non-amyloidogenic processing.

In addition, a more direct role of gangliosides in the formation of those insoluble Aβ aggregates that are extracellularly deposited, forming the amyloid plaques, has been as well suggested. The conversion of soluble, non-toxic Aβ into toxic Aβ fibrils is favored by a conformational transition from random coil or α-helix-rich to ordered β-sheet-rich structure that occurs during the interaction of Aβ with neuronal membranes [252, 253]. Diverse and compelling pieces of evidence indicate that gangliosides, highly enriched in neuronal plasma membranes, are responsible for specific interactions with Aβ that drive its conformational transition and Aβ fibrillogenesis. Membrane-bound Aβ tightly interacts with GM1 ganglioside [254]. GM1-bound Aβ has unique immunological properties [255], reflecting the occurrence of a conformational change associated with an increased surface protein density and with the ability to act as a "seed" for amyloid formation, i.e., to promote the formation and deposition of toxic Aβ aggregates in vitro and in living cells [256–258]. GM1-bound Aβ is endogenously generated in the brain [259] associated with amyloid plaques in cerebral cortices from AD patients [255, 260]. Moreover, GM1-bound Aβ formation is highly enhanced in synaptosomes prepared from aged mouse brains, bearing a high-density cluster of GM1 ganglioside [261]. APP-derived peptides bind to GM1 with different affinities (Aβ 1-42 showing the greatest affinity), and aged Aβ preparations have higher affinity than fresh ones. On the other hand, Aβ peptides bind not only to GM1 but also to a number of other gangliosides with different affinities, although not to various phospholipids or SM [256, 262–264]. The affinity studies revealed that the α2,3NeuAc residue is critical for binding and that the α2,6NeuAc residue linked to GalNAc in α-series gangliosides additionally contributes for the binding affinity to Aβ. Aβ seems to recognize ganglioside clusters in a density-dependent manner in artificial membranes [256], and GM1-Aβ interaction and Aβ aggregation are favored in a cholesterol-rich membrane environment [265, 266]. On the other hand, lipid rafts (that contain, by definition, clustered lipids, including gangliosides and cholesterol) are the preferential site for Aβ–ganglioside interactions, leading to Aβ conformational shift and aggregation [265, 267], and lipid rafts from brain cortices of AD patients contained higher levels of GM1 and GM2 gangliosides and were less rich in cholesterol with respect to age-matched controls [234]. Thus, the formation of insoluble Aβ fibrils seems a ganglioside- and lipid raft-dependent event.

Remarkably, the susceptibility to aggregation upon binding to gangliosides is somehow mutation-dependent [268]. The assembly of wild-, Arctic-, Dutch-, and Flemish-type Aβ were accelerated in the presence of GM1, GM2, GM3, and GD3 gangliosides, leading to different kinds of aggregates in the presence of a specific ganglioside [269–271]. For some hereditary Aβ variants, aggregation was accelerated in the presence of GM3 and GD3, the main gangliosides expressed in the cerebrovascular basement membrane [269–271]. On the other hand, amyloid deposition is significantly increased in the vascular tissue in brains of GM2 synthase KO mice, suggesting that ganglioside-mediated deposition of amyloid is relevant to AD-associated angiopathy as well [272].

More recently, it has been suggested that not only GM1 accumulated at the cell surface might contribute to GM1-induced amyloid fibril formation. In aged monkey brains, GM1-bound Aβ is preferentially accumulated in endosomes [273]. On the other hand, blockade of the endocytic pathway in PC12 cells resulted in accelerated extracellular release of exosome-associated GM1 that was able to induce Aβ aggregation [261]. These data suggest that abnormalities in the endocytic pathway contribute to Aβ-dependent pathology in AD.

In addition, GM1–Aβ interactions are also involved in plaque-independent neuronal death associated with AD: the incubation of Arctic Aβ in the presence of GM1-containing liposomes or neuronal membrane preparations led to the formation of a toxic, but soluble and non-amyloidogenic Aβ aggregate able to induce nerve growth factor-dependent neuronal death [274].

Huntington's Disease

Several early studies suggested that altered SL metabolism is associated with Huntington's disease (HD), and the potential benefits of using gangliosides for treating the behavioral deficits associated with HD have been recently

described [275]. Fatty acid composition of SM from human cerebral white matter was reported to be abnormal in patients with juvenile and adult HD, with a shift toward shorter chain fatty acid. However, this event does not seem to be specific for this disease, but rather an index of disturbed myelination and demyelination, since it has been associated with an immature myelin and detected also in young children and in several cases of non-specific brain damage associated with demyelination [276, 277]. On the other hand, changes in the GSL composition have been observed in erythrocytes from HD patients [278], and a marked reduction in the ganglioside concentration was detected in the striatum of HD human brains and in rat brains after lesioning by intrastriatal injection of kainic acid [279]. More recently, abnormal expression of the genes encoding several glycosyltransferases involved in ganglioside biosynthesis has been reported in the striatum of hexon 1 transgenic Huntington's disease mice (R6/1 mice) and in post-mortem caudate from human HD patients [280]. In particular, a significant decrease in the expression of the gene encoding GM2 synthase was found in both mice and humans, while other differences were not shared by the two models. Altered ganglioside levels were also observed, but the correlation between the changes in the gene expression and the resulting altered ganglioside profiles were not obvious, suggesting that regulation at the post-transcriptional level of genes involved in ganglioside synthesis is altered in HD. In the forebrain of R6/1 mice, total ganglioside content was unchanged while GM1 was significantly reduced respect to wild-type mice. On the other hand, in caudate samples from HD patients, total gangliosides were significantly reduced (−40%), with a similar loss of all major gangliosides, only in part compensated by a marked increase in GD3.

Parkinson's Disease

The treatment with the monosialoganglioside GM1 had a beneficial effect restoring neurochemical, pharmacological, histological, and behavioral parameters in different animal models of PD [281, 282] and reversing the dopaminergic deficits in nigrostriatal neurons of aged rats [283]. On the other hand, a possible role of an abnormal SL metabolism in the onset of the disease has been suggested by the observation that the deficiency of glucocerebrosidase in patients with GD might contribute to a vulnerability to PD [284]. In addition, a consistent portion of PD patients had increased levels of anti-GM1 antibodies of the IgM type [285]. Some light on the molecular mechanisms underlying the ameliorating effects of GM1 treatment on PD has been recently shed. A key step in the etiology of PD is probably the aggregation of α-synuclein followed by the formation of fibrils (intracellularly accumulated in PD and other neurodegenerative diseases as Lewy bodies or glial inclusion bodies), a process that shares some similarities with Aβ aggregation and fibrillation in AD. Inside the fibril-like structures α-synuclein specifically binds to GM1-containing liposomes [286]; furthermore, the binding with GM1 stabilizes α-synuclein in an α-helix-rich structure, prevents its fibrillation, and is abolished in the α-synuclein A30P mutant associated with a familial form of the disease. On the other hand, α-synuclein internalization into microglia was GM1- and lipid raft-dependent [287]. Trisialoganglioside GT1b that is abundantly expressed in CNS neurons, induced in vivo degeneration of nigral dopaminergic neurons in rats with a synuclein-independent mechanism [288]. The neurotoxic effect of GT1b was mediated by microglia activation, and it is worth to note that the release of proinflammatory or cytotoxic factors by activated microglia likely plays an important role also in other neurodegenerative diseases, including AD.

Prion Diseases

Prions containing prion proteins (PrP) are implicated in a large group of related neurodegenerative disorders, which affect both animals and humans. Prion diseases include Creutzfeldt–Jakob disease (CJD) and Gerstmann–Sträussler–Scheinker in humans, bovine spongiform encephalopathy in cattle, chronic wasting disease in mule deer and elk, and scrapie in sheep. All prion diseases, characterized by an unusually long incubation time and a rapid progression after the onset of clinical symptoms, are fatal with no effective form of treatment. The current dogma relates the etiology of these diseases to the formation of a proteinaceous infectious particle [289]. In this regard, the scrapie prion protein, PrP^{Sc}, is a disease-specific, conformationally modified isoform with amyloidogenic features of a normal cellular protein, PrP^{C} (cellular prion protein) or simply PrP, expressed at highest level in the CNS, whose exact cellular function remains unknown. SL (GalCer and SM) have been detected in highly purified preparations of infectious prion rods [290], and prion protein isoforms and prion protein-derived peptides bind to SL-containing artificial membranes [291–293], suggesting that PrP interacts with selected SL. Indeed, a common SL-binding motif has been identified in the human prion protein and Aβ peptide. As it is the case for the binding of Aβ with GM1, the binding of PrP with SL-rich membranes resulted in conformational changes that might favor the transition from PrP^{C} to PrP^{Sc} [292]. The process by which the protease-resistant PrP^{Sc} isoform is formed post-translationally from a protease-sensitive precursor remains uncertain. However, both PrP^{C} and PrP^{Sc} are localized in lipid rafts or SL-enriched membrane domains, and emerging evidence suggests that this localization is relevant for the physiological function of PrP^{C} and for the conversion of PrP^{C} to PrP^{Sc} [294]. Indeed, it has been shown

that the efficient conversion of PrP^{C} into PrP^{Sc} occurs after PrP^{C} reaches the plasma membrane, strictly requires the targeting of PrP^{C} (probably mediated by its by GPI-anchor) to lipid rafts [295] and is confined in this specific subcellular domains in scrapie-infected neuroblastoma cells [296]. Moreover, the localization of PrP^{C} to lipid membrane domains and PrP^{Sc} formation are inhibited by lovastatin, which reduces cell cholesterol content, presumably disrupting the lipid raft structure [297]. On the other hand, pharmacologically obtained SL depletion led to the increased formation of PrP^{Sc} in scrapie-infected neuroblastoma cells [116]. All these data suggest that lipid membrane domains represent the cellular site where prions are propagated and seem to imply that other components (proteins or lipids) of this compartment participate to the propagation of prions [297, 298]. In addition, within lipid rafts, other proteins seem to associate with PrP, likely representing functional partners of PrP [299]. Moreover, PrP is associated with a specific SL-rich membrane environment, whose regulated compositional changes are probably relevant for the biological function of PrP [300, 301]. In particular, we showed that PrP plasma membrane environment in differentiated neurons is a complex entity, whose integrity requires a network of lipid-mediated non-covalent interactions. Very little is known about the lipid raft structure in organisms affected by prion diseases or experimentally infected with PrP^{Sc}. However, dramatic alterations in ganglioside content and pattern have been reported in brain of patients and of chimpanzees with kuru and CJD [302–305], as well as in brains of experimentally infected guinea pigs [306] and Syrian hamsters [307]. In general, a marked decrease in ganglioside content with a shift from complex (GD1a, GD1b, GT1b, and GQ1b) to simpler gangliosides (GD3, GM3, GD2) has been observed in infected specimens. In addition, in scrapie-infected hamster brains, the appearance of a number of novel alkali-labile species has been observed [307], and alterations in the long-chain base composition of gangliosides with a strong decrease in C20-sphingosine containing species has been reported in CJD brains [303, 304].

Other Diseases

Given the importance of SL in the development and maintenance of the nervous system, it is not surprising that the number of conditions with neurological involvement found to be associated with anomalies in SL metabolism is continuously increasing. As mentioned above, several lysosomal storage diseases are due to defects in SL catabolism. Recently, the first example of a human disease associated with the disruption of ganglioside biosynthesis on a genetic base has been reported [308]. An autosomal recessive infantile-onset symptomatic epilepsy syndrome with a Mendelian mode of inheritance has been associated with a nonsense mutation (964C→T) in SIAT9 gene, leading to the synthesis of inactive GM3 synthase, the key enzyme in the biosynthesis of complex gangliosides of the a- and b-series. The analysis of plasma GSL in affected children revealed a complete lack of GM3 and of GM3-derived GSL, with a corresponding increase in the precursor of GM3, LacCer, and in alternative glycosylation products derived from LacCer (o-series gangliosides and globo- and neolacto-series GSL). Data on the brain GSL composition of the affected individuals are not available, but GM3 knockout mice predominantly expressed o-series gangliosides in the brain [309]. Remarkably, changes in brain ganglioside composition have been previously reported in several groups of epileptic patients [310–312], but a systematic investigation in epilepsy is still lacking.

Recently, we observed significant changes in the SL composition in the brain from the gray-lethal mouse (*gl*/*gl*) mutant, whose phenotype closely resembles the severe human malignant autosomal recessive OSTM1-dependent form of osteopetrosis, a disease showing a primary severe neurological defect (primary retinopathy and progressive cortical atrophy in addition to secondary neural defects) due to lysosomal storage disease [313]. In the brains of these mice, we found a low content of SM, sulfatide, and GalCer that is consistent with the immunohistochemical results showing significant depletion and disorganization of the myelinated fibers. In addition, we observed in *gl*/*gl* mouse brain a progressive accumulation of the monosialogangliosides GM3 and GM2. However, when we checked the enzyme activities of several lysosomal glycohydrolases, we found that all enzyme activities tested were higher or similar in the *gl*/*gl* mice brain homogenates with respect to the wild-type animals. Moreover, we tested the ability of cultured skin fibroblasts from wild-type and gray-lethal mice for their ability to catabolize exogenously added gangliosides, and no differences were observed in the uptake and catabolism of exogenous GM1 and GM2, nor accumulation of products deriving from the catabolism of gangliosides. Thus, the metabolic origin of the accumulation of GM3 and GM2 in *gl*/*gl* mice brain remains to be elucidated, but might be linked to a defect in the biosynthetic pathway. Remarkably, an accumulation of simpler gangliosides seems to be a feature shared by several neurological diseases of completely different origin, including AD, HD, NPA, and CJD, as reported above.

Targeting Sphingolipid Metabolism and Cellular Organization: a Novel Therapeutic Perspective for Neurodegenerative Disorders

The pieces of evidence presented in this review clearly suggest that SL and SL-related targets possess a high

potential for the intervention in a wide range of neurological and neurodegenerative disorders. On the other hand, the increasing level of complexity that is emerged for the various roles of SL in regulating the function of the nervous system indicates as well that the rationale design for a SL-based therapy is much more complicated than it was thought 20 years ago, when ganglioside-based drugs were licensed for the treatment of peripheral neuropathies.

The most obvious therapeutic application of SL is the use of exogenous gangliosides as neuroprotective agents that has been documented for AD, PD, and HD. Despite the several pharmacokinetic and safety problems associated with this approach, it is probably still a valuable option that deserves a critical re-evaluation for single pathologies in the light of the new knowledge reached in this field (for a recent discussion, see [275, 314]).

One of the main problems faced with the exogenous administration of gangliosides, i.e., the need to reach a significant concentration in the brain, the site of the lesions to be cured, could be overcome by the use of small molecules able to up-regulate ganglioside biosynthesis. In this sight, *L*-threo-PDMP, a synthetic ceramide analog able to up-regulate several glycosyltransferases involved in ganglioside biosynthesis, is very promising. *L*-PDMP preserves striatal dopamine levels in murine models of PD [315] and protects rats from the loss of spatial memory consequent to experimental brain ischemia [316].

Sphingolipid storage diseases, those disorders where the involvement of SL is better understood at the enzymatic, genetic, and molecular level, provide interesting lessons about how to target SL metabolism with a therapeutic perspective that in some cases have been validated by the application of successful clinical protocols. Remarkably, some of the strategies used for the cure of these diseases have the potential to be extended to other neurological diseases characterized by an impaired SL metabolism on a different basis.

The most logical approach for the treatment of SL storage disease implies the restoring of the detective lysosomal enzyme or activator protein. In principle, the most effective way to reach this goal would be somatic gene therapy, allowing delivery of the relevant genetic material to the defective cells. This should be relatively easy for these diseases for two reasons: (1) they are monogenic diseases; (2) in many cases, a small residual activity of the defective enzyme is present, leading to absent or very mild phenotype, so it can be predicted that even low levels of enzyme activity reached by gene therapy or other means are sufficient to ameliorate the disease. Several gene transfer methods have been applied to correct the gene defect in cultured cells; however, their translation into animal models or patients, despite the monumental efforts devoted in this direction, led to very modest results [231, 232, 317]. An opportunity to use genetically modified cells that overexpress the desired enzyme is represented by cross-correction. As example, in the case of Sandhoff disease, it has been shown that infection with a bicistronic lentiviral vector, containing both human HEXA and HEXB cDNAs, was able to restore hexosaminidase expression and activity in Sandhoff fibroblasts. Moreover, transduced fibroblasts secreted significant amounts of the enzyme in the culture medium that was taken up by the deficient cells via the mannose 6-phosphate receptor-mediated endocytosis. The internalized activity, even if low, was sufficient to restore proper GM2 catabolism. These [318] and similar [319] results suggest that this strategy is a useful alternative to direct gene therapy to cure these diseases.

A more conventional way to restore the defective enzyme is enzyme replacement therapy [320] that implies the direct delivery of the recombinant enzyme to the defective cells. At present, this represents the most successful therapeutic approach for the treatment of SL storage diseases, despite the problems related to the efficient targeting of the enzyme to the defective cells and to the stability and catalytic efficiency of the delivered recombinant enzymes, and enzyme replacement therapy-based protocols have been approved for the treatment of Gaucher and Fabry diseases [321, 322] and probably represent a valuable option for other sphingolipidoses, such as NPD [323].

In addition, it has been recently shown that small molecules interacting with the defective enzyme might act as "chemical chaperones" leading to the reactivation of the enzyme [215, 216]. In this sight, the results obtained with the reactivation of β-glucosidase by chemical chaperones in an animal model of GD are an important proof of principle about the validity of this approach, even if it has not yet been translated to a therapeutic option [324].

It is obviously tempting to speculate that targeting an SL metabolic enzyme with one of the above-mentioned approaches might be a valid solution to correct those alterations in SL metabolism that are associated with a wide range of neurodegenerative diseases. However, in most cases, the alterations in SL patterns associated with neurodegenerative diseases other than SL storage diseases do represent secondary biochemical pathways altered as a consequence of a non-related primary cause. Moreover, these alterations are usually the result of changes in the expression and/or activity of more than one single enzyme; in addition, they are often associated with anomalies in the traffic of the substrates and are the result of complex changes in the substrate/product concentrations in multiple cellular compartments, thus a strategy focused on a single enzyme activity might be less straightforward than in sphingolipidoses.

Substrate reduction therapy has been applied to the treatment of sphingolipidosis. The rationale for this

approach is based on the idea that the accumulation of undegraded substrates might be ameliorated by inhibiting their synthesis. *N*-butyl-deoxynojirimycin, an inhibitor of GlcCer synthase [325, 326], has been approved for the treatment of GD patients who cannot be treated with enzyme replacement therapy [327]. It is also effective in reducing the symptoms in NPD patients [328] and in Tay-Sachs disease mice models [329] and is under clinical trial (NCT00672022) for the treatment of the latter. This and similar compounds can be predicted to be effective in all sphingolipidoses where the accumulated substrate is a glycolipid. Their major advantage is represented by their ability to easily cross the blood–brain barrier and reach effective concentrations in the brain, and they are, in principle, useful for any disease that implies an increased synthesis of GlcCer-based SL. As mentioned above, in many neurological disorders (AD, PD, HD, CJD) a general decrease of ganglioside levels, accompanied by a marked increase in the levels of simpler ganglioside that are likely heavily affected by these compounds.

For neurodegenerative diseases other than SL storage diseases, the planning of strategies addressing SL metabolism is hampered by the heterogeneity of patterns observed in specific diseases and animal models and by the lack of information about the biochemical mechanism leading to the altered SL patterns. However, one common trait has emerged by the study of different diseases. In many cases, one of the key events underlying the development of the pathology is a conformational transition in a cellular protein that leads to the loss of its physiological function and to the acquisition of toxic, amyloidogenic properties. This is true for Aβ in AD, for α-synuclein in PD, for prion protein in CJD and other prion diseases. The conformational shift leading to the amyloidogenic folding of the protein seems to require or to be strongly accelerated by the associating of the protein with a ganglioside-rich membrane environment or by its binding with a ganglioside. The role of altered SL expression in the pathogenesis of the disease is surely at least in part explained on these bases. In principle, several strategies can be envisaged to prevent this event: (1) cellular ganglioside levels could be reduced by the use of one of the above-mentioned inhibitor of GSL biosynthesis that can reach the brain; (2) membrane organization of lipid rafts that in some cases seems to be essential for the pro-fibrillogenic ganglioside-protein interactions could be perturbed even without changing the cellular SL levels, for example, acting on the membrane levels of cholesterol that is important in maintaining the structure of lipid rafts, (3) binding between gangliosides and relevant proteins could be prevented, thus inhibiting the consequent amyloidogenic process. In this regard, it has been shown that drugs such as rifampicin are able to inhibit the binding of GM1 to Aβ, consequently preventing fibrils formation [225]; (4) the fibrillogenic properties of the ganglioside-protein aggregate could be inhibited by the use of antibodies able to bind the aggregate. For example, a monoclonal antibody raised against the GM1 Aβ complex purified from AD brains was able to prevent Aβ assembly in vitro and Aβ deposition in the brain when peripherally administered to transgenic mice expressing mutated APP [269–271].

In conclusion, SL-based therapeutic strategies toward neurodegenerative diseases today seem to be much more interesting than they were 20 years ago, when the neuroprotective role of gangliosides was emphasized.

Given the great relevance of these diseases in a constantly aging world population, and the substantial lack of definitive treatments for most of these diseases, it is reasonable to expect the appearance of successful SL-based drugs in this scenario in a not-far future.

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