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Review

Lysosomal disorders: From storage to cellular damage

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ABSTRACT

Lysosomal storage diseases represent a group of about 50 genetic disorders caused by deficiencies of lysosomal and non-lysosomal proteins. Patients accumulate compounds which are normally degraded in the lysosome. In many diseases this accumulation affects various organs leading to severe symptoms and premature death. The revelation of the mechanism by which stored compounds affect cellular function is the basis for understanding pathophysiology underlying lysosomal storage diseases. In the past years it has become clear that storage compounds interfere with various processes on the cellular level. The spectrum covers e.g. receptor activation by non-physiologic ligands, modulation of receptor response and intracellular effectors of signal transduction cascades, impairment of autophagy, and others. Importantly, many of these processes are associated with accumulation of storage material in non-lysosomal compartments. Here we summarize current knowledge on the effects that storage material can elicit on the cellular level.

1. Introduction

Many lysosomal storage diseases have already been recognized as clinical entities in the 19th century and this process continued well into the 20th century. At that time lysosomes were unknown and therefore it was not clear that the various diseases share the common feature of lysosomal storage. The classification as lysosomal storage diseases had to await the discovery of lysosomes by Christian de Duve in 1955 [1] and the development of the concept of lysosomal diseases by Hers ten years later [2]. Hers was interested in glycogen storage diseases and he noticed that one of these diseases was different from the others: In Pompe disease the deficient enzyme had an acidic pH optimum and glycogen was not stored in the cytoplasm but rather in an organelle surrounded by a membrane. Hers suggested that glycogen in Pompe disease is stored in lysosomes. This laid the ground for the classification of the already clinically recognized disorders as lysosomal storage diseases. In many of the diseases, however, the storage material was already identified long before lysosomes were discovered. Storage of glucosylceramide in Gaucher disease, for example, was already recognized in 1924. Analysis of the chemical structure of stored compounds allowed for working hypotheses on which enzyme could be responsible for the metabolic defect in the respective disease. In 60ties and 70ties of the last century the enzymatic defects in many of the various disorders were identified in this way. A prominent example is the recognition of glucocerebrosidase deficiency as the underlying defect in Gaucher disease [3].

Most lysosomal storage diseases are caused by deficiencies of soluble lysosomal proteins residing in the lumen of the lysosome (Table 1). A minority is caused by defects in lysosomal membrane proteins. Except for defects of transporters our knowledge of how deficiencies of lysosomal membrane proteins cause storage is still lagging behind. It should be emphasized that a number of lysosomal storage diseases are caused by the deficiencies of non-lysosomal proteins residing either in the endoplasmic reticulum, the Golgi apparatus or the endosomal pathway. We refer to the two reviews by Gasnier and Dierks in this issue which cover deficiencies of lysosomal membrane proteins and non-lysosomal proteins as a cause of lysosomal diseases. The neuronal ceroid lipofuscinoses representing a particular subgroup of lysosomal diseases are reviewed by Jalanko and Braulke in this issue.

Lysosomal diseases are most frequently classified according to the major storage compound. Clinically this classification is very useful and well accepted. Thus, disorders in which the accumulation of glycosaminoglycan fragments prevails are classified as mucopolysaccharidoses, those dominated by lipid storage as lipidoses. It must be emphasized, however, that in most lysosomal diseases more than one compound accumulates and in some disorders for various reasons the stored material can be rather heterogeneous. Thus, a number of lysosomal glycosidases are not specific for a certain substrate but rather for a sugar residue and the stereochemistry of its linkage. This residue and linkage may occur in glycosaminoglycans as well as in lipids, so that a deficiency of the enzyme results in storage of both. For example, β-galactosidase which is deficient in G_{M1} gangliosidosis is involved in the degradation of sphingolipids, oligosaccharides and keratan sulphate, all of which accumulate in patients. The degradation of many sphingolipids depends on activator proteins some of which

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Table 1
Lysosomal storage disorders

Disease	Defective protein	Storage materials
Mucopolysaccharidoses (MPS)		
MPS I (Hurler, Scheie, Hurler/Scheie)	α-Iduronidase	Dermatan sulphate and heparan sulphate, GM2, GM3, SCMAS
MPS II (Hunter)	Iduronate-2-sulphatase	Dermatan sulphate and heparan sulphate, GM2, GM3, SCMAS
MPS IIIA (Sanfilippo)	Heparan *N*-sulphatase (sulphamidase)	Heparan sulphate, GM2, GM3, GD2, SCMAS, ubiquitin
MPS IIIB (Sanfilippo)	*N*-Acetyl-α-glucosaminidase	Heparan sulphate, GM2,GM3, GD2, unesterified cholesterol, SCMAS
MPS IIIC (Sanfilippo)	Acetyl-CoA: α-glucosamide *N*-acetyltransferase	Heparan sulphate, GM2, GM3, GD2
MPS IIID (Sanfilippo)	*N*-Acetylglucosamine-6-sulphatase	Heparan sulphate, GM2,GM3, GD2
MPS IV A (Morquio-A)	*N*-Acetylgalactosamine-6-sulphate-sulphatase	Keratan sulphate, chondroitin-6-sulphate
MPS IV B (Morquio-B)	β-Galactosidase	Keratan sulphate, oligosaccharides
MPS VI (Maroteaux-Lamy)	*N*-Acetylgalactosamine-4-sulphatase (arylsulphatase B)	Dermatan sulphate, GM2, GM3, unesterified cholesterol
MPS VII (Sly)	β-Glucuronidase	Heparan sulphate, dermatan sulphate, chondroitin-4- and -6-sulphates, GM2, GM3, ubiquitin
Multiple sulphatase deficiency (Austin)	Formylglycine-generating enzyme	Heparan sulphate, dermatan sulphate, chondroitin-4- and -6-sulphates, sulpholipids
Spingolipidoses		
Fabry	α-Galactosidase A	Globotriaosylceramide, galabiosylceramide, globotriaosylsphingosine, blood-group-B glycolipids
Farber lipogranulomatosis	Ceramidase	Ceramide
Gaucher	β-Glucosidase	Glucosylceramide, GM1, GM2, GM3, GD3, glucosylsphingosine
Globoid cell leukodystrophy (Krabbe)	Galactocerebroside β-galactosidase	Galactosylceramide, psychosine lactosylceramide, globotriaosylceramide, globotetraosylceramide, fucosylneolactotetraosylceramide
Metachromatic leukodystrophy	Arylsulphatase A	Sulphatide, 3-O-sulpholactosylceramide, lysosulphatide, seminolipid, gangliotetraosylceramide-bis-sulphate, GM2
Niemann–Pick A and B	Sphingomyelinase	Sphingomyelin, cholesterol, bismonoacylglycerophosphate, GM2, GM3, glucosylceramide, lactosylceramide, globotriaosylceramide, globotetraosylceramide
GM1 gangliosidosis	β-Galactosidase	GM1, GA1, GM2, GM3, GD1A, lyso-GM1, glucosylceramide, lactosylceramide, oligosaccharides, keratan sulphate
GM2 gangliosidosis (Tay–Sachs)	β-Hexosaminidase A	GM2, GD1aGalNac, GA2, lyso-GM2
GM2 gangliosidosis (Sandhoff)	β-Hexosaminidase A and B	GM2, GD1aGalNac, globoside, oligosaccharides, lyso-GM2
Oligosaccharidoses and glycoproteinoses		
Aspartylglucosaminuria	Aspartylglucosaminidase	Aspartylglucosamine
Fucosidosis	α-Fucosidase	Fucose containing oligosaccharides and H-antigen–glycolipid
α-Mannosidosis	α-Mannosidase	Mannose-containing oligosaccharides, GM2, GM3
β-Mannosidosis	β-Mannosidase	Man(β1→4)GlcNAc disaccaride
Sialidosis	Sialidase	Sialyloligosaccharides and sialylglycopeptides
Schindler disease	α-*N*-Acetylgalactosaminidase	Glycopeptides with N- or O-linked oligosaccharides, oligosaccharides
Glycogenosis		
Pompe (glycogen-storage-disease type II)	α-Glucosidase	Glycogen

Examples of lysosomal storage diseases. Left column gives the name of the disease, middle column the deficient protein and the right column the stored compounds. GM1, GA1, GM2, GM3, GD1A are abbreviations for the respective gangliosides. SCMAS: subunit c of mitochondrial ATP synthase.

function as biological detergents presenting the lipids to the degrading enzymes (see the review of Schultze and Sandhoff in this issue). Since one activator can present different lipids to different enzymes its deficiency causes more than just one lipid to accumulate. In many diseases there is substantial secondary accumulation of compounds which cannot be explained by the underlying enzymatic defect (see the review of Vanier and Walkley in this issue). Thus, some gangliosides accumulate secondarily in mucopolysaccharidoses and accumulation of glucosylceramide – the storage compound of Gaucher disease – in Niemann-Pick Type C disease may reach levels of type 2 Gaucher patients. Therefore it must be kept in mind, that for various reasons in a number of diseases there is more than just one storage compound. From a pathophysiological point of view this is important since minor storage compounds may play major roles in pathogenesis. Thus, from a biochemical point of view the widely used classification according to the accumulating substrate is not fully systematic.

Research in the last ~25 years focussed on the genetics of lysosomal storage disorders. During this time period the majority of genes of lysosomal enzymes were cloned and a myriad of disease causing mutations were identified. Certainly identification of the underlying enzymatic defect, cloning and identification of the disease causing gene and characterization of mutations has contributed enormously to our understanding of lysosomal diseases. Nevertheless we still lack a clear description of the relevant events leading from the disease causing mutations to the symptoms of the disease which are determined by mechanisms operating not only at the cellular level but also in tissues and organs. The cellular consequences of substrate accumulation are determined by the type of storage material, the extent of storage, the type of storing cells, and the direct or indirect consequences that lysosomal storage has on basic cellular processes such as intracellular trafficking and autophagy. Certainly, it is a challenge to differentiate the extent by which each of these alterations quantitatively contributes to pathogenesis. Thus, revelation of pathophysiologic mechanisms in lysosomal storage diseases is a complex and demanding task which requires an integrated approach ranging from molecular genetics, biochemistry, cell biology and immunology, to name a few, as well as the use of animal models. It is expected that modern genomic, proteomic and system biology approaches will also play a role in the years to come.

In summary, even though lysosomal storage diseases were among the first genetic diseases for which the primary biochemical defects were elucidated, there is still a lot to learn about the underlying pathogenetic mechanisms. This review summarizes our current knowledge about the effects storage material elicits on the cellular level.

2. Alterations of signalling pathways

Compounds accumulating in lysosomal storage diseases can affect signal transduction pathways at different levels. Storage compounds can function as ligands of receptors (Krabbe disease,

mucopolysaccharidoses), modify receptor response (Hurler syndrome, Niemann Pick Type C disease), alter subcellular localization of receptors (Niemann Pick Type C disease), and alter activities of enzymes involved in signal transduction cascades (Krabbe disease). Since it may be difficult for the non-specialist reader to distinguish the various diseases which we address repeatedly but in different sections of this review we have summarized the main features of these diseases in Table 2.

2.1. Non-physiologic activation of signal transduction receptors

2.1.1. Glycosaminoglycan fragments activate the TLR4 receptor in mucopolysaccharidoses

Mucopolysaccharidoses are a group of lysosomal disorders caused by defects in the degradation of glycosaminoglycans such as heparan-, dermatan- or chondroitin sulphate, respectively. Most patients display severe central nervous system involvement, organomegaly, soft tissue disease, and affection of cartilage causes degenerative joint disease and reduced bone growth.

Mucopolysaccharidoses provide an example in which extracellularly accumulating storage compounds lead to non-physiologic activation of signal transduction receptors. Lipopolysaccharide (LPS) is an endotoxin of gram-negative bacteria which binds and activates the Toll like receptor 4 (TLR4). This leads to the secretion of a variety of proinflammatory cytokines eliciting a response of the innate immune system. Glycosaminoglycan breakdown products structurally resemble LPS [4]. Therefore it is not surprising that the accumulation of glycosaminoglycan breakdown products in mucopolysaccharidoses activate the TLR4 receptor [5]. Several genes involved in TLR signalling were found to elevated in mucopolysaccharidoses animals [6]. Among those are TLR4 itself, LPS binding protein and MyD88 which is an adapter protein acting downstream of TLR4. Accordingly, in dog, cat and rat models of mucopolysaccharidosis VII and mucopolysaccharidosis VI, respectively, glycosaminoglycan storing chondrocytes display higher NO levels, secrete enhanced amount of proinflammatory cytokines such as IL-1β, TNF-α and TGF-β. The expression of these cytokines increases with age of the animals and thus with development of pathology. NO and cytokines induce the expression of matrix metalloproteases which through their proteolytic activity may directly contribute to cartilage degeneration.

While there is degeneration of cartilage in mucopolysaccharidoses there is frequently hyperplasia of synovial tissue. TLR4 stimulation does not only lead to increased production of proinflammatory cytokines but also leads to alterations of ceramide levels in stimulated cells. Interestingly, chondrocytes and synovial cells appear to react differently to TLR 4 signalling with respect to ceramide levels. Mucopolysaccharidosis VI chondrocytes showed a substantially increased baseline ceramide level which could not be further stimulated by the addition of dermatan sulphate. Since ceramide is a proapoptotic signalling molecule this contributes to the increased apoptosis of chondrocytes in mucopolysaccharidosis models. In contrast, in synovial fibroblasts of mucopolysaccharidosis it was the prosurvival lipid sphingosine-1-phosphate which was elevated. This proliferative lipid explains that no apoptosis was seen in synovial fibroblasts but rather an increased proliferation [6].

Another example of non-physiologic activation of signal transduction receptors is the activation of the TDAG8 receptor by the lysolipid psychosine in a mouse model of Krabbe disease. Since lysolipids and in particular psychosine can affect various processes involved in signal transduction the effects of this lysolipid will be comprehensively reviewed in Section 2.4.

2.2. Modification of signal transduction receptor response

2.2.1. Impaired FGF-2 and BMP-4 signalling in Hurler syndrome

Hurler syndrome is caused by the deficiency of α-L-iduronidase which leads to the accumulation of heparan and dermatan sulphate fragments with reduced 6-O-sulphation [7]. Importantly, the accumulation of heparan sulphate oligosaccharides is not restricted to lysosomes but also occurs in the extracellular matrix. Of note, extralysosomal accumulation of storage compounds occurs in various lysosomal storage diseases and there are several examples in which

Table 2
Overview of major symptoms of the diseases frequently referred to in this review

Krabbe disease globoid cell leukodystrophy	Early apoptosis of oligodendrocytes and Schwann cells leads to dys/demyelination. In infantiles the disease starts in between 3 and 6 months of age with hyperirritability and progresses rapidly to severe, lethal neurologic impairment.
Gaucher disease	Three types (1, 2, 3) can be distinguished. Type 1 is the most common and most attenuated form. Lipid storing macrophages cause dysfunction of liver, spleen and bone marrow. Symptoms involve hepatosplenomegaly, thrombocytopenia, skeletal deformations and bone fractures. Importantly there is no nervous system involvement Type 2 In addition to visceral symptoms patients have severe neurologic involvement. Brain biopsies show extensive neuronal death. Type 3 is intermediate between type 1 and 2
Niemann Pick Type A	The disease manifests early in infancy and is characterized by hepatosplenomegaly and rapid progressive neurodegeneration. Lipid storing foam cells can be found in many visceral organs. In brain there are numerous swollen and vacuolated neurons in particular in the cerebellum.
Niemann Pick Type C	Children develop progressive ataxia, dystonia and dementia and variably hepatosplenomegaly. Pathologically there are foam cells in visceral organs and neuronal storage in the brain.
Hurler disease	Many organs are affected. Patients have skeletal deformities, hernias, coarse facial features, hepatosplenomegaly, cardiomyopathy, corneal clouding and severe mental retardation.
Multiple sulphatase deficiency	Deficiency of an enzyme modifying sulphatases posttranslationally causes loss of activity of a wide range of sulphatases. Clinically neurologic symptoms prevail. In addition patient have attenuated signs of mucopolysaccharidosis like hepatomegaly, dysostosis multiplex and coarse facial features. Pathologically neurologic symptoms are due to progressive demyelination as a consequence of sulpholipid storage in oligodendrocytes.
G_{M2} gangliosidosis Tay–Sachs diseases Sandhoff disease	The diseases are dominated by rapidly progressing neurologic symptoms manifesting in the first year of life. Pathologically there is extensive neuronal GM2 ganglioside storage.
G_{M1} gangliosidosis	Progressive neurologic symptoms start in the first year of life and are accompanied by facial dysmorphism, skeletal dysplasia and hepatosplenomegaly. Extensive neuronal storage, axonal degeneration and demyelination are found pathologically. Storing histiocytes are found in many visceral organs.
Fabry disease	Onset during childhood or adolescense painful paresthesias, angiokeratoma, renal disease, cardiomyopathy and stroke. There is no neuronal involvement. Pathologically there is widespread lipid storage predominantly in endothelial and smooth muscle cells of blood vessels.
Sanfilippo syndrome	Severe central nervous system involvement developing during childhood typically with hyperactivity and aggressive behaviour is characteristic. Compared to other mucopolysaccharidoses there is only mild visceral and skeletal disease.
Mucopolysaccharidosis VII	Patients have hepatosplenomegaly, hernias, dysostosis multiplex, short stature and delayed development.

this extralysosomal accumulation is of high pathophysiologic relevance. Glycosaminoglycans bind various growth factors. Binding is specific and determined by the type of glycosaminoglycan, its sequence, sulphation and three dimensional structure [8]. In some cases glycosaminoglycans act as growth factor reservoirs and as coreceptors in signal transduction. Although molecular details are still unclear, fibroblast growth factor 2 (FGF-2) depends on heparan sulphate in particular on its 6-O sulphation for binding to and efficient signalling through the FGF receptor [7]. Binding of FGF-2 to this receptor is reduced in cells derived from Hurler syndrome patients and consequently also the proliferative response of these cells to FGF-2 [9]. When heparan sulphate located on the surface of α-L-iduronidase deficient cells was enzymatically removed and replaced by heparan sulphate isolated from normal cells the binding defect was corrected and the proliferative response to FGF-2 was restored. In contrast, heparan sulphate obtained from cells of Hurler syndrome patients could functionally not replace enzymatically removed heparan sulphate on normal cells. Thus, heparan sulphate oligosaccharides accumulating extracellularly in Hurler syndrome interfere with the binding of FGF-2 to its receptor and impair signal transduction through this cascade. This reduces the survival promoting activity of FGF-2 and may explain the increased rate of apoptosis seen in cells of Hurler patients. FGF-2 acts proliferatively and protectively on a number of cell types among which are neurons and neuronal precursor cells [10]. Thus, it is conceivable that the impaired signalling through the FGF-2-FGF receptor/heparan sulphate complex in Hurler syndrome contributes to neurodegeneration occurring in this disease.

This concept of impaired growth factor signalling caused by the extracellular and cell surface accumulation of abnormal glycosaminoglycan fragments is likely to provide pathophysiologic explanations also for the other mucopolysaccharidoses. In this context it is interesting to note that proliferation of neural progenitor cells, which depends on FGF-2 [11] was reduced in a mouse model of Sanfilippo syndrome type B and a dog model of mucopolysaccharidosis VII, respectively [12,13].

The modulation of growth factor signalling may not only provide an explanation for the neurodegeneration seen in the respective diseases but also for skeletal pathology. Since many growth factors bind glycosaminoglycans, storage is likely to affect also signalling pathways important for development and maintenance of tissues other than the brain. Thus, also BMP-4 (bone morphogenetic protein 4) signalling is impaired in Hurler syndrome [14]. BMPs are growth factors which belong to the TGF-β growth factor superfamily and also bind to heparan sulphate [15]. They are known to control proliferation, differentiation and apoptosis in various tissues among which are the nervous system and the skeleton [16], two tissues predominantly affected in most mucopolysaccharidoses. Comparable to FGF-2, the accumulation of abnormal heparan sulphate (and possibly also dermatan sulphate) oligosaccharides impairs BMP-4 induced signalling in cells of Hurler patients. Multiple studies have shown that BMPs are important for bone and cartilage development [16] and therefore alterations in BMP signalling pathways are likely to contribute to the skeletal and cartilage abnormalities frequently found in patients with mucopolysaccharidoses.

2.2.2. Impaired insulin signalling in a mouse model of Niemann Pick Type C disease

Alterations of lipid composition of the plasma membrane due to lipid storage can also modify signal transduction through transmembrane receptors. Niemann–Pick type C disease is caused by deficiency of the NPC-1 protein involved in cholesterol trafficking. Patients accumulate non-esterified cholesterol as well as sphingolipids. These lipids are thought to play an important role in the formation of lipid rafts which have been shown to be critical for insulin receptor signalling in hepatocytes [17]. Cholesterol accumulation in Niemann–Pick type C disease does not only occur in the endosomal/lysosomal compartment but also in the plasma membrane itself [18]. Concomitantly, the fatty acyl chains of other membrane lipids were more saturated in murine NPC-1 deficient cells resulting in reduced plasma membrane fluidity [18]. Autophosphorylation of the insulin receptor through its tyrosine kinase activity was impaired in hepatocytes of a mouse model of Niemann–Pick type C disease [18]. Impaired signalling was also found in isolated plasma membranes proving that the mechanisms leading to reduced phosphorylation are effective at the plasma membrane. In addition, reduction of cholesterol in the plasma membranes isolated from Niemann–Pick type C hepatocytes improved insulin receptor signalling which demonstrates that plasma membrane lipid composition has a direct influence on signal transduction. Impaired insulin receptor signalling may cause insulin resistance comparable to diabetes type II. So far, however, there are no indications of insulin resistance in Niemann–Pick type C patients or the respective mouse model.

2.2.3. Insulin resistance in Gaucher disease type I

Insulin resistance, however, has been found in patients with Gaucher disease type 1 [19]. This lipidosis is caused by glucosylceramidase deficiency and results in the storage of glucosylceramide primarily in macrophages. For reasons which are not entirely clear, G_{M3} ganglioside levels are also increased in tissues of patients with Gaucher disease [20]. G_{M3} ganglioside is known to modify insulin receptor signalling substantially. In the absence of G_{M3} ganglioside, insulin receptor autophosphorylation is enhanced leading to increased insulin sensitivity [21]. In contrast, increased G_{M3} ganglioside levels impair insulin receptor signalling [22]. This increased level of G_{M3} ganglioside may explain the insulin resistance found in patients with Gaucher disease type 1 and would provide an example in which a minor storage compound is responsible for one of the symptoms found in the patients. Experimental prove, however, for this hypothesis is still missing.

2.3. Signalling from endosomes

2.3.1. Endosomal TLR4 receptor levels are increased in a mouse model of Niemann Pick Type C disease

Signalling through transmembrane receptors is not restricted to the plasma membrane but continues in endosomes after receptor internalisation [23]. Furthermore, delivery of receptors from the plasma membrane to the lysosome plays an important role in the physiologic downregulation of receptors and thus termination of signalling.

Primary fibroblasts from Niemann–Pick type C patients secrete increased amounts of cytokines such as IFN-β, IL-6- and IL-8. These cytokines may play a crucial role in sustaining the microglial activation which is thought to play a critical role in the neurodegeneration observed in this disease. Toll like receptors (TLR) are a family of 12 members and are important in the regulation of innate immune responses. In particular, activation of TLR-4 was shown to increase the production of IFN-β, IL6 and IL8 the very cytokines which among others are elevated in Niemann–Pick type C disease [24]. The TLR4 receptor is present on the plasma membrane as well as in endosomes and its signalling is switched off by lysosomal degradation [25]. In murine Niemann–Pick Type C cells the TLR4 level is substantially increased in particular in the intracellular endosomal fraction. Exposure to LPS a ligand of TLR4, caused increased cytokine production and secretion of Niemann–Pick type C cells indicating a more sensitive reaction towards this bacterial endotoxin [24]. These data suggest that the disturbed endosomal/lysosomal trafficking in Niemann–Pick type C (see Section 5) leads to increased endosomal accumulation and possibly decreased lysosomal degradation of TLR4 receptors which results in more intense signalling. This in turn leads to enhanced cytokine secretion contributing to the inflammatory component in pathogenesis of this disease. Ablation of TLR4, however,

in a mouse model of Niemann–Pick Type C disease decreased the secretion of IL6 only partially and the number of activated glial cells was not reduced. Thus, enhanced TLR4 receptor signalling may not be a major contributor to pathogenesis *in vivo*. Nevertheless this example provides an interesting pathogenic concept in which altered endosomal trafficking alters receptor signalling due to changes in receptor distribution. Since alterations of endosomal trafficking are not unique for Niemann–Pick Type C disease but also occur in other lipidoses (see Section 5) endosomal accumulation of signal transduction receptors is likely to play a role also in other lysosomal storage diseases, particularly lipid storage disorders.

2.4. Effects of psychosine and other lysosphingolipids on signal transduction

A number of lysosomal disorders are caused by defects in sphingolipid degradation. In these diseases storage of sphingolipids is frequently accompanied by accumulation of the respective lysolipid of the stored compound. Lysosphingolipids are derivatives of sphingolipids which have lost the amide linked acyl chain and are biologically active compounds [26]. Here we focus on psychosine the lysolipid of galactosylceramide, since it has been most thoroughly investigated.

2.5. Psychosine activates the TDAG8 receptor

Krabbe disease is caused by the deficiency of galactosylceramidase which results in the inability to degrade the sphingolipid galactosylceramide and its lysolipid derivative psychosine. Since galactosylceramide is abundant in myelin, severe myelin pathology and consequently severe neurologic symptoms are the pathological hallmark of this disease. It is in particular the concentration of psychosine (Fig. 1) which is dramatically increased in brains of Krabbe patients and the respective animal model the twitcher mice [27–29]. Krabbe disease is also known as globoid cell leukodystrophy, because of the presence of giant, multinuclear macrophage/microglia derived cells particularly in white matter which pathologists have described as globoid cells. When a myelomonocytic cell line was exposed to galactosylceramide and psychosine, only the latter induced the formation of large multinuclear cells [30] resembling globoid cells. In psychosine treated cells the cleavage furrow separating the cells during cellular division forms initially but then disappears. Thus, cytokinesis – the separation of cytoplasma following mitosis – cannot be completed whereas nuclear division proceeds which provides an explanation for the development of the giant, multinuclear globoid cells (Fig. 2). The signals inhibiting cytokinesis in these cells are transmitted through the orphan G-protein coupled receptor TDAG8 [T-cell associated gene 8] acting through an increase of cAMP. Psychosine and some structurally related lysosphingolipids such as glucosylsphingosine or sphingosylphosphorylcholine were identified as specific ligands for this receptor [31]. The K_m of psychosine for the TDAG8 receptor is around 3 μM, a concentration not reached under physiologic conditions. In Krabbe disease, however, psychosine accumulates to high micromolar concentration allowing activation of the TDAG8 receptor. Psychosine is an example in which a compound accumulating in a lysosomal storage disorder provides a non-physiologic ligand for a signal transduction receptor. The effects of the activation of this receptor give a direct explanation for one of the pathological hallmarks of the disease, the giant, multinuclear globoid cells.

2.5.1. Psychosine inhibits protein kinase C

Compounds accumulating in lysosomal storage diseases can directly affect components of signal transduction pathways downstream of receptor activation. Thus, lysosphingolipids in general are potent reversible inhibitors of proteinkinase C [26]. Proteinkinase C is activated by the lipid diacylglycerol which is generated from phosphatidylinositolbisphosphate in the course of signal transduction pathways mediated by phospholipase C. Lysosphingolipids interfere with the interaction of diacylglycerol with proteinkinase C preventing activation of the enzyme [26]. As mentioned above the lysolipid psychosine accumulates in Krabbe disease and causes apoptosis of oligodendrocytes and Schwann cells [32–34]. Platelet derived growth factor and glial growth factor elicit a proliferative response in Schwann cells by acting through a protein kinase C mediated pathway [35]. Cultured Schwann cells isolated from twitcher mice – the mouse model of Krabbe disease – respond less well to these growth factors than normal cells. Moreover, Schwann cells from twitcher mice are tenfold more sensitive to stauroporine – an inhibitor of protein kinase C – than normal cells indicating a preexisting inhibition of protein kinase C possibly by psychosine. Interference with proteinkinase C mediated growth factor signalling could therefore account partially for the loss of myelin producing cells in Krabbe disease.

2.5.2. Psychosine interferes with IGF-1 signalling

Insulin like growth factor I (IGF-1) is one of the growth factors acting on oligodendrocyte precursors. IGF-1 inhibits oligodendrocyte precursor apoptosis [36] and promotes oligodendrocyte development [37]. Mice deficient for the IGF-1 receptor have reduced number of oligodendrocytes and show substantial decrease of the size of corpus callosum and the anterior commissure two myelin rich regions. This underlines the importance of IGF-1 receptor signalling for normal oligodendrocyte development and myelination [38]. Thus, any impairment in the signal transduction pathways of IGF-1 can possibly contribute to the loss or malfunction of oligodendrocytes in Krabbe

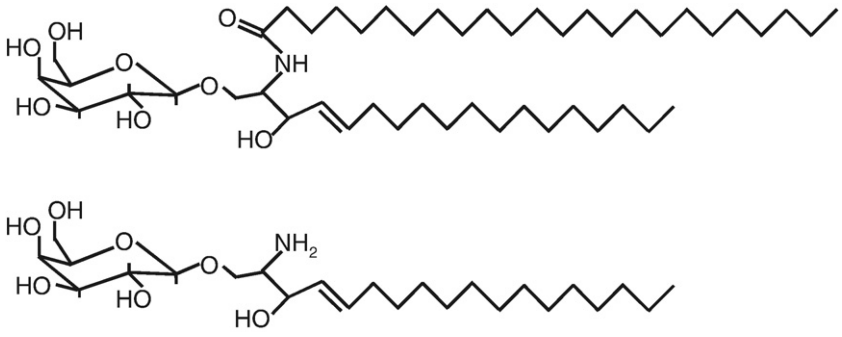

Fig. 1. Structure of psychosine. Lysosphingolipids accumulate in many sphingolipid storage diseases. They are derived from cerebrosides or gangliosides. In contrast to the original lipids lysosphingolipids have lost the acyl side chain attached to the amino group of the sphingosine backbone. As a prominent example the figure shows the structure of galactosylceramide (top) and its respective lysolipid psychosine (bottom).

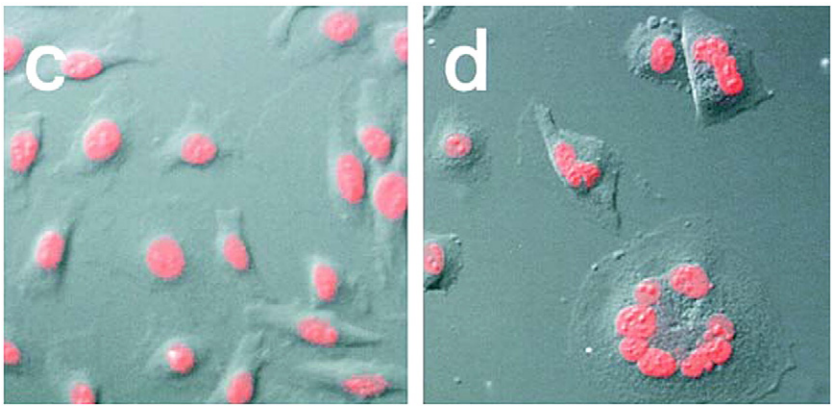

Fig. 2. Psychosine leads to the formation of multinuclear cells. HeLa cells were exposed to 35 μM psychosine and nuclei were stained with propidium iodide (c), control cells, (d) exposed cells. Modified from [30].

disease. In oligodendrocytes IGF-1 acts through the activation of the antiapoptotic PI3K-Akt/PKB or the MAPK/Erk1–2 signal transduction pathways, respectively [39]. In murine oligodendrocyte precursor cells psychosine causes a dose dependent decrease in both Akt und ERK1–2 phosphorylation accompanied by an activation of caspase-3 resulting in apoptosis. When psychosine treated cells were exposed to high doses of IGF-1, Akt phosphorylation and to a lesser extent also Erk1–2 phosphorylation was restored [39]. This led to a reduced cleavage of caspase-3 resulting in a reduced apoptotic rate in oligodendrocyte precursor cells [39]. Thus, the inhibition of IGF-1 mediated anti-apoptocic signalling pathways by psychosine may be one reason for the death of oligodendrocytes in Krabbe disease.

2.5.3. Psychosine activates phospholipase A2

Another major target of psychosine is phospholipase A_2 which cleaves the membrane lipid phosphatidylcholine into lysophosphatidylcholine and arachidonic acid. Both products are biologically highly active lipids involved in numerous physiological and pathophysiological reactions. Injection of lysophosphatidylcholine into the brain induces demyelination in vivo [40]. Importantly brain samples of Krabbe disease patients and twitcher mice show increased levels of lysophosphatidylcholine [41]. The second product of phospholipase A_2 arachidonic acid causes generation of reactive oxygen species and free radicals which pose a significant oxidative threat possibly contributing to pathogenesis.

Exposure of oligodendrocytes to psychosine increases production of lysophosphatidylcholine and release of arachidonic acid [41]. When secretory phospholipase A_2 is inhibited, psychosine dependent generation of arachidonic acid and lysophosphatidylcholine is abolished. Moreover inhibitors of secretory phospholipase A_2 prevented psychosine induced apoptosis and activation of caspase-3 whereas inhibitors of other phospholipase A_2 isoenzymes are not effective. Thus, psychosine activates secretory phospholipase A_2 the exact mechanisms, however, still needs to be resolved. Activation enhances the production of lysophosphatidylcholine. This lipid mediator induces caspase-3 mediated apoptosis which could be relevant for the oligodendrocyte loss in Krabbe disease.

2.5.4. Psychosine reduces AMP activated kinase activity

AMP activated protein kinase (AMPK) is considered as a crucial enzyme in the regulation of glucose and lipid metabolism. AMPK senses cellular energy levels and maintains the balance between ATP production and consumption. In a status of low energy it is activated, switches off anabolic pathways and activates catabolic pathways and vice versa. Exposure of cells to psychosine downregulates AMPK activity which leads to a preponderance of biosynthetic pathways in the treated cells. Oligodendrocytes treated with psychosine display enhanced synthesis of fatty acids and cholesterol while β-oxidation as a catabolic pathway was inhibited. Thus, psychosine may also influence the energy status of a cell by modulating the AMPK master switch in energy balance [42]. The inhibition of this kinase by psychosine favours energy consuming over energy generating pathways. The resulting lower energy load could also contribute to oligodendrocyte loss.

2.5.5. Lysolipids in other lipid storage disorders

Lysosphingolipids accumulate in various lipid storing diseases and may represent a factor of general importance for the pathogenesis of lipidoses. Glucosylceramide is the major storage compound in Gaucher disease. The respective lysosphingolipid glucosylsphingosine, however, also accumulates in the brain of Gaucher type 2 and 3 patients, which suffer from severe neurodegeneration. When cultured neurons were exposed to increasing concentrations of glucosylsphingosine the compound clearly had a toxic effect on these cells, implicating this lysolipid in the neurodegeneration occurring in this disease [43]. Similarly to psychosine, glucosylsphingosine binds the TDAG8 receptor [31] and activates phospholipase A_2 [41]. It also inhibits CTP:phosphocholine cytidylyl-transferase activity [44] (see Section 2.3). This enzyme is rate limiting in phosphatidylcholine synthesis. Since phosphatidylcholine is a major membrane lipid glucosylsphingosine, induced changes in lipid biosynthesis may be of pathogenic relevance. Similarly, lyso-G_{M2} ganglioside accumulates in Tay Sachs disease and lyso-G_{M1} ganglioside in G_{M1} gangliosidosis [45].

Fabry disease is a lipidosis caused by the deficiency of α-galactosidase. This enzyme is involved in the degradation of globotriaosylceramide. Fabry disease patients not only accumulate globotriaosylceramide but also substantial amounts of the respective lysolipid globotriaosylsphingosine [46]. One of the pathological signs of Fabry disease is increased intima media thickness of the carotids due to smooth muscle cell proliferation. Interestingly, when cultured smooth muscle cells were exposed to globotriaosylsphingosine at concentrations similar to those found in the plasma of patients, these cells showed an increased proliferation, providing a direct explanation for the increased intima media thickness [46]. How globotriaosylsphingosine on the cellular level causes an increased proliferation of smooth muscle cells remains to be determined.

3. Alterations of intracellular calcium homeostasis

3.1. Enhanced Ca^{++} release from the ER in Gaucher disease

Many pathways involve the release of calcium ions from the endoplasmic reticulum into the cytosol. Increase of cytosolic calcium

ion concentration triggers a variety of cellular responses like e.g. calcium/calmodulin dependent pathways. ER function is substantially altered in various lipidoses.

Gaucher disease is caused by the deficiency of glucosylceramidase which results in the storage of glucosylceramide. In the more severe type 2 form of this disease neurodegeneration is one of the dominating symptoms. Glucosylceramide not only accumulates in lysosomes but its concentration is 10 fold increased also in microsomes isolated from brain of patients suffering from the more severe neuronopathic forms of Gaucher disease [47].

Glucosylceramide storing neurons display an increased calcium release from the endoplasmic reticulum in response to a glutamate stimulus [48] (Fig. 3). This results in enhanced glutamate induced neurotoxicity which may explain partly the neurodegeneration seen in this disease. There are two endoplasmic reticulum located ligand gated Ca^{++} channels which release calcium into the cytoplasm: the inositol-1,4,5-trisphosphate (InsP3) receptor and the ryanodine receptor. The former opens upon binding of InsP3 which is generated in many different signal transduction pathways, the latter opens upon binding of cycloADP-ribose or palmitoyl-CoA, respectively, the role of which in signal transduction pathways is currently poorly understood. More importantly, the ryanodine receptor is controlled by cytoplasmic calcium levels. Increased cytosolic calcium opens the ryanodine receptor which leads to a further calcium release from the endoplasmic reticulum. Glucosylceramide accumulating in the membranes of the endoplasmic reticulum sensitizes the ryanodine receptor to agonist mediated calcium release from the endoplasmic reticulum. It does not affect the calcium release properties of the InsP3 receptor [48]. The sensitization occurs at concentrations of glucosylceramide which are well in the range of those found in brain microsomes of patients [47,48]. Thus, glutamate may induce an increase in cytosolic calcium either by opening of ionotropic calcium permeable glutamate receptors or through the increase of InsP3 by activation of metabotropic receptors. In both cases the cytoplasmic calcium increases and triggers calcium induced opening of the ryanodine receptor. Since the receptor is sensitized by glucosylceramide this results in an increased response to glutamate in storing neurons. Enhanced glutamate toxicity results in an increased rate of apoptosis which may explain partly the neurodegeneration occurring in the severe type 2 forms of Gaucher disease.

Alteration of ryanodine receptor mediated calcium release from the endoplasmic reticulum was also demonstrated in microsomes isolated from autoptic brain of Gaucher patients. Importantly, the degree of agonist induced release of calcium from the isolated microsomes correlated with disease severity: the strongest response was found in microsomes of patients suffering from the most severe neuronopathic form whereas less calcium was released from microsomes of patients suffering from the attenuated subacute or non-neuronopathic forms. These results provide strong evidence that alterations of calcium release from the endoplasmic reticulum are pathogenetically relevant in vivo [49].

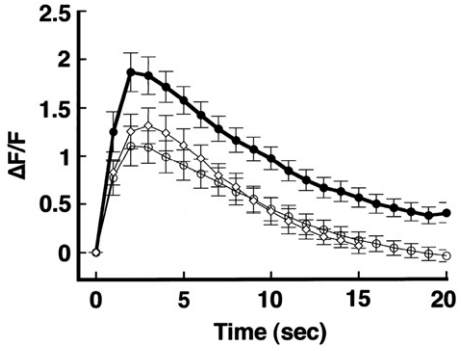

Fig. 3. Glucosylceramide accumulation alters neuronal response to glutamate. Primary rat control (open circles or rhombus) or glucosyceramide storing neurons (closed circles) were exposed to glutamate and the release of Ca^{++} from the ER was measured by using a fluorescent dye. Delta F/F is the change in fluorescence (F) intensity divided by basal fluorescence as a measure of calcium response. The response of glucosyceramide storing cells to glutamate is larger than the response of control cells. Figure from [48].

3.2. Reduced reuptake of Ca^{++} into the ER in GM1 and GM2 gangliosidosis

Alterations of calcium homeostasis in the endoplasmic reticulum have also been described for G_{M1} ganglioside, G_{M2} ganglioside and some lysosphingolipids. Surprisingly, the mechanisms through which the various lipids act are distinct. Whereas glucosylceramide sensitizes the ryanodine receptor, G_{M1} and G_{M2} ganglioside in contrast inhibit the reuptake of calcium into the endoplasmic reticulum by lowering the Vmax of sarco/endoplasmic reticulum Ca^{++} ATPase (SERCA). SERCA is the endoplasmic reticulum located calcium transporter which pumps calcium from the cytoplasm back into the endoplasmic reticulum [50] to terminate Ca^{++} mediated cellular responses. Since inhibition of SERCA activity has been shown to result in neuronal apoptosis [51] this may provide at least a partial explanation for neurodegeneration in the G_{M2} gangliosidoses, Tay–Sachs and Sandhoff disease, respectively. Since secondary accumulation of G_{M2} ganglioside is a widespread phenomenon in lysosomal storage diseases (see Table 1) alterations of SERCA activity may play a more general role in pathogenesis of lysosomal disorders. Reduced Ca^{++} reuptake by SERCA is also of importance in Niemann–Pick type A disease which is caused by the deficiency of sphingomyelinase which results in sphingomyelin storage. In this case, however, reduction of SERCA activity was caused by a severe reduction of SERCA expression [52] rather than inhibition. Moreover, this is accompanied by an almost complete loss of expression of the InsP3 receptor in the cerebellum [52]. The fact, that different structurally related lipids target important regulators of endoplasmic and cytosolic calcium levels points to a physiologically relevant regulation which is so far poorly understood.

3.3. Unfolded protein response in G_{M1} gangliosidosis

The dysregulated calcium homeostasis in the endoplasmic reticulum does not only lead to an increase in cytoplasmic calcium but also to a concomitant decrease of calcium in the endoplasmic reticulum. Proper folding of proteins inside the endoplasmic reticulum is guided by a number of chaperones – such as calnexin or calreticulin – which critically depend on calcium. If the cytosolic calcium concentration is increased at the expense of the calcium within the endoplasmic reticulum this interferes with proper protein folding. G_{M1} gangliosidosis is caused by the deficiency of β-galactosidase resulting in storage of G_{M1} ganglioside. G_{M1} ganglioside storing cells elicit an unfolded protein response as a consequence of altered calcium homeostasis in the endoplasmic reticulum [53]. This may be a direct consequence of the SERCA inhibition by accumulation of G_{M1} ganglioside in the endoplasmic reticulum [50]. Usually this unfolded protein response improves cell survival because the accumulation of protein aggregates due to external or internal effects is prevented. Cellular stress, however, elicited by a continuous unfolded protein response results in apoptosis which is among other pathways initiated by the activation of the endoplasmic reticulum located caspase-12. Thus, the alterations of endoplasmic reticulum calcium homeostasis provide two explanations for an enhanced apoptosis contributing to neurodegeneration in lipidoses. The increase in cytosolic calcium may trigger signal transduction pathways to an extent which is toxic for the cell and the depletion of calcium in the endoplasmic reticulum leads to a prolonged unfolded

protein response which results in activation of caspase-12 and apoptosis [53]. For a more detailed review of the GM_1 ganglioside storage induced pathways involved in the unfolded protein response the reader is referred to [54].

4. Alterations in lipid biosynthesis

Little is known about how cells measure and regulate the complex lipid composition of cellular membranes. It seems obvious that synthesis and degradation of the various lipids must be fine tuned to maintain membrane homeostasis. Therefore storage of a particular lipid is likely to affect the metabolism of other lipids.

4.1. Synthesis of phospholipids is reduced in G_{M2} gangliosidosis

Neurite growth of embryonic hippocampal neurons isolated from G_{M2} ganglioside storing Sandhoff mice is significantly decreased compared to controls [55]. Metabolic labelling experiments revealed a decreased level of phospholipids in the neurons from Sandhoff mice [56]. These findings were confirmed by *in vivo* labelling experiments in mice. Labelling of phosphatidycholine was decreased in the brain of GM_2 ganglioside storing Sandhoff mice whereas labelling in liver and spleen was unaffected. The same applies also for the steady state levels of these lipids in mice [56] and autopsy samples of patients [57]. Key enzymes in the synthesis of phosphatidylcholine and phosphatidylserine are CTP:phosphocholine cytidylyl-transferase and phosphatidylserine synthase, respectively. Compared to wild type mice, activities of both enzymes are decreased in the brain of Sandhoff mice. A direct inhibitory effect of G_{M2} ganglioside was excluded. Rather data suggest that regulation of activity of these enzymes occurs posttranslationally. This suggests that G_{M2} ganglioside storage affects synthesis of phospholipids which are major components of cellular membranes and precursors of important lipid second messengers. The reduced synthesis rate of these lipids may explain the impaired neurite growth of neurons derived from Sandhoff mice.

4.2. Enzymes of phospholipid synthesis are activated upon glucosylceramide storage

In contrast, glucosylceramide storing neurons from mice representing a model of Gaucher disease show an increase in the number of axonal branch points and significantly longer neurites than control mice [58]. In these cells the levels of various phospholipids were elevated. This elevation is most likely caused by a direct activation of CTP:phosphocholine cytidylyl-transferase the rate limiting enzyme in the synthesis of phosphatidylcholine by glucosylceramide. Although the relevance for neuronal pathology in the severe forms of Gaucher disease remains unclear the increased synthesis of phosphatidylcholine may explain partly the visceral pathology of Gaucher disease. Visceral pathology is mainly due to glucosylceramide storage in macrophages which are enlarged. Glucosylceramide stimulates phospholipid synthesis not only in storing neurons, but also in macrophages [59]. The increase in macrophage size upon lipid storage depends on the presence of CTP:phosphocholine cytidylyl-transferase since macrophages of mice deficient for this enzyme do not become enlarged when exposed to glucosylceramide. Thus, enlargement of macrophages is due to alterations in membrane lipid biosynthesis.

Furthermore, Gaucher patients display hepatosplenomegaly. Of note, the amount of glucosylceramide stored in the liver is too low to account for the increase in liver size [60]. Wild type mice in which the degradation of glucosylceramide was pharmacologically inhibited developed hepatomegaly due to increased cell proliferation [61]. Thus, hepatosplenomegaly in Gaucher patients may be due to alterations of phospholipid synthesis leading to enlarged macrophages and glucosylceramide induced cell proliferation enhancing the number of cells in the liver of Gaucher patients.

5. Alterations of trafficking

Since the endosomal and lysosomal pathway are functionally connected it is not surprising that lysosomal storage affects intracellular sorting events. In particular, in the sphingolipidoses it can be expected that accumulation of membrane lipids affects intracellular membrane flow and sorting.

5.1. Trafficking of lipids is altered in lipidoses

When fluorescently labelled short acyl chain derivatives of lactosylceramide were added to cultured normal cells and cells of patients suffering from various lipid storage diseases a remarkable difference was noted. Whereas in normal cells the lactosylceramide derivative is endocytosed and transported to the Golgi apparatus, in cells of patient with lipid storage diseases, it accumulates in the endosomal/lysosomal pathway, suggesting an alteration of the endosomal sorting common to all lipidoses [62]. Endocytosis of the lactosylceramide derivative is clathrin-independent and occurs through a caveolae-mediated pathway. This sorting is substantially influenced by cholesterol levels. Depletion of cholesterol from cells of patients suffering from lipidoses restores sorting of lactosylceramide derivatives to the Golgi apparatus. Similarly, when normal cells are overloaded with cholesterol the lactosylceramide derivative accumulates in the endosomal/lysosomal pathway as it does in lipidosis cells. This finding suggests that cholesterol homeostasis may generally be altered in patients with lipidoses [62]. The control and proper regulation of vesicle transport depends on ras-associated binding proteins (rab-proteins) which currently encompass a family of more than 60 proteins. Overexpression of the small rab 7 and 9 proteins in Niemann–Pick type C cells restores the abnormal sorting showing that these proteins have a role in the sorting of glycosphingolipids to the Golgi apparatus and that their function may be perturbed in lipid storage diseases [63].

5.2. Alterations of receptor trafficking in lipidoses

So far there is only little information on alterations of protein sorting in lysosomal storage disorders. Niemann–Pick disease type A is caused by the deficiency of sphingomyelinase. Storage of sphingomyelin occurs among other cells also in lung macrophages of patients. Lung macrophages isolated from a sphingomyelinase deficient mouse show a reduced endocytosis of the mannose-6-phosphate receptor whereas the endocytosis of the mannose receptor is unaltered [64]. The underlying mechanisms, however, by which lipid storage affects specifically the endocytosis of the mannose-6-phosphate receptor are unknown. Effects on the endocytosis of receptors, however, may depend on the accumulating lipid and the cell type investigated. Thus, in sulphatide storing kidney cells from a mouse model of metachromatic leukodystrophy both the endocytosis of the mannose-6-phosphate receptor and the transferrin receptor are enhanced. A more detailed examination revealed that whereas the internalisation rate of both receptors is enhanced, their recycling rate is reduced. The data are in accordance with a generally increased endosomal pool in sulphatide storing kidney cells [65]. The effect of the accumulated lipid on receptor sorting appears to be indirect, since accumulated sulphatide and mannose-6-phosphate receptors do not colocalize. An altered localization of the mannose-6-phosphate receptor towards the late endosomal compartment was also reported for cells from Niemann–Pick Type C patients [66]. Thus, the endosomal pool of receptors may be increased in several lipid storing diseases. This could also provide an explanation for increased concentration of the TLR4 receptor in endosomes of Niemann–Pick type C cells (see Section 2.3) [24].

6. Role of autophagy in pathogenesis

The lysosome plays a major role in an important degradation pathway, autophagy, which mediates the cellular turnover of proteins and organelles. Fusion between the autophagosome and the lysosome is a crucial step in this process [67]. The physiological relevance of autophagy was first identified in transgenic mice in which essential autophagy genes were disrupted, resulting in accumulation of polyubiquitinated protein aggregates and neurodegeneration, thus indicating that autophagy is required for neuronal survival [68,69]. Furthermore, protein aggregates accumulating in some common neurodegenerative diseases, such as Parkinson, Alzheimer and Huntington diseases, were found to be autophagy substrates [70], leading to the possibility of treating these diseases with autophagy-inducing drugs [71,72].

During autophagy a large portion of cytosol is sequestered in specific vesicles (autophagosomes, Avs) and then degraded upon fusion with lysosomes. Through basal autophagy the cell regulates also turnover of organelles, such as mitochondria, peroxisomes and endoplasmic reticulum. Beyond this basal activity, autophagy can be induced as response to many adverse circumstances: during nutrient depletion autophagy allows generation of ATP from catabolism of macromolecules; during oxidative stress, induction of autophagy allows the efficient removal of damaged organelles and proteins from the cytoplasmatic environment, acting as pro survival pathway [72]. Induced autophagy leads to abnormal overproduction of autophagosomes in the cells, a condition often associated to cell death. However, it is still unclear whether this represents an attempt to survive, or is itself the mechanism by which the cell dies (autophagic or type 2 cell death). It is reasonable to assume that beyond a certain threshold autophagic activity results in massive degradation of organelles and molecules that leads to cell death [73].

Recently, there has been an increased interest in investigating the autophagic pathway in lysosomal storage diseases based on the hypothesis that lysosomal storage may interfere with lysosomal contribution to the autophagic process. The rationale behind this interest hinges on the observation that neurons from animal models of lysosomal storage diseases show an accumulation of protein aggregates and of mitochondria, which are autophagy substrates. Table 3 summarizes the data obtained in the lysosomal storage diseases in which an involvement of autophagy was investigated.

The first evidence for an involvement of autophagy in lysosomal storage diseases was obtained in a mouse model of Danon disease, in which accumulation of autophagic vacuoles was observed in several tissues [74]. The accumulation was limited to early autophagosomes and, therefore, a defect in a maturation step was proposed [74]. Similarly, autophagosome accumulation was observed in neurons from murine models of Neuronal ceroid-lipofuscinoses (NCLs) [75,76]. Interestingly, the presence of activation of autophagy was suggested as a means to provide a pro-survival feedback response to the disease process [79]. In mucolipidosis type IV it was postulated that a defective autophagic recycling of mitochondria may lead to aberrant mitochondrial fragmentation that in turn activates an apoptotic-mediated cell death [77].

A profound disturbance of the autophagic pathway was demonstrated in a mouse model of Pompe disease in which an increase of autophagic-like vacuoles was detected. It is interesting to note that in this study the authors linked the autophagic build up to a deficiency of the trafficking/processing of the recombinant therapeutic enzyme along the endocytic pathway [78], suggesting that autophagy dysfunction may have an impact in determining the efficacy of enzyme replacement therapies [79].

The autophagic pathway was also studied in lysosomal storage diseases caused by defective lipid trafficking, such as Niemann Pick type C and the sphingolipidosis Sandhoff disease. A direct role of autophagy in the neuronal cell death observed in Niemann Pick type C was proposed [80]. Moreover, analysis of tissues derived from a Niemann Pick type C mouse model and of fibroblasts isolated from Niemann Pick type C and Sandhoff patients showed increased LC3 levels and elevated number of autophagic vacuoles [80]. Importantly, in this study the authors demonstrated an enhanced autophagic degradation of long-lived proteins associated with increased expression of Beclin-1, which is activated during autophagy induction [81].

A block of autophagy was recently demonstrated in two models of lysosomal storage diseases, multiple sulphatase deficiency (MSD) and mucopolysaccharidosis type IIIA [mucopolysaccharidosis-IIIA] [82,83]. Co-localization between autophagosomes and lysosomes was significantly reduced in cells derived from affected mice, suggesting a fusion defect (Fig. 4). Functional evidence for an impaired degradation of autophagic substrates was obtained by expressing exogenous aggregate-prone protein, such as expanded Huntingtin and mutated alpha-synuclein, in cultured cells from MSD mice, resulting in a decreased rate of their clearance. As a consequence of impaired autophagy, significant accumulation of polyubiquitinated proteins and aberrant mitochondria was detected in tissues from affected mice (Fig. 5). The multifunctional protein "p62/A170/SQSTM1", which is involved in the targeting of polyubiquitinated proteins to the autophagosomes and selectively degraded via the autophagic pathway, was also found to accumulate in cells from affected mice, further supporting a defect of autophagy [82].

Finally, increased autophagy and concomitant activation of beclin-1 were recently reported in another lipid storage disease, G_{M1}-gangliosidosis [84] in which autophagy dysfunction was proposed to be responsible of neuronal cell death.

Altogether these studies firmly establish the presence of autophagosome accumulation in lysosomal storage diseases. This may be the

Table 3
Autophagy involvement in lysosomal storage disorders

Disease	Autophagosomes accumulation (LC3-II increase)	Autophagy dysfunction in disease pathogenesis	Induction of autophagy (Beclin-1 activation)	Block of autophagosome–lysosome maturation	Reference(s)
Danon disease	+	+	?	+	Tanaka et al., *Nature* 2000 [74]
Neuronal ceroid-lipofuscinoses (NCL)	+	+	+	?	Koike et al., *Am. J. Pathol.* 2005; Cao et al., *J. Biol. Chem* 2006 [75,76]
Pompe	+	+	?	?	Fukuda et al., *Ann. Neurol.* 2006 [78]
Mucolipidosis type IV	+	+	?	+	Jennings et al., *J. Biol. Chem.* 2006 [77]
Niemann–Pick C (NPC)	+	+	+	−	Ko et al., *Plos Genetics*. 2005; Pacheco et al., *Hum. Mol. Genet.* 2007 [80,]
Multiple Sulphatase Deficiency (MSD)	+	+	−	+	Settembre et al., *Hum. Mol. Genet.* 2008 [82]
Mucopolysaccharidosis type IIIA (MPS-IIIA)	+	+	−	+	Settembre et al., *Hum. Mol. Genet.* 2008 [82]
GM1-gangliosidosis	+	+	+	?	Takamura et al., *Biochem. Biophys. Res. Commun.* 2008 [84]

Overview of alterations of autophagy in lysosomal storage disease.

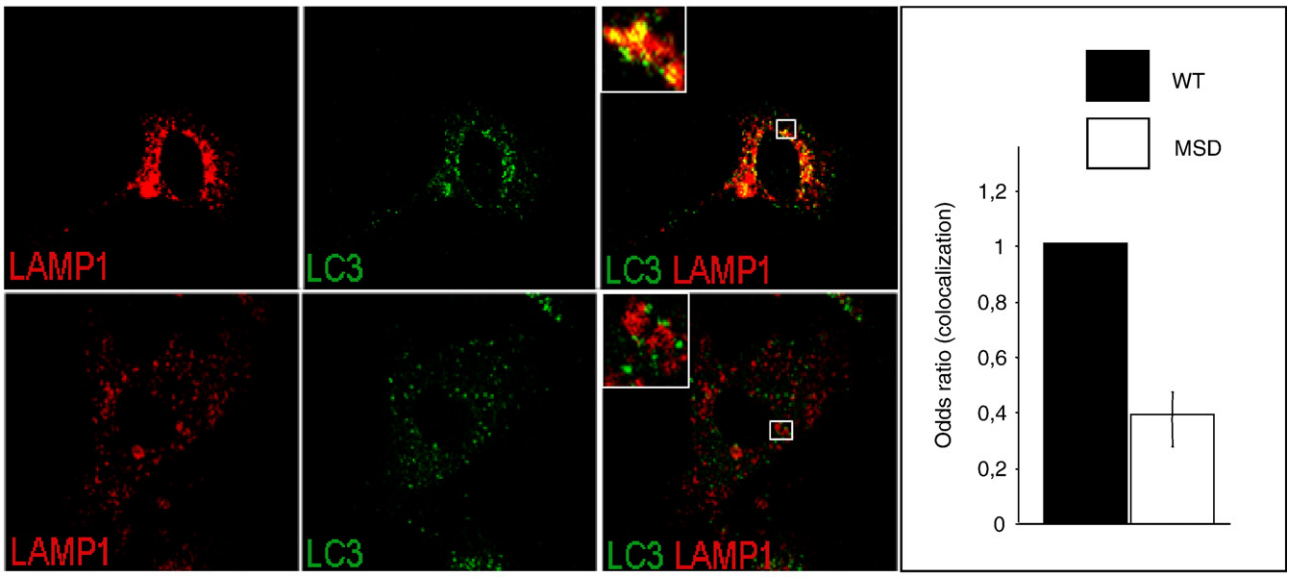

Fig. 4. Defective autophagosome–lysosome fusion in MSD MEFs. Co-localizazion of LAMP1 and LC3 in wild type and MSD MEFs stained for LAMP1 (red) and LC3 (green). Confocal microscopy shows a reduction in the extent of co-localization of LAMP1 and LC3 proteins in MSD cells indicative of a reduced autophagosome–lysosome fusion. From [82].

result of either an induction of autophagy, or of a defective autophagosome maturation. In some lysosomal storage diseases, such as NCL, Niemann Pick type C and GM1 gangliosidosis, autophagy was found to be activated as evident from increased levels of beclin-1 [75,76,81,82]. In other types of lysosomal storage diseases, such as Danon disease, Mucolipidosis IV, mucopolysaccharidosis-IIIA and MSD, evidences for a partial block of autophagosome maturation was observed [74,77,82]. More specifically, impaired autophagosome–lysosome fusion was demonstrated in MSD cells [82]. A unifying hypothesis that may solve this apparent discrepancy between a block and an induction of autophagy in lysosomal storage diseases is that lysosomal storage may affect fusion efficiency between autophagosomes and lysosomes,

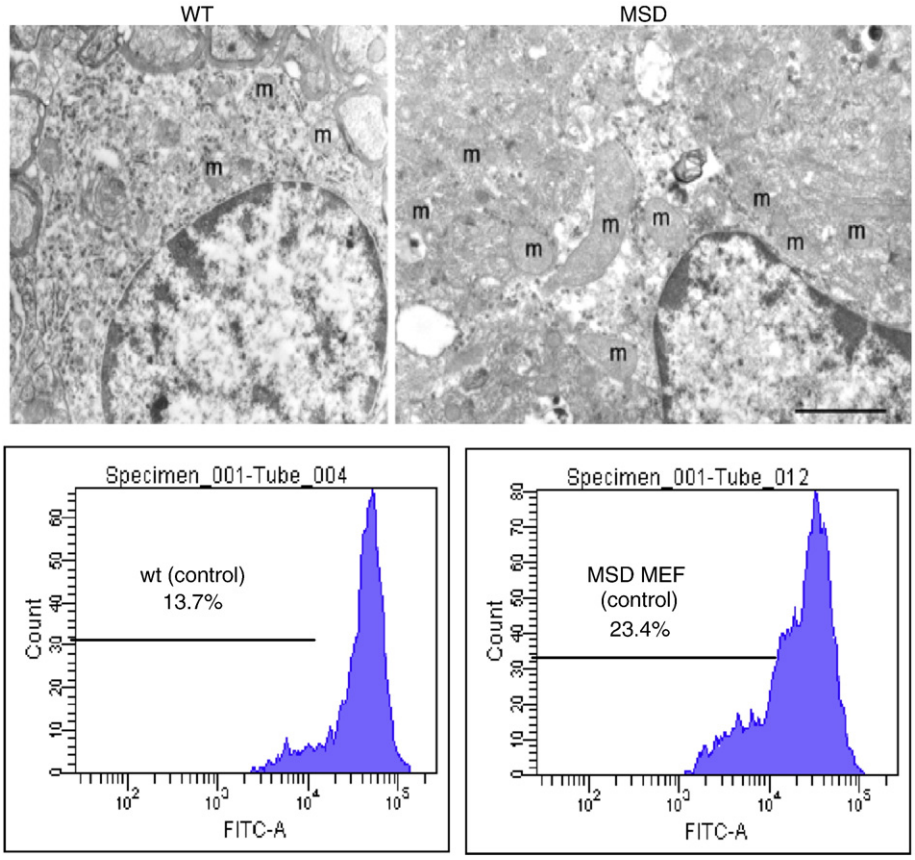

Fig. 5. Block in autophagy leads to accumulation of dysfunctional mitochondria. Electron microscopy analysis of the brain cortex neurons from MSD mice and wild type littermates (bar: wild type = 2.1 μm; MSD = 1.8 μm). MSD neurons contain a significantly higher number of mitochondria (m) compared to wild type neurons. The mitochondrial membrane potential ($\Delta\Psi$m) measured in MSD MEFs is reduced compared to wild-type cells, as evidenced by the increase in the percentage of cells that lost their $\Delta\Psi$m, thus indicating that mitochondria accumulating in MSD are dysfunctional. From [82].

leading to a partial block of autophagy. This may in turn activate a compensatory feedback mechanism through which autophagy is induced (most likely by beclin-1-mediated activation). Both processes, the block and the activation of autophagy, may mediate pathogenesis in different ways but they belong to the same dysfunctional process. In other words, the consequence of autophagy dysfunction on cellular physiology may depend on the severity of the block and on the ability of autophagy induction response to overcome this block without generating an excessive and deleterious build up of the autophagic/lysosomal compartment.

The evidence for a defective autophagy in lysosomal storage diseases suggests a model by which the secondary accumulation of autophagic substrates, such as polyubiquitinated proteins and aberrant mitochondria, has a leading role in LSD pathogenesis, possibly even more than the primary storage material (Fig. 6). This model, if correct, may represent an important point of intersection between lysosomal storage diseases and more common types of neurodegenerative diseases such as Alzheimer, Parkinson and Hungtinton, suggesting the possibility of overlapping therapeutic strategies.

7. Conclusion

Considering that it is less than 60 years ago that lysosomes were discovered research on lysosomal storage diseases has moved forward at a tremendous pace. The current challenge in the field is to understand how the stored material affects cellular and organ function. Recent years have shown that accumulating compounds can act as unphysiologic ligands of signal transduction receptors. Examples are the lysolipid psychosine and the glycosaminoglycan fragments in Krabbe disease and mucopolysaccharidosis, respectively. In Hurler syndrome glycosaminoglycan fragments can modulate growth factor receptor responses and altered membrane lipid composition in Niemann Pick type C disease impairs insulin receptor autophosphorylation. In addition, endosomal TLR4 receptor accumulation in this disease may result in enhanced signalling from this receptor. Psychosine accumulates in Krabbe disease and is the most thoroughly examined lysolipid. It affects various enzymes involved in signal transduction pathways and in all likelyhood this is also relevant for lysolipids accumulating in other lipidoses. Glucosylceramide, G_{M1} and G_{M2} ganglioside enhance calcium release from the ER or inhibit the reuptake, respectively. Consequently altered Ca^{++} homeostasis in Gaucher disease and G_{M1}or G_{M2} gangliosidosis may be of high pathogenic relevance. It should be emphasized that most of these alterations in cellular processes are not caused by storage material present in the lysosome, but rather by accumulation at other intracellular and extracellular locations. Some disease mechanisms are not unique to specific disorders but are of relevance in various diseases (Table 4). This applies for impaired autophagy, inflammation, alteration of Ca^{++} homeostasis and lysolipid accumulation.

We are currently experiencing the era of enzyme replacement therapy. Although for most diseases it will not be curative it must be appreciated as an important step towards treatment of these diseases. The scientific basis of this therapy was worked out in the 70ies and 80ies of the last century. Similarly, the current revelation of pathogenic pathways is likely to identify therapeutic targets which can be used for the benefit of the patients in future.

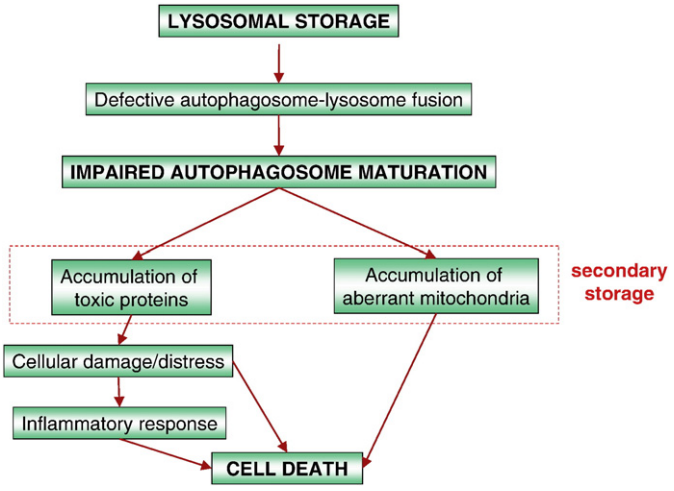

Fig. 6. A proposed model for the pathogenesis of LSDs. Lysosomal storage leads to a reduced ability of lysosomes to fuse with autophagosomes. This results in a block (at least partial) of autophagy maturation and defective degradation. Consequently autophagy substrates such as polyubiquitinated protein aggregates and dysfunctional mitochondria accumulate and promote cell death. The inflammatory response to cell damage further contributes to cell death.

Table 4
Common mechanisms in various lysosomal disorders

Disease	Autophagy Dysfunction	Inflammation	Altered Calcium homeostasis	Lysolipid Accumulation
Mucopolysaccharidoses (MPS)				
MPS I (Hurler)		+		
MPS III (Sanfilippo)	+	+		
MPS VI (Maroteaux-Lamy)		+		
MPS VII (Sly)		+		
MSD	+	+		
Spingolipidoses				
Fabry				+
Gaucher		+	+	+
Globoid cell leukodystrophy (Krabbe)		+		+
Niemann–Pick A			+	
Niemann–Pick C	+	+		
G_{M1} gangliosidosis	+	+	+	+
G_{M2} gangliosidosis (Sandhoff)		+	+	+
Multiple sulphatase deficiency	+	+		
Glycogenosis				
Pompe (glycogen-storage-disease type II)	+			

Table summarizes pathogenically relevant mechanisms likely to play a role in various lysosomal storage disorders. The role of inflammation was not discussed in this review. The reader is reffered to a recent review of Castaneda et al. on immune system alterations in lysosomal disorders [85].

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