

Chapter 12

Neuronal Ganglioside and Glycosphingolipid (GSL) Metabolism and Disease



Cascades of Secondary Metabolic Errors Can Generate Complex Pathologies (in LSDs)

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Abstract Glycosphingolipids (GSLs) are a diverse group of membrane components occurring mainly on the surfaces of mammalian cells. They and their metabolites have a role in intercellular communication, serving as versatile biochemical signals (Kaltner et al, *Biochem J* 476(18):2623–2655, 2019) and in many cellular pathways. Anionic GSLs, the sialic acid containing gangliosides (GGs), are essential constituents of neuronal cell surfaces, whereas anionic sulfatides are key components of myelin and myelin forming oligodendrocytes. The stepwise biosynthetic pathways of GSLs occur at and lead along the membranes of organellar surfaces of the secretory pathway. After formation of the hydrophobic ceramide membrane anchor of GSLs at the ER, membrane-spanning glycosyltransferases (GTs) of the Golgi and Trans-Golgi network generate cell type-specific GSL patterns for cellular surfaces. GSLs of the cellular plasma membrane can reach intra-lysosomal, i.e. luminal, vesicles (ILVs) by endocytic pathways for degradation. Soluble glycoproteins, the glycosidases, lipid binding and transfer proteins and acid ceramidase are needed for the lysosomal catabolism of GSLs at ILV-membrane surfaces. Inherited mutations triggering a functional loss of glycosylated lysosomal hydrolases and lipid binding proteins involved in GSL degradation cause a primary lysosomal accu-

Dedicated to Professor Kunihiko Suzuki on the occasion of his 90th birthday.

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mulation of their non-degradable GSL substrates in lysosomal storage diseases (LSDs). Lipid binding proteins, the SAPs, and the various lipids of the ILV-membranes regulate GSL catabolism, but also primary storage compounds such as sphingomyelin (SM), cholesterol (Chol.), or chondroitin sulfate can effectively inhibit catabolic lysosomal pathways of GSLs. This causes cascades of metabolic errors, accumulating secondary lysosomal GSL- and GG- storage that can trigger a complex pathology (Breiden and Sandhoff, *Int J Mol Sci* 21(7):2566, 2020).

Keywords Ganglioside · Glycosphingolipid · Glycolipid · Metabolism · Membrane-surface · Development · Neuron · Receptor · Topology · Ganglio-series · Glycosyltransferase · Organelle · Secretory pathway · Endosomal pathway · Lysosome · Catabolism · Degradation · Intra-lysosomal luminal vesicle (ILV) · Hydrolase · Sphingolipid-binding protein (SAP) · Sphingolipid-transfer protein · Lysosomal storage disease (LSD) · Secondary storage · Neurodegenerative disease · Genetic disease · Parkinson · Alzheimer · Frontal lobe dementia

Abbreviations

ASA	Arylsulfatase A
ASM	Acid sphingomyelinase
BMP	Bis(monoacylglycero)phosphate
CAD	Cationic amphiphilic drug
Cer	Ceramide
CerS	Ceramide synthase
CERT	Ceramide transfer protein
Chol	Cholesterol
CNS	Central nervous system
ER	Endoplasmic reticulum
FA	Fatty acid
GalCer	β -galactosylceramide; Ganglioside names are abbreviated according to Svennerholm (1962, 1994) as recommended by IUPAC (Chester 1997)
GBA1	lysosomal β -glucocerebrosidase
GlcCer	β -glucosylceramide
GM2AP	GM2 activator protein
GRN	Granulin
GT	Glycosyltransferase
Hex A/B/S	β -hexosaminidase A/B/S
KDS	3-keto-dihydrospingosine
KDSR	Keto-dihydrospingosine reductase
LacCer	Lactosylceramide
NEU	Neuraminidase
NPC	Niemann–Pick disease type C protein

PA	Phosphatidic acid
PD	Parkinson disease
PG	Phosphatidylglycerol
PI	Phosphatidylinositol
PiP	Phosphatidyl inositol phosphate
PL	Phospholipid
PM	Plasma membrane
PS	Phosphatidylserine
Sa	Sphinganine
Sap	Saposin
SAP	Sphingolipid activator protein
SL	Sphingolipid
SM	Sphingomyelin
SM4g	Seminolipid, i.e. 3-sulfogalactosyl-1-alkyl-2-acylglycerol
SM4s	3-sulfogalactosylceramide
So	Sphingosine
S1P	Sphingosine 1-phosphate
SPT	Serine palmitoyltransferase
TGN	Trans Golgi network

1 Historical Aspects of Structure, Location and Function of GSLs

Sphingolipids were identified and analyzed in extracts of postmortem brains in 1884 by Johannes L. W. Thudichum (1884). He named the previously unknown basic key structures “sphingosine”, “ceramide” and “sphingomyelin” and identified some small GSLs, glucocerebroside and galactocerebroside, now called glucosyl- and galactosylceramide (For their biosynthesis see Fig. 12.1). Gangliosides (GGs) were isolated and identified as acidic glycolipids of postmortem human brain tissue obtained from infantile patients suffering of amaurotic idiocy by Ernst Klenk (Klenk 1939, 1942). They are enriched in ganglion cells and contain N-acetylneuraminic acid, now also named sialic acid, as an acidic component. As anionic and amphiphilic GSLs, gangliosides form huge micelles in aqueous solution. The first structure of a complex brain GG, GM1a (Fig. 12.2b), became known in 1963 (Kuhn and Wiegandt 1963), allowing the identification of GSL and GG structures accumulating in ganglioside storage diseases (Jatzkewitz and Sandhoff 1963; Sandhoff et al. 1971; Wiegandt 1995). The investigation and analysis of lysosomal storage diseases was a pacemaker for the elucidation of cellular GSL metabolism, its intracellular pathways and locations, its enzymes and the essential cofactors for lysosomal GSL degradation (Conzelmann and Sandhoff 1978; Kolter and Sandhoff 1999; Mehl and Jatzkewitz 1964). This allowed identification of the catabolic blocks caused by inborn errors in GSL and GG storage diseases (Sandhoff et al. 1971). The

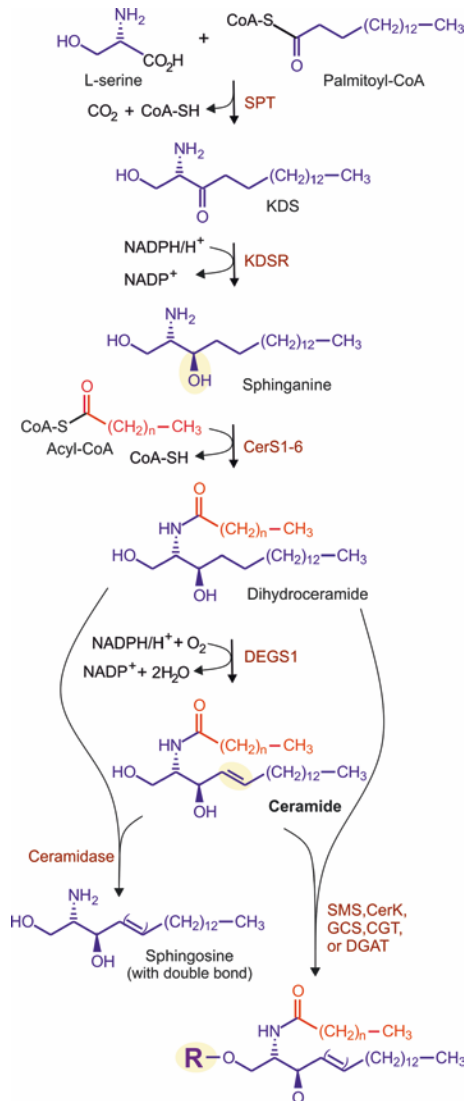


Fig. 12.1 Sphingolipid neo-biosynthesis in mammals. Serine palmitoyl transferase (SPT) initiates sphingolipid biosynthesis by condensing activated fatty acids (most commonly palmitic acid) with L-serine. After reduction by keto-dihydrosphingosine reductase (KDSR), sphinganine is produced, which can be converted to various dihydroceramides by one out of six ceramide synthases (CerS1-6) in mammals. The CerS have different specificities towards activated fatty acids of various chain length. The sphingosine double bond is then introduced by a dihydroceramide desaturase (DEGS1) and subsequent cleavage by ceramidases will release sphingosine, which can be used for sphingolipid synthesis by the salvage pathway. (Dihydro)ceramides are then substrates for sphingomyelin synthases (SMS, R: phosphorylcholine), ceramide kinases (CerK, R: phosphate), glucosylceramide synthase (GCS, R: β -glucosyl), galactosylceramides synthase (ceramide galactosyltransferase or CGT, R: β -galactosyl), or diacylglycerol acyltransferases (DGAT, R: acyl) adding corresponding head groups to the primary hydroxyl group

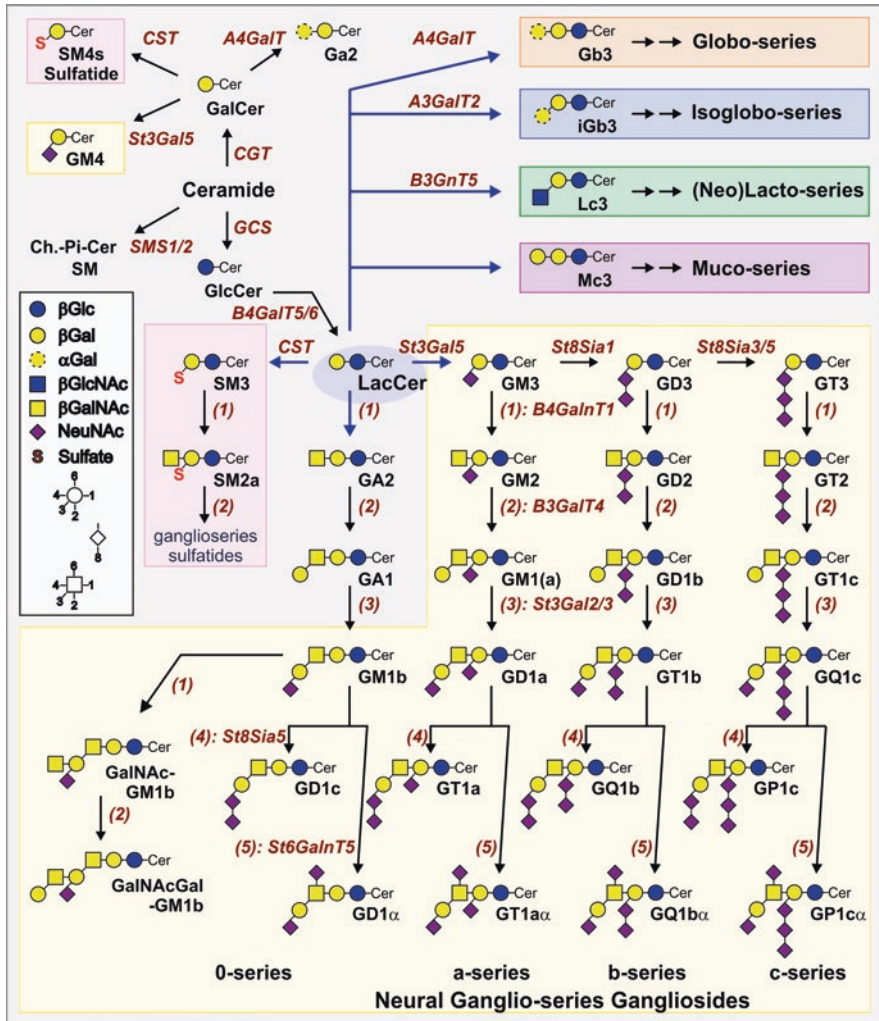


Fig. 12.3 Pathways of combinatorial ganglioside biosynthesis as adopted from (Sandhoff and Sandhoff 2018). The majority of GSL/GG structures are based on the initial attachment of glucose in β -anomeric linkage to the 1-O-position of ceramides (\diamond GlcCer). Lactosylceramide (LacCer) represents a fundamental branching point in GSL synthesis, highlighted by blue arrows radiating from LacCer. It is substrate for at least six different glycosyltransferases and a sulfotransferase guiding GSL synthesis into different GSL series. GM3 synthase (St3gal5) is one of them, guiding GSLs into a-, b-, and c-series of neuronal GGs. Cer, ceramide; GalCer, β -galactosylceramide; GlcCer, β -glucosylceramide. Ganglioside names are abbreviated according to Svennerholm (Svennerholm 1962, 1994) as recommended by IUPAC (Chester 1997)

laboratories of Klenk (Klenk 1942; Klenk et al. 1967), Yamakawa (Yamakawa 2005; Yamakawa and Nagai 1978) and Hakomori (Hakomori 1981; Regina Todeschini and Hakomori 2008) introduced and developed concepts that location,

distribution and pattern of GSL and GG are species-, cell type- and organelle specific.

2 Significance and Function of Neuronal GGs

In mammals, most cells of the visceral organs and the skin generate GSLs of the globo-series on their surfaces (see GSL pattern of fibroblasts in Fig. 12.2a). This is also true for neuronal stem cells which, however, substitute them by a molecular switch during neuronal differentiation with the expression of the ganglio-series of GGs (Russo et al. 2018). GGs then become the most abundant GSLs on the neuronal plasma membrane of the developing and ageing nervous system. It has been speculated that the specific combination of hydrophobic and hydrogen bonding elements of GGs may even hide an interaction code, e.g. with signaling receptors, for determining neuronal functions (Kaltner et al. 2019; Lunghi et al. 2021). It is noteworthy that globo-series GSLs repress the epigenetic regulator of neuronal gene expression AUTS2, which otherwise would activate the promotor of GM3 synthase, the first and rate-limiting enzyme of ganglioside-biosynthesis (Russo et al. 2018). Other cells also express different GG during differentiation. For example, male murine germ cells switch between GGs of the a- and the 0-series (Fig. 12.3), when they cross the blood-testis barrier during differentiation (Rabionet et al. 2015; Sandhoff 2010; Sandhoff et al. 2005). This switch also depends on increased activity of GM3 synthase. Another shift occurs in small intestinal epithelial cells during suckling-to-weaning transition (Yoneshige et al. 2010). It was also demonstrated that different GSLs support differentiation of human myeloid HL-60 cells into either monocytes/macrophages or granulocytes (Nojiri et al. 1986, 1988; Sandhoff 1993) as well as the activation of different T cell types (Inokuchi et al. 2015).

GGs are abundant sialic acid containing GSLs of the nervous system (Posse de Chaves and Sipione 2010), where they are enriched in neuronal plasma membranes (Rahmann 1983), especially in nerve endings. They form cell-type-specific patterns on the surfaces of neuronal cells that change with differentiation (Kotani et al. 1993, 1994, 1995; Kotani and Tai 1997). Neuronal GGs contain a ceramide anchor composed mainly of C18-sphingosine with little C20-sphingosine in combination almost exclusively with a saturated stearyl residue. The percentage of C20-sphingosine containing GGs increases steadily with differentiation and age throughout life (Sonnino and Chigorno 2000), but the potential benefit or function of this process remains to be elucidated. GGs, together with high levels of sphingomyelin and cholesterol, stabilize neuronal plasma membranes (Jennemann et al. 2005; Mori et al. 2012), while their hydrophilic glycan head groups on the cellular surfaces contribute to cell-to-cell adhesion processes (Handa and Hakomori 2012; Schnaar 2016). As amphiphilic lipids, GGs and GSLs are anchored onto the outer leaflet of plasma membranes with a hydrophobic ceramide moiety and correspondingly onto anti-cytosolic leaflets of cellular organelles. Thus, the glycan moiety of GGs is part of the glycocalyx at the cellular surface.

GGs are GSL that contain one or more of up to 50 different sialic acid residues, placing negative charges onto cellular surfaces (Schauer 2016). They can serve as ligands for lectins and modulate the activity of membrane proteins, such as insulin receptor, leptin and EGF receptor and serve as binding sites for surface proteins on neighboring cells (Allende and Proia 2014; Furukawa et al. 2012; Hakomori and Handa 2015; Inokuchi 2010; Ledeen et al. 2012; Lipina and Hundal 2015; Nordstrom et al. 2013; Ohmi et al. 2012; Regina Todeschini and Hakomori 2008). These interactions depend on the structure of the hydrophilic oligosaccharide head group on the cell surface and that of the hydrophobic ceramide anchor within the outer leaflet of the plasma membrane (PM) bilayer, both of which can vary significantly with cell type. Ceramide anchors of neuronal GGs of the A- and B- series contain almost exclusively a saturated fatty acid residue, the stearyl moiety (Kishimoto and Radin 1966) ($n = 16$ in Fig. 12.1), whereas GM3 and many GGs of visceral organs, like liver, spleen and intestine, prefer a variety of long to very long chain fatty acids in their ceramide moieties (Hara et al. 1984; Iwamori et al. 1984; Jennemann et al. 2010, 2012a; Keranen 1976; Nakamura et al. 1987; Riboni et al. 1992). The acyl chain length may also affect uptake, endosomal and intracellular trafficking of GM1 in cell culture as observed by the incorporation of synthetically prepared GM1 molecules containing different acyl chain lengths (Chinnapen et al. 2012; Saslowsky et al. 2013). In *C. elegans*, glycolipids with a C22-acyl chain are required for proper membrane localization of clathrin and clathrin-dependent autophagic lysosome reformation, which subsequently activated TOR and suppressed longevity (Wang et al. 2021a). CerS2 deficiency, which decreases sphingolipids with very long acyl chains (C22 and C24) in favor of those with long acyl chains (C16 and C18), reduced the rate of clathrin-mediated endocytosis in isolated mouse astrocytes apparently through indirect oxidative stress mediated mechanisms (Volpert et al. 2017). Whether the acyl-chain length of GGs would also affect vesicular transport mechanisms in neurons and eventually longevity is not yet clear.

Neuronal stem cells express globo-series GGs. However, when separated from blood and the rest of the body by the blood brain barrier during neuronal differentiation, they substitute them with the expression of ganglio-series GGs of the A- and B-series (Russo et al. 2018). These GGs carry a hydrophilic tetraosyl moiety with a varying number of sialic acids. The head group of axonal GGs like GM1a, GD1a, GT1b stabilize the axon – myelin interaction by binding the myelin-associated glycoprotein of the surrounding inner myelin sheet (Collins et al. 1997; Schnaar 2016; Yang et al. 1996).

As indicated by the analysis of mutant mice and patients with inherited defects in GG biosynthesis pathways, GGs are important for stability of neuronal structures and signaling. Although the molecular mechanisms facilitating these GG functions are still poorly understood (Proia 2003), it became clear that complex GGs GD1a and GT1b are specific ligands for the myelin associated glycoprotein MAG (Schnaar 2019) and support the interaction of axonal membrane with myelin sheaths at the node of Ranvier (McGonigal and Willison 2021). The importance of sialylation for this interaction, which establishes the terminal Neu5Ac α 2,3Gal β 1,3GalNAc-binding domain for MAG on GD1a and GT1b, was further underlined by

investigations on St3gal2/3 double-null mice, which displayed among other things disruptions at nodes of Ranvier (Yoo et al. 2015). The complex a- and b- series GGs play a central role in preventing dysfunction of the CNS in aging (of mice). The systemic elimination of complex GG of both series in GalNAc-transferase (GalNAc-T) deficient mice (which then overexpress the simple GGs GM3 and GD3, see Fig. 12.3) leads to an age-dependent neurodegeneration. Mice that express only GG GM3, however, suffer from a markedly accelerated neurodegeneration (disintegration of axons and disruption of node of Ranvier architecture) and reduced survival. Reintroduction of both a- and b-series specifically into neurons of GalNAc-T-deficient mice is sufficient to rescue the age-dependent neurodegenerative phenotype. Similarly, reconstituting neuronal a-series GGs but not b-series GGs – such as GD3 – in GM3-only mice by reintroducing neuronal GalNAc-T expression, abrogated the adult lethal phenotype (McGonigal et al. 2021). These results support recent findings that structural glycosphingolipids may be more relevant to achieve the centenarian condition than signaling sphingolipids (Pradas et al. 2022).

The b-series ganglioside GD3 and its key biosynthetic enzyme, GD3-synthase (GD3S), also seem to be relevant in the regulation of phagocytosis in an ischemic stroke model. Both were upregulated in the microglia of mouse hippocampus from 2 to 7 days after global cerebral ischemia, whereas the phagocytic capacity of the GD3S-KO microglia exhibited decreased amoebic morphology, reduced engulfment of neuronal material, and lower expression of the phagolysosome marker CD68. Also, microglia isolated from GD3S-KO mouse brain at 2 days after global cerebral ischemia were less neurotoxic to co-cultured hippocampal neurons than the WT-global cerebral ischemia microglia. Moreover, the impaired phagocytic capacity of GD3S-KO microglia could be partially restored by pre-treatment with exogenous ganglioside GD3 (Wang et al. 2021b).

The b-series GGs apparently play a critical role in regulating the structure and function of the mouse visual system. The absence of these b-series GGs in the retinas of GD3S-KO mice triggered a reduction of retinal ganglion cell density, of axons in the optic nerve and a 15% reduction of photoreceptor nuclei, a 30% reduction of light responsiveness and a reduced visual acuity and contrast sensitivity (Abreu et al. 2021).

A negative function of GGs is that they also provide effective binding sites and receptors for lectin-type bacterial exotoxins such as botulinum toxins, shiga, cholera and tetanus toxin (Cuatrecasas 1973; Dong et al. 2007; Eidels et al. 1983; Hamark et al. 2017; Lingwood 1999; Melton-Celsa 2014; Sandvig et al. 2014; Van Heyningen and Miller 1961) and viruses (Schneider-Schaulies et al. 2021).

Various aspects of glycosphingolipid metabolism and function have been reviewed recently (Ashida and Li 2014; Aureli et al. 2014; Breiden and Sandhoff 2019b; Dunn et al. 2019; Furukawa et al. 2014; Ladisch and Liu 2014; Quinville et al. 2021; Sandhoff and Sandhoff 2018; Schengrund 2015; Schneider 2014; Seyfried et al. 2014; Yu and Itokazu 2014). This review is an update and adaption of our previous review (Sandhoff and Sandhoff 2018).

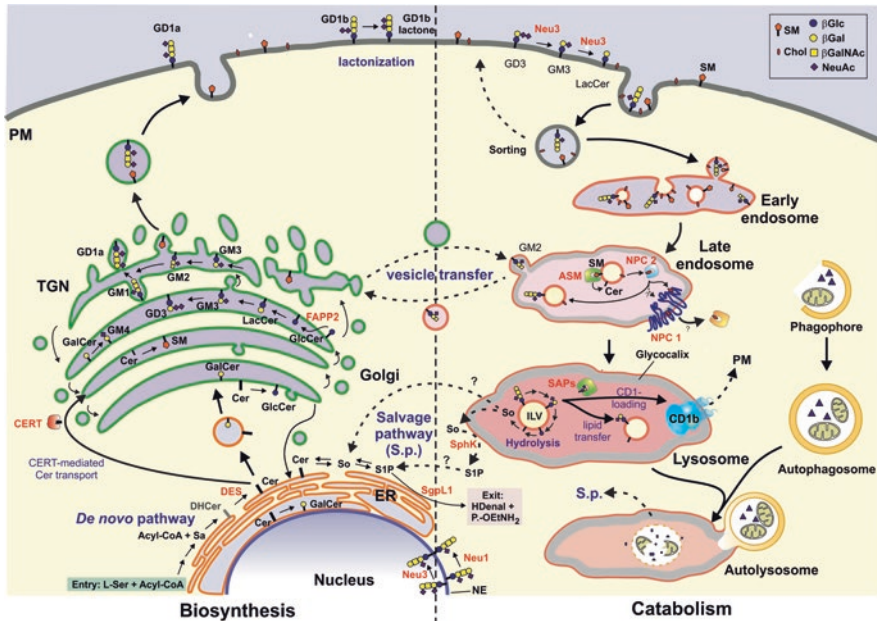


Fig. 12.4 Cellular compartmentalization and trafficking in GSL and GG metabolism modified from (Sandhoff and Sandhoff 2018) and (Breiden and Sandhoff 2020). Ganglioside biosynthesis and secretion starts at the endoplasmic reticulum (ER) and finalizes in the lumen of the Golgi system before gangliosides are carried to the plasma membrane by vesicular transport (anabolism, left side). Besides vesicular transport, some sphingolipids such as Cer and GlcCer are also transported between the ER and the Golgi system by lipid transfer proteins like CERT and FAPP2, respectively (Yamaji and Hanada 2015). Upon endocytosis and incorporation into intraendolysosomal luminal vesicles (ILVs), GG are degraded into monosaccharides, free fatty acids, and sphingoid bases (catabolism, right side), which are recycled for sphingolipid synthesis by the salvage pathway (modified after Sandhoff et al. 2018). Functional defects of any catabolic step cause an accumulation of the undegradable substrates in the lysosomes. The increasing lysosomal storage can trigger a reduced ability of lysosomes to fuse with autophagosomes, attenuating autophagy. ASM acid sphingomyelinase, Cer ceramide, CERT ceramide transfer protein, DES dihydroceramide desaturase, DHCer dihydroceramide, FA fatty acid, GlcCer glucosylceramide, ER endoplasmic reticulum, FAPP2 (PLEKHA8) four-phosphate adaptor protein 2 (pleckstrin homology domain containing A8), LacCer lactosylceramide, NE nuclear envelope, NEU neuraminidase, NPC Niemann–Pick disease type C protein, PM plasma membrane, Sa sphinganine, SAP sphingolipid activator protein, SgpL1 S1P lyase, SM sphingomyelin, So sphingosine, S1P sphingosine-1-phosphate, SphK sphingosine kinase, TGN trans Golgi network, HDenal hexadecenal, P-OEtNH₂ phosphoryletanolamine

3 Intracellular Pathways of GG and GSL Metabolism

Metabolic steps of GSL and GG metabolism occur at intracellular membranes and are intimately connected with intracellular trafficking steps between organellar membranes of the secretory and endocytic pathways (Fig. 12.4) (De Duve and Wattiaux 1966; Kolter and Sandhoff 1999). The main routes of GSL and GG biosynthesis (Figs. 12.1 and 12.3) and catabolism (Fig. 12.5) proceed in a stepwise

manner through the same membrane-bound lipophilic intermediate structures, which occur, however, at different organellar membranes of different intracellular routes: mainly the secretory pathways in case of biosynthesis and endocytotic pathways in case of degradation (Fig. 12.4). Biosynthesis starts with the formation of sphingoid bases and the hydrophobic ceramide anchor at the cytoplasmic leaflet of ER membranes (Stoffel 1971). Ceramides are substrate for various enzymes including cerebroside galactosyltransferase at the site of the ER and glucosylceramide synthase at the cytosolic site of the early Golgi. The products GlcCer and to some extent GalCer are then substrates for the luminal activity of glycosyltransferases (GTs) localized in Golgi and trans Golgi network (TGN) membranes of the secretory pathway. Remodeling and trimming of GGs has been observed by sialidases and other hydrolases of the PM and endosomal membranes, and by GTs at the TGN and PM, whereas the constitutional catabolism of GSLs and GGs takes place at intraendosomal and intralysosomal vesicles (ILVs) of late endosomes and lysosomes (Fig. 12.4) (Breiden and Sandhoff 2019b; Sandhoff et al. 2018).

4 Emerging Concepts of GSL & GG Metabolism at Organellar Membranes

The metabolism of amphiphilic, membrane-bound glycolipids is catalyzed at the membrane-water interphase

- (a) By membrane-spanning GTs in case of biosynthetic pathways or
- (b) By soluble catabolic hydrolases with the essential help of soluble lipid-binding protein cofactors at the surface of ILV membranes.

Concentration of the reaction partners within the plane of the same membrane should increase the reaction rate of the biosynthetic processes. Lipid substrates and membrane-bound enzymes should interact within the plane of the membrane by diffusion following a two-dimensional form of the Michaelis-Menten equation (Scheel et al. 1982).

Indeed, biosynthetic reaction rates are significantly increased by concentrating membrane-bound enzymes, the GTs or multi-GTs complexes, and membrane-bound GSL substrates together within the same organellar membranes of the secretory pathways, thereby avoiding needless molecular waste in the cellular environment. Catabolic reaction rates are sped up by binding cationic, positively charged lysosomal hydrolases and lipid binding proteins by forces of enzyme-substrate affinity and above all by electrostatic attraction to the negatively charged surfaces of the GSL-substrate containing ILVs in the lysosomal compartment (Breiden and Sandhoff 2019b).

5 Emerging Topology of Glycolipid Biosynthesis

The Golgi is the main glycosylation site of the cell. It harbors a stack of discontinuous cisternae that contain biosynthetic glycosyltransferases (GTases) in specific cisternae along the cis-trans axis of the organelle. A matrix protein, GRASP55, regulates the polarized location and distribution of the GTases in the Golgi and controls GSL biosynthesis (Pothukuchi et al. 2021).

Synthesis of sphingoid bases and the rather hydrophobic dihydroceramides and ceramides occurs at the cytosolic leaflet of the ER membranes and is catalyzed by enzymes that are integral membrane proteins, bound to the same ER membranes as their lipid substrates. Generated (dihydro)ceramides are then transferred to the cytosolic leaflet of Golgi membranes to serve as substrates of the glucosylceramide synthase. From here, the generated glucosylceramides are transferred to the luminal leaflet of the Golgi membranes by ATP-binding cassette transporters. At the luminal side they can be converted to lactosylceramide (LacCer). Multiple ABC transporters apparently provide distinct but overlapping GlcCer and LacCer pools at the luminal surface of Golgi membranes for anabolism of different GSL series by metabolic channeling (Budani et al. 2021). LacCer can be formed by one of two lactosylceramide synthases (Lannert et al. 1994; Nishie et al. 2010; Tokuda et al. 2013). LacCer itself is substrate for six transferases guiding GSL synthesis into different GSL series. Two of them, the GM3-synthase as well as the GA2-synthase, will guide anabolism into ganglio-series GSLs/GGs. These integral membrane-spanning GTs exhibit their catalytic activity at the luminal site of the Golgi and TGN membranes. The lumenally oriented GSLs/GGs reach the plasma membrane by vesicular transport and are therefore mainly located in the extracellular leaflet of the PM (van Meer and Hoetzel 2010; van Meer et al. 2008).

Contrary to the above mentioned neuronal GGs, the smallest ganglioside, GM4, is derived from galactosylceramides, just as is the case for sulfatide (SM4s) (Fig. 12.3). All three lipids, GalCer, SM4s, and GM4, are typical components of myelin and are produced by oligodendrocytes in the central nervous system. GM4 hardly occurs in neurons but has been detected in low concentrations in myelin (Ledeen et al. 1973; Yates 1986), erythrocytes, kidney, and intestine (Iwamori et al. 1984; Tadano and Ishizuka 1980). Its biosynthesis along the secretory pathway has been discussed before (Sandhoff and Sandhoff 2018).

6 Generation of Cell-Type-Specific Ganglioside Patterns

There are several factors contributing to the generation of cell type specific patterns on the surface of mammalian cells, which may be summarized as combinatorial biochemistry. Initially surprisingly, most of the enzymes involved in biosynthetic

pathways of GGs and GSLs have a rather poor specificity for their lipid and glycolipid substrates, e.g. ceramide synthases accept sphingoid bases and activated fatty acids of different chain length, saturation and hydroxylation grade. Likewise GTs exhibit a rather low substrate specificity. GalNAc-transferase for example converts LacCer as well as the GGs GM3, GD3, and GT3 into higher GGs of the ganglio-series (Pohlentz et al. 1988) (Fig. 12.3). In this way, the combinatorial activity of only a few promiscuous GTs, the variable levels of which are mostly controlled by transcriptional regulation, allows for the formation of cell type specific GSL patterns on cellular surfaces (Kolter et al. 2002). The combinatorial action of GTs is mirrored by the interplay of ceramide synthases with the cell type specific machinery for synthesis of sphingoid bases and fatty acids to define their ceramide anchor pattern (Kihara 2016). Whereas the sphingoid base C18-sphingosine is present in all cell gangliosides, C20-sphingosine containing gangliosides are rather minor, or absent. They appear, however, during differentiation of neurons and their ratio increases in the brain throughout the life span (Sonnino and Chigorno 2000). During neural differentiation, dynamic changes occur in the pattern of carbohydrate-rich molecules, including in the ganglioside composition. GG GD3 is the predominant ganglioside species in neural stem cells and modulates their proliferation as well as their long-term maintenance (Itokazu et al. 2018).

Cell specific GG and GSL patterns and metabolism have even been observed for different types of neurons, including granule neurons, pyramidal neurons and Purkinje cells (Furuya et al. 1996; Kotani et al. 1992, 1994; Molander-Melin et al. 2004; Tai et al. 1999; Taniike et al. 1995). Cell type- and cell-specific expression of GG and GSL are obviously obtained by a combination of several factors. Major players are (a) the cell type specific expression of often promiscuous biosynthetic enzymes (SPT subunits, ceramide synthases and GTs) and (b) the availability of local acyl-CoA donors and glycolipid acceptors at the organellar membranes of the secretory pathway. Of course, further well-known factors like pH value, ionic strength, ion composition and the availability of soluble enzyme substrates like activated sugar nucleotides in the lumen of the Golgi and TGN compartment are also of great importance.

Emerging factors like membrane fluidity, lipid and protein composition of organellar membranes, and nutritional state will presumably increasingly be recognized as important for the regulation of biosynthetic and remodeling steps in GG and GSL metabolism. For example, the anionic lipid phosphatidylglycerol (PG) stimulates GG biosynthesis in detergent-free in vitro assays (Yusuf et al. 1983a, b) and an increase of membrane fluidity as triggered by the addition of general anesthetics (e.g. halothan, Xenon gas) or a series of fatty acids with declining acyl chain lengths stimulates desialylation of oligosialo-GGs by membrane bound sialidases (Scheel et al. 1982). Finally, high fat or the so called western diet may cause increased production of C16-sphingolipids (Hla and Kolesnick 2014) including for example an incorporation into ganglioside GM3, which in turn can downregulate insulin receptor activity thereby supporting metabolic syndrome and type 2 diabetes mellitus (Lipina and Hundal 2015).

7 Enzyme Catalysis at Membrane Surfaces

Biosynthetic steps are catalyzed by integral membrane-bound enzymes, which can interact with their lipid substrates by diffusion within the plane of the membrane. This is the case for enzymes needed for sphinganine (Sa) and ceramide formation in the cytosolic leaflet of the ER and their membrane-bound lipid substrates, whereas the soluble serine substrate can interact with the membrane-bound SPT through the aqueous space (Fig. 12.4) (Braun et al. 1970; Braun and Snell 1968; Kolter and Sandhoff 1999; Stoffel et al. 1968a; Williams et al. 1984). GTs are also membrane spanning proteins, which interact with their lipid substrates by lateral diffusion within the plane of the membrane, and directly with the water-soluble activated sugar nucleotides in the lumen of the Golgi and TGN.

The kinetics of lipid substrate turnover by membrane-bound GTs at the lipid-water interphase, however, has been only poorly studied, mostly in the presence of detergents, which profoundly obscure the physiological interaction between membrane-bound substrate and enzyme. A model case has been investigated, however, for the interaction of plasma membrane-bound sialidases (presumably Neu3) and the radiolabeled GG GD1a in isolated synaptosomal membranes (Durrrie et al. 1988; Ohman 1971; Preti et al. 1980; Saito et al. 1995; Tettamanti et al. 1980). As expected, GG hydrolysis does not follow the well-known Michaelis-Menten kinetics, but its two-dimensional version. A reproducible Michaelis-Menten constant was obtained only when the substrate concentration was presented as amount of GD1a per membrane-surface available in the incubation assay and not as amount of GD1a per incubation volume, as usually done for solutes (Sandhoff and Pallmann 1978; Scheel et al. 1982).

Analysis also revealed a stimulation of GG hydrolysis by sialidase in isolated neuronal membranes with increasing membrane fluidity (Sandhoff and Pallmann 1978; Scheel et al. 1982, 1985) modulated by the addition of free FAs with different chain lengths or general anesthetics like halothane or Xenon gas (Sandhoff and Pallmann 1978; Scheel et al. 1985). The concept of two-dimensional kinetics, however, does not apply to the lysosomal GG catabolism, driven mostly by water-soluble, protonated and positively charged mammalian exo-hydrolases in the lysosomal compartment. Their interaction with membrane-bound substrates is mainly facilitated by electrostatic attraction to the surface of negatively charged anionic ILV-membranes carrying GSL and other lipid substrates. Degradation of GSLs, GGs, SM and other complex lipids is mediated by hydrolases at ILV-surfaces in cooperation with essential small promiscuous lipid binding proteins, the sphingolipid activator proteins (SAPs) (Breiden and Sandhoff 2019b).

8 GSL Biosynthesis and Salvage Pathways

8.1 Ceramide Synthesis

De novo synthesis of sphingolipids (SLs) starts with the condensation of an activated fatty acid (mainly palmitoyl-CoA) with L-serine at the cytosolic leaflet of the ER membrane (Figs. 12.1 and 12.3). The rate limiting step is catalyzed by the serine-palmitoyl-CoA-transferase (SPT) (Hornemann et al. 2009), which is regulated in a feedback loop by inhibitory ORM proteins in yeast (Breslow et al. 2010; Han et al. 2010), and ORMDL proteins in mammals (Gupta et al. 2015; Siow and Wattenberg 2012). Interestingly, the chain length of the product 3-keto-sphinganine also known as 3-keto-dihydrosphingosine (KDS) is influenced by the presence of either of the two small subunits SPT_{ssa} and SPT_{ssb} (Han et al. 2009; Zhao et al. 2015). KDS is reduced to Sa by the 3-ketodihydrosphingosine reductase (KDSR) with the help of NADPH; this, in turn, is a substrate of ER-bound ceramide synthases (CerSs). They convert sphingoid bases, e.g. Sa and sphingosine (So), from de novo and salvage pathways to the respective dihydroceramides and ceramides at the ER. In mammals, there are 6 genes leading to different CerS isoforms, which are cell-type specifically expressed. In combination with the available acyl-CoA profile, the expressed CerS pattern leads to characteristic ceramide anchor profiles of SLs and GGs in different cell types and organs (Levy and Futerman 2010; Morell and Radin 1970; Pewzner-Jung et al. 2006; Rabionet et al. 2014; Sandhoff 2010; Sassa et al. 2016; Venkataraman and Futerman 2002). The activities of CerS are regulated at the transcriptional and the protein level (D'Angelo et al. 2007; Yamaji and Hanada 2015). The ceramide anchors of neuronal GGs contain mainly stearic acid due to high expression levels of CerS1 (Jiang et al. 1998; Riebeling et al. 2003; Sambasivarao and McCluer 1964). Enzyme activities of CerS2-6 are regulated in several ways (Wegner et al. 2016), e.g. by phosphorylation. They contain a HOX-domain (Venkataraman and Futerman 2002), which may have a regulatory role as a nuclear DNA-binding protein as shown for the single CerS of drosophila (Sociale et al. 2018; Voelzmann et al. 2016). Transcripts of several genes involved in lipid metabolism and cell division in mouse liver are also regulated by CerS2 activity, suggesting a role of very long acyl chain ceramides in the nucleus for the transcriptional regulation of target genes (Bickert et al. 2018; Pewzner-Jung et al. 2010).

Dihydroceramide (DHCer) formed by the acylation of Sa is subsequently converted to ceramide (e.g. in neurons) by the dihydroceramide desaturase 1 with the cosubstrates NAD(P)H and oxygen by generating a trans-double bond in the 4-position of the sphingoid base (Fig. 12.1) (Geeraert et al. 1997; Michel et al. 1997). DHCer can also be converted to phytoceramides, especially in intestinal tissue (Enomoto et al. 2006; Omae et al. 2004; Ternes et al. 2002).

8.2 *Ganglioside Synthesis and Function*

Synthesis of complex GSLs and GGs occurs by the stepwise addition of monosaccharides (Fig. 12.3) to ceramides at the membranes of the Golgi and TGN (Daniotti and Iglesias-Bartolome 2011; Kolter and Sandhoff 1999) (Fig. 12.4). Ceramides reach the Golgi membranes either by vesicular transport or by the inter-organelle transfer protein CERT, preferentially transporting ceramides with C14 to C30 acyl chains (Yamaji and Hanada 2015).

GSL formation is initiated by the addition of glucose to ceramide by UDP-glucose ceramide glucosyltransferase, the glucosylceramide (GlcCer) synthase at the cytosolic leaflet of early Golgi membranes (Futerman and Pagano 1991; Ichikawa et al. 1998; Jeckel et al. 1992) and thus GlcCer may be present on the cytosolic leaflet of cell membranes contrary to all other GSLs and GGs. Generated glucosylceramide can be translocated to the luminal face of Golgi membranes (D'Angelo et al. 2007), where it is converted to lactosylceramide (LacCer) by one of two lactosylceramide synthases (Nishie et al. 2010; Tokuda et al. 2013).

LacCer is the precursor of different GSL series, i.e. ganglio-, asialoganglio-, sulfoganglio-, globo-, isoglobo- and (iso)lacto series (Fig. 3 and Tab. 1 of reference Sandhoff and Sandhoff 2018). These series are formed by cell type specific glycosyltransferases, integral membrane proteins at the Golgi and TGN membranes, which are organized in distinct multi-enzyme complexes (Daniotti et al. 2017; Giraudo and Maccioni 2003).

In neurons, the biosynthesis of a- and b-series gangliosides starts with the transfer of sialic acid from CMP-sialic acid to the galactosyl residue of LacCer in 2,3 linkage to generate the simple ganglioside GM3 (Ishii et al. 1998) (Figs. 12.3 and 12.4). Further sialylation of GM3 yields GD3 and finally GT3 (Kono et al. 1996; Yoshida et al. 1995), the precursors of b- and c-series gangliosides, respectively.

As suggested by Saul Roseman, biosynthesis of GG is often mediated by multi-glycosyltransferase complexes (Bagatolli and Gratton 2000; Roseman 1970; Simons and Gerl 2010; Simons and Toomre 2000; Yusuf et al. 1983b, 1984) located in Golgi and TGN membranes (Fig. 12.4), which stabilize enzymes and improve glycolipid synthesis (Maccioni 2007; Spessott et al. 2012). GG glycosyltransferases (GGTs) are type II membrane glycoproteins (Martina et al. 2000), some of them are S-acylated at conserved cysteine residues, which may be involved in the formation of homodimers through disulfide bonds (Chumpen Ramirez et al. 2017).

Enzymatic steps for the synthesis of GGs had already been identified in the 1960s and 70s (Kaufman et al. 1968; Keenan et al. 1974). It was expected that the formation of a- and b-series gangliosides would be catalyzed by different GTs. Kinetic competition experiments between the lipid acceptors GM3 (of the a-series) and GD3 (of the b-series), however, proved that both lipid substrates competed for the same active site of a quite unspecific GalNAc-transferase (Pohlentz et al. 1988). All GTs involved in the biosynthesis of complex gangliosides turned out to be rather

promiscuous for their lipid substrates (Iber and Sandhoff 1989; Iber et al. 1991, 1992), resulting in the assembly line given in Fig. 12.3. Further modifications of the formed gangliosides (see Fig. 12.3) by different forms of sialylation, O-acetylation and lactonization have been reviewed (Sandhoff et al. 2018). In vivo labeling of lipids from 14-day-old rat brain with (^{35}S)-sulfate also allowed the identification of sulfated, sialic acid containing lipids, gangliosides in intact brain tissue, e.g. in oligodendrocytes (Farrer and Quarles 1997). Biosynthesis of gangliosides can be promoted by a low-carbohydrate ketogenic diet via transcriptional regulation of genes related to ganglioside biosynthesis and downregulation or even suppression of the GM2AP-gene, coding for the GM2-activator protein, an essential cofactor for lysosomal ganglioside catabolism (Okuda 2019).

GG biosynthesis is cell-type specific and regulated at the transcriptional and posttranslational protein level (Gupta et al. 2015; Han et al. 2010; Harmon et al. 2013; Hornemann et al. 2009; Kishimoto and Radin 1966; Kolter et al. 2002; Pewzner-Jung et al. 2006; Sandhoff 2010, 2012; Sandhoff and Pallmann 1978; Sassa et al. 2016; Siow and Wattenberg 2012; Tettamanti et al. 1980; Yusuf et al. 1983b) as well as by a sequential organization and coordination of glycosyltransferases with the traffic of biosynthetic intermediates through the membranes of the secretory pathway (Yu et al. 2004). The recent development of high resolution mass spectrometry for the analysis of complex ganglioside patterns of the human motor cortex identified an unexpected complexity of at least 83 different gangliosides, exhibiting a higher degree of sialylation than known before, including fucogangliosides and glycan chains elongated by either-acetylation and /or acetate anion attachment (Ica et al. 2021).

Gangliosides are enriched in the outer leaflet of neuronal plasma membranes. Though their detailed organization is still elusive, a model based on dynamic simulations proposes a dynamic membrane structure composed of transient domains with liquid-ordered character that form and disappear on the microsecond time scale. Nano-domains consisting of gangliosides were observed in the outer leaflet (Ingolfsson et al. 2014). These domains can be small and transient or larger and more persistent and their location can correlate with PIP- and cholesterol rich domains on the inner leaflet of the membrane (Ingolfsson et al. 2017), suggesting the existence of lipid cross-talk between leaflets. Using a freestanding, planar lipid membrane system, Hyun-Ro Lee and Siyoung Q Choi analyzed sphingomyelinase (SMase of *Bacillus cereus*) mediated membrane remodeling in vitro (Lee and Choi 2021). Their results indicated that SMase activation at the surface of the sphingomyelin and GM1 containing leaflet of the lipid bilayer can induce GM1 clustering by at least 2 different spatio-lateral scales. First, ceramide rich domains are derived from less ordered phases, which grow to a few micrometers in size. Second, SMase triggers a reduction of the area covered by lipid aggregates (“rafts”) thereby condensing GM1 clusters to nanosized objects. The obtained data suggest that similar GM1 condensation processes may occur also in cellular plasma-membranes, which might facilitate and modify signal transmission processes.

GSL expression presents a great potential for regulation of neural development and progression of neurological pathology (Bottai et al. 2019). Different authors

recently reviewed the role of neutral GSLs as neuroinflammatory signaling molecules in neurodegeneration (Mutoh 2021) and the physiology of GGs and of anti-GG antibodies in human diseases (Cuttillo et al. 2020) as well as the role of GGs in peripheral pain mechanisms (Santha et al. 2020). GSLs may also play a role in neuronal polarity (Cui et al. 2019).

Neuronal development is associated with dynamically changing GG patterns. Neuronal stem/progenitor cells express stage-specific antigen-4 (SSEA-4), a complex ganglioside of the globoseries (Barraud et al. 2007; Brochner and Mollgard 2016; Kannagi et al. 1983), which is overexpressed in some cancers, linked to disease progression, and defines spontaneous loss of the epithelial phenotype in human solid tumor cells (Sivasubramaniyan et al. 2015). The simple GGs GM3 and GD3 are associated with neural tube formation and neural stem cell proliferation. They decline with neural differentiation, the time point at which complex ganglioseries GGs increase to reach adult levels during axonal/dendritic arborization and synaptogenesis (Olsen and Faergeman 2017). Adult neuronal complex GGs mainly contain a neutral tetraacylceramide backbone (i.e. GM1a, GD1a, GD1b, GT1b) but almost no detectable GGs with a triaacylceramide backbone (i.e. GM2, GD2). Especially GD2 is a target of tumor therapy as it has limited expression in normal tissues but is overexpressed in a variety of tumors (Nazha et al. 2020) including neuroblastoma, which develops from neural crest cells (Schengrund 2020).

The roles of gangliosides as modulators of receptors (Ledeen and Wu 2018a) and in inflammation (Furukawa et al. 2018) have recently been reviewed. Neurostatin, (a derivative of GD1b O-acetylated on the outer sialic acid) and other O-acetylated GGs were described as neuroprotective regulators and can reduce microglia activation (Yanguas-Casas et al. 2019). Different classes of gangliosides are expressed in nociceptive primary sensory neurons and play a role in peripheral pain mechanisms (Santha et al. 2020). Endothelial cells and cancer cells express their own specific ganglioside pattern, especially ganglioside GM3. At least in human breast cancer, GM3 synthase is highly expressed in immune cells and in vascular endothelial cells. In GM3-synthase-KO mice, tumor growth and angiogenesis is increased, suggesting that ganglioside GM3 attenuates tumor angiogenesis (Suzuki et al. 2021). GM3 in the plasma membrane can interact with a transmembrane segment of the EGF receptor (EGFR) laterally as detected by FRET (Förster resonance energy transfer) technology in lipid bilayers. It suppresses cell growth by preventing the autophosphorylation of epidermal growth factor and the formation of active EGFR dimers (Nakano et al. 2021).

9 Inherited Errors of GG Biosynthesis

Blocks in GG biosynthesis, as generated in GM3-only KO mice and other mouse models indicate **no** feedback inhibition due to accumulating intermediates (Sandhoff 2012). With respect to defects in GG biosynthesis, mouse models and inherited human defects do not necessarily correlate: Inherited defects of ganglioside GM2

biosynthesis cause severe infantile epilepsy and spastic paraplegia (Harlalka et al. 2013), whereas mice lacking major gangliosides due to a GM2/GD2 deficiency develop manifestations of Parkinsonism (Wu et al. 2011). Human defects in GM3 synthesis (Fragaki et al. 2013; Simpson et al. 2004) cause infantile-onset symptomatic epilepsy syndrome or refractory epilepsy and mitochondrial dysfunction, while GM3 synthase-deficient mice appeared rather normal but are deaf (Yoshikawa et al. 2009, 2015). Congenital disorders of ganglioside biosynthesis have been reviewed recently (Dunn et al. 2019; Li and Schnaar 2018; Trinchera et al. 2018).

10 Remodeling and Recycling of Cell Surface Gangliosides

Complex GSLs and GGs reach the cell surface by vesicular transport. They are enriched in the extracellular leaflet of PMs, GGs especially at synaptic surfaces, where they undergo lateral associations and trans interactions (Mori et al. 2012). GG interactions may be regulated by PM located sialidases, like the PM bound Neu3 (Rodriguez-Walker and Daniotti 2017) and sialyltransferase activity, both of which are present in synaptosomal membranes (Durrie et al. 1988; Ohman 1971; Preti et al. 1980; Saito et al. 1995; Tettamanti et al. 1980).

Polysialogangliosides expressed on cellular surfaces can serve as ligands for lectins and substrates for enzymes (Sonnino and Prinetti 2016). Their composition can be adjusted to changing membrane functions by desialylation mostly catalyzed by Neu3 (Miyagi and Yamaguchi 2012; Pan et al. 2017; Shiozaki et al. 2015) and by recycling. Radiotracer techniques and ESR spectroscopy studies on cultured GM2 gangliosidosis fibroblasts demonstrated that the exogenously added radiolabeled GG GM2 can be incorporated into the PM and directly glycosylated to form radiolabeled GGs GM1 and GD1a despite a complete cellular block in GM2 degradation, thus excluding transfer through the endo-lysosomal compartment. A direct glycosylation of labeled GGs GM2 and GM1 was also observed in normal control cells, presumably after trafficking from endosomes to the TGN compartment (Schwarzmann et al. 1983; Sonderfeld et al. 1985) (Fig. 12.4), which may involve the retromer complex (Lucas et al. 2016).

Intracellular trafficking, especially of GG GM1 having unsaturated or shorter ceramide anchors (Chinnapen et al. 2012; Saslowsky et al. 2013), was observed as an important aspect of metabolism and cellular uptake of cholera toxin. Recycling of fluorescently labeled glucosylceramide was also observed from the PM through early endosomes and back (Kok et al. 1989) as well as the glycosylation of non-degradable glucosylceramide analogs involving trafficking from proximal to distal Golgi cisternae to form complex gangliosides (Schwarzmann et al. 1995). Gangliosides were also identified in the nucleus, their metabolism and functions, however, remain mostly unknown to date. GGs GM1 and GD1a are located in both membranes of the nuclear envelope together with two neuraminidases (Ledeen and Wu 2011).

11 Concepts of the Constitutive Degradation of Gangliosides and Glycosphingolipids at ILVs

11.1 Location and Topology of Sphingomyelin, Glycosphingolipids and Ganglioside Catabolism

The constitutive degradation of membrane lipids like sphingolipids (SLs) and phospholipids (PLs) takes place at the surface of ILVs in the lysosomal compartment. SLs and GGs of the PM can reach ILV membranes by pathways of endocytosis (Fig. 12.4). The concept is supported by biochemical and immune-electro-histochemical studies on the endocytosis of biotinylated and radiolabeled ganglioside GM1 (Mobius et al. 1999b) and allows one to understand mechanisms of drug induced phospholipidosis. Most steps of membrane lipid catabolism are catalyzed by rather promiscuous hydrolases (lipases, phospholipases, glycosidases), for SLs it is often with the essential help of lipid binding proteins, the SAPs.

11.2 Endocytosis of Gangliosides

Desialylation of complex polysialylgangliosides is catalyzed by membrane bound sialidases, Neu1, Neu4 and Neu3, to generate ganglioside GM1 (Smutova et al. 2014; Timur et al. 2015). This redundancy may explain why complex polysialogangliosides do not accumulate in the brain tissue of 2 months old mice deficient in either Neu1, Neu3, Neu4 or Neu3 and Neu4 (Pan et al. 2017). Desialylation is an initial step in ganglioside degradation in mammalian tissues (Monti et al. 2010) and can take place at the plasma membrane or later in the endosomes and lysosomes. The lysosomal sialidase Neu1 is a member of a multi-enzyme complex together with protective protein/cathepsin A (a stabilizing protein for sialidase Neu1), and the GM1 cleaving β -galactosidase (Nicoli et al. 2021; Sandhoff et al. 2018). Inherited defects of Neu1 leading to sialidosis cause accumulation of sialylated metabolites (d'Azzo et al. 2015), including an amyloidogenic processing of an over-sialylated amyloid precursor protein in lysosomes and an extracellular release of A β -peptides (Annunziata et al. 2013), whereas genetic defects in protective protein/cathepsin A trigger a storage of GM1 besides oligosaccharides and other glycolipids (Bonten et al. 1996; d'Azzo and Bonten 2010).

Lysosomes degrade a variety of macromolecules and complex lipids and release their components into the cytosol as nutrients for use in energy metabolism and biosynthetic salvage pathways (Kolter and Sandhoff 2005). Macromolecules and complex lipids can reach the lysosomes and the intra-lysosomal luminal vesicles (ILVs) by endocytotic pathways (Kolter and Sandhoff 2005), and probably by phagocytosis and autophagy as well (Florey and Overholtzer 2012). Metabolic studies and immuno-electron microscopic observations indicate that PM-bound radio- and biotin-labeled ganglioside GM1 reaches intralysosomal luminal vesicles (ILVs)

for final degradation (Mobius et al. 1999a) (Fig. 12.4). Released components like So, fatty acids, and monosaccharides can leave the lysosomes to reach the ER and the cytosol. Released So can be incorporated again into newly synthesized SLs and GGs (Gillard et al. 1996; Sonderfeld et al. 1985; Tettamanti 2004; Tettamanti et al. 2003), but may also be phosphorylated to generate SIP for final degradation of the sphingoid base by SIP-Lyase (Merrill Jr. et al. 1997; Stoffel et al. 1968b).

11.3 Generation of ILVs During Endocytosis and GSL-Catabolism

As proposed (Furst and Sandhoff 1992), ILVs are generated by an inward budding of the endosomal membrane and budding off of ILVs into the luminal space, which is catalyzed by ESCRT proteins (Wollert and Hurley 2010) (Fig. 12.4). Components of the endosomal membrane sorted into ILVs can be catabolized by the digestive juice in lysosomes. ILV degradation will destabilize and destroy the bilayer structure of ILV membranes and transiently generate micellar and other amphiphilic aggregates, which either may dissolve or fuse with new incoming ILVs.

Catabolism of GSLs, SLs and GGs in ILVs can be blocked by genetic defects affecting lysosomal proteins (Kolter and Sandhoff 2005), causing lysosomal lipid and membrane storage diseases. An inherited defect of prosaposin, the precursor of four lipid binding proteins, the saposins A, B, C and D, triggers an excessive accumulation of GSLs, SLs and intra-lysosomal ILVs with an average diameter of 90 nanometers within the lumen of late endosomes and lysosomes (see insert in Fig. 12.5). The blocked lipid catabolism can be restored and the pathological lipid and ILV accumulation can be completely reversed by feeding nanomolar concentrations of the missing prosaposin to patients' cultured fibroblasts (Bradova et al. 1993; Burkhardt et al. 1997; Harzer et al. 1989; Mobius et al. 1999a; Sandhoff et al. 2018; Schnabel et al. 1992). ILVs are obviously the main location for degradation of SLs including GGs and membranes.

Progranulin acts as a key regulator of lysosomal function. It is a large glycoprotein, proteolytically cleaved in the lysosomal compartment or in the extracellular matrix to generate up to eight small granulin glycopeptides. In 2006, the discovery of the heterozygous mutation of the granulin (GRN) gene leading to progranulin haploinsufficiency in patients with frontotemporal dementia stimulated the search for its many properties and functions (Wikipedia 2021). Progranulin enhances memory in normal aging and Alzheimer's disease mice (Farr et al. 2021). Delivering progranulin to neuronal lysosomes protects against excitotoxicity (Davis et al. 2021). Both, glucocerebrosidase and progranulin are possible targets in the treatment of Parkinson's disease (Rodrigues and Kale 2021).

Homozygous GRN gene mutations cause a neuronal ceroid lipofuscinosis with accumulation of auto-fluorescent lipofuscin, impairment of lysosomal activity, microgliosis and brain degeneration (Nguyen et al. 2013; Paushter et al. 2018).

Defective lysosomal lipid catabolism is a common pathogenic mechanism for dementia. Defective lipid clearance from endo-lysosomes is a central driver of Alzheimer's disease. Impaired cholesterol turnover or sphingolipid catabolism is sufficient to produce the pathological hallmarks of dementia (Lee et al. 2021; Wang et al. 2021c). Progranulin mutations result in impaired processing of prosaposin and reduced glucocerebrosidase activity (Valdez et al. 2020). GRN variants were identified that may contribute to lifetime risk of multiple neurodegenerative diseases. Expression quantitative trait locus analyses and genome-wide association studies identified functional associations between increasing genetic risk in the GRN region and decreased expression of the GRN gene in Parkinson's, Alzheimer's and amyotrophic lateral sclerosis. GRN expression mediates neuroinflammation functions related to multiple neurodegenerative diseases (Nalls et al. 2021). The lysosomal storage disorder caused by GRN loss of function can be rescued with a brain penetrant progranulin biologic (Logan et al. 2021).

11.4 Maturation of Intra-Lysosomal Luminal Vesicles (ILVs) and Lipid Sorting

Whereas ILVs can be attacked by the lysosomal juice, the lysosomal perimeter membrane is protected by a thick glycocalyx, which covers its luminal surface and prevents hydrolases and SAPs from reaching and digesting the perimeter membrane. The coat consists of integral glycoproteins, heavily N-glycosylated with almost digestion-resistant poly lactosamine units (Eskelinen et al. 2003). Defects in the biosynthesis of the lysosomal perimeter membrane glycoproteins, however, increase their turnover (Eskelinen et al. 2003) and may attenuate their barrier function. Most of the degradation products released within the lysosomal compartment need membrane carriers to move from the lysosol to the cytosol (Eskelinen et al. 2003; Sandhoff et al. 2018), where they can be used for biosynthesis of new macromolecules or as fuel for energy metabolism.

The degradation of complex lipids by lysosomal enzymes is substantially facilitated by a lipid sorting process leading to a maturation of ILVs at the level of late endosomes (Gallala and Sandhoff 2011; Kolter and Sandhoff 2005): membrane stabilizing lipids of the plasma membrane, such as sphingomyelin (SM) and cholesterol, are removed. Acid sphingomyelinase (ASM) cleaves SM and other phospholipids, releasing ceramide and diacylglycerol (Breiden and Sandhoff 2021; Oninla et al. 2014; Quintern et al. 1987). Reconstitution studies in vitro suggest that the decrease of inhibiting SM levels and the concomitant increase of stimulating ceramide levels are prerequisites for an effective secretion of the non-degradable cholesterol from the ILVs by the transfer- and sterol-binding glycoprotein NPC2 (Abdul-Hammed et al. 2010). The anionic lysophospho-glycerolipid bis(monoacylglycerol)phosphate (BMP) contributes to a negative surface charge on the ILV membranes that attracts and binds the protonated NPC2 protein facilitating

its cholesterol transfer function. Reversely, increasing SM levels in ILVs of ASM deficient cells in NPA & B disease inhibit cholesterol secretion from the lysosomal compartment (Abdul-Hammed et al. 2010; Enkavi et al. 2017; Oninla et al. 2014). In addition to BMP, other anionic phospholipids like phosphatidic acid (PA), PG and phosphatidylinositol (PI) were also found, using in vitro studies, to stimulate cholesterol transfer by NPC2. BMP itself is a lysosome-specific lipid as it is a transient intermediate of PG degradation within the ILVs. As BMP is catabolized rather slowly, for example by ASM (Breiden and Sandhoff 2021), significant BMP levels can build up within lysosomes (Abdul-Hammed et al. 2010; Gallala and Sandhoff 2011; Sandhoff et al. 2018).

In summary, lowering SM and cholesterol levels and generating anionic BMP during maturation of ILVs appear to be essential to reach physiological rates of GSL and SL degradation in the endo-lysosomal pathway (Sandhoff 2013).

11.5 BMP Formation Contributes Significantly to the Quintessential Negative Surface Charge on ILVs

Reaching ILVs in the lysosol, membrane lipids are usually readily degraded in active lysosomes. The catabolism of PG, however, generates an intermediate, the anionic bis-lysolipid BMP, which is only slowly catabolized and therefore increases to make up to 40-60 mol% of ILV-phospholipids (Gallala and Sandhoff 2011; Kobayashi et al. 1999; Mobius et al. 2003; Sandhoff et al. 2018). Like other phospholipids (PA, PG and PI), BMP is negatively charged, even at pH values as low as pH 4 (Oninla et al. 2014; Wilkening et al. 1998). Reconstitution studies in vitro reveal it to generate a negative surface potential on ILVs (Oninla et al. 2014). This potential should force binding and concentration of lysosomal enzymes and SAPs on the surface of ILVs by electrostatic interaction as these proteins are protonated in the acidic lysosol with pH values around 4–5 (Kolzer et al. 2004; Oninla et al. 2014). Importantly, surface bound lysosomal enzymes like ASM (Hurwitz et al. 1994), acid ceramidase (Elojeimy et al. 2006), hexosaminidases A and B (HexA, HexB) and other glycoproteins are partially protected against premature proteolytic digestion in the lysosome. Compensation of the negative surface charge of ILVs favors release of these enzymes and activator proteins and subsequently their proteolytic digestion. This is observed when feeding cationic amphiphilic drugs (such as desipramine) to cultured fibroblasts, which causes a drop in lysosomal degradation activity and triggers a phospholipid storage disease, i.e. an induced phospholipidosis (Hurwitz et al. 1994; Kolzer et al. 2004; Lullmann et al. 1978; Sandhoff et al. 2018). The catabolism of membrane lipids by the concerted action of lipases and glycosidases is supported by the mixture of five known lipid binding and membrane disturbing SAPs and will destroy the lipid bilayer and topology of ILVs. Micelles and smaller lipid aggregates may well be formed as intermediates of ILV degradation.

11.6 Regulation of GG Catabolism and the Removal of Inhibitory Lipids from Intraendosomal Vesicles and ILVs

The plasma membrane stabilizing lipids SM and cholesterol are inhibitors of GSL and GG catabolism in the lysosomes and are therefore removed from ILVs during endocytosis by a NPC2-mediated cholesterol efflux and an ASM mediated SM digestion. The inherited NPC2-deficiency and the reduced ASM activity in the lysosomes of Niemann-Pick disease type C2 patients may well cause a lysosomal cholesterol and sphingomyelin accumulation (Vanier 1983, 2015), which could strongly inhibit the BMP-enhanced hydrolysis of GM2 and glucosylceramide (Abdul-Hammed et al. 2017; Anheuser et al. 2015). In wild type cells, however, hydrolysis of sphingomyelin by ASM generates stimulatory ceramide, which enhances cholesterol transfer by NPC2 tremendously (Oninla et al. 2014). Sphingomyelin degradation is obviously an initial step required for physiological secretion of cholesterol from the late endosomal compartment, which in turn is a prerequisite for lysosomal GSL and GG digestion (Sandhoff et al. 2018). The secondary storage of LacCer and ganglioside GM3 in NPC patients (Vanier 2015) might also be explained by a cholesterol mediated inhibition of several SAPs, which are important for the digestion of LacCer and ganglioside GM3, Sap A (Locatelli-Hoops et al. 2006), Sap B (Rommel et al. 2007) and GM2 activator protein (GM2AP) (Anheuser et al. 2015). The clinical course of the disease can be improved by reducing GSL storage with Miglustat, a drug inhibiting glucosylceramide synthase, the first step of GSL biosynthesis (Bowman et al. 2015; Patterson et al. 2015).

11.7 Catabolism of Gangliosides at ILVs

Catabolism of gangliosides, SM and other SLs and their lipophilic catabolites proceeds at the surface of ILVs (Figs. 12.4 and 12.5) (Kolter and Sandhoff 2005). In contrast to GG biosynthesis at the membranes of the secretory pathways, no membrane spanning enzymes are directly involved in lysosomal phospholipid and GG catabolism. The lipids of the ILVs are catabolized by soluble lysosomal glycoproteins, lysosomal hydrolases and lipid-binding sphingolipid activator proteins (SAPs).

Stepwise degradation of complex gangliosides in mammalian tissues starts with the removal of terminal sialic acid units from the oligosaccharide chain by neuraminidase(s) to generate GM1 (Fig. 12.5). GM1 degradation continues with the removal of the terminal galactose by GM1- β -galactosidase, supported by the GM2 activator protein or saposin B to yield GM2 (Sandhoff et al. 2018; Wilkening et al. 2000). Thereafter, the terminal N-acetylgalactosamine residue is split off by β -hexosaminidase A with the help of the GM2 activator protein, to form GM3

(Fig. 12.5), which can be degraded to LacCer by an α -sialidase together with Sap B. In mouse, the first two steps can be bypassed by the removal of sialic acid from GM1 and GM2, producing the corresponding asialo-derivates GA1 and GA2, respectively. The latter are degraded to form LacCer by a β -galactosidase and β -hexosaminidase A and B, respectively, with the assistance of the GM2 activator protein (Sandhoff et al. 2018; Sango et al. 1995; Seyrantepe et al. 2018). Consequently, HexA-deficiency in mice causes a very mild gangliosidosis and does not correlate with the infantile form of Tay-Sachs disease, which is caused by this deficiency in humans (Phaneuf et al. 1996; Sango et al. 1995). Additional deficiency of Neu3 in mice on top of the loss of HexA function blocks this bypass quite efficiently resulting in strong GM2 gangliosidosis (Seyrantepe et al. 2018). Sap B or Sap C-assisted action of β -galactosidase degrades LacCer to GlcCer, whose glucosyl residue is removed by Sap C-assisted β -glucosidase. Finally, ceramide is cleaved by acid ceramidase aided mainly by Sap D resulting in the sphingoid base (mainly sphingosine) and a free fatty acid (Sandhoff et al. 2018). The speed of hydrolytic steps of GM2 catabolism is supported by several factors, such as low pH values (e.g. only a narrow range of pH 3.8–4.5 allows hydrolysis of membrane bound GM2 by HexA and GM2AP (Bierfreund et al. 1999)), surface tension, and electrostatic binding of protonated and positively charged enzymes and SAPs to negatively charged surfaces of ILVs (Fig. 12.6). The optimal combination of all these regulating and essential factors restricts ganglioside catabolism almost exclusively to the ILVs in the lysosol and protects cells from spontaneous self-degradation. Most regulating mechanism of ganglioside homeostasis, however, are still poorly understood, e.g. the influence of ethanol on brain gangliosides and their turnover. In rats, even maternal alcohol consumption can impact the ganglioside content and the ganglioside catabolizing hydrolases in the brain of the offspring (Prasad 1992). The pups exposed to alcohol developed a brain with a reduced wet weight, protein, and DNA content, but with an increased concentration and content of gangliosides as assayed by the total ganglioside sialic acid content. Region-specific alterations in the ganglioside pattern were observed.

The salvage pathway, which utilizes lysosomal degradation products for biosynthesis, e.g. sphingosine for the formation of the growth factor sphingosine-1-phosphate, plays an important role in many cells and may explain that ablation of acid ceramidase blocks cell cycle progression and fertilization of Oocytes (Eliyahu et al. 2012; Lai et al. 2017).

A schematic overview of these and other degradation steps and corresponding inherited diseases, which are described elsewhere in more details is given in Fig. 12.5 (d'Azzo et al. 2019; Desnick et al. 2019; Gieselmann and Ingeborg 2019; Grabowski et al. 2019a, b; Gravel et al. 2019; Kolter and Sandhoff 2006; Levade et al. 2019; Patterson et al. 2019; Sandhoff et al. 2019; Schuchman and Desnick 2019; Schuchman et al. 2019; Suzuki et al. 2019; Valle et al. 2021; Wenger et al. 2019).

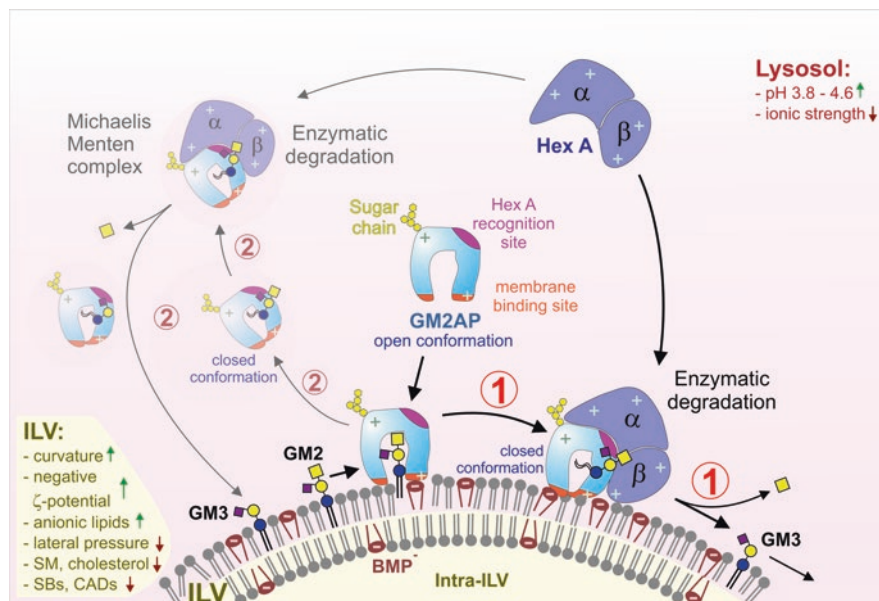


Fig. 12.6 Model of GM2 degradation by human β -hexosaminidase A (Hex A), and the cofactor GM2AP. GM2AP contains a hydrophobic cavity lined by surface loops and a single short helix. The cavity is suitable for the ceramide anchor of GM2 and other lipids. In its open conformation, GM2AP binds to the membrane using the hydrophobic loops (orange) and penetrates to a certain extent into the hydrophobic region of the bilayer. The lipid recognition site of the activator then interacts with the substrate, and its ceramide portion can move inside the hydrophobic cavity. At this point, the lipid-loaded activator may change to its closed conformation, thus the complex becomes more water soluble and can stay (1) or leave the membrane (2), exposing GM2 to the water-soluble Hex A for degradation (modified after Sandhoff and Sandhoff 2018). Besides the cofactor GM2AP, lysosomal degradation is stimulated (green arrow pointing upwards) by the acidic lysosomal pH leading to a net positive charge of hydrolases and activator proteins as well as increasing curvature, negative ζ -potential, and anionic lipids of ILVs, whereas increasing lysosomal ionic strength, too high lateral pressure, and the presence of sphingomyelin (SM), cholesterol, free sphingoid bases (SBs), and cationic amphiphilic drugs (CADs) inhibit (red arrow pointing down) lysosomal lipid (GM2) catabolism. The lysosomal-specific anionic lipid BMP serves the net negative charge, reduced lateral pressure, and increased negative ζ -potential of ILVs. The ILV-associated effects suggest digestion taking place mainly at the ILV surface (1) in vivo

11.8 SAPs Are Essential Cofactors for Lysosomal Ganglioside Catabolism

Soluble lysosomal hydrolases hardly attack lipophilic membrane components of ILVs directly due to a solubility barrier between the aqueous and the lipid phase, also known as the lipid phase problem: Hydrophobic lipids or lipid moieties of SLs like their ceramide residues are insoluble in aqueous phases, whereas hydrophilic molecules like water soluble proteins, carbohydrates or oligosaccharide chains of GSLs and GGs are insoluble in lipid phases.

Therefore the catabolism of GSLs and GGs by water soluble enzymes needs the help of membrane perturbing lipid binding proteins, the SAPs (saposin A, B, C and D, and GM2AP), small glycoproteins with amphiphilic properties, some with lipid transfer and others with fusiogenic functions at low pH values (Kolter and Sandhoff 2005). Their inherited defects cause rare, but fatal, often degenerative brain diseases (Kolter and Sandhoff 2006; Sandhoff 2012; Sandhoff and Harzer 2013) with a clinical picture quite similar to that of the respective enzyme deficiencies (Abdul-Hammed et al. 2010; Sandhoff and Harzer 2013; Schulze and Sandhoff 2014).

The GM2 activator protein (GM2AP) and the dimeric saposin B (Sap B) are promiscuous lipid binding and intervesicular lipid transfer proteins (Breiden and Sandhoff 2019b). GM2AP is an essential cofactor for the catabolism of GM2 by hexosaminidase A (HexA) and of GM1 by beta-galactosidase (Figs. 12.5 and 12.6). Its inherited deficiency causes a fatal GM2 gangliosidosis (variant AB of GM2-gangliosidosis) with a clinical picture almost indistinguishable from Tay-Sachs disease. GM2AP can extract GGs from vesicular membranes at low pH values, forming a stoichiometric and soluble GG-protein complex, which is recognized by HexA as substrate, forming a Michaelis-Menten complex (Fig. 12.6). Furthermore, it facilitates the degradation of GA2 (Liu et al. 1997) and of its sulfated derivative, SM2a, which appeared in the liver of a Tay-Sachs patient (Sandhoff et al. 2002). Film balance experiments show that amphiphilic GM2AP inserts into lipid monolayers only when the lateral surface pressure is below a critical value of about 25 mN/m (Giehl et al. 1999), a value significantly below that of most biological membranes, which ranges between 30–35 mN/m. Due to their small diameter of around 90 nanometer and high curvature, ILVs are more susceptible to an enzymatic attack, as demonstrated for the cleavage of membrane bound ceramide by acid ceramidase in cooperation with saposin D (Linke et al. 2001). These membrane factors, lateral pressure and membrane curvature obviously ensure that GM2AP interacts preferentially with ILVs (Anheuser et al. 2019b). Besides well-known factors like pH value and ionic strength, GM2AP facilitated hydrolysis of membrane bound GM2 by HexA is strongly stimulated by anionic lipids in the GM2-carrying membranes like BMP, PA, PG, PI, fatty acids, and also by ceramide, lyso-phosphatidylcholine and diacylglycerol. The stimulatory role of these lipids argues for degradation of GM2 directly at the surface of ILVs by the GM2AP/HexA complex *in vivo* (Fig. 12.6). In contrast, hydrolysis of membrane bound GM2 is effectively inhibited by membrane stabilizing lipids like SM and cholesterol, cationic lipids like sphinganine and sphingosine and CADs like desipramine, chlorpromazine, imipramine and chloroquine, as well as mucopolysaccharides as primary storage compounds in mucopolysaccharidoses like chondroitin sulfate, dermatan-sulfate and hyaluronan (Anheuser et al. 2019a). These factors also affect the ability of GM2AP to solubilize and mobilize membrane lipids, but hardly affect the hydrolysis of water-soluble synthetic substrates like 4-methyl-umbelliferyl- β -D-N-acetylglucosaminide-6-sulfate (MUGS), which are often used for the diagnosis of HexA deficiency in patients with inherited GM2 gangliosidosis.

The homodimeric saposin B is also a promiscuous lipid binding and transfer protein. It is an essential cofactor in the lysosomal hydrolysis of sulfatide SM4s by

arylsulfatase A, binding sulfatides in soluble stoichiometric complexes, which are recognized by arylsulfatase A as substrates (Fischer and Jatzkewitz 1975; Mehl and Jatzkewitz 1963). On the other hand, saposin C (Ahn et al. 2003; Rossmann et al. 2008; Schwarzmann et al. 2015) and D (Graf et al. 2017) are lipid binding proteins, which can fuse lipid vesicles at low pH values, thereby eventually bringing newly formed ILVs together with already processed, hydrolase loaded and BMP containing ILVs. The four saposins A, B, C and D are generated in the lysosomal compartment by proteolytic cleavage of their precursor protein, prosaposin (Kolter et al. 2005). They share a similar folding pattern containing four helices stabilized by three conserved disulfide bridges. They are also involved in loading lipid antigens for presentation by the Cluster of Differentiation 1 (CD1) receptor protein (Fig. 12.4) (Garrido-Arandia et al. 2018).

All hydrolases and SAPs are N-glycosylated and glycosylation regulates their function, as has been shown for Sap B. The un-glycosylated mutant Sap B causes a fatal sphingolipid storage disease just as in patients with classical metachromatic leukodystrophy, despite the presence of the un-glycosylated protein in the lysosomal compartment and its ability to stimulate the enzymatic hydrolysis of sulfatides *in vitro* even better than the glycosylated wild type Sap B. However, un-glycosylated Sap B is unable to extract membrane lipids at acidic pH, which is essential for SL degradation at ILVs *in vivo* (Rommel et al. 2007). An inherited deficiency of prosaposin, the precursor of four SAPs, the saposins A, B, C and D, triggers a perinatal fatal disease. Here, ILVs, ceramide and several GSLs accumulate (Fig. 12.5). In addition, there is a loss of the water permeability barrier of the skin, since SAPs are required in the extracellular processing of barrier lipids, which is required for maturation of water barrier-essential extracellular lipid lamellae between corneocytes (Breiden and Sandhoff 2014; Doering et al. 1999; Hulkova et al. 2001; Sandhoff et al. 2018). Further information on the function of SAPs and corresponding diseases is given in (Sandhoff et al. 2018).

11.9 Membrane Lipid Modifiers Regulate GSL and Ganglioside Catabolism

In contrast to membrane spanning GTs catalyzing the biosynthesis of GGs at the ER-, Golgi and TGN-membranes, water-soluble hydrolases are the main players in the catabolism of GGs. Due to their insolubility in the lipid phase of the substrate carrying membranes, the soluble hydrolases cannot interact properly with GSL and GG substrates that have short oligosaccharide chains of up to four monosaccharides and are incorporated in the ILV-membranes. They need the support of homo-dimeric SAPs as lipid binding, membrane disturbing and lipid extracting glycoproteins to allow physiologically relevant catabolic rates. The lipid-splitting activity of soluble lysosomal hydrolases depends heavily on posttranslational modifiers such as SAPs, stimulating and inhibiting membrane lipids, pH-values, electrostatic attraction to ILVs, their curvature etc.

Some SAPs, (Sap B and GM2AP) can bind lipids at low pH values, lift and extract them – like sulfatides and ganglioside GM2 – to form stoichiometric soluble complexes, which they present as Michaelis Menten substrates to their respective hydrolases for degradation (Fischer and Jatzkewitz 1978; Wendeler et al. 2006). Other SAPs, Sap A, C and D, disturb membrane structures, trigger vesicle fusion and are needed for the catabolism of GalCer, GlcCer and ceramides, respectively (Abdul-Hammed et al. 2017; Anheuser et al. 2015; Kolter and Sandhoff 2005; Sandhoff 2016; Schwarzmann et al. 2015). However, GSLs and GGs with long hydrophilic oligosaccharide chains of more than five monosaccharides extending far enough from the membrane surface can be attacked directly by a hydrolase as observed for GG IV⁴-GalNAc-GD1a, which is hydrolyzed by HexA (Meier et al. 1991).

Despite the presence of activator proteins, membrane-bound complex GSLs like GM1 and GM2 are hardly hydrolyzed at all by their respective hydrolases in the absence of ceramide and anionic lipids, such as bis(monoacyl-glycerol)phosphate (BMP), phosphatidic acid, phosphatidylglycerol, PI, or PS, and even less so in the absence of both, SAPs and anionic lipids (Fig. 12.7) (Anheuser et al. 2015; Sandhoff et al. 2018; Werth et al. 2001). Addition of ceramide and anionic lipids to substrate carrying liposomal membranes can stimulate the catabolic rate immensely *in vitro*. Quantitative data on the synergetic stimulation of ganglioside GM1 hydrolysis by β -galactosidase in the presence of SAPs (GM2AP and Sap B) and anionic lipids are given in Fig. 12.7.

A model of GM2 degradation by human β -hexosaminidase A, stimulated by GM2AP, is presented in Fig. 12.6. Results of reconstitution experiments using liposomes to mimic ILVs of the late endosomal/lysosomal compartment show the essential role of anionic membrane lipids like BMP, PI, and PG in the vesicular GM2-carrying membranes to achieve physiologically relevant catabolic rates of GM2 degradation (Anheuser et al. 2015, Sandhoff et al. 2018, Werth et al. 2001) (Fig. 12.7). *In vivo*, however, the lipid composition of ILVs within endosomes and lysosomes is changing constantly due to ongoing lipid-digestion and sorting out of cholesterol along the endocytic pathway. Therefore, *in vivo* rates of GG catabolism should also change continuously depending on the momentary conditions within the lysosomes.

The degradation of sphingolipids with short glycan chains is also regulated by the surrounding lipids of the substrate carrying vesicular membrane. Glucosylceramide is the primary storage lipid in the lysosomes of Gaucher patients and a secondary one in Niemann-Pick disease types A, B, and C. Glucosylceramide can be cleaved by β -glucosidase in the absence of SAPs when anionic lipids are present (Abdul-Hammed et al. 2017; Sandhoff et al. 2018; Wilkening et al. 1998). Anionic lipids stimulate its hydrolysis up to 1000-fold (Fig. 12.7), mostly by transglucosylation (to generate Glc-Chol) while cationic lipids inhibit its degradation, especially sphingoid bases and the minor cytotoxic storage compound glucosylsphingosine (Sarmientos et al. 1986), a biomarker for Gaucher disease (Murugesan et al. 2016). Since sphingoid bases are the final product of lysosomal SL degradation, they need to be exported from lysosomes efficiently. Ceramides, fatty acids,

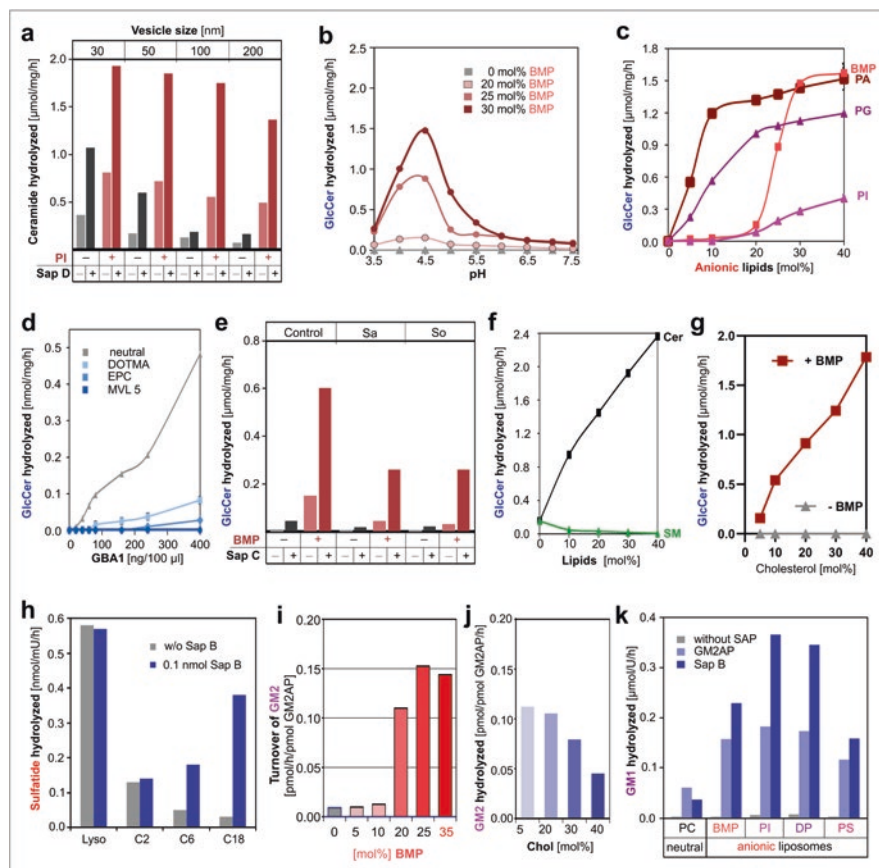


Fig. 12.7 (Glyco)sphingolipid degradation is regulated by (a) vesicle size, (b) pH, (c–g and i–k) lipid composition of the vesicles, and (h) acyl chain length of lipids. The presence of anionic lipids [(b, c, e, g, i, k) BMP; (a, c, k) PI; (k) PS; (c) PA and PG] and (f) ceramide enhances the degradation of GSLs. Sphingolipid activator proteins stimulate the hydrolysis of GLSs: (a) Sap D the hydrolysis of ceramide by acid ceramidase, (h) Sap B the hydrolysis of micellar sulfatide by the arylsulfatase A, (k) Sap B and GM2AP the hydrolysis of GM1 by β -galactosidase, and (e) Sap C the hydrolysis of GlcCer by GBA1. The presence of cationic substances [(d) cationic lipids or cationic amphiphilic drugs], (j) cholesterol, (f) sphingomyelin, and (e) sphingoid bases sphingosine and sphinganine inhibits the digestions of GSLs. Liposomes in panels j and f contained additional 20 mol% BMP. GBA1 and many other beta-glucosidases are also transglucosidases that catalyze the transfer of the glucose moiety from glucosylceramide to cholesterol generating 1-O-cholesteryl-beta-D-glucopyranoside (GlcChol) (Akiyama et al. 2013, Marques et al. 2016). Such transglucosylation can speed up GlcCer degradation supporting the strong turnover observed in panel f in the presence of 5% cholesterol and g; Cholesterol is an acceptor substrate for Gba1 in g but not for HexA in j, which would explain the seemingly contrary results obtained in these two experiments. Figure 12.7 was modified according to references (Breiden and Sandhoff 2019b; Sandhoff and Sandhoff 2018). Abbreviations: BMP bis(monoacylglycero)phosphate, Cer ceramide, Chol cholesterol, DOTMA 1,2-di-O-octadecenyl-3-trimethylammonium propane, DP dolicholphosphate, EPC 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine, GBA1 lysosomal β -glucocerebrosidase, GlcCer glycosylceramide, GM2AP GM2 activator protein; MVL 5 multivalent cationic lipid, PA phosphatidic acid, PG phosphatidylglycerol, PI phosphatidylinositol, PS phosphatidylserine, Sa sphinganine, SAP sphingolipid activator protein, Sap saposin, SM sphingomyelin, So sphingosine

monoacylglycerol, and diacylglycerol also stimulate glucosylceramide hydrolysis in the presence of anionic lipids while sphingomyelin, sphingosine, and sphinganine play strong inhibitory roles, thereby explaining the secondary storage of glucosylceramide in Niemann-Pick diseases (Abdul-Hammed et al. 2017; Sandhoff et al. 2018).

12 GSL and GG Metabolism and Disease

Dysfunctional genes in SPT involved in sphinganine biosynthesis can lead to neurodegeneration and sensory and autonomic neuropathy type 1 (HSAN1) (Penno et al. 2010). These mutations, either in subunit SPTLC1 or in subunit SPTLC2, cause a higher incorporation rate for other amino acids than L-serine, mainly L-alanine and cause increased levels of 1-deoxy-SLs, mainly 1-deoxy-dihydroceramides, which cannot be converted to sphingomyelin or GSLs/GGs and appear to cause mitochondrial dysfunction (Alecu et al. 2017). The big subunits of SPT are associated with a small subunit, either SPTssa or SPTssb, which confer distinct acyl-CoA substrate specificities. In Stellar mice, a mutation in SPTssb results in increased production of C20-sphingoid bases and corresponding GGs in the brain causing neurodegeneration (Zhao et al. 2015). A human correlate is so far unknown.

Downstream of SPT, KDSR reduces KDS to sphinganine. Mutations of this gene have recently been reported to cause a spectrum of keratinization disorders (Boyden et al. 2017; Takeichi et al. 2017), although the overall production of normal epidermal ceramides appeared to not be dramatically affected. Some of these mutation however lead to a metabolic bypass of KDSR: a substantial amount of otherwise not observed ketodihydroceramides is synthesized by ceramide synthases directly from KDS. These ketodihydroceramides were detected in the stratum corneum of the skin, but not in blood of the patients (Pilz et al. 2022). These finding might also explain the absence of systemic failures.

In contrast to KDSR, ceramide synthases comprise a family of six genes in mammals, CERS1-6. In humans, mutations of CERS3, which is expressed mainly in stratified epithelia and male germ cells, were described to cause autosomal recessive ichthyosis (ARCI) (Eckl et al. 2013; Radner et al. 2013) and CerS3-deficient mice die after birth due to breakdown of the lipid based skin barrier (Jennemann et al. 2012b; Rabionet et al. 2014). In mice, germ cell specific deletion of CerS3 affects SLs, including complex GGs and GSLs of the 0- and a-series resulting in a differentiation arrest of spermatids and male infertility (Rabionet et al. 2015). A homozygous nonsynonymous mutation in CERS1, abundantly expressed in neurons, causes progressive myoclonus epilepsy. Expressed *in vitro*, the mutant CERS1 did not cause additional ceramide production in contrast to the expression of the wild type CERS1 gene. How this mutation affects neuronal GG pattern in the patients, however, remains to be elucidated (Vanni et al. 2014). Corresponding

mouse models revealed reduced levels of neuronal ceramides (Zhao et al. 2011), sphingomyelins and GGs with stearic acid, leaving about half the neuronal GG levels (Ginkel et al. 2012). Deficiencies of CerS2 (Ali et al. 2013, 2015; Barthelmes et al. 2015; Bickert et al. 2018; Imgrund et al. 2009; Kim et al. 2017; Marsching et al. 2014; Oertel et al. 2017; Park et al. 2013a, b, 2014, 2015; Petrache et al. 2013; Pewzner-Jung et al. 2010; Rieck et al. 2017; Saroha et al. 2017; Shin et al. 2021; Zigdon et al. 2013), of CerS4 (Ebel et al. 2014; Peters et al. 2015), of CerS5 (El-Hindi et al. 2020; Gosejacob et al. 2016), and of CerS6 (Barron et al. 2021; Ebel et al. 2013; Eberle et al. 2014; Hammerschmidt et al. 2019; Helke et al. 2018; Turpin et al. 2014) as well as that of glucosylceramide synthase (Jennemann et al. 2005, 2010, 2012a; Nordstrom et al. 2013; Rabionet et al. 2015; Watanabe et al. 2010; Yamashita et al. 1999) were analyzed so far only in genetically engineered mice and are reviewed briefly elsewhere (Sandhoff et al. 2018).

Inherited defects in GG biosynthesis cause a severe infantile epilepsy syndrome (GM3-S deficiency) (Boccutto et al. 2014; Fragaki et al. 2013; Simpson et al. 2004) and progressive hereditary spastic paraplegia (GM2-S deficiency) (Harlalka et al. 2013). A wide spectrum of defects has been described in mutant mice (Sandhoff et al. 2018) and it will be a matter of time until corresponding inherited human diseases will be identified.

Ganglioside degradation can be blocked at almost every catabolic step, either by an inherited deficiency of a hydrolase or the respective activator protein. The rare and fatal gangliosidoses are dominated by neuronal ganglioside, GSL and lyso-GSL storage, triggering neurodegeneration (Kolter and Sandhoff 2006; Regier et al. 2016; Sandhoff and Harzer 2013). GG and GSL storage diseases have been briefly reviewed in (Rosenberg and Pascual 2020; Sandhoff et al. 2018).

13 Cascades of Metabolic Errors in LSDs and in Defective GSL Biosynthetic Pathways Generate Complex Pathologies

Some membrane lipids such as SM and Cholesterol emerge as inhibitors of lysosomal lipid catabolism (Breiden and Sandhoff 2020). As primary storage compounds, insoluble lipids and soluble storage compounds like chondroitin 6 sulfate can trigger cascades of secondarily accumulating sphingolipids inducing complex pathologies in LSDs. In reconstitution experiments, primary storage compounds were identified as strong inhibitors of SAPs (GM2AP, SapA & SapB), which are essential for the catabolism of many GSLs. They were furthermore identified as inhibitors of specific catabolic steps in lysosomal ganglioside catabolism and cholesterol release from the late endosomal compartment. In particular, accumulating SM triggers a massive secondary accumulation of cholesterol, and SM and cholesterol together induce increasing levels of ganglioside GM2 and glucosylceramide in

Niemann-Pick disease type A, B, and C (Anheuser et al. 2019b; Oninla et al. 2014). Chondroitin-6-sulfate effectively inhibits GM2 catabolism in mucopolysaccharidoses like Hurler, Hunter, Sanfilippo and Sly syndrome and causes a secondary neuronal ganglioside GM2 accumulation, triggering neurodegeneration (Anheuser et al. 2019a). Secondary storage of simple monosialo-gangliosides, GM2 and GM3, and lactosylceramide can cause neurological forms of several mucopolysaccharidoses (Viana et al. 2020).

Accumulating metabolic modifiers within the lysosomal compartment can dysregulate cellular metabolism. These include SM, cholesterol, positively charged toxic lyso-sphingolipids (glucosylsphingosine (Sarmientos et al. 1986), galactosylsphingosine), other cationic bases like sphingosine and sphinganine and the primary storage compound chondroitin-6-sulfate in MPS, Hurler, Hunter, Sanfilippo and Sly syndrome (Anheuser et al. 2019a).

Furthermore, secondary ganglioside, GSL and lipid accumulation is known in many cellular and lysosomal storage diseases, often without known molecular basis (Grassi et al. 2019; Hornemann 2021). Secondary lysosomal storage of minor GGs and GSLs like GM2, GM3, glucosylceramide and/or lactosylceramide were observed in prosaposin deficiency, muco-lipidoses, glyco-proteinoses, neuronal ceroid lipofuscinoses and in hereditary spastic paraplegia (Breiden and Sandhoff 2020).

Juvenile neuronal ceroid lipofuscinosis is caused by mutations of the CLN3 gene. A genomic deletion in the CLN3 gene triggered a significant increase of the GG GM3 levels, apparently caused by a reduction in activity of GM2 synthase that converts GM3 to GM2 in cerebellar precursor cell lines. A significant reduction of the major α -series GGs GM1a and GD1a in brain cells seems to be a consequence of the blockade of the biosynthetic pathway (Somogyi et al. 2018).

In a form of Batten disease, carrying CLN3 mutations, the levels of various soluble, lysosomal proteins, which are involved in the degradation of diverse macromolecules and sphingolipids, were also reduced. In particular, GM3 and lactosylceramide appeared to accumulate while hexosylceramides further downstream in catabolism decreased (Schmidtke et al. 2019).

A secondary but deleterious GG-accumulation can also be caused by an inhibition of lysosome membrane recycling in human hereditary spastic paraplegia 11 (SPG11) and corresponding mouse models (Boutry et al. 2018). The primary lysosomal accumulation of GG GM1 in GM1-gangliosidosis can spill out slowly, contaminate and interfere with the function of membrane contact sites, for example between lysosomes and mitochondria, leading to neuronal cell death and neurodegeneration (Annunziata et al. 2018).

Long-term treatment of patients, animals or cultured cells with cationic amphiphilic drugs (CADs) can also lead to an intralysosomal accumulation of phospholipids, GGs and GSLs. CADs are trapped in the lysosomal compartment, where they interfere with negatively charged ILVs, the major platforms of cellular degradation of complex lipids, attenuating their catabolism (Breiden and Sandhoff 2019a).

14 Gangliosides and Glycosphingolipids as Secondary Storage Compounds

GGs are the primary and dominant storage compounds in GM1 and GM2 gangliosidoses (Sandhoff and Harzer 2013). Small GGs like GM2 and GM3 and small GSL like GlcCer and LacCer, however, can also accumulate as secondary storage compounds in different forms of Niemann-Pick disease (Vanier 1983) and other LSDs. A review on sphingolipid metabolism and the disruption of sphingolipid homeostasis was published recently (Quinville et al. 2021). The lysosomal catabolism of small GSLs is apparently inhibited by primary storage compounds (Breiden and Sandhoff 2020), generating a secondary ganglioside, GSL and lipid accumulation. For instance in Niemann Pick type A and B diseases (NPA and NPB, respectively), caused by defects in acid sphingomyelinase, the primarily accumulating sphingomyelin (SM) strongly inhibits the sterol transfer protein NPC2 and thereby the secretion of cholesterol from the late endosomal and lysosomal compartment (Abdul-Hammed et al. 2010; Oninla et al. 2014). The combined primary and secondary storage lipids in Niemann Pick diseases, SM and cholesterol, respectively, subsequently inhibit the catabolism of GM2 (Anheuser et al. 2015), GlcCer (Abdul-Hammed et al. 2017), and the function of several SAPs, GM2AP (Anheuser et al. 2015), Sap A (Locatelli-Hoops et al. 2006) and B (Remmel et al. 2007) together, and thereby the catabolism of further GSLs (Breiden and Sandhoff 2020).

Soluble primary storage compounds like chondroitin-6-sulfate and other mucopolysaccharides can also inhibit GG-catabolism and trigger a secondary ganglioside accumulation in Hurler, Hunter, Sanfilippo and Sly syndrome by impacting for instance the interaction between GM2AP and the surface of GM2-carrying ILV-membranes (Anheuser et al. 2019a).

15 Accumulation of Lyso-Sphingolipids, Likely Attenuating Lysosomal SL-Catabolism

Lysosomal SL-storage is often accompanied by increasing levels of cationic lyso-sphingolipids in the endo-lysosomal compartment. Their analysis can be applied to diagnose patients with sphingolipidoses using LC-MS/MS (Polo et al. 2017). Though the pathways of their generation are poorly understood, recent evidence points to an active role of acid ceramidase in the deacylation of lysosomal glycosphingolipids, glucosylceramide in Gaucher disease and globotriaosylceramide in the case of Fabry disease, to generate lysosphingolipids (Ferraz et al. 2016). Besides other factors deregulating lysosomal functions (Hoglinger et al. 2015; Lloyd-Evans et al. 2008), accumulating lyso-sphingolipids may well have a pathological potential by attenuating lysosomal lipid degradation. It is expected that lipophilic sphingoid bases and lysolipids can easily integrate into the lipid bilayers of the ILVs and get their free amino-groups protonated in the acidic environment of the lysosomes.

Cationic lysolipids in the membranes of ILVs should reduce the negative surface charge of the SL-substrate carrying ILVs and reduce their electrostatic capability to attract protonated catabolic hydrolases. As observed for cationic amphiphilic drugs, the increasing desorption of enzymes from the substrate-carrying ILV-surfaces (Breiden and Sandhoff 2019a) should attenuate the lysosomal degradation of many SLs.

Krabbe disease is caused by an inherited deficiency of galactosylceramidase (Igisu and Suzuki 1984), which normally degrades both, the lysolipid galactosylsphingosine and galactosylceramide, the latter with the help of the lipid binding protein, saposin A (Matsuda et al. 2001). The monogenic deficiency of galactosylceramidase causes predominantly a toxic accumulation of the cationic lysolipid galactosylsphingosine (also called psychosine) probably exclusively in the myelin forming oligodendrocytes of the brain, triggering their dysfunction and death, followed by an arrest of myelination and myelin degeneration (Suzuki 2003). It was postulated, that the accumulation of toxic psychosine is responsible for the rapid loss of oligodendrocytes in Krabbe disease, which explains the initially surprising absence of galactosylceramide storage despite its blocked degradation. (Suzuki 1998).

16 GSL Metabolism and Parkinson Disease

The neuropathology of Parkinson disease (PD) is characterized by fibrous inclusions of Lewy-bodies in the degenerating dopaminergic neurons of the substantia nigra, which are enriched in aggregates of alpha-synuclein (α -Syn). α -Syn binds GG GM1 and other SLs, inhibiting fibril formation. The pathological changes lead to motor dysfunctions. Reduced GM1 levels due to a heterologous disruption of GM2/GD2 synthase in mice present an accurate PD phenotype including elevation of α -Syn aggregates, motor impairment and non-motor symptoms characteristic of PD, all of which can be corrected by GM1 replacement therapy (Ledeen and Wu 2018b; Wu et al. 2020). GM1 triggers expression of neuroprotection genes in astrocyte-neuron co-cultures, a novel mechanism mediated by astrocytes, thereby exerting its metabolic and neuroprotective effects (Finsterwald et al. 2021). On the other hand, neuroinflammation in PD seems to be linked with a dysregulation of GSL metabolism, especially with an impaired lysosomal catabolism of GSLs and SLs (Abdelkarim et al. 2018; Belarbi et al. 2020; Kim et al. 2018; Nelson et al. 2018). Elevated levels of lysosomal proteins such as SAPs, Sap C and LAMP1, have also been found in Alzheimer disease mouse brains during (A beta) plaque growth (Sharoar et al. 2021). Mutations in the GBA1 gene coding for the glucocerebrosidase (GCase) are the most common genetic risk factor for the development of PD. Pharmacological inhibition of the GCase and the D409V GBA1 mutation enhance the accumulation of GSLs and insoluble phosphorylated α -Syn. Accumulation of GlcCer with very long acyl chains (\geq C22) appeared to especially promote pathological α -syn aggregation (Fredriksen et al. 2021). On the other hand,

inhibition of glucosylceramide synthase (GCS) by a novel inhibitor (benzoxazole 1) attenuates α -Syn pathology and lysosomal dysfunction in preclinical models of synucleinopathy, suggesting that reduction of GSLs by GCS inhibition may impact progression of synucleinopathy (Cosden et al. 2021). Most PD patients show significant deficiency of GGs, including GM1, in the brain. Supplementation of GGs, such as GD3 and GM1 via intranasal infusion could reduce neurotoxic proteins and restore functional neurons via modulating chromatin status by nuclear GGs (Itokazu et al. 2021). Recent evidence has shown that Parkinson disease can be reversed also by feeding a hydrophobic isoprenoid, farnesol, to several mouse models of PD. Farnesylation of the protein PARIS has been reported to prevent the build-up of damaging reactive oxygen species and thereby the loss of dopamine-releasing neurons. Thus, farnesol treatment may turn out to be beneficial for PD patients (Jo et al. 2021).

Therapeutic approaches for lysosomal GG-storage diseases have been summarized recently (Blumenreich et al. 2021; Nicoli et al. 2021; Platt et al. 2018). It is important to know that white matter deficits, well known components of gangliosidosis pathology, are partially resistant to correction by gene therapy (Maguire and Martin 2021).

17 Conclusions and Perspectives

Gangliosides (GGs) and glycosphingolipids (GSLs) are especially enriched in neuronal plasma membranes. Their functional significance became more visible recently by studies of their metabolic pathways in cell cultures and animals including those affected by monogenetic diseases. As GGs, GSLs and their lipophilic metabolic intermediates are poorly soluble amphiphilic lipids, they are bound to cellular membranes. Therefore, biosynthetic and catabolic pathways occur mostly at membrane surfaces either of the secretory pathway organelles or at intraluminal vesicles, the ILVs, within endosomes and lysosomes of the catabolic pathway. Though we know only a little about the complex regulation of individual metabolic steps, it seems to be clear that the main biosynthetic and catabolic pathways can be regulated at genetic as well as posttranslational levels. Many of the known biosynthetic steps involve an interaction between membrane-bound sphingolipids (SLs) and glycosyltransferases within the plane of the lipid bilayer following a kind of two-dimensional Michaelis-Menten kinetic (Scheel et al. 1982). On the other hand, the major catabolic steps of GGs and GSLs are catalyzed by protonated hydrolases with the help of lipid binding proteins (SAPs), basically soluble cationic glycoproteins in the lumen of the lysosomes. They are – besides other still unidentified forces – electrostatically attracted to the SL-substrate carrying negatively charged ILVs to enable SL catabolism. The dominant role of electrostatic attraction for the lysosomal degradation of SLs, SM, GSL and glycerophospholipids has been summarized recently (Breiden and Sandhoff 2019a). A negative surface potential is generated on ILV-membranes by a high content of anionic phospholipids, especially of

the ILV-specific BMP and a few other anionic phospholipids, that exists even at low lysosomal pH values (Oninla et al. 2014). The negative surface potential of ILVs, however, can be strongly reduced by feeding CADs (cationic amphiphilic drugs) to cultured cells that integrate into ILV-membranes (Oninla et al. 2014). The mostly antidepressant CADs have not been identified as specific inhibitors of the ASM-catalyzed degradation of SM to ceramide, but as protonated cationic lipophilic molecules, that reduce and eventually compensate the negative surface of the ILVs in the lumen of lysosomes. This concept, often ignored in the medical literature, is supposed to be valid for the interaction of many lysosomal hydrolases with their SL- and PL-substrates carried by the ILV-membranes. We believe that terms like “functional inhibitors of acidic sphingomyelinase” disguise the major general impact of CADs on lysosomal functions and may hamper correct interpretation of scientific results. Detailed molecular studies on the molecular action of anti-depressive CADs in the brain are still missing. Whereas most glycerophospholipids can be degraded by different catabolic pathways, lysosomal SL-catabolism follows a stepwise and strictly sequential one. As a consequence, monogenetic functional defects of lysosomal SL catabolism typically trigger an accumulation of its substrates, generating a storage disease and modifying cellular metabolism. Lysosomal SL-catabolism is regulated by genetic and by posttranslational modifiers as outlined in Figs. 12.5, 12.6, and 12.7. Surprisingly, lipids of the SL-substrate carrying membranes are not only essential building blocks of their ILVs-membrane, but can also effectively stimulate SL-hydrolysis – like anionic lipids – or reduce SL-substrate turnover – like cationic sphingolipid bases. This novel concept of intra-lysosomal regulation can easily explain a secondary accumulation of GSLs and a few other lipids in LSDs. They accumulate despite the absence of any genetic defects in their catabolic pathways. An interesting illustration is the secondary accumulation of cholesterol (Chol) and several GSLs in ASM-deficient Niemann-Pick disease. This is due to the primary storage of SM, which specifically inhibits the NPC2-protein, which exports Chol from the lysosomal compartment. Both, SM and the strongly accumulating Chol, effectively trigger a cascade of secondary and tertiary accumulations by inhibiting the enzymatic hydrolysis of GlcCer and GM2 ganglioside as well as the action of GM2AP and of several other lipid binding proteins. Cascades of secondarily accumulating lipids aggravate the clinical course of the disease. Therefore, ASM appears to be a key player for the regulation of cellular homeostasis. GM2-catabolism is also inhibited by primary storage compounds in some mucopolysaccharidoses, Hurler-, Hunter-, Sanfilippo- and Sly-disease, especially by chondroitin-6-sulfate, accumulating GM2 and triggering a neurodegenerative disease. Dysregulation of the cellular lipid composition and cascades of accumulating lipids may well dysregulate cellular metabolism, which may especially affect patients with obesity, Alzheimer and Parkinson disease.

In recent years, several neuronal GSL-functions have been uncovered in the brain. GGs and GSLs are involved in metabolic, neurologic, neurodegenerative, psychiatric, and psychological features of the CNS. In many studies, however, the molecular and cellular basis of their involvement, for example in Alzheimer,

Parkinson and Frontal lobe dementia, remains unknown, which leaves important starting points for further studies.

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