



# Lysosomal Lipid Storage Diseases

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Lysosomal lipid storage diseases, or lipidoses, are inherited metabolic disorders in which typically lipids accumulate in cells and tissues. Complex lipids, such as glycosphingolipids, are constitutively degraded within the endolysosomal system by soluble hydrolytic enzymes with the help of lipid binding proteins in a sequential manner. Because of a functionally impaired hydrolase or auxiliary protein, their lipid substrates cannot be degraded, accumulate in the lysosome, and slowly spread to other intracellular membranes. In Niemann-Pick type C disease, cholesterol transport is impaired and unesterified cholesterol accumulates in the late endosome. In most lysosomal lipid storage diseases, the accumulation of one or few lipids leads to the coprecipitation of other hydrophobic substances in the endolysosomal system, such as lipids and proteins, causing a “traffic jam.” This can impair lysosomal function, such as delivery of nutrients through the endolysosomal system, leading to a state of cellular starvation. Therapeutic approaches are currently restricted to mild forms of diseases with significant residual catabolic activities and without brain involvement.

Lysosomal lipid storage diseases are a group of inherited catabolic disorders in which typically large amounts of complex lipids accumulate in cells and tissues. Macromolecules such as complex lipids and oligosaccharides are constitutively degraded in the acidic compartments of the cell, the endosomes, and lysosomes, into their building blocks. The resulting catabolites are exported to the cytosol and reused in cellular metabolism. When lysosomal function is impaired because of a defect in a catabolic step, degradation cannot proceed normally and undegraded compounds accumulate. Lysosomal lipid storage diseases comprise mainly the sphingolipidoses, Niemann-Pick type C disease (NPC), and Wolman disease, including the

less severe form of this disease, called cholesteryl ester storage. NPC is a complex lipid storage disease mainly characterized by the accumulation of unesterified cholesterol in the late endosomal/lysosomal compartment (Bi and Liao 2010). The sphingolipidoses are caused by defects in genes encoding proteins involved in the lysosomal degradation of sphingolipids (Kolter and Sandhoff 2006). First reports on these diseases were given more than a century ago. Already in 1881, Warren Tay described the clinical symptoms of a disease, which is today called Tay-Sachs disease (Tay 1881). After Christian de Duve discovered the lysosome in 1955 (de Duve 2005), Henri-Géry Hers established the first correlation between an enzyme

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deficiency and a lysosomal storage disorder (Pompe's disease) in 1963 (Hers 1963). In the following decades, the enzymes and cofactors deficient in the sphingolipidoses have been identified. Though lysosomal lipid storage diseases have been known for a long time, treatment is only available for a few mild forms of the diseases, such as the adult forms of Gaucher disease (Barton et al. 1991). For several lysosomal storage diseases, therapies like enzyme replacement or bone marrow transplantation are in the clinical trial stage (Platt and Lachmann 2009). For a long time, lysosomal diseases have been considered a problem of superabundance (storage) in which the storage material can slowly spread to other cellular membranes, impairing their function. More recently, it came into focus that massive storage prevents lysosomal functions such as nutrition delivery through the endolysosomal system, leading to a state of cellular starvation. In mouse models of both GM1 and GM2 gangliosidoses iron is progressively depleted in brain tissue. Administration of iron prolonged survival in the diseased mice by up to 38% (Jeyakumar et al. 2009).

### LYSOSOMES AS STOMACHS OF THE CELL PROVIDE CELLS WITH NUTRIENTS

Lysosomes provide cells with nutrients, and should be thought of as stomachs of the cell (Kolter and Sandhoff 2010). Export of metabolites from the lysosome is mediated by transport proteins within the lysosomal perimeter membrane (Sagné and Gasnier 2008). Defective transport across the lysosomal membrane can lead to intralysosomal storage and starvation of the cell, as in Salla disease, where sialic acid is accumulated (Ruivo et al. 2009). Cobalamin uptake takes place via endocytosis and release from the lysosomes. Defects in the presumed lysosomal membrane exporter for cobalamin, LMBD1, lead to the accumulation of the vitamin in the lysosomes, reducing its conversion to enzyme cofactors (Rutsch et al. 2009). Furthermore, lysosomes play an important role in iron metabolism (Kurz et al. 2008), supplying the cytosol with  $\text{Fe}^{2+}$  either by autophagy or

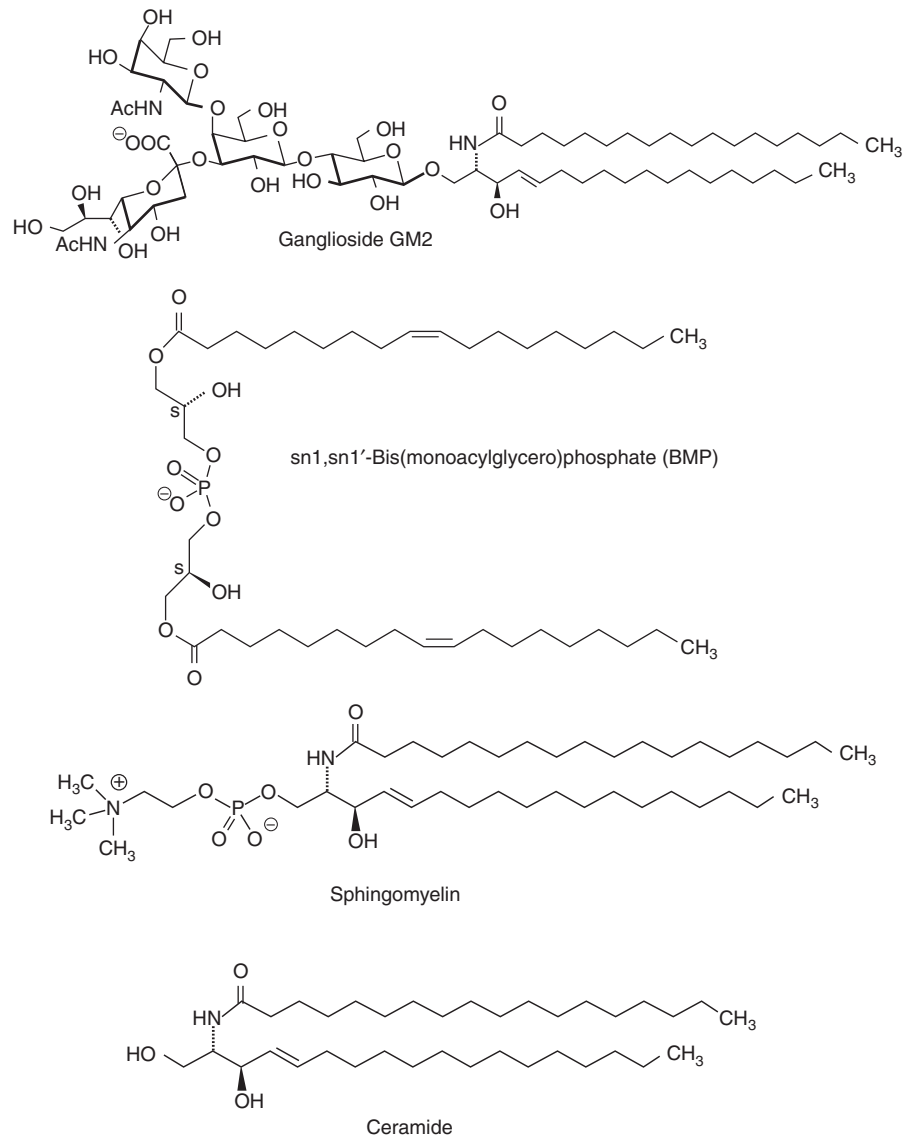
by release from endocytosed transferrin. Many autophagocytosed proteins such as ferritin, and proteins from the electron transport chain, contain iron. Nondividing cells, (i.e., neurons), might fulfill their need in iron ions largely by reuse of catabolites of autophagocytosed iron-containing proteins.

Mutations in the human *TRPML1* gene, coding for a predicted late endosomal and lysosomal iron channel protein, cause mucopolidosis type IV disease. Impaired iron transport may contribute to hematological and degenerative symptoms of mucopolidosis type IV patients (Dong et al. 2008).

Besides the degradation of defective proteins, the supply with nutrients such as iron ions even under nonstarving conditions is an essential function of autophagy. Mice lacking *Atg7*, a gene essential for autophagy, show massive neurodegeneration (Komatsu et al. 2006). Uptake of exogenous iron by dividing cells is mediated through endocytosed transferrin (and the transferrin receptor). The iron is released in the endosomes at decreased pH-values and can leave the compartment to the cytosol by the divalent metal transporter-1 and may reach the outer mitochondrial membrane by temporary close contact of the organelles (Zhang et al. 2005). However,  $\text{Fe}^{2+}$  ions should always be protein bound because free  $\text{Fe}^{2+}$  ions can promote the formation of very reactive hydroxyl-radicals via the Fenton reaction (1.  $\text{Fe}^{2+} + \text{O}_2 \rightarrow \text{Fe}^{3+} + \text{O}_2^{\bullet-}$ ; 2.  $2\text{H}^+ + 2 \text{O}_2^{\bullet-} \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$ ; 3.  $\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^{\bullet}$ ).

### SPHINGOLIPIDS

Sphingolipids and glycosphingolipids are ubiquitous components of mammalian cell membranes. They are characterized by the presence of a hydrophobic membrane anchor, ceramide, and a sphingoid base linked via the amino group to a fatty acid. Its terminal hydroxyl group is bound to a hydrophilic headgroup, phosphorylcholine in the case of sphingomyelin or a carbohydrate headgroup in the case of glycosphingolipids (GSL) (Fig. 1). Biosynthesis of glycosphingolipids starts with the formation of ceramide at the cytoplasmic face of the



**Figure 1.** Structures of ganglioside GM2, sphingomyelin, ceramide, and BMP.

endoplasmatic reticulum (ER) membrane (Merrill 2002; Kolter and Sandhoff 1999). De novo biosynthesis competes with sphingolipid formation by salvage pathways using building blocks (e.g., sphingoid bases) released from the lysosomal compartments. Depending on the cell type, 50%–90% of glycosphingolipids are derived from the salvage pathways (Gillard et al. 1998; Tettamanti et al. 2003). During

biosynthesis, ceramides are transferred to the cytosolic leaflet of the Golgi membrane by secretory vesicular flow and by the lipid transfer protein CERT (Hanada et al. 2003), where glucosylceramide is formed and translocated to the luminal face. Subsequent glycosylation reactions give rise to the complex carbohydrate pattern of gangliosides. After their biosynthesis, complex glycosphingolipids 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 (Tafesse et al. 2007).

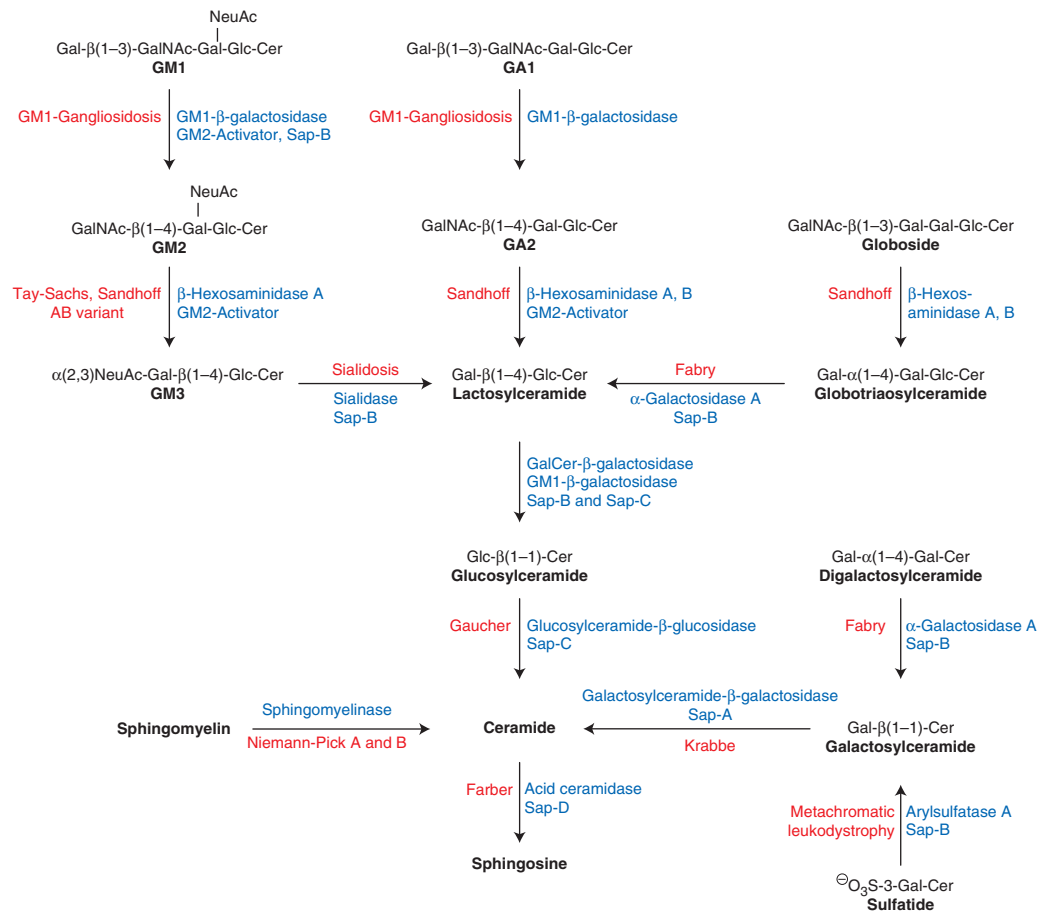
To date, only very few diseases associated with impaired sphingolipid biosynthesis are known. Partial deficiency of the biosynthetic enzyme lactosylceramide  $\alpha$ 2, 3 sialyltransferase (GM3 synthase) causes an autosomal recessive infantile-onset symptomatic epilepsy syndrome (Simpson et al. 2004). Mutations in the *SPTLC1* gene coding for a subunit of the serine palmitoyltransferase, lead to enhanced neuronal apoptosis because of elevated levels of deoxyceramides (Dawinks et al. 2001; Bejaoui et al. 2002). They cause an adult-onset, hereditary sensory, and autonomic neuropathy type I (HSAN1). The mutations alter amino acid selectivity of the serine palmitoyltransferase enzyme, leading to condensation of palmitate with alanine and glycine, in addition to serine, and resulting in the accumulation of two atypical neurotoxic deoxysphingoid bases (Penno et al. 2010).

### SPHINGOLIPIDOSES

The sphingolipidoses are inherited lipid storage diseases caused by defects in genes encoding proteins of the lysosomal catabolism. All sphingolipidoses are inherited in an autosomal recessive mode, with the exception of Fabry disease, which follows an X-linked recessive mode of inheritance (Desnick et al. 2001). GSLs are degraded along a strictly sequential pathway in humans (Fig. 2). For almost every degradation step, a disease has been described in which the correlated enzyme or activator protein is defective. Lactosylceramide can be degraded by two enzyme/activator systems (Zschoche et al. 1994). Therefore, no single enzyme defect is known that leads to isolated lactosylceramide storage. However, lactosylceramide accumulates, together with other sphingolipids, when several cofactors are absent simultaneously, as it is the case in prosaposin deficiency (Bradova et al. 1993).

### LIPID SORTING AND FORMATION OF INTRAENDOLYSOSOMAL VESICLES

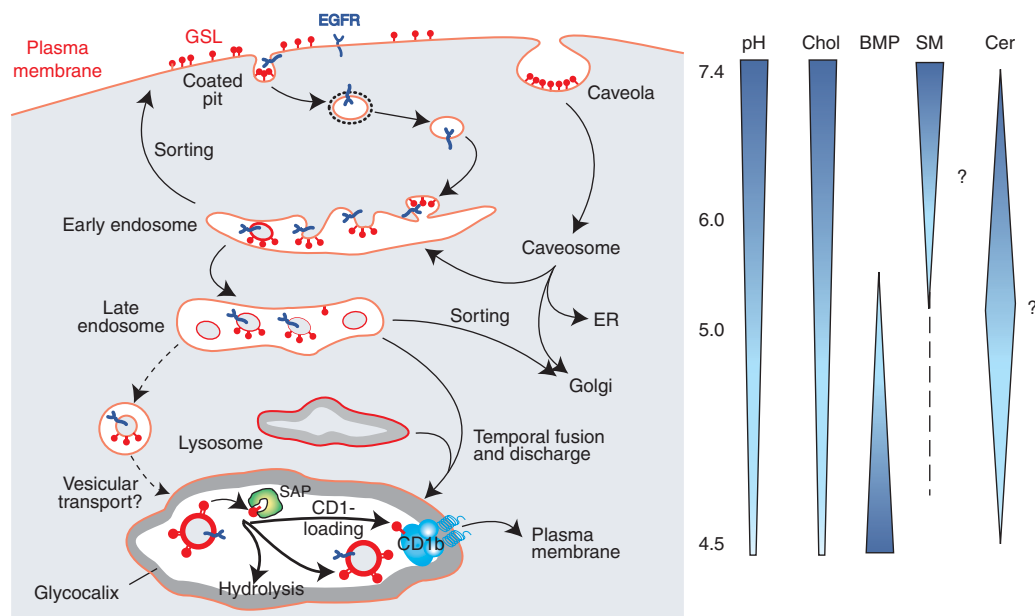
Water-soluble macromolecules such as proteins and oligosaccharides can easily be reached by soluble enzymes and be degraded in the endolysosomal system. However, degrading membrane-lipids in an organelle without destroying the integrity of its perimeter membrane requires more complex sorting and disintegration systems. This led to the assumption that two distinct pools of membranes exist in the late endolysosomal compartment, which differ in lipid and protein composition (Fürst and Sandhoff 1992; Kolter and Sandhoff 2010). Lipids reach the lysosomal compartment either as part of the limiting membrane, or as part of intraendosomal membranes, the main site of sphingolipid degradation. The lysosomal perimeter membrane is protected from degradation by a glycocalyx facing the lumen of the organelle and composed of glycoproteins heavily glycosylated with lactosamine units (Eskelinen et al. 2003). Intralysosomal membranes have initially been observed in cells of patients with sphingolipid storage diseases such as GM1 gangliosidosis (Suzuki and Chen 1968) or combined sphingolipid activator protein deficiency (Harzer et al. 1989), where nondegradable lipids accumulate in multivesicular storage bodies. Multivesicular bodies are formed by inward budding of the limiting endosomal membrane, mediated by the sequential action of three endosomal sorting complexes required for transport, ESCRT-I, -II, -III (Saksena et al. 2007; Wollert and Hurley 2010). During endocytosis and maturation of endosomes, the luminal pH value decreases, and lipid composition of the internal membranes is adjusted for degradation (Fig. 3). Membrane-stabilizing cholesterol is sorted out and a main activator of enzymatic sphingolipid degradation, bis-(monoacylglycerol)-phosphate (BMP), is formed (Möbius et al. 2003). BMP is a characteristic anionic lipid on the surface of intralysosomal membranes, which is negatively charged even at lysosomal pH values. The perimeter membrane does not contain BMP (Möbius et al. 2003). Because of its unusual sn1, sn1'-configuration, BMP is only slowly



**Figure 2.** Degradation of selected sphingolipids in the lysosomes of the cells. The eponyms of individual inherited diseases are given. Activator proteins required for the respective degradation step in vivo are indicated. Variant AB, AB variant of GM2 gangliosidosis (deficiency of GM2-activator protein); Sap, saposin (adapted from Kolter and Sandhoff [2005] and reprinted here with permission from Annual Review of Cell and Developmental Biology ©2005).

degraded by lysosomal phospholipases (Matsuzawa and Hostetler 1979). BMP derives from phosphatidylglycerol generated in the ER and from cardiolipin, which reaches the lysosomes presumably as part of mitochondria by macroautophagy (Brotherus and Renkonen 1977; Amidon et al. 1996). Together with smaller amounts of phosphatidylinositol (Kobayashi et al. 1998) and dolichol phosphate (Chojnacki and Dallner 1988), BMP causes a negative charge of intralysosomal membranes. Because of their isoelectric points, most activator proteins and hydrolytic enzymes, such as acid sphingomyelinase, are positively charged at the acidic pH

values of the lysosomes. As polycations, they should adhere to the surfaces of intralysosomal vesicles. Binding of the cationic lysosomal proteins to the negatively charged surface of the inner vesicles allows degradation of lipids at the membrane-water interphase. Some cationic amphiphilic drugs, such as the antidepressant desipramine, can interfere with the negatively charged surface, leading to release and subsequent proteolysis of the hydrolytic enzyme. In the case of acid sphingomyelinase, this leads to a drug induced lipidosis (Kölzer et al. 2004). Based on in vitro experiments we assume that acid sphingomyelinase is already quite active in



**Figure 3.** Model of endocytosis and lysosomal digestion of membranes. Glycosphingolipids (GSL) are highlighted on the plasma membrane (PM) and on internal membranes, and gradients of pH in the lumen of the organelles and lipids in the intraendosomal vesicles; cholesterol (Chol), BMP, sphingomyelin (SM; hypothetical), and ceramide (Cer; hypothetical) are shown (adapted from Kolter and Sandhoff [2005] and reprinted here with permission from Annual Review of Cell and Developmental Biology ©2005). EGFR epidermal growth factor receptor, SAP sphingolipid activator proteins.

late endosomes and converts sphingomyelins of the intraendosomal vesicles and lipid aggregates into ceramides (Abdul-Hammed et al. 2010). This would stimulate the cholesterol transfer mediated by the NPC-2 protein. This transfer is stimulated by BMP and ceramide in the vesicular membranes and inhibited by sphingomyelin (Abdul-Hammed et al. 2010).

### LYSOSOMAL LIPID BINDING PROTEINS

In addition to hydrolyzing enzymes and anionic lipids, especially BMP, lysosomal degradation of glycosphingolipids requires auxiliary proteins, the lysosomal lipid binding proteins (LLBP). Other membrane compounds such as phospholipids can apparently be degraded without their help. GSLs with short carbohydrate chains of four or less sugars bound to intralysosomal membranes are not sufficiently accessible to the water-soluble enzymes present in the

lysosomal lumen. LLBP bind, solubilize, and present membrane-lipids to their respective hydrolases for degradation (Fürst and Sandhoff 1992). They encompass five sphingolipid activator proteins, the saposins A-D (Sap-A-D), and the GM2 activator protein (Conzelmann and Sandhoff 1979; Sandhoff et al. 2001). Sap-A-D derives from one common precursor protein, the prosaposin (p-Sap).

### MOLECULAR AND CELLULAR PATHOGENESIS OF SPHINGOLIPID STORAGE DISEASES

#### Organ and Cell Specificity of Sphingolipid Storage

Sphingolipid storage diseases are caused by defective catabolic activities in the endolysosomal system of the cells. Lysosomal accumulation occurs predominantly in cells and organs that have the highest rates of biosynthesis or uptake



of the undegradable sphingolipids and their precursors. For example, blocks in ganglioside catabolism result predominantly in neuronal degeneration, whereas blocks in sulfatide and galactosylceramide (GalCer) degradation lead to myelin diseases. Blocks in glucosylceramide (GlcCer) catabolism primarily lead to GlcCer and glucosylsphingosine storage in macrophages (in blood, spleen, and in Kupffer cells of the liver), thus generating Gaucher cells, because they have the highest load of GSLs to degrade, their own synthesized GSLs and all the GSL material they ingest, (e.g., from red blood cells) (Kolter and Sandhoff 2010).

### Threshold Theory

Genetic mutations may well result in a complete functional loss of the encoded lysosomal hydrolase or LLBP, leading to severe clinical forms, usually infantile (Tay-Sachs disease, Niemann-Pick disease type A) or even prenatal fatal disease (“Collodian Babies,” p-Sap deficiency), whereas the generation of variant lysosomal proteins may well cause protracted forms of the disease (juvenile, adult, chronic forms). The level of residual catabolic activity is one out of several factors contributing to the molecular pathogenesis and clinical form of the disease. In the threshold theory, a correlation between functional residual catabolic activity and the progression of the lipid storage disease has been formulated (Conzelmann and Sandhoff 1983–1984), which was basically confirmed for different clinical forms of diseases such as metachromatic leukodystrophy (Leinekugel et al. 1992; Tan et al. 2010), GM2-gangliosidosis (Leinekugel et al. 1992), Gaucher (Gieselmann 2005), and Niemann-Pick type A and B diseases (Ferlinz et al. 1995).

### FORMATION OF TOXIC COMPOUNDS AND CELLULAR PATHOGENESIS (LYSOSPHINGOLIPIDS AS CATIONIC AMPHIPHILES)

Cationic lysocompounds (galactosylsphingosine (GalSo), glucosylsphingosine (GlcSo), sphingosine (So), sphinganine (Sa), but also lysoGM2

and lysosulfatides) are toxic. They are micelle-forming inhibitors of catabolic enzymes, and presumably also compensate negative charge of inner membranes in lysosomes.

GalSo is specifically formed in oligodendrocytes. Its accumulation kills these myelin-forming cells in Krabbe disease, leading to an impaired myelination (Suzuki 2003).

GlcSo is toxic. It inhibits glucosylceramide- $\beta$ -glucosidase (Sarmientos et al. 1986) and accumulates in severe forms of Gaucher disease. “Collodian babies” with no residual glucosylceramide- $\beta$ -glucosidase activity have a severe skin phenotype with no functional water barrier because of a block of ceramide formation in the extracellular space of the epidermis. These babies lose dramatic amounts of water through the skin and die within two hours after birth.

A moderate accumulation of sphingosine and sphinganine also contributes to the molecular pathology of Niemann-Pick type C disease (Rodríguez-Lafrasse et al. 1994; Lloyd-Evans and Platt 2010).

Complex lysoglycolipids (lysosulfatide, lysoGM2, etc.) are minor storage compounds and their contribution to the pathogenesis of their respective disease is presumed to be small (Neuenhofer et al. 1986; Rosengren et al. 1989).

### ACCUMULATION OF SPHINGOLIPIDS IN CELLULAR MEMBRANES OUTSIDE THE ENDOLYSOSOMAL SYSTEM

Storage of GlcCer (in Gaucher disease) (Jmoudiak and Futermann 2005), Globotriaosylceramide (Gbose3) (in Fabry disease), GM2, and GM1 (Tessitore et al. 2004) has also been identified in other cellular membranes besides the endolysosomal system. During months and years of disease progress, storage compounds spill over from endolysosomal membranes to other cellular membranes by membrane-flow, membrane contact, or probably also by protein transport.

Accumulation of these storage compounds in ER membranes affects several functions of the organelle, (e.g.,  $\text{Ca}^{2+}$  homeostasis) (LaPlante et al. 2002; Pelled et al. 2003) and signaling cascades (Takamura et al. 2008).



## LABILIZATION OF LYSOSOMAL PERIMETER MEMBRANES

The integrity of the limiting lysosomal membrane is essential for cell survival. It has been shown that Hsp70 can bind to BMP and stabilize lysosomes and ASM activity (Kirkegaard et al. 2010). Cationic amphiphilic drugs (CADs) are lysosomotropic agents. They increase the permeability of lysosomal perimeter membranes, and cause a “traffic jam” by secondary accumulation of further lipid compounds. As neutral amphiphiles, they penetrate membranes, and accumulate as protonated, membrane impermeable compounds in the acidic lysosomal compartment. This traffic jam attenuates autophagy and could also impair uptake of nutrients and removal of damaged organelles and proteins, as has been observed in GM1 gangliosidosis, Niemann-Pick disease type C, and Sandhoff disease. Sphingosine storage in Niemann-Pick type C disease reduces lysosomal  $\text{Ca}^{2+}$  ion content and impairs membrane trafficking (Lloyd-Evans and Platt 2010).

Defective processing of transferrin bound  $\text{Fe}^{2+}$  could also cause oxidative stress in lysosomes. Generation of free, not protein bound  $\text{Fe}^{2+}$  ions could trigger the formation of radical oxygen species (ROS) by the Fenton reaction and may give rise to the formation of lipofuscin or “age pigment.” Accumulation of lipofuscin seems to hinder normal autophagy and may be an important factor behind aging and age-related pathologies (Kurz et al. 2008). Enhanced oxidative stress causes lysosomal membrane permeabilization (Kurz et al. 2008).

NPC1 knockout mice show increased levels of many potentially atherogenic cholesterol auto-oxidation products (e.g., with hydroxyl groups in positions 5, 6, or 7) (Tint et al. 1998). However, levels of enzymatically formed 27-hydroxycholesterol are decreased (Zhang et al. 2008).

## GM1 GANGLIOSIDOSIS (AND MORQUIO TYPE B DISEASE)

GM1-gangliosidosis is caused by an inherited deficiency of the lysosomal enzyme GM1- $\beta$ -galactosidase (Suzuki et al. 2001; Sano et al. 2005).

In the presence of either the GM2-activator protein or Sap-B, GM1- $\beta$ -galactosidase catalyzes the cleavage of terminal  $\beta$ -D-galactose from ganglioside GM1 resulting in GM2. The reaction is stimulated by anionic phospholipids such as BMP (Wilkening et al. 2000).

Similarly, to other sphingolipidoses, three clinical forms of GM1-gangliosidosis can be distinguished: In infantile (type 1) GM1-gangliosidosis, developmental arrest and progressive deterioration of the nervous system occur in early infancy. The late infantile/juvenile form (type 2) is characterized by progressive neurologic symptoms in children, and the adult/chronic form (type 3) occurs in young adults. Besides spontaneous animal models of the disease (Suzuki et al. 2001), an engineered mouse model resembling the neurological phenotype of human GM1 gangliosidosis has been analyzed (Hahn et al. 1997).

Because of its changed substrate specificity, defective GM1- $\beta$ -galactosidase can also lead to Morquio type B disease. Morquio type B disease clinically resembles a mild phenotype of Morquio A disease, where keratan sulfate accumulates because of N-acetylgalactosamine-6-sulfatase deficiency.

## GM2 GANGLIOSIDOSES

The GM2 gangliosidoses are a group of three sphingolipidoses that result from defects in degradation of ganglioside GM2 and related glycolipids (Sandhoff 1969; Sandhoff et al. 1971; Gravel et al. 2001). In vivo, the degradation of GM2 requires the presence of the GM2-activator protein. Three lysosomal  $\beta$ -hexosaminidases, which differ in the combination of their two subunits ( $\alpha$  and  $\beta$ ) and their substrate specificity have been described.  $\beta$  - Hexosaminidase A (consisting of the  $\alpha$  and  $\beta$  subunits) cleaves terminal  $\beta$ -glycosidically linked N-acetylglucosamine- and N-acetylgalactosamine residues from negatively charged and uncharged glycoconjugates.  $\beta$ -Hexosaminidase B ( $\beta\beta$ ) cleaves uncharged substrates such as glycolipid GA2 and oligosaccharides with terminal N-acetylhexosamine residues.  $\beta$ -Hexosaminidase S ( $\alpha\alpha$ ) contributes to the degradation of



glycosaminoglycans and sulfated glycolipids. The inborn deficiency of the GM2-activator as well as the deficiency of the  $\alpha$ - or  $\beta$ -chain of the  $\beta$ -hexosaminidase isoenzymes leads to one of the three different variants of this disease that are named according to the isoenzyme remaining intact. Mouse models for Tay-Sachs and Sandhoff disease surprisingly differ severely in their phenotypes. The Sandhoff mouse, lacking hexosaminidases A and B, shows a severe neurological phenotype, corresponding to the human infantile onset variant. However, the Tay-Sachs mouse model, lacking hexosaminidases A and S, showed no significant neurological phenotype. The reason for the difference is the specificity of the sialidase, which is different in mouse and human (Sango et al. 1995). Mouse sialidase, in contrast to the human enzyme, accepts GM2 as a substrate and converts it slowly to GA2, which is further degraded by the still intact  $\beta$ -hexosaminidase B in the Tay-Sachs mice.

#### Tay-Sachs Disease (B-Variant)

The B-variant of the GM2 gangliosidosis is because of an  $\alpha$ -chain deficiency, and the subsequent deficiency of hexosaminidases A and S, but with normal hexosaminidase B. In B1 variant, the patient hexosaminidase A lost its catabolic activity against ganglioside GM2 but not against neutral substrates (Kytzia and Sandhoff 1985; Tanaka et al. 1990). Clinically, the B-variant of GM2 gangliosidosis can be subclassified into infantile, juvenile, chronic, and adult forms, corresponding to increasing residual enzyme activity (Leinekugel et al. 1992).

The infantile form, known as Tay-Sachs disease (Filho and Shapiro 2004), has a higher prevalence among Ashkenazi Jews with a heterozygote frequency of 1:27.

#### Sandhoff Disease

The 0-variant of GM2-gangliosidosis was the first gangliosidosis for which the underlying enzymatic defect, a functional loss of both hexosaminidases A and B, was identified. It is characterized by storage of negatively charged glycolipids characteristic for Tay-Sachs disease,

but also by elevated levels of uncharged glycolipids such as glycolipid GA2 in the brain and globoside in visceral organs (Sandhoff 1969; Sandhoff et al. 1971).

#### AB-Variant of GM2-Gangliosidosis

The AB-variant is characterized by normal  $\beta$ -hexosaminidase A, B, and S activities, but a deficient lipid binding protein, the GM2-activator protein. The clinical picture resembles that of Tay-Sachs disease.

#### FABRY DISEASE

Fabry disease is an X-chromosomal-linked lysosomal storage disorder with a recessive mode of inheritance. The disease is caused by a deficient  $\alpha$ -galactosidase A enzyme that results in intracellular accumulation of neutral glycosphingolipids (predominantly Gbose3). The disease manifests itself primarily in affected hemizygous males and to some extent in heterozygous females (“carrier”) and is characterized by progressive clinical manifestations and premature death from renal failure, stroke, and cardiac disease (Linhart and Elliott 2007; Zarate and Hopkin 2008). Gbose3 accumulates in cardiomyocytes, conduction system cells, valvular fibroblasts, endothelial cells, and vascular smooth muscle cells.

#### GAUCHER DISEASE

Gaucher disease is the most common form of the sphingolipidoses (Beutler et al. 2001). It is caused by the deficiency of glucosylceramide- $\beta$ -glucosidase (also called glucocerebrosidase) leading to accumulation of glucosylceramide. Three different types of Gaucher disease are distinguished: The attenuated form, Gaucher disease type I, has a nonneuropathic course and is the most frequent form of this disease. It has a frequency of 1: 50,000–200,000 births, but is higher amongst the Ashkenazi Jewish population (1:1000). The life expectancy of these patients ranges between 6 and 80 years. Brady developed an enzyme replacement therapy for this type of Gaucher disease (Barton

et al. 1990; Brady 2006). Gaucher disease type II, the acute form, is a very rare panethnic disease characterized by an additional storage of the toxic glucosylsphingosine and the involvement of the nervous system with early onset and a life expectancy of less than two years. The subacute or juvenile form, Gaucher disease type III, is an intermediate variant of the other two types. In all variants, patients may show hepatosplenomegaly, anemia, thrombocytopenia, and bone damage. The severity of these symptoms differs widely, but is inversely correlated to the residual enzyme activity determined in skin fibroblasts of Gaucher patients (Meivar-Levy et al. 1994). Complete glucosylceramide- $\beta$ -glucosidase deficiency leads to a perinatal fatal form, the “collodion baby” phenotype with a severe impairment of skin function (Liu et al. 1988).

#### NIEMANN-PICK DISEASE TYPES A AND B

Accumulation of sphingomyelin in Niemann–Pick disease type A and B (NPD A and B) is caused by mutations in the sphingomyelin phosphodiesterase 1 gene (*SMPD1*) encoding for acid sphingomyelinase (ASM) (Ferlinz et al. 1991). Niemann-Pick disease type C shows a similar clinical appearance and sphingomyelin accumulation, but is caused by impaired cholesterol transport. The modular structure of acid sphingomyelinase includes a Sap-like domain and a catalytic domain (Schuchman and Desnick 2001; Lansmann et al. 2003). Type A NPD is a fatal disorder of infancy caused by an almost complete ASM deficiency and results in a life expectancy of 2 to 3 years. Type B NPD is a phenotypically variable disorder with residual ASM activities of up to 4% of normal and with little or no involvement of the nervous system.

#### KRABBE DISEASE

Krabbe disease or globoid cell leukodystrophy is caused by an inherited deficiency of galactosylceramide- $\beta$ -galactosidase (Suzuki and Suzuki 1970; Pastores 2009). This membrane-associated enzyme hydrolyzes galactosylceramide, which

occurs predominantly in oligodendrocytes and kidney cells, to ceramide and galactose. This enzyme is stimulated in vivo by Sap-A and Sap-C it also cleaves the toxic galactosylsphingosine to galactose and sphingosine. Although there is some storage, especially in oligodendrocytes of the (globoid cells), the enzyme deficiency does not lead to substantial substrate accumulation, because of the rapid loss of oligodendrocytes producing and accumulating the toxic galactosylsphingosine (Suzuki 2003).

#### METACHROMATIC LEUKODYSTROPHY

Metachromatic leukodystrophy (MLD) is a lysosomal storage disease caused by the deficiency of arylsulphatase A (ASA) (Mehl and Jatzkewitz 1965; Gieselmann 2008) resulting in the accumulation of sulfatides in several tissues. Arylsulfatase A is essential for the conversion of sulfatides into galactosylceramides and sulfate in the presence of Sap-B (Mehl and Jatzkewitz 1964). Sulfated glycolipids occur mainly in the myelin sheaths in the white matter of the brain, in the peripheral nervous system, and in the kidney tissue. MLD can be classified into a late infantile, a juvenile, and an adult form, correlating with increasing residual catabolic activities (Leinekugel et al. 1992). The clinical and histopathologic manifestations of MLD are fundamentally caused by a demyelination process. This phenomenon appears to be secondary to sulfatide-induced changes in oligodendrocytes and Schwann cells. Deficiency of Sap-B, the cofactor required for sulfatide cleavage by ASA in vivo, leads to a clinical picture similar to MLD although ASA activity is normal (Schlote et al. 1991). In contrast to the human disease, the mouse model of MLD shows no demyelination (Hess et al. 1996). Enzyme replacement therapy has been successfully evaluated in the animal model: In ASA knockout mice, intravenous ASA injection restored sulfatide metabolism in peripheral tissues and the central nervous system (Matzner et al. 2005). The related disease multiple sulfatase deficiency is caused by a defective formation of a formylglycine residue in the active sites of all sulfatases (Dierks et al. 2005).

## FARBER DISEASE

Farber disease is a rare ceramide storage disease caused by the inherited deficiency of lysosomal acid ceramidase (AC). AC is a heterodimeric enzyme composed of two subunits (Bernardo et al. 1995), which are derived from a common precursor that is processed within late endosomes and lysosomes (Koch et al. 1996; Shtraizent et al. 2008). AC catalyses the degradation of ceramide to sphingosine and a fatty acid in the lysosomes, the reaction requires the presence of Sap-D (Klein et al. 1994). The enzyme is also able to catalyze the reverse reaction (Okino et al. 2003). The most characteristic clinical manifestation is the development of painful and progressive joint deformations, subcutaneous nodules (lipogranulomas), and progressive hoarseness. AC is an essential factor required for embryonic survival. AC knockout mice do not survive beyond the 2-cell stage and undergo apoptotic death (Eliyahu et al. 2007). Recent findings show that AC improves the quality of oocytes and embryos and the outcome of in vitro fertilization (Eliyahu et al. 2010).

## SPHINGOLIPID AND MEMBRANE STORAGE CAUSED BY DEFECTIVE LLBP

### Prosaposin-Deficiency

The prosaposin deficiency is a fatal perinatal sphingolipid and membrane storage disorder characterized by hepatosplenomegaly and severe neurological symptoms. Prosaposin, a 70 kDa glycoprotein, is proteolytically processed to four lipid-binding proteins, the mature activator proteins Sap-A-D in the late endosomes and lysosomes (Fürst et al. 1988; Kolter and Sandhoff 2005). Prosaposin is intracellularly targeted to the lysosomes via mannose-6-phosphate receptors and sortilin. Rare mutations in the start codon of the prosaposin gene lead to a complete deficiency of the protein and of all four mature saposins (Schnabel et al. 1992; Bradova et al. 1993). Prosaposin deficiency in human patients and mice causes simultaneous storage of many sphingolipids, including ceramide, glucosylceramide, lactosylceramide, ganglioside GM3, galactosylceramide,

sulfatides, digalactosylceramide, and globotriaosylceramide, accompanied by a massive accumulation of intralysosomal membranes (Fujita et al. 1996). In cultured fibroblasts, the lipid storage can be completely reversed by treatment with human prosaposin, as demonstrated in prosaposin deficient fibroblasts (Burkhardt et al. 1997).

### Sap-A

Sap-A is required for the degradation of galactosylceramide by galactosylceramide- $\beta$ -galactosidase. Genetically engineered mice and patients that carry a mutation in the saposin A-domain of the saposin precursor accumulate galactosylceramide and suffer from a late-onset variant of Krabbe disease (Matsuda et al. 2001).

### Sap-B

Sap-B was the first activator protein identified, and was called the sulfatide-activator (Mehl and Jatzkewitz 1964). It mediates the degradation of sulfatide by arylsulfatase A, globotriaosylceramide and digalactosylceramide by  $\alpha$ -galactosidase A, as well as other glycolipids, (e.g., ganglioside GM2 together with the GM2-activator protein) (Wilkening et al. 2000). Glycosylated saposins bind to lipid bilayers in vitro at acidic pH and are able to extract lipids.

### Sap-C

Sap-C was initially isolated from the spleen of patients with Gaucher disease (Ho and O'Brien 1971). It is required for the lysosomal degradation of glucosylceramide by glucosylceramide- $\beta$ -glucosidase (Alattia et al. 2007). Sap-C deficiency leads to an abnormal juvenile form of Gaucher disease with an accumulation of glucosylceramide (Schnabel et al. 1991).

### Sap-D

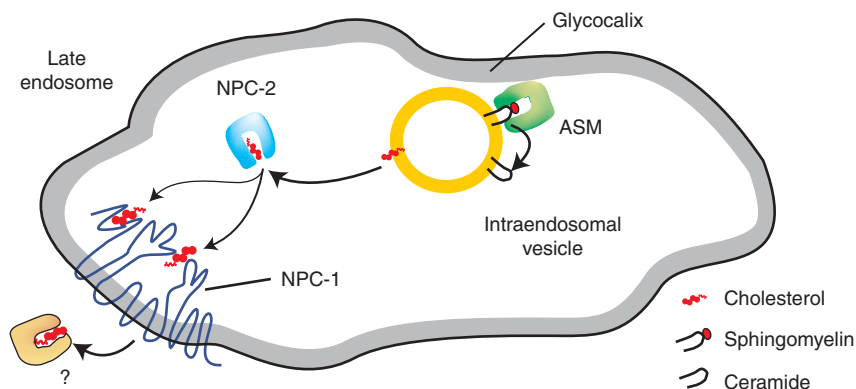
Sap-D stimulates lysosomal ceramide degradation by acid ceramidase. It is able to bind to vesicles containing negatively charged lipids and to solubilize them at an appropriate pH (Ciaffoni et al. 2001). Saposin D-deficient

mice accumulate ceramides with hydroxylated fatty acids mainly in the brain and in the kidney (Matsuda et al. 2004).

### NIEMANN-PICK DISEASE TYPE C

Niemann-Pick disease type C (NPC) is a complex neurodegenerative lipid storage disease characterized by the accumulation of unesterified cholesterol and a broad range of other lipids in the late endolysosomal compartment (Patterson et al. 2001). The disease is caused by mutations in either of the genes of the NPC1 or the NPC2 protein, which leads to impaired cholesterol transport out of the late endosomes. Cells can take up cholesterol via receptor mediated endocytosis, (e.g., of low-density lipoprotein (LDL) rich in cholesteryl ester). In the endosomal compartments, cholesteryl esters are hydrolyzed by cholesterol esterase to fatty acid and cholesterol (Brown and Goldstein 1986). The cholesterol is not degraded in the lysosome, but is rapidly transported out of the late endosome to induce homeostatic responses by downward regulation of the de novo synthesis of the LDL-receptor, thus regulating the cellular cholesterol uptake and de novo synthesis of cholesterol (Ikonen 2008). Transport of cholesterol from the endosomal

system requires two cholesterol-binding proteins, Niemann-Pick C1 (NPC1) and Niemann-Pick C2 (NPC2) (Infante et al. 2008). NPC1 is a late endolysosomal glycoprotein with 13 transmembrane domains (Carstea et al. 1997). NPC2 is a glycosylated, soluble protein (Naureckine et al. 2000). In a proposed model (Abdul-Hammed et al. 2010; Gallala et al. 2010), soluble NPC2 removes cholesterol from inner endosomal/lysosomal vesicles and delivers it to NPC1 in the limiting membrane of endosomes/lysosomes for cholesterol egress from the late endocytic compartments (Karten et al. 2009; Storch and Xu 2009) (Fig. 4). Liver and brain of NPC patients show accumulation of cholesterol in the late endosomes and lysosomes. Additionally, sphingomyelin, neutral glycolipids (e.g., glucosylceramide, lactosylceramide), gangliosides GM3 and GM2 (Zervas et al. 2001a; teVruchte et al. 2004), BMP (Kobayashi et al. 1999), and sphingosine (Rodriguez-Lafrasse et al. 1994) also accumulate. This secondary storage can be explained by a type of traffic jam that occurs in the late endosomal compartments when lipids such as cholesterol accumulate and might contribute to the clinical features associated with each lysosomal storage disorder (Simons and Gruenberg 2000) as increasing cholesterol levels have an inhibitory



**Figure 4.** Proposed model for lipid sorting at the stage of late endosomes. At the surface of intraendosomal vesicles acid sphingomyelinase degrades sphingomyelin to ceramide. The resulting decrease of sphingomyelin and the increase of ceramide levels stimulate the removal of cholesterol from BMP containing inner endosomal vesicles and its transfers to NPC1 in the limiting membrane of the late endosome (Infante et al. 2008). NPC1 mediates cholesterol egress through the glycocalyx (adapted from Abdul-Hammed et al. [2010] and reprinted with permission from the American Society for Biochemistry and Molecular Biology ©2010).



effect on the activity of some lysosomal lipid binding and transfer proteins such as Sap-A and -B (Locatelli-Hoops et al. 2006; Rimmel et al. 2007). NPC1-mutant cells show a substantial reduction of the calcium levels in the acidic compartment (Lloyd-Evans et al. 2008). Furthermore, cholesterol levels are increased in the cell bodies of cultured murine neurons lacking functional NPC1 and are decreased in their distal axons. This altered cholesterol distribution suggests that transport of endogenously synthesized cholesterol, from cell bodies to distal axons is impaired in NPC1-deficient neurons (Karten et al. 2002, 2003).

### WOLMAN DISEASE AND CHOLESTERYL ESTER STORAGE DISEASE

Deficiency of lysosomal acid lipase (LAL, also called acid cholesteryl ester hydrolase) leads either to Wolman disease or to the less severe cholesteryl ester storage disease (CESD) (Assmann and Seedorf 2001). Wolman disease is nearly always fatal in infancy, whereas CESD may go undetected until adulthood. In contrast to CESD, the more severe course of Wolman disease is caused by genetic defects of LAL that leave no residual enzyme activity (Aslanidis 1996). These diseases follow an autosomal recessive mode of inheritance. LAL hydrolyzes a variety of substrates such as cholesteryl esters and triglycerides, which are the main lipid storage material. There is no specific treatment available. Bone marrow transplantation might preserve the hepatic and cognitive functions of Wolman disease patients (Krivit et al. 2000; Tolar et al. 2009), although success seems to be inconsistent (Gramatges et al. 2009). Enzyme replacement and gene therapy have been applied to LAL deficient mice (Tietge et al. 2001; Du et al. 2008).

### THERAPEUTIC APPROACHES

In addition to symptomatic treatment, therapies addressing the underlying metabolic defect of LLD have been in development over the last three decades. Therapies include enzyme replacement therapy (ERT), bone marrow

transplantation (BMT), hematopoietic stem cell transplantation, gene therapy, enzyme stabilization, and substrate reduction therapy (Platt and Lachmann 2009). Enzyme replacement was first developed for the adult nonneuropathic form of Gaucher disease (Barton et al. 1990). Glucosylceramide- $\beta$ -glucosidase (glucocerebrosidase), purified from human placenta, was modified in the carbohydrate part to contain targeting information for the mannose receptor on macrophages such as Kupffer cells (Barton et al. 1990). Today, ERT with recombinant enzymes is available for Gaucher and Fabry disease. ERT is restricted to the nonneuropathic forms of the diseases, because the proteins cannot pass the blood-brain barrier. However, ERT alleviated CNS storage in an arylsulfatase A knockout mouse model of metachromatic leukodystrophy (Matzner et al. 2005). Allogenic BMT has been used in a multiplicity of lysosomal sphingolipid storage diseases. Early BMT can even halt neurodegeneration in some cases as described for Wolman disease (Krivit et al. 1999). Microglial cells producing the deficient enzyme in the brain derive from stem cells from the donor bone marrow.

Gene therapy to target the central nervous system has been evaluated in the animal models of some lysosomal storage diseases such as Gaucher disease (Enquist et al. 2006), metachromatic leukodystrophy (Biffi et al. 2006), and Tay-Sachs disease (Cachón-González et al. 2006).

Enzyme stabilization, or pharmacological chaperone therapy, is based on the application of small molecules that enhance folding or prevent premature degradation of the defective enzyme. Lysosomal storage diseases are suitable candidates for enzyme stabilization treatment, as the levels of enzyme activity needed to prevent substrate storage are often relatively low (Fan 2008).

In substrate reduction therapy, the glucosylceramide synthase inhibitor *N*-butyldeoxynojirimycin is used to reduce GSL biosynthesis, thus lowering the amount of accumulated GSL in the lysosome (Platt et al. 1994; Platt and Butters 2004). Possible residual activity might then



be able to cope with the smaller GSL load. In clinical trials, substrate reduction therapy for type 1 Gaucher patients was effective (Elstein et al. 2004) and studies on mouse models of Tay-Sachs (Platt et al. 1997), Sandhoff (Jeyakumar et al. 1999), Fabry (Heare et al. 2007), GM1 gangliosidosis (Elliot-Smith et al. 2008), and NPC (Zervas et al. 2001b) demonstrated the usefulness of this approach in a wide range of lysosomal lipid storage diseases (Lachmann and Platt 2001).

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