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Review

Sphingolipids and lysosomal pathologies ☆

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ABSTRACT

Endocytosed (glyco)sphingolipids are degraded, together with other membrane lipids in a stepwise fashion by endolysosomal enzymes with the help of small lipid binding proteins, the sphingolipid activator proteins (SAPs), at the surface of intraluminal lysosomal vesicles. Inherited defects in a sphingolipid-degrading enzyme or SAP cause the accumulation of the corresponding lipid substrates, including cytotoxic lysosphingolipids, such as galactosylsphingosine and glucosylsphingosine, and lead to a sphingolipidosis. Analysis of patients with prosaposin deficiency revealed the accumulation of intra-endolysosomal vesicles and membrane structures (IM). Feeding of prosaposin reverses the storage, suggesting inner membrane structures as platforms of sphingolipid degradation. Water soluble enzymes can hardly attack sphingolipids embedded in the membrane of inner endolysosomal vesicles. The degradation of sphingolipids with few sugar residues therefore requires the help of the SAPs, and is strongly stimulated by anionic membrane lipids. IMs are rich in anionic bis(monoacylglycero)phosphate (BMP). This article is part of a Special Issue entitled New Frontiers in Sphingolipid Biology.

1. Introduction

(Glyco)sphingolipids (GSLs) are degraded in a strictly sequential pathway by endolysosomal enzymes with the assistance of small lipid binding proteins [1]. Inherited metabolic defects in one of the steps lead to a block of degradation and accumulation of the corresponding substrates, resulting in a group of lysosomal storage diseases, the sphingolipidoses. Detailed descriptions, the underlying defects, and clinical manifestations of the diseases have been described elsewhere at greater length [2–7]. We will here focus on basic concepts and new developments.

2. Sphingolipid biosynthesis

Sphingolipids are essential plasma membrane components of eukaryotic cells and important bioactive cell signaling molecules.

GSLs are expressed in a cell type specific pattern on cellular surfaces, also depending on the stage of differentiation [8–10]. Typical components of neuronal plasma membranes are gangliosides [11]. Galactosylceramide (GalCer) and sulfatides are found in myelin and kidney, globosides in visceral organs and ceramides with very long chain fatty acid residues of up to 36 carbon atoms, as well as glucosylceramides with very long chain fatty acyl chains in keratinocytes and sperm cell [12–14].

De novo biosynthesis of sphingolipids starts with the formation of their hydrophobic membrane anchors, the ceramides, which takes place at the cytoplasmic side of the endoplasmic reticulum (ER) and is controlled by six different ceramide synthases [15–17]. Ceramides are then transferred to the Golgi membrane by secretory vesicular flow and by the lipid transfer protein CERT [18], where glucosylceramide (GlcCer) is formed at the cytosolic leaflet of the Golgi membrane and is translocated to the luminal surface of Golgi membranes to get converted to lactosylceramide (LacCer) [19]. GlcCer reaches the luminal site of the Golgi membrane by multidrug transporters [20,21] or *via* the action of the cytosolic GlcCer-transfer protein FAPP2. FAPP2 transports GlcCer back to the ER, where it may flip to the luminal site and reaches the Golgi lumen for LacCer synthesis by vesicular transport [22,23].

Subsequent glycosylation reactions give rise to the complex carbohydrate pattern of gangliosides and other GSLs. After their biosynthesis, complex GSLs reach the outer surface of plasma membranes by vesicular exocytotic membrane flow. Sphingomyelin is formed from ceramide and phosphatidylcholine at the luminal side of the *trans*-Golgi network and at the plasma membrane [24].

Depending on the cell type the reutilization of building blocks (*e.g.* sphingoid bases) released from the lysosomal compartments may predominate the *de novo* biosynthesis by far. Salvage pathways can contribute to 50–90% of glycosphingolipid generation in fully differentiated cells *e.g.* neurons [25,26]. Inherited defects in sphingolipid biosynthesis

Abbreviations: ASM, acid sphingomyelinase; BMP, bis(monoacylglycero)phosphate; Cer, ceramide; ER, endoplasmic reticulum; ERT, enzyme replacement therapy; ESCRT, endosomal sorting complex required for transport; FA, fatty acid; GalCer, galactosylceramide; GlcCer, glucosylceramide; GM2-AP, GM2 activator protein; GSL, glycosphingolipid; IM, inner membranes; HSAN1, hereditary sensory neuropathy type I; LacCer, lactosylceramide; LIMP-2, lysosomal integral membrane protein type 2; NPC-1, Niemann–Pick C1 protein; NPC-2, Niemann–Pick C2 protein; p-Sap, prosaposin; Sap-A, saposin A; Sap-B, saposin B; Sap-C, saposin C; Sap-D, saposin D; SAPs, sphingolipid activator proteins (GM2-AP and Sap-A, -B, -C, -D)

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have been studied in genetically manipulated mice, where they cause a broad spectrum of pathological phenotypes [27,28]. In human patients only 3 diseases, have been described so far [29–32].

Deficiency of ganglioside GM3 synthase causes an infantile onset refractory epilepsy [29] or a complex hereditary spastic paraplegia [30]. Mutations in the SPTLC1 gene coding for a subunit of the serine palmitoyltransferase lead to adult-onset, hereditary sensory neuropathy type I (HSAN1) [30,33] (see Table 1).

3. Luminal vesicles and inner membranes as platforms of membrane degradation

Inborn errors of lysosomal function comprise mucopolysaccharidoses, glycoprotein-, glycogen storage disorders, mucolipidosis, sphingolipidoses, and others. This review focuses on the lysosomal pathologies, caused by disturbed sphingolipid degradation.

Located in the outer (exoplasmic) leaflet of the plasma membrane, sphingolipids are degraded within the process of endosomal/lysosomal membrane digestion. Together with other macromolecules and membrane components they are transferred into the lysosomal compartment by autophagy [34,35], phagocytosis [34,36] and endocytosis [37].

At the level of endosomes, sphingolipids reach luminal intraendosomal vesicles or intraendosomal membranes for digestion (Fig. 1) [38–40]. These intraendolysosomal vesicles are generated during endocytosis by successive steps of vesicle budding and fission controlled by the endosomal sorting complex required for transport (ESCRT) [41]. They are platforms for lipid and membrane degradation as demonstrated in prosaposin deficient cells [38,39]. We assume that luminal vesicles or IMs are prepared for lysosomal digestion by a lipid sorting process beginning at the level of endosomes [42,43].

Studies suggest that at least subpopulations of exosomes, intraluminal vesicles, which can be secreted after fusing with the plasma membrane, are formed in an ESCRT independent pathway [44,45].

Membrane-stabilizing cholesterol is sorted out mainly by two sterol binding proteins, NPC-2 and NPC-1 [46]. Inherited defects of NPC-1 or NPC-2 cause the Niemann–Pick disease type C, a cholesterol trafficking disease in which cholesterol is accumulating in the lysosome [47–51]. *In vitro*, cholesterol of liposomal membranes inhibits lipid solubilization by Sap-A and -B [52,53]. NPC-1 is a transmembrane protein of the endosomal perimeter membrane, whereas NPC-2 is a small soluble glycoprotein [50,54]. NPC-2 might remove cholesterol from IM and deliver it to NPC-1 which exports the lipid from endosomes. *In vitro*, the cholesterol transfer between liposomes, mimicking endolysosomal vesicles, is strongly inhibited by sphingomyelin (SM). Degrading liposomal SM with acid sphingomyelinase releases the inhibition [55,56]. This might explain that in Niemann–Pick disease types A and B, patients deficient of acid sphingomyelinase accumulate not only SM but also large amounts of cholesterol [57]. During endosomal maturation the pH decreases and the degradation resistant anionic lipid bis(monoacylglycero)phosphate (BMP) is formed from phosphatidylglycerol in the IM [58] (Fig. 1). *In vitro* BMP stimulates cholesterol transfer between liposomes and sphingolipid degradation substantially.

In contrast to the luminal vesicles, the lysosomal limiting membrane is protected on the inner leaflet by a thick glycocalix containing hardly digestable polylactosamine structures. The glycocalix is formed by highly N-glycosylated integral membrane proteins [59,60], and stabilized by high cholesterol levels [61,62], and chaperone HSP70 [63]. The high lateral pressure of the limiting membrane presumably also attenuates the insertion of the GM2 activator protein (GM2-AP), a lipid binding protein essential for ganglioside catabolism [64].

Sphingolipids are degraded in a stepwise fashion by hydrolytic enzymes with the help of lipid binding SAPs on the surface of luminal intralysosomal vesicles and membrane structures [42]. The lysosomal degradation starts with the stepwise release of monosaccharide units from the nonreducing end of the oligosaccharide chain (Fig. 2).

Table 1
Inherited defects in sphingolipid biosynthesis in human patients and mouse models.

Diseases associated with biosynthesis of sphingolipids involved in cutaneous permeability barrier formation are summarized in [14].

Inherited defects in sphingolipid biosynthesis in human patients			
Protein	Gene	Human disease	Reference
Serine palmitoyl-CoA transferase (SPT)	*SPTLC1*	Hereditary sensory neuropathy type I	[32,33]
	SPTLC2	Hereditary sensory and autonomic neuropathy type I	[30]
Lactosylceramide α2,3 sialyltransferase (GM3-synthase)	*SIAT9/ST3GAL5*	Infantile-onset-symptomatic epilepsy syndrome	[29]
		Salt & Pepper Syndrom	[178]
Ceramide synthase 3	*CERS3*	Autosomal recessive congenital ichthyosis	[179,180]
Mouse models of defect sphingolipid biosynthesis			
Protein	Gene	Knock-out mouse	Reference
Serine palmitoyl-CoA transferase (SPT)	*Sptlc1/Sptlc2*	Homozygous: embryonic lethal, heterozygous: reduced sphingolipid levels	[181]
Ceramide synthase 1	*Cers1*	Cerebellar ataxia and Purkinje cell degeneration	[17,182]
Ceramide synthase 2	*Cers2*	Myelin sheath defects, cerebellar degeneration, and hepatocarcinomas	[183,184]
Ceramide synthase 3	*Cers3*	Early lethality due to transepidermal water loss	[185]
Ceramide synthase 6	*Cers6*	Altered sphingolipid metabolism and behavioral abnormalities	[186]
Sphingosine Kinase	*Sphk1*	Sphk1 and Sphk2 act redundant. Double-deficient Sphk1/Sphk2 embryonic lethal	[187,188]
Isoform 1 (Sphk1)	*Sphk2*		
Isoform 2 (Sphk2)			
Cerebroside sulfotransferase	*Cst*	Disturbed paranodal junction formation and spermatogenesis	[189]
Galactosylceramide synthase	*cgt*	Severe dysmyelinosis	[190,191]
Glucosylceramide synthase	*Ugcg*	Embryonic lethal	[192–195]
		Cell-specific deletion in brain, skin, and liver	
β-1,4-GalNAc transferase (GM2/GD2 synthase)	*B4galnt1*	No complex gangliosides,	[196,197]
		elevated levels of GM3 and GD3, male infertility	
GD3 synthase	*St8ia1*	No b series gangliosides (GD3, GD2, GD1b, GT1b, GQ1b)	[197,198]
GM3 synthase	*St3gal5*	Enhanced insulin sensitivity, early death,	[199]
		0-series gangliosides formed	
β-1,4-GalNAc transferase and GD3 synthase	*B4galnt1 and St8ia1*	GM3 only	[197,200]
β-1,4-GalNAc transferase and GM3 synthase	*B4galn and St3gal5t1*	No ganglio-series of glycosphingolipids,	[201]
		neurodegeneration,	
		formation of lactosylceramide-3-sulfate	

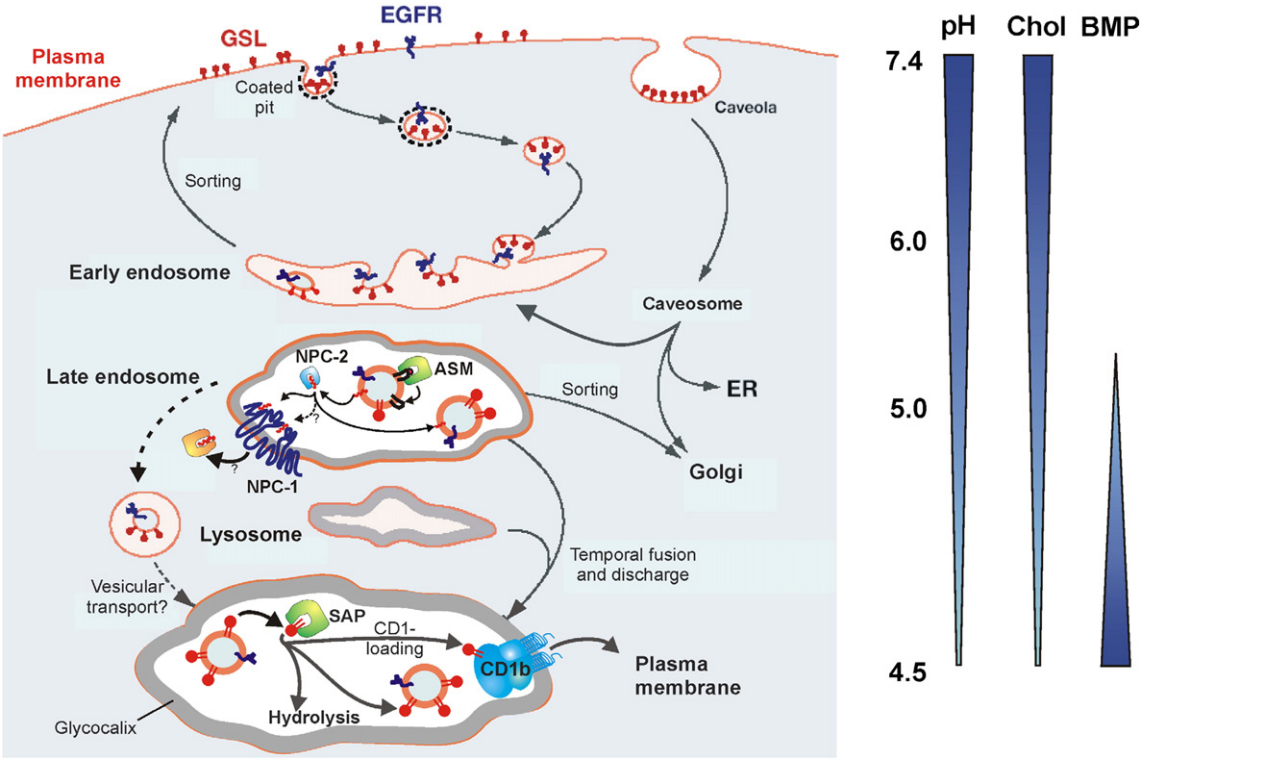

Fig. 1. Model for the maturation of luminal endolysosomal membranes as platforms for sphingolipid catabolism. Glycosphingolipids (GSL) are highlighted on the plasma membrane (PM) and on internal membranes (IM). Gradients of the luminal pH, cholesterol (Chol), and bis(monoacylglycero)phosphate (BMP) of the IM are shown (modified from [176]). ASM, acid sphingomyelinase; CD1, Cluster of differentiation antigen 1; CD1b, Cluster of differentiation antigen 1b; EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; SAP, sphingolipid activator protein; NPC-1, Niemann–Pick C1 protein; NPC-2, Niemann–Pick C2 protein.

4. Anionic lipids control sphingolipid degradation

In vitro experiments have shown that sphingolipid degradation requires not only enzymes, SAPs and an acidic lysosomal pH, but also the presence of anionic lipids such as BMP in the substrate carrying liposomes. Ganglioside GM2 is degraded by hexosaminidase A in the lysosome with the help of GM2-AP. *In vitro* GM2 in liposomal membranes containing no anionic lipids, however, is hardly degraded by hexosaminidase A and the GM2-AP. Addition of BMP or other anionic phospholipids, such as phosphatidic acid, phosphatidylglycerol, phosphatidylinositol, phosphatidylserine etc., in the liposomal membrane stimulates the reaction more than 100-fold [65] (Fig. 3). With more than 10 mol% anionic phospholipids in the liposomal membrane GlcCer is degraded by β-glucosidase [66] even in the absence of SAP. Under these conditions ceramide is cleaved by acid ceramidase in the absence of an activator protein. Addition of saposin D (Sap-D) stimulates the reaction rate up to 3fold. The rate also increases with the curvature of the liposome [67].

5. Sphingolipids are degraded at the lipid–water interface

Sphingolipids are degraded stepwise by hydrolytic enzymes with the help of SAPs on the surface of luminal intralysosomal vesicles and membrane structures releasing monosaccharide units from the nonreducing end of the oligosaccharide chain.

Water soluble glycosidases can hardly attack sphingolipids with short oligosaccharide chains embedded in the vesicular membranes. Therefore, they require the help of small lysosomal membrane perturbing and lipid binding proteins, the SAPs. The SAPs comprise five sphingolipid activator proteins, the saposins A–D (Sap-A–D) and GM2-AP [1,68]. Sap-A–D derive from a common precursor protein, the prosaposin (p-Sap) by proteolysis in the endolysosomes [69]. Proteases, like cathepsin D cleave p-Sap at three different sites located in the interdomain region between Sap-A and -B, -B and -C, and -C and -D [70]. Diseases caused by a defect in one of the proteins are known for Sap-A, Sap-B, Sap-C, Sap-D and GM2-AP. A single deficiency of Sap-A causes a variant form of Krabbe disease [71]. Genetic defects of Sap-B lead to variant forms of metachromatic leukodystrophy with juvenile or late infantile onset [72,73]. Sap-C and Sap-D deficiency cause Gaucher disease [74,75]. The AB-variant of GM2 gangliosidosis is caused by GM2-AP deficiency [68].

Absence of all four saposins due to prosaposin deficiency leads to a neonatal fatal disease with simultaneous storage of many sphingolipids, including ceramide, glucosylceramide, lactosyceramide, ganglioside GM3, galactosyceramide, sulfatides, digalactosylceramide, and globotriaosylceramide, accompanied by a massive accumulation of luminal, intralysosomal vesicles and membranes [76]. Prosaposin deficiency also leads to the loss of the water permeability barrier in the skin of prosaposin knock-out mice [77,78]. The mice show an ichthyotic skin phenotype with red and wrinkled skin similar to β-glucocerebrosidase-deficient Gaucher mice [79–81].

6. Sphingolipidoses

6.1. Gangliosidoses

The gangliosidoses comprise GM1-, and the GM2-gangliosidoses (Tay-Sachs disease, B1-variant, 0-variant (Sandhoff disease), and AB-variant of GM2-gangliosidoses). The diseases are characterized by the accumulation of complex glycosphingolipids in the nervous system and other tissues [82,83].

Degradation of gangliosides occurs at the surface of endolysosomal vesicles and IM, rich in anionic lipids like BMP. Soluble enzymes and membrane perturbing lipid binding SAPs with isoelectric points above the surrounding lysosomal pH values are positively charged. The

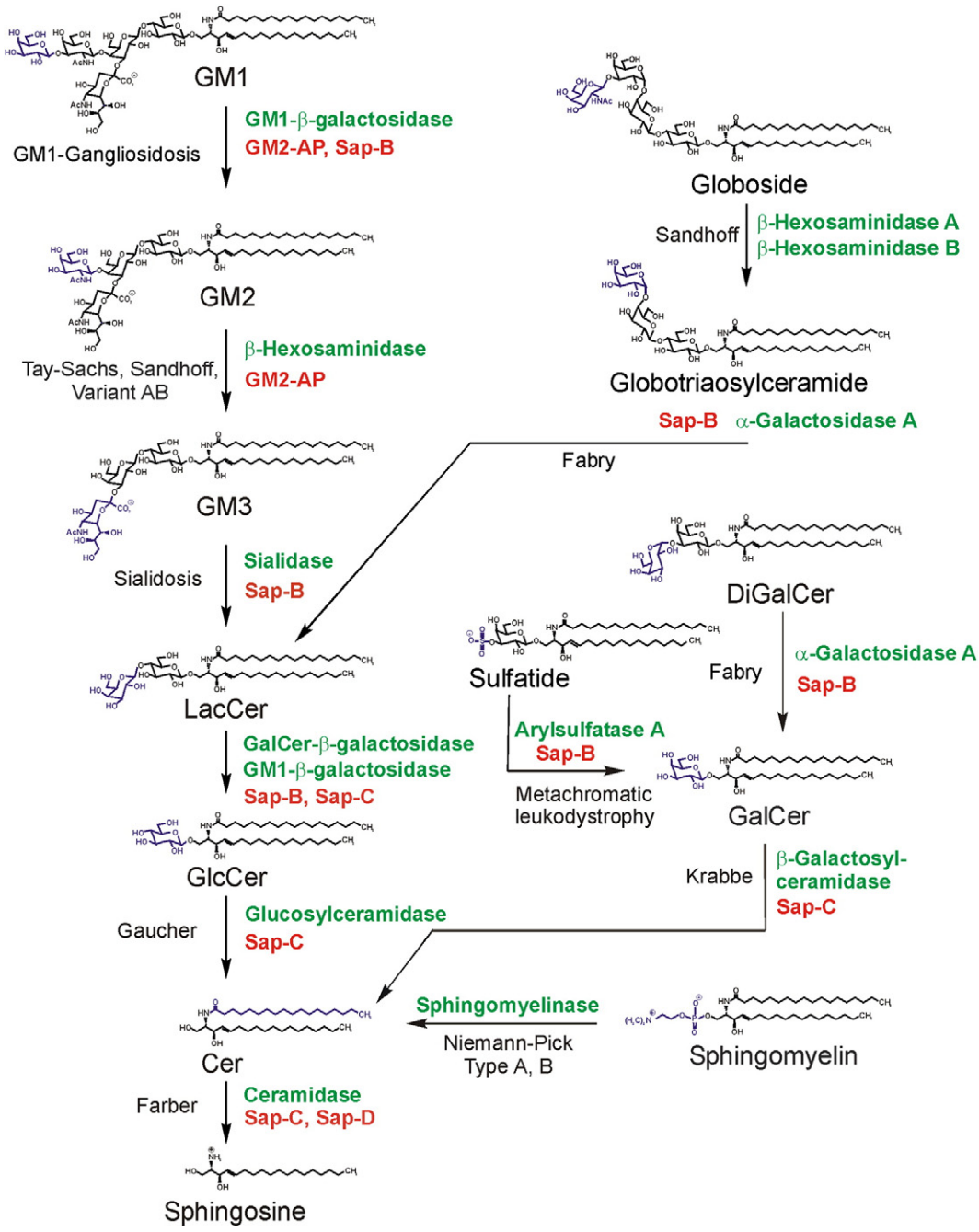

Fig. 2. Sphingolipid degradation and sphingolipidoses. The names of individual inherited diseases are indicated. Degrading enzymes are given in green, activator proteins required for the respective degradation step *in vivo* in red. Variant AB, variant AB of GM2 gangliosidosis [83].

protonated cationic amphiphilic proteins can therefore bind to the anionic, BMP rich membrane surfaces (Fig. 4). Cationic amphiphilic drugs such as desipramine reach the IM in the lysosomal compartment and compensate their anionic surface charge. They interfere with the electrostatic binding of lysosomal proteins to the anionic surface of IM, and attenuate the lysosomal catabolism [84]. Cationic amphiphilic drugs release lysosomal proteins from the IM surface and trigger their proteolysis, *e.g.* that of ASM [85].

GM1 gangliosidoses [86] is caused by inherited deficiency of GM1 degrading β-galactosidase [87]. Hydrolysis of GM1 to GM2 requires the presence of either GM2-AP or Sap-B [88]. β-Galactosidase is part of a lysosomal multienzyme complex, containing sialidase, cathepsin A (the so-called protective protein [89]) and N-acetylaminogalacto-6-sulfate sulfatase [90]. An inherited defect in GM1- β-galactosidase can lead to a changed substrate specificity of the enzyme, resulting in major accumulation of galactose containing keratansulfate and oligosaccharides, a phenotype called Morquio syndrome, type B (MPS IV b) [91,92].

GM2-gangliosidoses are caused by defective hydrolysis of GM2. The ganglioside is degraded by the cooperation of two proteins, β-hexosamindase A and the GM2-AP [6]. The lysosomal β-hexosaminidases differ in the composition of their two subunits and in their substrate specificity. The formation of catalytically active enzyme requires the dimerization of two subunit chains. β-Hexosamindase A consists of the subunits α and β. The enzyme has two active sites at the interface of the subunits α and β [93,94] and releases terminal β-

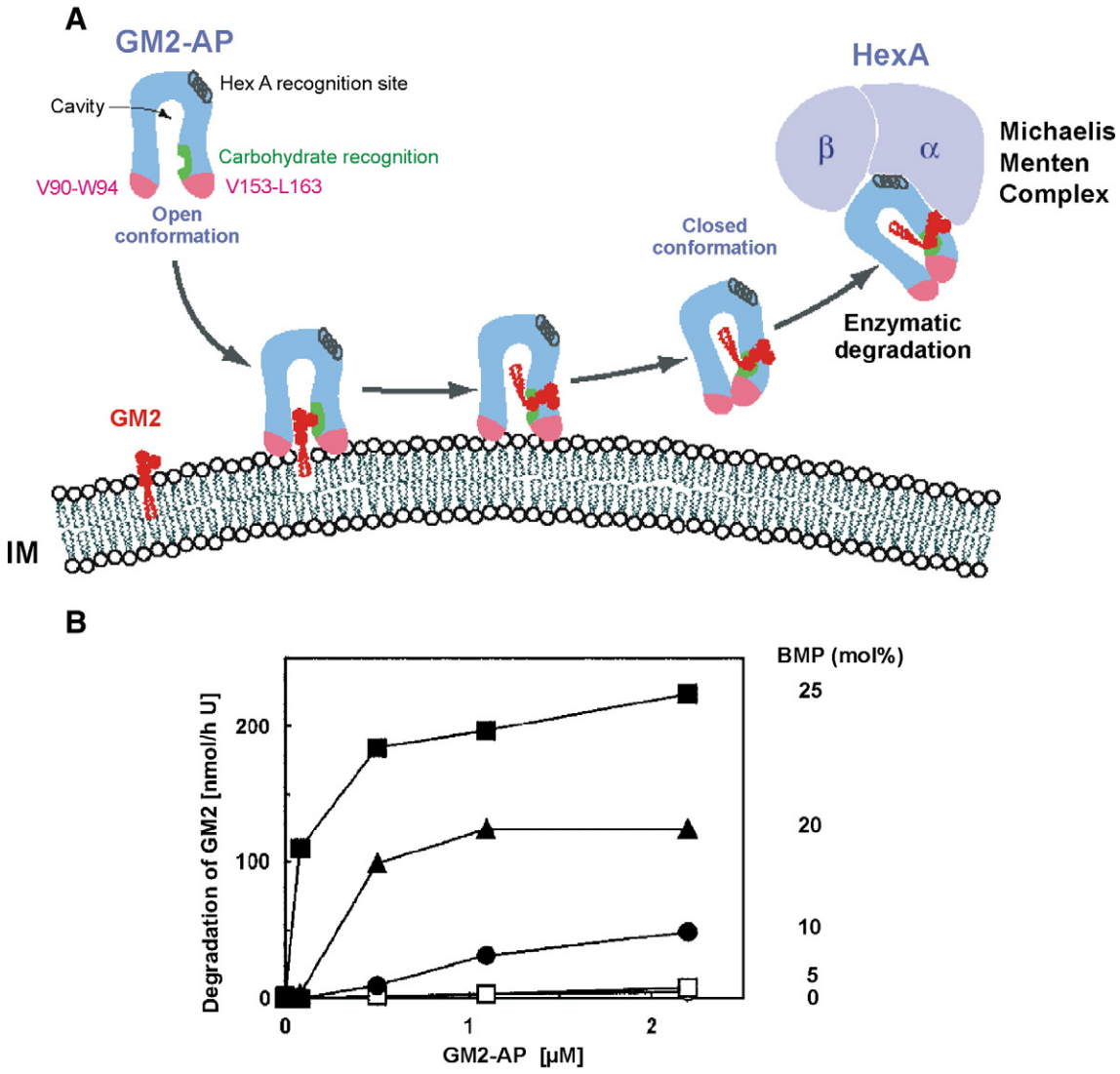

Fig. 3. Molecular mechanism of ganglioside GM2 catabolism. A) Model for the interaction of GM2 activator protein (GM2-AP) with luminal lysosomal membranes in the degradation of ganglioside GM2 (modified after [177]). Two exposed hydrophobic loops of the GM2-AP, V90-W94 and V153-L163, interact with the inner membranes (IM). GM2 is recognized by specific carbohydrate recognition sites at the rim of the cavity. In open protein conformation, the large hydrophobic area reaching from the apolar phase of the membrane to the activator's cavity lowers the energy barrier for lipids to leave the membrane. After the ceramide tail has moved inside the activator's cavity, the conformation changes to the closed form, may leave the membrane and interact with the degrading enzyme [76]. HexA, hexosaminidase A. B) Bis(monoacylglycero)phosphate (BMP) (and other anionic phospholipids) and GM2-AP stimulate the hexosaminidase A catalyzed hydrolysis of ganglioside GM2, which is inserted in liposomal membranes, containing different BMP concentrations [65]. In the absence of anionic phospholipids proteins essential for GM2 degradation (GM2-AP and Hex A) cannot reach a physiological needed rate of GM2 catabolism.

glycosidically linked *N*-acetylglucosamine- and *N*-acetylgalactosamine residues from negatively charged and uncharged glycoconjugates. β-Hexosamindase B (subunits ββ with two active sites at the interface of the two β subunits [95]) cleaves uncharged substrates like glycolipid GA2 and oligosaccharides with terminal *N*-acetylhexosamine residues. The labile β-hexosaminidase S (subunits αα) has a substrate specificity like hexosaminidase A and also degrades sulfated glycolipids [96]. Mutations in the gene encoding for the α-subunit lead to hexosaminidase A and S deficiency in Tay-Sachs disease. Hexosaminidase A mutations that still allow the formation of the heterodimeric hexosaminidase A (α, β) and affect only its activity against anionic substrates result in B1 variant of GM2-gangliosidosis [83,94,97]. The underlying cause of Sandhoff disease is an inherited defect in the hexosaminidase B gene, the gene coding for the β-subunit, leading to the loss of HexA (α,β) and HexB (β,β). The combined deficiency of both hexosaminidase isoenzymes causes the additional storage of neutral glycolipids, especially of globoside in the visceral organs, and of oligosaccharides [98]. GM2-AP deficiency leads to the AB-variant of GM2 gangliosidosis, which is characterized by an accumulation of ganglioside GM2 and glycolipid GA2 [68]. In mice deficiency of all three lysosomal β-hexosaminidases causes a mucopolysaccharidosis-like pathology [99].

6.2. Fabry disease

In Fabry disease an inherited deficiency of alpha-galactosidase A causes the deposition of globotriaosylceramide and digalactosylceramide in lysosomes. Especially affected are endothelial, perithelial, and smooth-muscle cells of blood vessels. In contrast to the autosomal recessive mode of inheritance of the other sphingolipidoses, Fabry disease is an X-chromosomal-linked inherited disorder about as frequent as Gaucher disease [100]. Hemizygous males are severely affected by renal, cardiac, and cerebral complications, heterozygous females have an attenuated form of the disease [101].

6.3. Gaucher disease

Gaucher disease is the most common sphingolipidosis [102]. Deficiency of glucosylceramide-β-glucosidase (β-glucocerebrosidase) leads

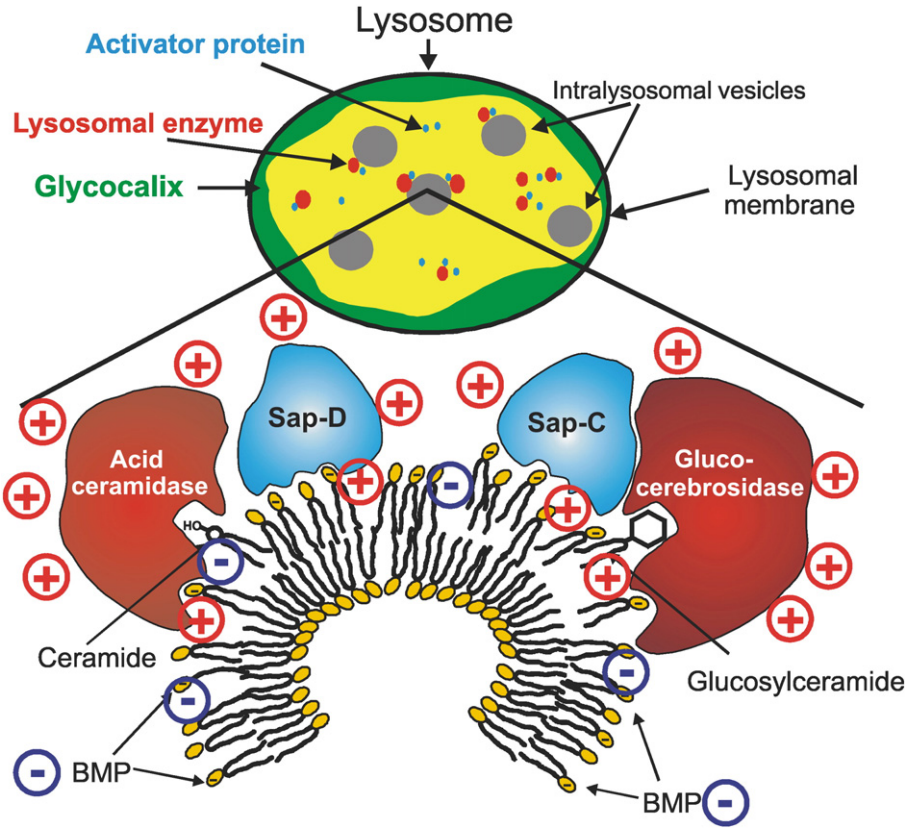

Fig. 4. Sphingolipid degradation at membrane–water interface of luminal lysosomal vesicles. At acidic pH values (4–5) cationic lysosomal lipid binding proteins and enzymes bind to the negatively charged surface of bis(monoacylglycero)phosphate (BMP) containing inner membranes (IM) (modified from [66]). Sap-D, saposin-D; Sap-C, saposin-C.

to accumulation of glucosylceramide [103,104] and of the cytotoxic and cationic glucosylsphingosine especially in the severe forms of the disease [105–107]. The rarely diagnosed deficiency of the lipid binding and transfer protein saposin C causes a variant form of Gaucher disease [108,109]. Also Sap-D deficiency causes Gaucher disease [75]. Plasma levels of glucosylsphingosine can be used as markers to control the efficiency of enzyme replacement therapy in Gaucher type I patients [110]. As in most sphingolipidoses, three different courses of the disease are distinguished. Type I is the most frequent form of Gaucher disease with a frequency of 1:50 000–200 000 births, with a higher frequency amongst Ashkenazi Jewish population (1:1000) [6]. This adult or late onset form is characterized by a chronic, nonneuropathic course of the disease due to a residual catabolic activity of the patients defective β-glucosidase. It can be treated with enzyme replacement therapy (see Section 8). Gaucher type II is an infantile disease with involvement of the nervous system and life expectancy of less than two years. Gaucher type III is an intermediate, juvenile form of the disease. A complete loss of β-glucosidase activity causes a perinatal fatal disease of the "collodion baby" type with a loss of the skin permeability barrier [81,111]. Additionally mutations in the glucosylceramide-β-glucosidase gene are a risk factor for Parkinson's disease and other dementia with Lewy bodies [112]. Although the nature of this connection is not fully understood, it has been observed that α-synuclein, a protein implicated in Parkinson disease interacts under lysosomal conditions with glucosylceramide-β-glucosidase and inhibits its enzymatic activity [113]. It has been shown that Sap-C can protect glucosylceramide-β-glucosidase from α-synuclein inhibition by competing with α-synuclein binding at the enzyme [114].

Glucosylceramide-β-glucosidase is stimulated by anionic lipids [105] and targeted to the lysosome in a mannose-6-phosphate receptor independent pathway. The enzyme binds the lysosomal integral membrane protein type 2 (LIMP-2) and the glucosylceramide-β-glucosidase/LIMP-2 complex is transported to the lysosomal compartment [115].

Lyso(glyco)sphingolipids have been identified as storage compounds besides sphingolipids in several sphingolipidoses, glucosylsphingosine and glucosylsphinganine in Gaucher disease [106], galactosylsphingosine and galactosylsphinganine in Krabbe disease [116], lyso-GM2 in Tay-Sachs disease [117], and globotriaosyl-sphingosine and -sphinganine in Fabry disease [118] (psychosine hypothesis). They all contain a free secondary aminogroup which gets protonated in acidic environment of the late endosomes and lysosomes, and therefore neutralize the anionic surface of the luminal vesicles. As cationic amphiphilic lipids they should decrease the electrostatic interaction between the surfaces of luminal lysosomal vesicles and polycationic lysosomal proteins, namely the hydrolytic enzymes and lipid binding proteins SAPs. Lyso(glyco) sphingolipids thereby interfere with the lipid degradation in the lysosomal compartment at the surface of intraluminal vesicles and membrane structures, as analyzed and elucidated for desipramine, a cationic amphiphilic drug [84]. Lyso-sphingolipids are micelle forming lipids, labilize bilayer structures and are toxic (psychosine hypothesis [119]) when they occur at higher concentrations in vesicles and membranes. Cationic glucosyl-sphingosine (and -sphinganine) is catabolized by glucosylceramide-β-glucosidase and competes as a substrate with the main storage glucosylceramide in Gaucher disease [105], whereas the lipid binding protein Sap-C and anionic membrane lipids like phosphatidylglycerol, phosphatidic acid, phosphatidylinositol, phosphatidylserine, and BMP stimulate GlcCer degradation by glucosylceramide-β-glucosidase by up to two orders of magnitude [66,105] (Fig. 5). In contrast to the water-soluble synthetic substrates, *e.g.* 4-methylumbelliferyl-β-D-glucopyranoside (4-MU-β-Glc), widely used for glucosylceramide-β-glucosidase assays in hospitals, the catabolic rate of the water-insoluble storage compound GlcCer by glucosylceramide-β-glucosidase is in the absence of endogenous stimulators, Sap-C and anionic membrane lipids negligible using a liposomal assay system mimicking the *in vivo* situation. It would not allow a physiological turnover. Indeed, the inherited absence of Sap-C

or Sap-D result in a fatal glucosylceramide storage even in the presence of wild type active glucosylceramide-β-glucosidase, causing late infantile/juvenile forms of Gaucher disease [75,108,109].

6.4. Metachromatic leukodystrophy

Inherited deficiency of arylsulfatase A leads to the accumulation of sulfatide in several tissues of patients with metachromatic leukodystrophy (MLD) [120]. *In vivo* the reaction requires the presence of Sap-B, its deficiency causes a late infantile form of MLD with a pronounced sulfatide storage [72,121]. Sap-B forms stoichiometric soluble complexes with sulfatides which are forming a Michaelis–Menten complex with arylsulfatase A [121,122]. Although this implies a narrow specificity, Sap-B seems to be a rather promiscuous lipid binding protein [123,124]. Even in the presence of plant and bacterial enzymes, human Sap-B stimulates the hydrolysis of numerous microbial glycolipids [125].

6.5. Krabbe disease

The underlying cause of Krabbe disease, also called globoid cell leukodystrophy, is an inherited deficiency of galactosylceramide-β-galactosidase [126,127]. *In vivo* hydrolysis of galactosylceramide to ceramide and galactose, catalyzed by galactosylceramide-β-galactosidase requires the help of Sap-A. Deficiency of the sphingolipid activator protein likewise causes Krabbe disease [71]. In cell culture experiments Sap-C stimulates galactosylceramide degradation [128], possibly not enough to ensure sufficient turnover in human tissues. Its mild storage is not the main feature of Krabbe disease. The impaired degradation of the cationic and cytotoxic galactosylsphingosine (psychosine) which is also formed in the myelin producing oligodendrocytes causes their rapid destruction and the consequent paradoxical analytical finding, the lack of accumulation of the primary substrate, galactosylceramide, in patients' brain (psychosine hypothesis). Meanwhile the psychosine hypothesis (see Section 6.3), the idea that the lyso-derivatives play a key role in sphingolipidoses pathology, has been extended to other sphingolipidoses, like Gaucher, MLD, GM1-gangliosidosis, Niemann–Pick type A, and Tay-Sachs disease [119].

6.6. Niemann–Pick disease, types A and B

Niemann–Pick disease types A and B are caused by an inherited deficiency of acid sphingomyelinase (ASM) [129,130]. The enzyme catalyzes the hydrolysis of sphingomyelin to ceramide in a phospholipase C reaction [131,132]. *In vivo* the reaction does not require an additional SAP, since the modular structure of acid sphingomyelinase includes a Sap-like domain and a catalytic domain [133]. Niemann–Pick disease type A is caused by an almost complete loss of ASM activity. The disease is a fatal disorder of infancy with a life expectancy of 2 to 3 years.

In Niemann–Pick type B patients have a higher residual ASM activity [134]. The disease is phenotypically variable with little or no involvement of the nervous system.

ASM does not only catalyze the cleavage of sphingomyelin, it also shows phosphodiesterase activity against several phospholipids, such as phosphatidylcholine, phosphatidylglycerol, and at slow rate phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine

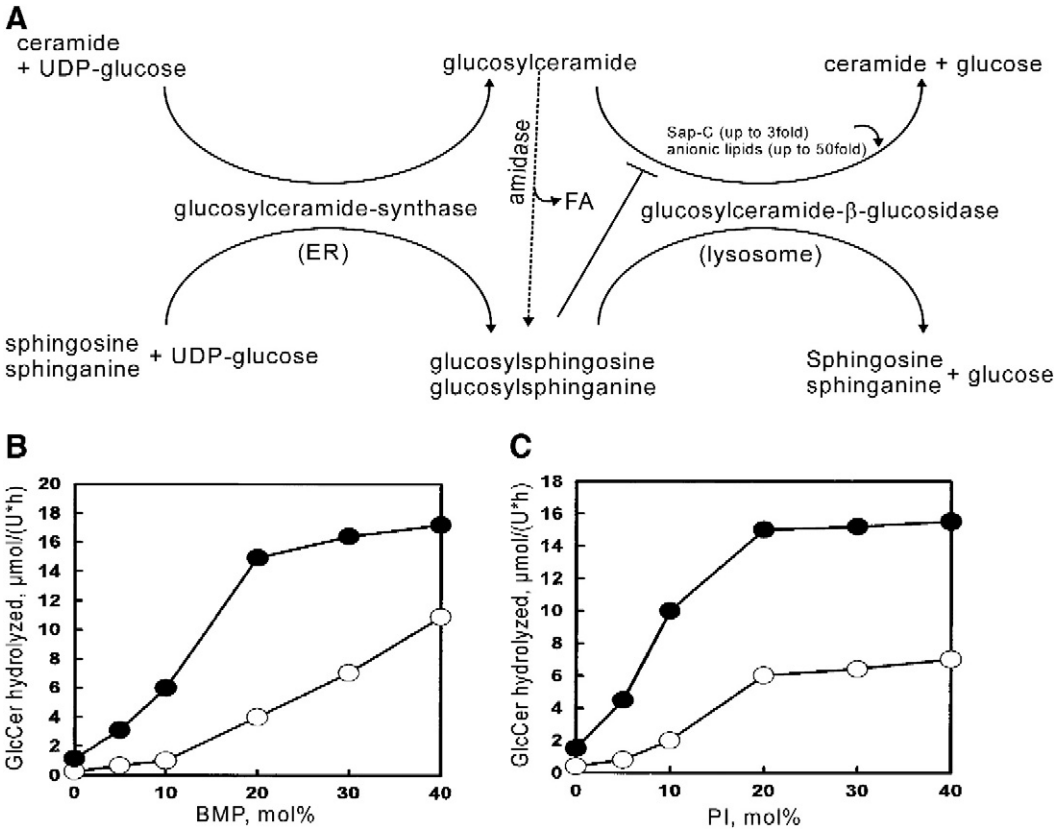

Fig. 5. Proposed formation of glucosylsphingosine and -sphinganine. A: Glucosylceramide is generated as a component of ER membranes from ceramide and UDPglucose by glucosylceramide synthase, or in the endosomal compartment by catabolism of glycosphingolipids. Glucosylsphingosine and -sphinganine may be generated at the endoplasmic reticulum (ER) biosynthetically from sphingosine, sphinganine and UDP-glucose by a minor side specificity of glucosylceramide synthase, or in the endolysosomal compartment by an acid ceramidase like amidase. FA, fatty acid. B + C: Assays were conducted with glucosylceramide (GlcCer) as substrate in the absence (o) and presence of saposin-C (Sap-C) (2.5 µmol) (•) using large unilamellar vesicles with various proportions of synthetic bis(monoacylglycero)phosphate (BMP) (B) or phosphatidylinositol (PI) (C) (0–40 mol%), keeping the total lipid concentration in the assay constant [66].

[131]. The ASM reaction is *in vitro* stimulated by Sap-C and by anionic phospholipids [56,135].

The hydrolysis of sphingomyelin by ASM yields phosphorylcholine and ceramide, a highly bioactive lipid [136]. Ceramide is further degraded to sphingosine, which can be phosphorylated to sphingosine-1-phosphate, a potent signaling molecule [137]. The key position of ASM for the dynamic balance between these lipids has implications for various pathological conditions. Increased levels of ASM activity have been found in major depression [138]. Elevated acid sphingomyelinase and acid ceramidase expression was found in Alzheimer's disease [139]. Increased Secretory ASM activity is implicated in alcohol-induced lipid alterations [140].

In an experimental model of hepatocellular carcinoma the use of recombinant ASM as an adjuvant treatment with sorafenib lowered tumor volume, increased tumor necrosis, and decreased tumor blood vessel density compared to sorafenib alone [141].

6.7. Farber disease

Inherited deficiency of acid ceramidase leads to Farber disease, also called Farber lipogranulomatosis. Acid ceramidase catalyzes the last step of sphingolipid degradation, the cleavage of ceramide to sphingosine and fatty acid. The reaction requires the presence of Sap-D [142]. At a distinct pH acid ceramidase is also able to catalyze the reverse reaction, the formation of ceramide [143]. The clinical manifestation of Farber disease is the development of painful and progressive joint deformations, subcutaneous nodules (lipogranulomas), and progressive hoarseness. Acid ceramidase knockout mice do not survive beyond the 2-cell stage [144]. The enzyme is an essential component of oocyte and embryonic survival *in vivo*. Supplementing culture medium with recombinant acid ceramidase improves the outcome of *in vitro* fertilization [145].

7. The threshold theory

The almost complete loss of catabolic activities usually gives rise to the fatal infantile forms of storage diseases. Allelic mutations which lead to proteins with some residual catabolic activities result in the protracted clinical forms often described as late infantile, juvenile or chronic diseases. Usually the severity of the clinical symptoms correlates inversely with the residual catabolic activities. Indeed, residual ganglioside GM2 cleaving activities of 10–20% of the control value already appear to be sufficient to prevent a clinical manifestation of the storage disease [146] (Fig. 6). On the basis of a greatly simplified kinetic model [147], the threshold theory, these observation can be understood as a fragile balance between substrate influx into the lysosomal compartment, the steady-state concentration and the turnover, the degradation of the substrate. When the degradative capacity falls below a critical threshold, the lysosomal turnover of the substrate is reduced and it will accumulate, leading to a storage disease. This model is not limited to GM2 cleavage, it can be used for every storage disease which manifests itself in an infantile, juvenile and adult (chronic) form, such as Gaucher, MLD, or Farber [6].

8. Therapeutic approaches

Therapeutic approaches addressing the underlying metabolic defect of the diseases are enzyme replacement therapy (ERT), substrate reduction therapy (SRT), cell mediated therapies, enzyme stabilization by chemical chaperones, and gene therapy.

ERT was first developed by Brady and coworkers for the adult nonneuronopathic type (type 1) of Gaucher disease [148]. They modified the oligosaccharide chains of glucosylceramide-β-glucosidase (glucocerebrosidase) to expose mannose residues, targeting the enzyme for the mannose receptor on macrophages, like Kupffer cells [149]. In Gaucher type 1 ERT does not only attenuate the disease process, but also reverses the lysosomal storage of glucosylceramide in patient's reticuloendothelial system, providing the proof of principle for ERT [149,150]. Today ERT with recombinant enzymes is available for Gaucher, Fabry, Pompe disease, and mucopolysaccharidosis I, II, and VI [2,151]. Up to now ERT was limited on nonneuronopathic forms of the diseases since it is generally assumed that the enzymes cannot pass the blood–brain barrier. Nevertheless results indicate that therapeutic enzymes can be delivered across the blood–brain barrier in the adult mucopolysaccharidosis type VII mouse if administered in high doses [152]. It also improves nervous system pathology and function in a mouse model for metachromatic leukodystrophy [153].

In case of patients with advanced Fabry disease ERT did not prevent progression towards fatal organ failure and death [154]. The reason for the poor outcome is still unclear. In a mouse model of GM1-gangliosidosis, GM1 accumulates not only in the lysosomes, but also in mitochondria-associated ER membranes, or MAMs, which would be inaccessible to ERT [155].

In substrate reduction therapy orally available small molecule drugs are inhibiting sphingolipid biosynthesis [156]. Substrate influx into the lysosome is reduced and potentially available residual catabolic enzyme activity can degrade the reduced storage amounts of lipids and attenuate clinical manifestation of the disease. In Gaucher type I patients the glucosylceramide synthase inhibitor N-butyl-deoxynojirimycin (Zavesca) improved key clinical features [157,158]. However negative side effects might be a problem in long-term treatment [159].

Another therapeutic approach so far with limited success is based on small molecules, chemical chaperones, also called enzyme enhancement

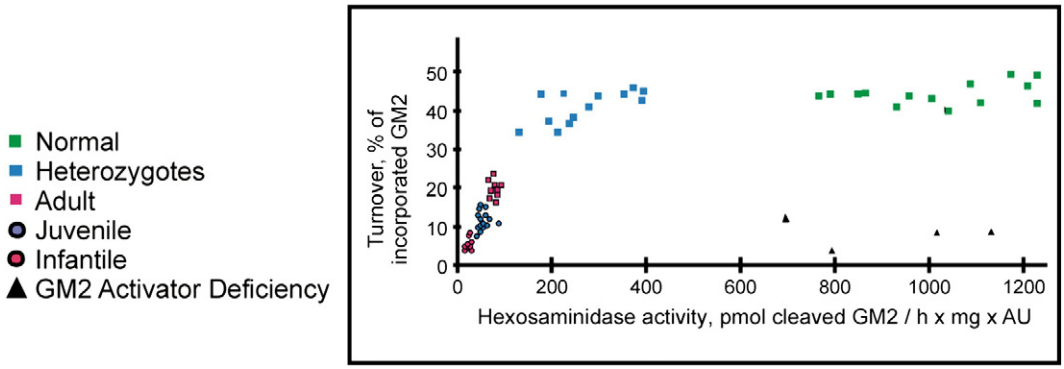

Fig. 6. Threshold theory. Residual catabolic activity correlates with clinical forms of GM2 gangliosidoses (modified after [147]). Radiolabeled ganglioside GM2 was added to cultured skin fibroblasts of patients and probands with different activities of beta-hexosaminidase A and its turnover measured. In agreement with the threshold theory the degradation rate of GM2 increased with residual activity. At a residual beta-hexosaminidase A activity of approximately 10–20% of normal, the cells reached a physiologically normal turnover rate for ganglioside GM2 (the critical threshold). According to this feeding experiments, GM2 gangliosidosis does not manifest itself in heterozygotes of beta-hexosaminase deficiency, since they have a residual enzyme activity above the critical threshold.

therapy [160]. Active site-binding molecules can stabilize the functional conformation of patient's variant enzymes with residual activity. Chemical chaperones, mostly active site inhibitors have been used in Fabry disease [161,162], Gaucher disease [163], GM1 gangliosidosis [164], and GM2 gangliosidosis [165].

Gene therapy might have the potential to treat sphingolipidoses with CNS involvement. The insertion of a functional copy of a mutated gene with suitable vector systems has been tested in several animal models of sphingolipidoses [166], such as Gaucher disease [167], metachromatic leukodystrophy [168], and Tay-Sachs disease [169]. However, intracranial delivery of recombinant adeno-associated viral vectors to Sandhoff mice can increase survival only when the therapeutic genes are expressed either before the disease is apparent or during its early manifestations [169]. Up to now the experimental technique of gene therapy of sphingolipidoses is limited to animal models, their feasibility in human patients may arise in the distant future.

Cell mediated therapies of sphingolipidoses include bone marrow transplantation and the implantation of neural progenitor cells [170]. Bone marrow transplantation has been applied to human patients, *e.g.*, of Krabbe disease and MLD [171–173].

In the mouse model of Niemann–Pick disease type A the intracerebral transplantation of transduced neural progenitor cells into the brain led to a reversal of lipid storage [174]. In adult symptomatic Sandhoff mice the intracranial injection of neural stem cells restored β-hexosaminidase enzyme activity, improved neurological function and slowed disease progression [175].

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