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GM1 Ganglioside: Past Studies and Future Potential

Massimo Aureli · Laura Mauri · Maria Grazia Ciampa · Alessandro Prinetti · Gino Toffano · Cynthia Secchieri · Sandro Sonnino

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Abstract Gangliosides (sialic acid-containing glycosphingolipids) are abundant in neurons of all animal species and play important roles in many cell physiological processes, including differentiation, memory control, cell signaling, neuronal protection, neuronal recovery, and apoptosis. Gangliosides also function as anchors or entry points for various toxins, bacteria, viruses, and autoantibodies. GM1, a ganglioside component of mammalian brains, is present mainly in neurons. GM1 is one of the best studied gangliosides, and our understanding of its properties is extensive. Simple and rapid procedures are available for preparation of GM1 as a natural compound on a large scale, or as a derivative containing an isotopic radionuclide or a specific probe. Great research interest in the properties of GM1 arose from the discovery in the early 1970s of its role as receptor for the bacterial toxin responsible for cholera pathogenesis.

Keywords GM1 · GM1 Chemistry and Physico-chemistry · GM1 and Cholera toxin · Cellular organization of GM1 · Neurotrophic and neuroprotective properties of GM1 · GM1 and the neurodegenerative diseases · GM1 and GBS

Introduction

In this article, we review and speculate on various aspects of GM1 structure and function that are relevant to future studies of the physiological roles of endogenous gangliosides and the potential therapeutic effects of GM1 administration.

M. Aureli · L. Mauri · M. G. Ciampa · A. Prinetti · S. Sonnino (✉)
Department of Medical Biotechnology and Translational Medicine, University of Milan, Via Fratelli Cervi 93, 20090 Segrate, Milan, Italy
e-mail: sandro.sonnino@unimi.it

G. Toffano · C. Secchieri
FIDIA Farmaceutici, Abano Terme, Padua, Italy

Structure and Nomenclature of GM1

The structure of ganglioside GM1 (referred to hereafter simply as "GM1"; Fig. 1a) was established in 1963 [1]. GM1 is a monosialo-glycosphingolipid (GSL) belonging to the gangliotetrahexosyl series, with formula β-Gal-(1–3)-β-GalNAc-(1–4)-[α-NeuX-(2–3)-]β-Gal-(1–4)-Glc-(1–1)-Cer, or II^3-α-NeuX-Gg_4Cer according to International Union of Pure and Applied Chemistry (IUPAC)–International Union of Biochemistry (IUB) nomenclature [2]. "NeuX" in these formulas signifies any sialic acid structure. Sialic acid is the trivial name used for all derivatives of neuraminic acid, the 5-amino-4,6,7,8,9-pentahydroxy-2-oxononanoic acid also known as 5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid. Many sialic acid structures have been identified. In mammals, the most common are the 5-*N*-acetyl-derivative (Neu5Ac), the 5-*N*-glycolyl-derivative (Neu5Gc), and the 5-*N*-acetyl-9-*O*-acetyl-derivative (Neu5,9,Ac_2) [3]. Neu5Gc is not present in healthy humans. The term "GM1" was proposed by Svennerholm [4] in 1980 as a replacement for the original term "Gtet1," was subsequently approved by IUPAC–IUB, and is now commonly used worldwide [2]. In the term GM1, G indicates a ganglioside structure (i.e., a sialic acid-containing GSL), M indicates that the ganglioside contains a single sialic acid unit, and 1 indicates the Gg_4 neutral series, i.e., β-Gal-(1–3)-β-GalNAc-(1–4)-β-Gal-(1–4)-Glc oligosaccharide structure. The alternative term "GM1a" is sometimes used to avoid confusion following the discovery of α-NeuX-(2–3)-β-Gal-(1–3)-β-GalNAc-(1–4)-β-Gal-(1–4)-Glc-(1–1)-Cer, and IV^3-α-NeuX-Gg_4Cer structure ("GM1b"). Nomenclature and formulas of major ganglioside series structures are summarized in Table 1.

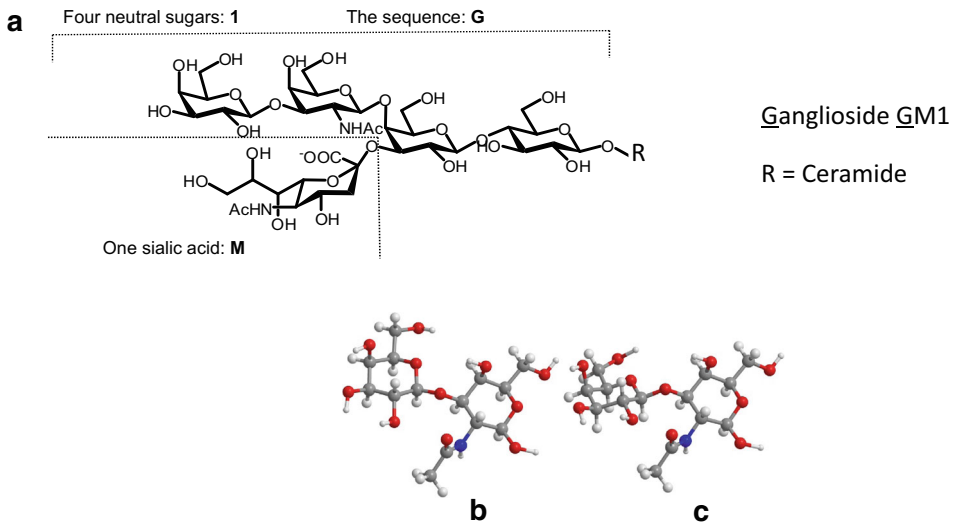

Fig. 1 **a** Structure of the GM1 ganglioside; **b**,**c** the two conformers of disaccharide portion Gal/GalNAc

The term GM1 refers to the general structure as above, but provides no information on the structures of the sialic acid and ceramide components.

The hydrophobic moiety of all sphingolipids, including GM1, is ceramide. "Ceramide" is a trivial name for a family of structures with various numbers of carbon atoms, hydroxyl groups, and unsaturations. Naturally occurring GM1 comprises a number of species that differ in their ceramide structure. Ceramide consists of a basic long hydrophobic chain (hereafter termed long-chain base [l.c.b.]; trivial name: sphingosine [Sph]), linked to a fatty acid. In the nervous system, l.c.b. comprises a mixture of C18- and C20-l.c.b. [5, 6] (trivial names: C18- and C20-Sph), 2-amino-1,3-dihydroxy-octadec-4-ene, and 2-amino-1,3-dihydroxy-eicos-4-ene present in a variable ratio. C20-Sph is barely detectable in the fetus [7] but becomes the major Sph species in the adult [6]. In tissues outside the nervous system, C20-Sph is absent or a very minor species. Of the four possible configurations of Sph, only the 2*S*,3*R* structure (Fig. 2) occurs in nature [8, 9]. Small percentages of sphinganines (2-amino-1,3-dihydroxy-octadecane and 2-amino-1,3-dihydroxy-eicosane) are sometime present.

In the nervous system, stearic acid accounts for >90 % of total fatty acid content. In extranervous tissues, a variety of fatty acid structures with 14–24 (or more) carbons may be present.

Table 1 Nomenclature and formulas of major glycosphingolipid series structures

Series	Abbreviation	Structural formula
	GalCer	ß-Gal-(1–1)-Cer
	GlcCer	ß-Glc-(1–1)-Cer
	LacCer	ß-Gal-(1–4)-ß-Glc-(1–1)-Cer
Ganglio-3	Gg_3Cer	ß-GalNAc-(1–4)-ß-Gal-(1–4)-ß-Glc-(1–1)-Cer
Ganglio-4	Gg_4Cer	ß-Gal-(1–3)-ß-GalNAc-(1–4)-ß-Gal-(1–4)-ß-Glc-(1–1)-Cer
Ganglio-5	Gg_5Cer	ß-GalNAc-(1–4)-ß-Gal-(1–3)-ß-GalNAc-(1–4)-ß-Gal-(1–4)-ß-Glc-(1–1)-Cer
Globo-3	Gb_3Cer	α-Gal-(1–4)-ß-Gal-(1–4)-ß-Glc-(1–1)-Cer
Globo-4	Gb_4Cer	ß-GalNAc-(1–3)-α-Gal-(1–4)-ß-Gal-(1–4)-ß-Glc-(1–1)-Cer
Globo-5	Gb_5Cer	ß-Gal-(1–3)-ß-GalNAc-(1–3)-α-Gal-(1–4)-ß-Gal-(1–4)-ß-Glc-(1–1)-Cer
Isoglobo-3	iGb_3Cer	α-Gal-(1–3)-ß-Gal-(1–4)-ß-Glc-(1–1)-Cer
Lacto		(Paraglobo)
Lc_4Cer	ß-Gal-(1–3)-ß-	GlcNAc-(1–3)-ß-Gal-(1–4)-ß-Glc-(1–1)-Cer
Neolacto-4	nLc_4Cer	ß-Gal-(1–4)-ß-GlcNAc-(1–3)-ß-Gal-(1–4)-ß-Glc-(1–1)-Cer
Neolacto-6	nLc_6Cer	ß-Gal-(1–4)-ß-GlcNAc-(1–3)-ß-Gal-(1–4)-ß-GlcNAc-(1–3)-ß-Gal-(1–4)-ß-Glc-(1–1)-Cer

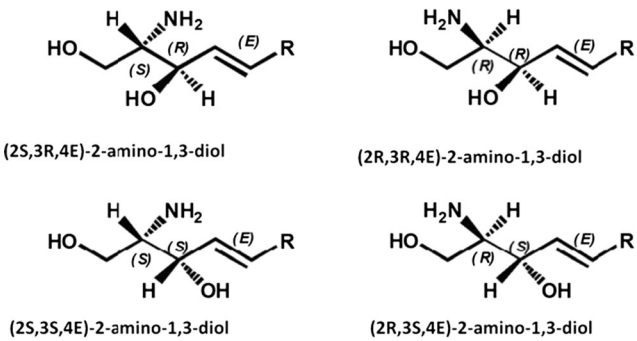

Fig. 2 Four possible configurations of the sphingosine molecule. Only the 2*S*,3*R*,4*E* configuration occurs in mammals

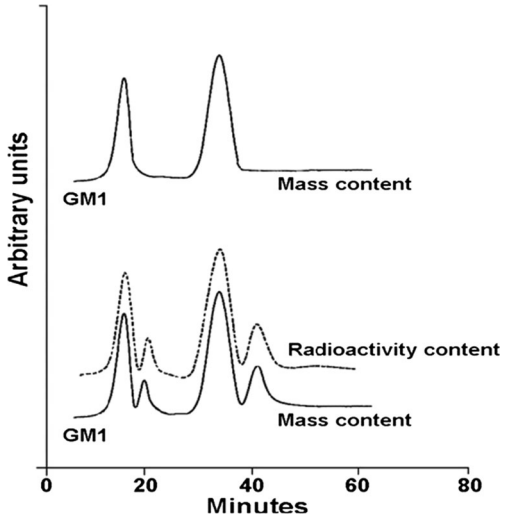

Fig. 3 Reversed-phase HPLC of GM1 containing C18:1 and C20:1 Sph and >90 % stearic acid. *Upper curve*: Natural GM1. GM1 is separated into two species containing C18:1 Sph (*left peak*) and C20:1 Sph (*right peak*). *Lower curve*: Separation of natural GM1 by oxidation at position 3 of Sph followed by reduction with ^{3}H-labeled sodium borohydride. This chemical procedure results in partial inversion of the configuration at position 3, yielding the unnatural 2*S*,3*S*,4*E* compound. GM1 is separated into four species containing 2*S*,3*R*4*E*-C18:1 Sph (*left peak*), 2*S*,3*S*4*E*-C18:1 Sph, 2*S*,3*R*4*E*-C20:1 Sph, and 2*S*,3*S*4*E*-C20:1 Sph, respectively. Chromatography was performed using a preparative C18 reversed-phase column and acetonitrile/sodium phosphate (5 mM, pH 7) buffer (3:2 v/v) at a flow rate of 7.5 ml/min. Mass detection at 195 nm; radioactivity detection by solid scintillator

Chemistry and Availability of GM1

Research on the biological properties of GM1 in cells is based on its availability as a natural compound or as a synthetic molecule containing a specific tracer. Techniques have been developed for preparation of GM1 per se, of GM1 with homogeneous ceramide moiety (see Fig. 3), of isotopically labeled GM1, and of GM1 containing photoreactive, fluorescent, or paramagnetic probes (see Fig. 4) [10–37].

Methods for synthesis of GM1 and many other GSLs have been developed, but yields are very low and practical synthesis of large amounts has not been achieved. Large-scale preparation of GM1 is, therefore, still based on extraction of total ganglioside mixture from tissues [12, 13], followed by ganglioside fractionation and GM1 purification by diethylaminoethanol or silica gel column chromatography [10, 12–17]. GM1 is present in all mammals, but there is no tissue in which GM1 is the sole ganglioside component. Within mammalian tissues, the brain contains the higher ganglioside quantity, 1–2 g gangliosides per kg fresh tissue [38], and is, therefore, the best source for preparation of gangliosides, particularly GM1. The major gangliosides found in the mammalian central and peripheral nervous systems are summarized in Table 2.

The major ganglioside components of mammalian brain (~95 % of total content) are GM1, GD1a, GD1b, GT1b, and GQ1b [39, 40]; the remaining ~5 % consists of minor components such as GM4, GM3, GD3, GM2, GD2, Fuc-GM1, Fuc-GD1b, GT1a, and GP1c, whose proportions vary depending on species [41, 42]. Gangliosides containing *N*-glycolyl neuraminic acid (Neu5Gc) are present in some animal species [43]. Gangliosides [10, 44] and ganglioside lactones [11] containing 9-*O*-acetyl-*N*-acetyl neuraminic acid (Neu5,9Ac$_2$) are components of some brain ganglioside mixtures, but are often lost as a result of alkaline treatments during ganglioside mixture preparation and purification. GM1 is 10–20 % of total brain ganglioside content, and after sialidase treatment, the value is near 95 % [18, 19].

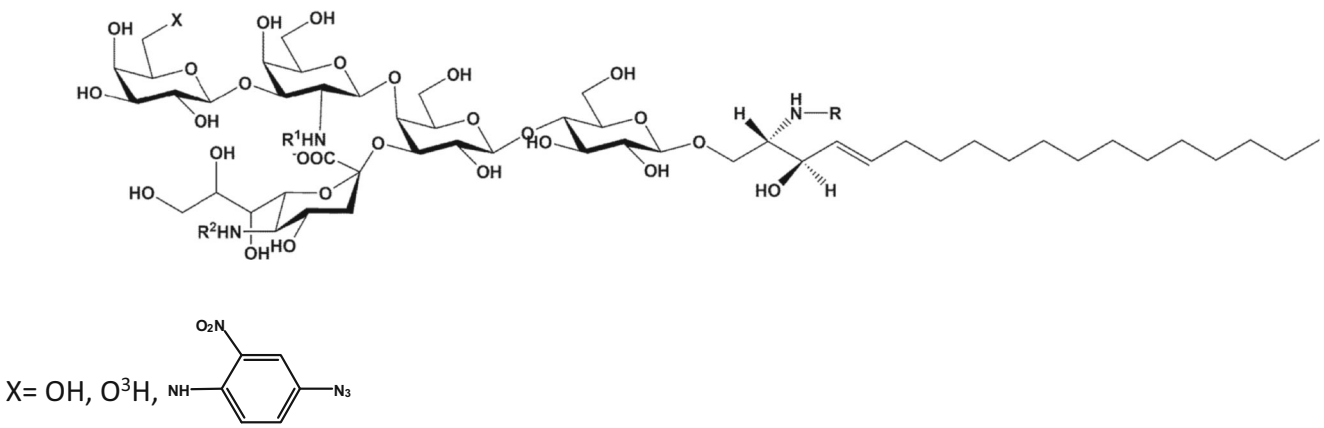

Fig. 4 Examples of synthetic GM1. *FA* fatty acid, *NBD* nitrobenzodiazol, *NR* Nile Red

Table 2 Structures of gangliosides in the mammalian nervous system

GM4	Neu5AcGalCer
GM3	II^3Neu5AcLacCer
GD3	II^3(Neu5Ac)$_2$LacCer
O-acetyl-GD3	II^3[Neu5,9Ac$_2$-(2–8)-Neu5Ac]LacCer
GM2	II^3Neu5AcGg$_3$Cer
GD2	II^3(Neu5Ac)$_2$Gg$_3$Cer
GM1a	II^3Neu5AcGg$_4$Cer
GM1b	IV^3Neu5AcGg$_4$Cer
Fuc-GM1	IV^2αFucII^3Neu5AcGg$_4$Cer
GalNAc-GM1	II^3Neu5AcGg$_5$Cer
3′-LM1	IV^3nLc$_4$Cer
GD1a	IV^3Neu5AcII^3Neu5AcGg$_4$Cer
GalNAc-GD1a	IV^3Neu5AcII^3Neu5AcGg$_5$Cer
GD1α	IV^3Neu5AcIII^6Neu5AcGg$_4$Cer
GD1b	II^3(Neu5Ac)$_2$Gg$_4$Cer
GD1b-lactone	II^3[Neu5Ac-(2–8,1–9)-Neu5Ac]Gg$_4$Cer
Fuc-GD1b	IV^2αFucII^3Neu5Ac$_2$Gg$_4$Cer
GT1a	IV^3(Neu5Ac)$_2II^3$Neu5AcGg$_4$Cer
GT1b	IV^3Neu5AcII^3(Neu5Ac)$_2$Gg$_4$Cer
9-*O*-acetyl-GT1b	IV^3Neu5AcII^3[Neu5,9Ac$_2$-(2–8)-Neu5Ac]Gg$_4$Cer
GT1c	II^3(Neu5Ac)$_3$Gg$_4$Cer
Chol-1α-a	IV^3Neu5AcIII^6Neu5AcII^3Neu5AcGg$_4$Cer
Chol-1β	III^6Neu5AcII^3(Neu5Ac)$_2$Gg$_4$Cer
GT1α	IV^3Neu5AcIII^6(Neu5Ac)$_2$Gg$_4$Cer
GQ1b	IV^3(Neu5Ac)$_2II^3$(Neu5Ac)$_2$Gg$_4$Cer
9-*O*-acetyl-GQ1b	IV^3(Neu5Ac)$_2II^3$[Neu5,9Ac$_2$-(2–8)-Neu5Ac]Gg$_4$Cer
GQ1c	IV^3Neu5AcII^3(Neu5Ac)$_3$Gg$_4$Cer
GQ1α	IV^3(Neu5Ac)$_2III^6$(Neu5Ac)$_2$Gg$_4$Cer
Chol-1α-b	IV^3Neu5AcIII^6Neu5AcII^3(Neu5Ac)$_2$Gg$_4$Cer
GP1c	IV^3(Neu5Ac)$_2II^3$(Neu5Ac)$_3$Gg$_4$Cer

Structural Properties of GM1

In relevant biological environments, GM1 has a negative charge because of the presence of sialic acid. The p*K*a value for free sialic acid is 2.6 [45]. The exact p*K*a value for the sialic acid residue of GM1 has not been reported. The number of negative charges present in GM1 micelles in water solution was experimentally determined to be only 16 % of the expected number [46], most likely as a result of polyelectrolyte effect and masking of negative charges by positive ions. In any case, the GM1 pentasaccharide chain is very hydrophilic because of the presence of sialic acid and the general hydrophilicity of sugars. The hydrophobic lipid moiety in combination with the hydrophilic saccharide chain gives the GM1 ganglioside structure a generally well-balanced amphiphilic character. The amphiphilic equilibrium may be disrupted by minor structural differences in the hydrophilic or hydrophobic moiety. Gangliosides, in general, display characteristics very different from those of glycerophospholipids, amphiphilic compounds with a strong hydrophobic character essential for the organization of membrane bilayers. The physicochemical properties of gangliosides play an important role in defining and modulating membrane organization (see *Cellular organization and topology of GM1*) [47–49].

GM1 is soluble in water, in which it forms aggregates through a hydrophobic effect. The geometry of the GM1 monomer is dictated by the large hydrophilic head and requires the strong curvature allowed by micellar aggregates [46, 50]. GM1 forms small ellipsoidal micelles, also termed toroidal-like micelles, whose parameters [46] are summarized in Table 3. GM1 micelles are formed at very low concentrations, with a critical micellar concentration (c.m.c.) in the range of 10^{-8}–10^{-9} M according to both experimental and theoretical determination [49, 51]. At any GM1 concentration, the free monomers in equilibrium with the aggregates cannot exceed the c.m.c. value.

Aggregative properties are related to GM1 structure. The hydrophilic chain of GM1(Neu5Gc) is more flexible and packable than that of GM1(Neu5Ac). The structure of ceramide determines the amphiphilic balance and, consequently, the physicochemical properties of GM1. In a change from C18 to C2 acyl chain, the c.m.c. increases to 2.3×10^{-5} M, accompanied by over threefold order of magnitude increase in the monomer concentration [49]. Very minor changes in ceramide structure are sufficient to modify ganglioside aggregative properties. Aggregative properties are highly relevant to the effect of exogenously administered gangliosides on cell membranes. Exogenous GM1 is associated rapidly with cells, becoming a component of the plasma membrane bilayer and then entering the natural GSL metabolic pathway. Only GM1 monomers enter the plasma membrane, whereas GM1 micelles bind to the cell surface through interactions with proteins and reach lysosomes following endocytosis [52]. GM1 micelles bind rapidly with a variety of proteins, forming fairly stable complexes (Fig. 5). GM1 complexes with albumin have been studied in detail [53, 54]. An increase of c.m.c. reduces the half-life of GM1 micelles and increases the rate of release of monomers. The half-life of micelles of calf brain GM1 was estimated as ~8–10 h [55].

Gangliosides are stably inserted into the outer layer of plasma membranes through lipid–lipid interactions. The position of gangliosides at the water/lipid interface is stabilized by hydrogen bonds with neighboring glycerophospholipids, involving both ceramide amide and carbonyl protons.

The conformation of the GM1 oligosaccharide chain has been studied in detail by nuclear magnetic resonance (NMR) and molecular modeling [56–60]. NMR studies have been performed on GM1 monomer, small micelles of lipid-modified GM1, and mixed micelles of natural GM1

Table 3 Parameters for GM1 aggregates

Ceramide composition		*M* (kDa)	N	Rh (Å)	a_0 (Å^2)	Axial ratio	*P* value
l.c.b., %	Fatty acids, %						
GM1(Neu5Ac), 25 °C		470	301	58.7	95.4	2.3	0.428
Heated to 60 °C and cooled to 25 °C		*320*	*205*	*52.8*	*99.5*		0.411
*d*18:1, 49.7	16:0, 3.1						
*d*20:1, 45.7	18:0, 93.5						
*d*18:0, 3.0	18:1, 0.4						
*d*20:0, 1.6	20:0, 3.0						
GM1(Neu5Gc), 25 °C		576	365	62.4	93.8	2.5	0.437
Heated to 60 °C and cooled to 25 °C		488	309	59.0	93.8	2.5	0.437
*d*18:1, 49.7	16:0, 3.1						
*d*20:1, 45.7	18:0, 93.5						
*d*18:0, 3.0	18:1, 0.4						
*d*20:0, 1.6	20:0, 3.0						
GM1(Neu5Ac), 25 °C after catalytic hydrogenation		529	339	61.2	96.3	2.5	0.433
*d*18:0, 52.7	16:0, 3.1						
*d*20:0, 47.3	18:0, 93.9						
	20:0, 3.0						
GM1(Neu5Ac), 25 °C		570	366	60.0	93.0	2.6	0.438
*d*18:1, 49.7	α-OH-18:0, 100						
*d*20:1, 45.7							
*d*18:0, 3.0							
*d*20:0, 1.6							
GM1(Neu5Ac), 25 °C		102	76	34.0	64.8	1.2	0.370
*d*18:1, 49.7	C2, 100						
*d*20:1, 45.7							
*d*18:0, 3.0							
*d*20:0, 1.6							

M molecular mass, *N* aggregation number, *Rh* hydrodynamic radius, *a0* surface of the monomer in aggregate, *P* packing parameter, *l.c.b.* long-chain base

(Neu5Ac) and GM1 (Neu5Gc). The micellar surface was considered as a cluster of gangliosides, with single monomers anchored in a carbohydrate-enriched model membrane matrix. These studies indicate that Gal-GalNAc and Gal-Glc linkages in GM1 oligosaccharide are much more dynamic than Neu5Ac-Gal and GalNAc-Gal linkages. β-Gal-(1–3)-β-GalNAc is represented mainly by the two conformers shown in Fig. 1b, c) that satisfy the Gal-H1/GalNAc-H2 and Gal-H1/GalNAc-NH nuclear Overhauser effect (NOE) averaged contacts and the Gal-H1/GalNAc-H4 contact. The trisaccharide core -β-GalNAc-(1–4)[(α-Neu5Ac-(2–3)]β-Gal- shows a single stable conformation corresponding to the minimum energy conformation observed for the same trisaccharide in a number of ganglioside systems [56, 61, 62]. This trisaccharide is generally considered to be a rigid block. Some molecular dynamics calculations predict the possible existence of two structures, although it is not possible to obtain quantitative information on the ratio of the conformers. The few NOEs between glucose and ceramide suggest a single conformation with the total oligosaccharide structure perpendicular to the cell surface. GM1 monomers inserted into a surface are apparently present as a group of conformers that confer high dynamics to the structure. This property accounts for the ability of GM1 to interact with a variety of proteins and membrane receptors and produce different, in some cases opposite, physiological effects.

GM1 monomers inserted into and segregated in the membrane can interact with each other through two different processes. At the water/lipid interface, the ceramide amide acts as both proton acceptor and donor, allowing formation of a network of hydrogen bonds that reduces dynamics at the interface. The oligosaccharide chains can also interact each other through hydrogen bonds. NMR spectroscopic studies indicated that these interactions are mediated by water molecules that act as linking bridges between two chains [58, 63]. Calorimetric analysis suggested that ~40–50 water molecules interact with a chain [64].

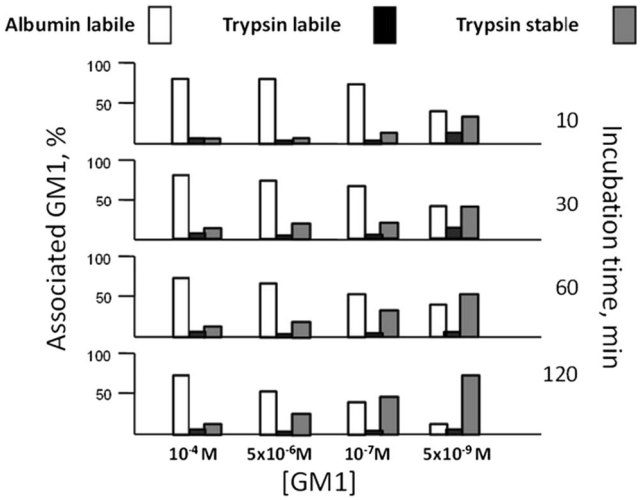

Fig. 5 Association of exogenous GM1 with cultured neurons. Distribution of various forms of associated GM1 is shown as a function of added ganglioside and incubation time. Albumin labile: portion of GM1 removed by washing with albumin. Trypsin labile: portion of GM1 removed by mild trypsin treatment after washing with albumin. Trypsin stable: portion of GM1 that remains associated with cells (considered to be a cellular component) following albumin and trypsin treatment

GM1 and Cholera Toxin

Studies in the early 1970s revealed that cholera pathogenesis begins with interaction between the *Vibrio cholerae* exotoxin (cholera toxin [CT]) and the mucosal surface [65]. The receptor structure for the toxin was identified as oligosaccharide β-Gal-(1–3)-β-GalNAc-(1–4)-[α-Neu5Ac-(2–3)-]β-Gal-(1–4)-Glc, i.e., GM1 oligosaccharide. This finding initiated major research interest in GM1.

CT belongs to the AB5 family of bacterial toxins [66, 67]. It consists of a single catalytically active component A and a nontoxic pentamer of identical B subunits (B5). Cholera disease involves passage of complete AB5 through the epithelial barrier of the intestine. This process is mediated by GM1, acting as the CT membrane receptor [68]. Passage of AB5 through the membrane may occur by caveolae-dependent [69], clathrin-dependent [70], or noncaveolae/nonclathrin-mediated [71] endocytosis of AB5–GM1 complex into the apical endosome, followed by retrograde transport into Golgi/ER (Fig. 6). In Golgi/ER, the complex dissociates, and active A is transported to the basolateral membrane, where it catalyzes ADP ribosylation of α subunits of stimulatory G proteins, leading to persistent activation of adenylate cyclase and continuous production of cAMP. Increased intracellular cAMP in intestinal epithelial cells results in the severe diarrhea and fluid loss characteristic of cholera.

The B pentamer binds to five GM1 monomers on the external membrane. This binding capability does not require the presence of the A subunit or the ceramide moiety. The binding constant is very low: in the range of 10^{-8}–10^{-12} M, depending on the procedure used [72, 73].

CT derivatives and anti-CT antibodies for analytical immunostaining are available. Procedures for GM1 analysis and separation using CT have been developed. Enzyme-linked immunosorbent assay and thin layer chromatography procedures combining sialidase hydrolysis and CT binding are available for detection and quantitation of ganglioside mixture components [74–77]. CT was used in electron microscopic studies to detect GM1 enrichment at nerve endings [78]. These studies provided the first information on GM1 topology in the brain (see "Cellular Organization of GM1" section) and on GM1 clusters in cell membranes.

Exogenous addition of purified B5 to cells results in formation of B5–GM1 complexes but does not lead to the pathological cascade. This approach has been used to study certain physiological functions of GM1. The sequestration of GM1 by B5-CT and resulting inhibition of processes indicate that GM1 is a regulator of cell growth and proliferation, through (i) its interaction with the protein channel necessary for extracellular calcium influx and (ii) changes in its intracellular trafficking and catabolism [79, 80].

Although the GM1-to-CT binding constant is very low, other GSLs and glycoprotein oligosaccharides have been found to bind CT with similar binding constants, e.g., α-fucose-(1–2)-GM1 [72]. Great care is, therefore, necessary in using CT immunostaining approaches for identification of

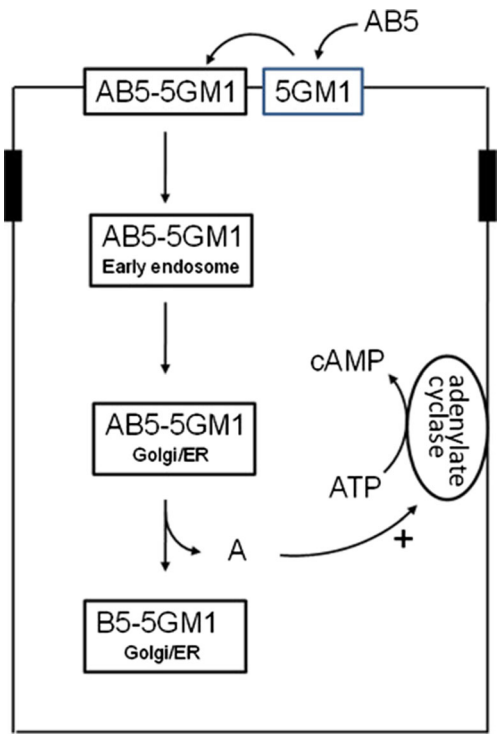

Fig. 6 Proposed internalization (schematic) of AB5–5GM1 complex and subsequent effect on adenylate cyclase activity

GM1 and in binding experiments focused on specific roles of GM1 in plasma membrane. In in vitro experiments, it is crucial to ensure that subunit B preparations are completely free of subunit A [81].

Two procedures for cholera therapy are based on the interaction of GM1 with B5 of CT: (i) administration of GM1 adsorbed on charcoal to block toxin released in the intestinal lumen, and (ii) administration of purified B5 to block the GM1 receptor and prevent interaction with AB5 [82].

Cellular Organization of GM1

Subcellular Topology of GM1

GM1, like other GSLs, is characterized by a large, bulky polar head group, and a critical packing shape in any aggregate in the form of a cone or truncated cone. GM1 molecules can aggregate to form micelles, but can also be inserted into a bilayer, with some important geometric constraints [83]. Glycerophospholipids and cholesterol are the bulky components of cellular membranes. GM1 concentration in different membrane compartments varies greatly and is high in certain membrane environments. The highest ganglioside concentrations are found in the central nervous system of mammals. The content of lipid-bound sialic acid in the frontal and temporal cortices is 3.5-folds to fourfolds higher than that of protein-bound sialic acid [84]. Neuronal cells in brain are particularly enriched in gangliosides, as reflected by the much higher concentration of gangliosides in gray vs. white matter [84]. Significant amounts of gangliosides, including GM1, are also found in glial cells (astrocytes and oligodendrocytes) and brain macrophages.

GM1 is synthesized by the action of glycosyltransferases in Golgi and trafficked to the plasma membrane [85], the intracellular site having the highest GM1 concentration. Cell subfractionation studies on brain cortex suggested ultrastructural localization of GM1 at the plasma membrane level in neurons [86], which was confirmed by high-resolution detection of tissue-bound CT using immunoelectron microscopy [77]. GM1 was shown to be concentrated at the presynaptic and postsynaptic membranes of nerve endings and confined to the external membrane surface. This was the first demonstration of nonhomogeneous distribution of GM1 in neuronal plasma membranes. These and subsequent supporting studies [38, 87] were the starting point for clarifying the biological roles of GM1 and gangliosides, in general, and their essential conclusions are still valid today [88].

However, the quantitative aspects of GM1 subcellular localization remain poorly understood, and certain important pieces of information are still missing. The original reports of the absence of CT binding at limiting toxin dilutions on neuronal membranes outside synaptic terminals and of an efficient mechanism of axonal transport of newly synthesized gangliosides to nerve terminals [89] led, in the late 1970s, to a prevailing dogma that gangliosides were concentrated at the synapse, consistently with proposed specific functions of GM1 in nerve conduction and/or synaptic transmission. However, newly available data on ganglioside concentrations in isolated synaptosomes soon revealed the presence of gangliosides on the entire neuronal surface, with concentrations lowest in the neuronal perikaryon and highest in the synaptic terminal [38]. There are striking differences in turnover of various pools of GM1 associated with neuronal plasma membrane, with highest turnover in dendritic membranes [90]. Minor (but not negligible) pools of gangliosides, including GM1, are present in several intracellular loci besides plasma membrane.

The presence of these pools, in some cases, was predictable and not surprising in view of the complex trafficking routes followed by GM1 and other gangliosides during their metabolism, and the proposed biological roles of gangliosides. The synaptic vesicles of nerve endings have significant ganglioside content [91]. GM1 is associated with internal membranes of late endosomes and lysosomes [92, 93], reflecting its transport from plasma membrane to sites of intracellular degradation along the endocytic pathway. The association of GM1 with the endosomal/lysosomal compartment changes during the aging process in brain. GM1 bound to amyloid β peptide is accumulated in the endosomal compartment of aged monkey brain [94].

A retrograde, transcytotic transport of GM1 has been described in polarized epithelial cells [95, 96], and a similar event may occur in neurons [97, 98]. A portion of plasma membrane GM1 internalized to recycling endosomes is recycled back to plasma membrane [97], and this process may contribute to the uneven distribution of GM1 in various domains of neuronal plasma membrane. The presence of soluble gangliosides (particularly GM1) associated specifically with cytosolic proteins has been reported in fibroblasts [99], cultured cerebellar neurons [100], and rat brain [101]. A GSL transfer protein that efficiently transfers GM1 was isolated from rat brain cytosol [102]. Gangliosides associated with soluble proteins (e.g., GM1 associated with GLTP [103]) presumably reflect nonvesicular intracellular transport among different membrane compartments.

The association of a certain amount of GM1 with intracellular membranes and soluble intracellular proteins is well documented. A recent report indicates that 40–60 % of the cellular pool of GM1 is associated with a raft-like intracellular membrane fraction enriched in *trans*-Golgi network and endosomal membranes [104]. The size of this intracellular pool of GM1 can hardly be explained in terms of a pool of trafficking molecules. GM1 in the pool may help control transcriptional programs affecting lipid homeostasis through epigenetic regulatory mechanisms [105].

GM1 is present in mitochondria-associated endoplasmic reticulum membranes (MAMs) in a mouse model of GM1 gangliosidosis [106]. The most intensively studied and best characterized pool of intracellular GM1 is that of nuclear GM1. The presence of notable amounts of GM1 in nuclear membranes of various organs has been known since the late 1980s [107] and was first reported in neuronal cells in 1995 [108]. The complexity of the nuclear envelope has become clear only in recent decades, and the role of nuclear sphingolipids is evidently important but still under investigation [109]. GM1 in the nuclear envelopes of neuronal cell lines and primary neurons is closely associated with a specific Na^+/Ca^{2+} exchanger, NCX, whose functional activity is strongly enhanced by the association and plays a crucial role in regulation of nuclear Ca^{2+} concentration. The nuclear complex between NCX and GM1 appears to have a cytoprotective and antiapoptotic role and to be an important part of the general neuroprotective role of GM1 (see Ref. [110] for a recent review).

GM1 Organization in the Plasma Membrane

Sphingolipids, in general, and GSLs, in particular, have several structural features that promote their segregation and phase separation with respect to the fluid phase of glycerophospholipid bilayers, with formation of membrane areas having properties similar to those of liquid-ordered (lo) phases as observed in membrane model systems [111, 112]. The simultaneous presence of an amide linkage and a hydroxyl group in the ceramide allows sphingolipids to act as both proton donors and acceptors in hydrogen bond formation [47] and to help form a dense hydrogen bond network at the water/lipid interface. This ability is one of the driving forces for the tendency toward lateral segregation of sphingolipids within the membrane bilayer [113–115].

A second feature that promotes segregation of sphingolipid-rich phases within a fluid phospholipid bilayer is the unique composition of the hydrophobic portion. Ceramide is highly heterogeneous in the length and degree of unsaturation of its fatty acyl chains. Within the nervous system, saturated acyl chains such as palmitic and stearic acid predominate in certain classes of lipids, including gangliosides and sphingomyelin [116]. Brain gangliosides, highly enriched in stearic acid, are typical lo phase lipids. The distribution of GM1 in the fluid phase of two-component, two-phase phosphatidylcholine bilayers [27] (as revealed by freeze-etch electron microscopy following labeling with CT) is correlated inversely with acyl chain length and correlated directly with degree of unsaturation.

The final and most important factor determining the segregation ability of GSLs is the bulkiness of the oligosaccharide hydrophilic head group, which strongly affects the molecular shape and packing of GSLs in aggregates and, consequently, the large surface area occupied by GSL oligosaccharide chains. Theoretical calculations of minimum energy conformation for the hydrophilic oligosaccharide head group of GM1 [56] indicate that it occupies a volume much larger than that of phosphocholine, which has the bulkiest head group among the phospholipids and is present in phosphatidylcholine and sphingomyelin. Predictions based on packing parameters and critical packing shapes of gangliosides [49] suggest that separation of a ganglioside-rich phase in a phospholipid bilayer and concomitant acquisition of positive membrane curvature are strongly favored on an energetic basis because they imply minimization of the interfacial free energy required to accommodate amphipathic molecules such as gangliosides in the bilayer. The geometric properties of single ganglioside molecules, which depend on the bulkiness of the hydrophilic head group, greatly affect the local lateral organization and geometry of biological membranes, favoring phase separation and spontaneous membrane curvature [49, 117, 118]. Recent studies have re-addressed the importance of heterogeneous ganglioside distribution in determining the three-dimensional structure of membranes, distinct from flat geometry (see [83] for review).

As a general rule, the volume occupied by the head group increases with the complexity of the oligosaccharide chain. In view of the heterogeneity and complexity of nervous system structures, this volume varies greatly for different GSLs. The degree of ganglioside phase separation in phospholipid bilayers depends on the surface area occupied by oligosaccharide chains, which is correlated directly with the number of sugar residues in the oligosaccharide [119–121]. Studies on a variety of model systems confirm the ability of GM1 to form phase-separated clusters with respect to bulk fluid membrane regions, distinct from other types of phase-separated clusters. GM1 is associated preferentially with gel-phase regions in multilamellar liposomes of phospholipid mixtures, which have laterally separated fluid- and gel-phase regions [122]. Analysis of ternary sphingomyelin/GM1/cholesterol vesicles by differential scanning calorimetry revealed formation of separate GM1-enriched and cholesterol-enriched domains and of GM1-enriched domains in sphingomyelin bilayers [123].

A major obstacle to research progress in this field, i.e., lack of experimental approaches capable of identifying and studying phase separation in more complex, physiologically relevant model systems or living cells is being progressively overcome by new or improved imaging techniques. Stimulated emission depletion (STED) microscopy is a fluorescence-based technique able to overcome the limit imposed by the diffraction barrier, thus making possible nanolevel resolution [124, 125]. STED studies demonstrated that putative lipid raft markers, including GPI-anchored proteins, sphingomyelin, and GM1, are confined to transient, cholesterol-dependent molecular complexes that cover membrane areas with diameters<20 nm and have an average lifespan of 10–20 ms [126, 127]. The use of order-sensitive probes revealed the existence

of GM1- and cholesterol-rich domains in spheres obtained from plasma membrane of A431 cells by a cell swelling procedure. This model is not equivalent to living cells, but may be the best possible approximation. Such domains have a high degree of lateral order (higher than that of the surrounding fluid membrane environment, although lower than that of the lo phase of two-phase giant unilamellar vesicles), are highly dynamic (e.g., can coalesce to form micrometer-scale structures by cross-linking with pentavalent CT), and are able to recruit typical lipid raft proteins [128, 129].

In regard to simpler model systems, phase separation was observed in mixed micelles comprised of two different gangliosides (GM2 and GT1b [130], GD1b and GD1b-lactone [131], and GM1 and GD1a [132]) with identical hydrophobic moiety composition. The resulting suggestion that different ganglioside-enriched domains may coexist in a cell membrane was subsequently confirmed by a series of elegant studies. An experiment using CT as a probe for GM1, and GD3-specific mAb, showed that GM1 and GD3 form distinct, separate clusters in artificial supported monolayers, intact cerebellar granule neurons, and isolated membrane rafts from adult rat cerebellum [133]. GD3, but not GM1, coclustered with the neuronal raft marker Lyn. GM1 in supported myelin membrane monolayers coclustered with its functional ligand, the myelin-associated glycoprotein MAG [134]. The earliest evidence for GSL clustering in cell membranes, obtained from immunoelectron microscopy, was instrumental in development of the lipid raft hypothesis [135]. GM1 clustering was demonstrated in dorsal root ganglion neurons using an anti-GM1 mAb as a probe [88]. There has been persistent criticism of immuno-EM methods for investigation of membrane lipid topology, because they require extensive sample manipulation, multivalent probes (e.g., IgM antiglycolipid antibodies and CT B-subunit for GM1), and organic solvents and/or chemical fixatives, all of which may produce experimental artifacts. Most membrane lipids do not react with the aldehyde fixatives commonly used in EM and can, therefore, be redistributed within or removed from the membrane during sample handling [136, 137]. Chemical fixatives do not preserve the in situ localization of membrane lipids [138]. This method has been successfully applied for nanoscale analysis of membrane lipid distribution in the outer leaflet (GM3 and GM1) or inner leaflet (phosphatidylinositol 4,5-bisphosphate) of the plasma membrane, demonstrating that GM3 and GM1 form independent clusters at the cell surface [139, 140]. To date, this method has not been applied for studies of ganglioside distribution in neuronal cell membranes.

Neurotrophic and Neuroprotective Properties of GM1

The first studies on brain lipids were reported in the late 1800s [141], and brain gangliosides were separated from other lipids in the 1930s [142, 143]. Research on gangliosides during the next few decades was slow because of difficulty in identifying their structures. The structures of Sph, sialic acid, and GM1 were elucidated respectively in 1947 [8], 1955 [144], and 1963 [1].

Rapidly expanding research beginning in the 1970s showed clearly that gangliosides were involved in a variety of physiological processes, in addition to their role as bulky membrane components separating the cell interior from the exterior. The ganglioside research groups were located worldwide and included specialists in chemistry, physicochemistry, enzymology, genetics, pathology, and biology and biochemistry of the nervous system and extranervous system. The neurotrophic and neuroprotective properties of GM1 were first reported during this burst of activity.

Members of the ganglioside family vary widely in composition and number of oligosaccharide chains. Mammalian differentiated neurons, whose ganglioside content is up to tenfolds higher than that of nonneuronal cells, have primarily gangliotetraose series oligosaccharide chains with 1 to 5 sialic acid residues. Ganglioside content and ganglioside pattern change during differentiation, aging, and neurodegenerative diseases. In contrast, the ganglioside pattern in nonneuronal cells is generally less heterogeneous, and lactose series oligosaccharide chains are predominant. Sialyllactosylceramide (GM3) is the major ganglioside component of the human body. GM3 is abundant in proliferating neurons, but its proportion in the total ganglioside mixture declines following differentiation. Thus, lactose series vs. ganglio series gangliosides evidently play differing roles in proliferating vs. nonproliferating cells, i.e., nonneuronal vs. neuronal cells.

Numerous studies involving exogenous addition of GM1 or ganglioside mixtures to cultured neurons indicate that changes of membrane ganglioside content and pattern alter brain responses to signals from the surrounding environment. This effect evidently results from modulation of membrane enzyme and membrane receptor activities through specific molecular interactions. Thus, gangliosides are associated with functional plasticity of the brain [145, 146].

Neobiosynthesis of gangliosides occurs in the Golgi apparatus where sugar units are added sequentially to ceramide by specific glycosyltranferases working on nucleotide activated sugars. To reveal and to determine a specific role of gangliosides in the brain, several KO mice were developed removing glycosphingolipid and ganglioside biosynthetic enzymes [147]. Synthesis of glucosylceramide is the first step for the Golgi synthesis of complex gangliosides and removal of the glucosylceramide synthase lead to total deletion of glycosphingolipids and gangliosides [148]. This lead to embryonic lethality. Removal of the GM2/GD2 synthase depleted GM2 and GD2 and all the gangliosides belonging to the gangliotetraose series. The mice developed parkinsonism, and manifestations were largely attenuated by administration of

GM1 derivatives that are capable to penetrate the blood–brain barrier reaching then neurons [149].

GM1 modifies differentiation processes, amplifies responses to neurotrophic factors, protects against excitatory amino acid-related neurotoxicity by limiting the downstream consequences of receptor overstimulation, and reduces acute nerve cell damage by blocking excitotoxicity and potentiating neurotrophic factors [150]. Administration of ganglioside mixtures affected recovery processes of both cholinergic and adrenergic nerve fibers in experimental models of peripheral sympathetic regeneration and reinnervation (preganglionic and postganglionic anastomosis). A large body of studies have elucidated the role of gangliosides (including GM1) in induction of neurite sprouting, and confirmed the involvement of GM1 [151–153] and membrane-bound sialidase Neu3 (which produces GM1 from polysialogangliosides) in the process of neuritogenesis.

Clustering of GM1 in specialized membrane domains, as described above, is evidently responsible for the molecular interactions that underlie the neurotrophic and neuroprotective effects of GM1. These GM1 effects can de facto replace or potentiate the actions of neurotrophins in several experimental contexts [154] and are largely (although not exclusively) mediated by modulation of neurotrophin receptors. For example, GM1 exerts a neurotrophic effect on dopaminergic neurons by interacting with a GDNF (glia cell-derived neurotrophic factor) receptor complex [155].

The best studied functional interactions between GM1 and neurotrophin receptors involve Trk family receptors. Exogenous GM1 stimulates Trk kinase activity, receptor autophosphorylation, and dimerization in various cell types [156–160]. A significant proportion of Trk receptors in neurons is typically associated with lipid rafts or GM1-enriched membrane domains [161–166]. Colocalization of GM1 and Trk receptors is a prerequisite for GM1–receptor interaction, and GM1 binds specifically and tightly to Trk in cultured cells [167], brain tissues [168], and live rats [169, 170]. Conversely, formation of high-affinity complexes between fully glycosylated Trk and GM1 is essential for targeting of Trk into GM1-enriched domains [171] and for GM1-induced activation of the receptor [157], suggesting that receptor glycosylation is a crucial regulatory mechanism for functional modulation of Trk mediated by its interaction with GM1. Lateral interactions between oligosaccharide chains of the receptor and of GM1 within lipid rafts (*cis* carbohydrate–carbohydrate interactions) may be necessary for "fine tuning" of Trk activity. This idea is supported by findings that activation of lipid raft-enriched, ganglioside-specific Neu3 sialidase, which is highly expressed in the cerebral cortex and cerebellum [172], is essential for axon specification and neuronal polarization [173]. Anti-GM1 antibodies from Guillain–Barré syndrome patients abolished nerve growth factor (NGF)-induced Trk activation and altered association of the receptor with lipid rafts [174].

GM1 and Neurodegenerative Diseases

Experimental studies based on exogenous addition of GM1 to cultured cells (see "Neurotrophic and Neuroprotective Properties of GM1" section) suggested that various neuropathologies are associated with changes in plasma membrane organization and that GM1 may be useful in therapy of central and peripheral nervous system disorders such as neurodegenerative diseases, stroke, subarachnoid hemorrhage, and spinal cord injury [175–180].

Data on the role of GM1 in neurodegenerative diseases are abundant but sometimes contradictory. A characteristic common to many neurodegenerative diseases is the misfolding of a cellular protein (e.g., α-synuclein in Parkinson's disease, amyloid β peptide (Aβ) in Alzheimer's disease, scrapie prion protein, PrP^{Sc}, in transmissible encephalopathies), with consequent loss of normal function of the protein and/or formation of a toxic form that leads in nervous tissue to formation of poorly soluble fibrils or particles whose intracellular or extracellular accumulation is causally linked with disease onset. The etiopathogenesis of neurological (particularly dementing) diseases is extremely complex, and the importance of harmful effects by amyloidogenic proteins remains controversial [181]. The mechanisms leading to formation of pathological forms of these proteins vary depending on the protein, but a common trait appears to be interaction with lipid-rich membrane regions having anomalous composition and organization. Altered plasma membrane organization of GM1 may be associated (either as a cause or consequence) with neurological pathologies. Alterations in sphingolipid metabolism potentially leading to anomalous membrane organization have been reported for several important nervous system diseases, including most of the neurodegenerative diseases and major forms of dementia [175–177].

α-synuclein, a cytosolic protein, contains two distinct cholesterol-binding domains and a glycolipid-binding domain [182, 183], and binds to membrane-associated GM1 in the presynaptic regions of neurons [184]. Aβ (see preceding paragraph) also binds directly to cholesterol and GM1, interacting with GM1 oligosaccharide chains through sugar-specific mechanisms [185–188]. The role of GM1 in Alzheimer's disease (AD) is much more complex. Deregulation of ganglioside metabolism has been reported in brain of AD patients and in transgenic mouse models of AD (for review, see Ref. [189]). Reduced ganglioside concentrations (associated with altered ratios of a-series to b-series gangliosides) have been reported for most brain regions of patients with AD or dementia of the Alzheimer type (DAT) [190–195] in comparison with age-matched controls. This finding is consistent with reported age-associated ganglioside loss during human physiological senescence [84]. On the other hand, concentrations of GM1 and GM2 in lipid rafts from the frontal and temporal cortex were reported to be higher in AD patients

than in controls [196], and higher levels of anti-GM1 antibodies were found in AD patient brains [197].

Lipid rafts from cultured cells and mammalian brains contain amyloid precursor protein (APP) and also proteolytic fragments derived from APP (including Aβ) and several proteolytic enzymes involved in APP processing [189, 198] (including active β-secretases and γ-secretases. Lipid rafts are evidently the major cellular site for amyloidogenic processing of APP leading to production of Aβ [199–201]. Following formation of soluble Aβ, the next step toward the toxic effect that underlies AD is conversion of Aβ into aggregated forms (favored by a conformational transition from random coil or α-helix-rich to ordered β-sheet-rich structure) necessary for formation of insoluble amyloid fibrils. The conversion of soluble, nontoxic Aβ into toxic Aβ fibrils requires interaction of Aβ with neuronal membranes [202, 203], particularly the strong interaction of membrane-bound Aβ with GM1 in GM1-rich domains at the neuronal surface [204].

GM1-bound Aβ has unique immunological properties [205] that reflect a conformational transition in the protein resulting from interaction with the ganglioside and has been proposed to act as a "seed" for amyloid formation that promotes formation and deposition of toxic Aβ aggregates in vitro and in living cells [206–209]. GM1-bound Aβ is associated with amyloid plaques in cerebral cortex of AD patients [205]. Its level is high in synaptosomes from aged mouse brains, which also display high-density GM1 clusters [210]. Aβ binds to ganglioside clusters in a density-dependent manner in artificial membranes [206], and GM1–Aβ interaction and Aβ aggregation are enhanced in a cholesterol-rich membrane environment [211, 212]. Thus, the membrane organization and clustering of GM1 are crucial factors for GM1-Aβ formation.

Recent studies suggest that GM1 accumulated at locations other than the cell surface may contribute to GM1-induced amyloid fibril formation. In aged monkey brains, GM1-bound Aβ is accumulated preferentially in endosomes [94]. Blocking of the endocytic pathway in PC12 cells accelerated the extracellular release of exosome-associated GM1, with consequent induction of Aβ aggregation [213]. These findings indicate that abnormalities in the endocytic pathway contribute to Aβ-based pathology in AD.

GM1–Aβ interactions are also involved in neuronal death associated with AD. Incubation of Arctic Aβ in the presence of GM1-containing liposomes or neuronal membrane preparations resulted in formation of a toxic but soluble and nonamyloidogenic Aβ aggregate that induced NGF-dependent neuronal death [214]. On the other hand, interaction of GM1 with Aβ may lead to formation of toxic soluble oligomers that exert their harmful effects through high-affinity binding with cellular PrP, another protein enriched in lipid rafts [215].

The findings described above may seem contradictory to the beneficial effects of GM1 on disease progression in AD patients. The neurotrophic and neuroprotective effects of GM1 observed in in vitro and in some in vivo experimental models suggested that GM1 could be used to treat patients with neurodegenerative diseases, but the results of early studies along this line were ambiguous. The pharmacokinetics of intramuscularly or subcutaneously injected GM1 in humans has been carefully quantified. GM1 enters the bloodstream rapidly, reaches a maximal level after 48–72 h, and has an elimination half-life of 60–75 h. In rat studies, injected GM1 reached all types of brain tissue. The majority of injected GM1 accumulated in liver, where its metabolism was slower than in brain. GM1 in brain was rapidly metabolized to water and carbon dioxide, and a portion was recycled to yield GD1a following sialylation. In liver, catabolic sugars and lipids picked up by lysosomes are recycled for de novo glycolipid biosynthesis. Recycling of sialic acid, Sph, and the GSL portion of GM1 has been studied in detail and is evidently a general process that occurs in all body tissues. A small quantity of GM1 is associated with brain and with subcellular fractions that do not display activity of γ-glutaminyltransferase, an enzyme characteristic of microvessels. These findings suggested that GM1 passed through the "blood–brain barrier" [180, 216, 217]. However, the actual benefit of GM1 treatment to the central nervous system was unclear, in view of the fact that only a small quantity of GM1 reached brain neurons. To increase the quantity of GM1 in brain, an experimental group of 5 AD patients were continuously administered GM1 (20–30 mg/24 h) through brain ventricles. After 12 months of treatment, deterioration had stopped, and the patients had improved motor performance and neuropsychological assessments. They became more active, had improved reading comprehension, and were able to perform activities such as writing reports and short letters on a computer.

In view of the above finding, it was proposed that GM1 treatment and its insertion into neuronal plasma membranes led to formation of new GM1–Ca^{2+} complexes. Following membrane depolarization, the presence of these complexes would allow increased calcium influx and neurotransmitter release. It is not surprising that gangliosides can use Ca^{2+} as a counterion to form a network of associations in vitro. Gangliosides in vivo are diluted into glycerophospholipids, which also show a higher association constant with Ca^{2+}. It has been proposed that the glycerophospholipid–calcium interaction network favors exclusion of gangliosides and thereby formation of new ganglioside-enriched domains. Ganglioside–protein interactions within lipid rafts are responsible for the cascade of events that follows insertion of GM1 into plasma membranes.

A portion of GM1 inserted into the membrane can enter the cell and reach subcellular compartments, where it modulates various protein functions. At the level of the cell nucleus,

gangliosides induce synthesis of mRNAs specific for production of cytoskeletal proteins. Cytosolic GM1–protein complexes may also be formed following GM1 administration.

We cannot rule out the possibility that GM1, prior to entering the membrane, forms a complex with soluble Aβ and prevents its deposition and oligomerization at the cell surface. Formation of soluble complexes between GM1 and proteins associated with Parkinson's disease (PD) could account for beneficial effects of GM1 treatment reported for this disease. Daily administration of a large quantity of GM1 to several PD patients [180] resulted in a general improvement of motor symptoms and reduced rate of symptom progression.

Concluding Remarks

The major localization of GM1 ganglioside at the neuronal plasma membrane, its uneven distribution at the neuron surface, and its enrichment in specialized subdomains of the plasma membrane, are reflected in the multifaceted roles of GM1 in neuronal physiology and pathology. GM1 has been studied in laboratories worldwide in a variety of experimental models and clearly shown to exert neurotrophic and neuroprotective effects in various situations, including promotion of neuronal survival, neurite outgrowth, and neuronal differentiation in vivo and in cultured neurons, and protective and therapeutical effects against several types of neuronal damage [160, 218–224].

GM1 in neurons helps transfer information from the exterior to the interior of the cell, through specific recognition and binding of biologically active molecules (membrane receptors and ion channels), and has specific functions in nerve conduction and/or synaptic transmission. The mechanisms underlying the effects of GM1 remain unclear in many cases, but it appears that these effects are often due to specific interactions between GM1 and proteins involved in signaling processes, within GM1-enriched lipid rafts in the plasma membrane. The membrane-associated proteins proposed as interaction partners for GM1 include neurotrophin receptors, opioid receptors, integrins, and Ca^{2+} channels. The interaction of GM1 with such molecules is evidently facilitated or synergized by their cosegregation in specialized membrane domains.

GM1 is a major component of total ganglioside mixtures from mammalian brains, from which it can be extracted and purified in large amounts by relatively simple procedures. Alternatively, GM1 can be produced on a large scale by sialidase hydrolysis of total ganglioside mixtures.

GM1 was widely used in the past as a therapeutic drug for a wide variety of neurological disorders. A series of studies in the 1990s suggested that GM1 and other gangliosides injected for therapeutic purposes were immunogenic and led to production of antibodies that promoted peripheral neuropathies such as Guillain–Barré syndrome (GBS) [225, 226]. This event prevented the completion of some trials designed for better understanding of the therapeutic properties of GM1. In some of these trials, patients received GM1 in large amount (see previous article), but no one developed peripheral neuropathies or anti-GM1 antibody titers in serum. Later, GBS was found to be strongly associated with bacterial infection, particularly *Campylobacter jejuni* infection. Contact of host cell membranes with *C. jejuni* lipooligosaccharides stimulates the immune system to produce specific antibodies against oligosaccharides that mimic those present in normal glycoconjugates of human neuronal membranes [227]. Nevertheless, it is necessary to recall that the oligosaccharide of GM1, and that of many other gangliosides, acts as an antigen for serum antibodies from patients with neuropathies [228, 229].

A possible relationship between ganglioside therapy and GBS was conclusively ruled out by a systematic comparison performed by the Local Health District (LHD) of Ferrara, Italy of the incidence of GBS during 1988–1993 (when ganglioside-based drugs were widely prescribed) vs. 1994–2001 (following withdrawal of such drugs). The analysis showed no difference in the incidence of GBS cases between the two periods [230, 231].

Further studies and trials based on the therapeutic properties of GM1 are of great interest and importance. Novel procedures for effective administration of GM1 are under investigation, and trials of its ability to counteract peripheral neurotoxicity related to chemotherapy [232, 233] are in progress [234].

Conflict of Interest None

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