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Glycosphingolipidoses: Beyond the enzymatic defect

Annick Raas-Rothschild[1,2], Irene Pankova-Kholmyansky[1], Yaacov Kacher[1] and Anthony H. Futerman[1]

[1]*Department of Biological Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel,* [2]*Department of Human Genetics, Hadassah Hebrew University Hospital, Jerusalem, 91120, Israel*

The glycosphingolipid lysosomal storage diseases are a group of monogenic human disorders caused by the impaired catalytic activity of enzymes responsible for glycosphingolipid catabolism. Clinical presentation of the diseases is heterogeneous, with little obvious correlation between the kind of accumulating glycosphingolipid and disease progression or pathogenesis. In this review, we discuss clinical symptoms of this group of diseases, and attempt to link disease progression and pathology with the biochemical and cellular pathways that may be potentially altered in the diseases.
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Introduction

The lysosomal storage diseases (LSDs) include more than 40 different human disorders and have been classified into broad categories such as the sphingolipidoses, oligosaccharidoses, and mucopolysaccharidoses, based on the nature of the stored material [1,2]. Among the LSDs, the glycosphingolipid (GSL) storage diseases (GSDs) comprise a small group of monogenic disorders caused by the impaired catalytic activity of one or other lysosomal hydrolase responsible for the stepwise degradation of GSLs, which leads to accumulation of undegraded GSLs in the lysosome. A complete list of the GSDs, the enzymatic defects, and the nature of the accumulating material, is given inTable 1.

The clinical presentation of GSDs is very heterogeneous and progressive, with the rate of progression varying between disorders and also within each disorder. The reason for this heterogeneity is not known, but presumably depends on the level and nature of the accumulating GSL, and on the down-stream biochemical and cellular pathways that are altered. However, few if any direct links have been provided to connect clinical progression and pathology with the biochemistry of the diseases. This is somewhat surprising since many GSDs were described decades ago and a wide variety of functions have been proposed for GSLs in both cell survival and death [3]. A lack of basic scientific research into GSD pathogenesis is immediately apparent from reading the literature (reviewed in [2]).

In this review, we provide an overview of the clinical symptoms of the GSDs, and discuss whether the symptoms can be correlated with what is known about the normal physiological and pathophysiological roles of GSLs particularly in the nervous system. A rather unique aspect of this review is that it is written by a physician who regularly treats GSD patients, and by three basic scientists who work on the physiology of GSLs. We believe that this kind of cooperative effort is essential if progress is to be made in connecting clinical aspects of GSDs with basic biochemical mechanisms.

Clinical description of the GSDs

GSDs as a group occur with a relatively high frequency [4,5]. The variability in clinical presentation of the different diseases and even of the same disease, together with the non-specific clinical presentation and the lack of good diagnostic tools, render it likely that the suggested disease frequency might actually be an underestimate. In this section we summarize the main clinical features of the GSDs with an emphasis on neurological symptoms. It should be noted that GSDs are unique among the LSDs inasmuch as all GSDs display neurological involvement, with the exception of type 1 Gaucher disease. The propensity of neurological involvement in the GSDs is almost certainly due to the enrichment of GSLs in the central nervous system where they have been proposed to play a variety of essential

To whom correspondence should be addressed: Anthony H. Futerman, Department of Biological Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel. Tel.: +972-8-9342704; Fax: +972-8-9344112; E-mail: tony.futerman@weizmann.ac.il

Table 1. Sphingolipid, glycosphingolipid and lysosomal storage diseases

Disease	*Enzymatic defect*	*GSL storage material*[a]
		GSD
Gaucher	β-glucosidase, saposin C activator	Glucosylceramide, GM1, GM2, GM3, GD3, glucosylsphingosine
Sphingolipid activator deficiency	Sphingolipid activator protein	Glycolipids
GM1 gangliosidosis	β-Galactosidase	GM1, GM2, GM3, GD1A
Tay Sachs	β-Hexosaminidase A	GM2, other glycolipids
Sandhoff	β-Hexosaminidase A and B	GM2, other glycolipids
GM2 activator deficiency	GM2 activator protein	GM2, other glycolipids
Krabbe	β-Galactosidase	Galactosylceramide
Fabry	α-Galactosidase A	Globotriaosylceramide, and blood group B substances
Metachromatic leukodystrophy	Arylsulfatase A, saposin B activator	Sulphated glycoproteins and glycolipids, and GM2
		SLSD
Farber	Ceramidase	Ceramide, GM3
Niemann-Pick A & B	Sphingomyelinase	Sphingomyelin, GM2, GM3
		Other LSDs
Niemann-Pick C	NPC1, NPC2	Cholesterol and sphingolipids including GM2 & GM3
Fucosidosis	α-Fucosidase	Fucosides and glycolipids
Mucolipidosis II & III	GlcNac transferase	Oligosaccharides, mucopolysaccharides, lipids, GM1
Mucopolysacharidosis I, II, III, VII	Various enzymes[b]	GM2 and GM3
Alpha mannosidosis	α-mannosidase	GM2, GM3
Galactosialidosis	Protective protein cathepsin A	GM1, GM2, GM3

LSDs are classified according to the enzymatic defect rather than according to the nature of the accumulating substrate. In the GSDs, the defect is in an enzyme or protein involved in GSL catabolism. In the sphingolipid storage diseases (SLSDs), the enzymatic defect is in an enzyme or protein involved in SL catabolism, but GSLs often accumulate as secondary storage materials. This latter aspect is discussed in more detail in the text.
[a]A complete list of secondary GSL storage products can be found in a number of reviews [2,7].
[b]Mucopolysacharidosis are caused by the impaired function of 1 of any 11 lysosomal enzymes involved in glycosaminoglycan degradation.

roles [3,6]. This is particularly important when considering that GSLs also accumulate as secondary metabolites in a number of other LSDs (Table 1 and [7]), which might suggest common pathological pathways leading from GSL accumulation to clinical symptoms, particularly neurological symptoms.

The major clinical symptoms in the GSDs are summarized inTable 2. All the diseases listed therein are inherited as autosomal recessive disorders, except Fabry disease which is an X-linked disease [8]. Some additional clinical information for a number of the diseases is given below, which should be read in conjunction with Table 2. A glossary of medical terms is given in Appendix 1.

Gaucher disease

Gaucher disease occurs in three forms, type 1, the non-neuronopathic form, type 2, the acute neuronopathic form, and type 3, the juvenile neuronopathic form [9]. Type 1, Gaucher disease patients can be asymptomatic, or present with hepatosplenomegaly, abnormal hematological features such as anemia and thrombocytopenia, and/or bone lesions, but do not normally display neurological features. In contrast, neurological symptoms are observed in type 2 and 3 patients [10]. Type 2 patients generally die as infants since the disease has an acute progression, while type 3 patients have a more chronic neuropsychiatric involvement. Type 1 Gaucher disease is the only GSD which does not present with neurological symptoms.

GM1 gangliosidosis

In classical cases the disease is recognized in early infancy. This is a progressive neurological disease with 3 clinical subtypes, infantile (with death occurring during the first years), juvenile (death by the age of 10 years), or adult. Patients with early presentation have a rapid deterioration while adult patients have a more protracted course and present with cerebellar and upper motor neuron damage [11].

GM2 gangliosidoses (Tay-Sachs disease, Sandhoff disease, and GM2 activator deficiency)

The phenotypes of these three diseases are variable. They are often classified based on the time of disease onset into infantile, juvenile and adult forms [12]. However, some clinicians prefer to classify the diseases based on disease progression, into infantile acute, corresponding to the classical presentation of each individual GM2 gangliosidoses, subacute, including late infantile and juvenile, and chronic with adult onset; note that there are no known adults with late onset GM2 activator deficiency. The infantile acute forms of all three GM2 gangliosidoses present

Table 2. Clinical features of sphingolipid storage diseases

Disease	*Psycho-motor delay*	*Ataxia*	*Hypotonia/ Weakness*	*Other CNS features*	*Psychiatric/ behavioral Changes*	*Storage Features*	*Opthalmological features*	*Other features*
Gaucher types 1, 2 & 3	Yes (2,3)	Yes (3)	Yes (2,3)	Pseudo bulbar palsy, laryngeal spasm (2, 3)	Dementia (3)	HSM[a] (1, 2, 3)	Supranuclear gaze palsy, Strabismus (2, 3)	Anemia, thrombocytopenia (1, 2, 3), Bone lesions (1, 2, 3), Mitral valve calcification (3)
Sphingolipid-activator deficiency	Yes	No	No	Myoclonus	Hyperkinetic behavior	HSM		Respiratory insufficiency
GM1 gangliosidosis (Infantile onset)	Yes	No	Yes	Exaggerated startle response	No	Coarse facies, HSM, Macrocephaly, Joint stiffness, Gingival hyperplasia	CRS, Blindness	Hypertrichosis, Inguinal hernia, Kyphosis Scoliosis, Skeletal dysplasia
GM1 gangliosidosis (Late onset)	Yes	Yes	No	Dystonia, dysarthria	PM regression			
Tay-Sachs, Sandhoff and GM2 activator deficiency (Early onset)	Yes	No	Yes	Hyperacusis, Exaggerated startle reaction	PM Regression	Doll-like face Macrocephaly, macroglossia	CRS, Early blindness	Cardiomegaly
Tay-Sachs and Sandhoff (Late onset)	No	Yes	No	Dysarthria, Dystonia	Psychoses, regression			
Krabbe (Infantile onset)	Yes	No	Yes	Irritability	No	Deafness	Optic atrophy	FTT, Vomiting, Fever of unknown origin
Krabbe (Late onset)		Yes	Yes	Paraparesis, Loss of speech, peripheral neuropathy	Yes		Blindness, optic atrophy	
Fabry	No	No	No	Stroke, Burning paresthesias	Yes	No	Corneal opacities	Angiokeratomas, Kidney disease, Cardiomyopathy, Conduction defects

(*Continued on next page.*)

Table 2. (*Continued*).

Disease	*Psycho-motor delay*	*Ataxia*	*Hypotonia/ Weakness*	*Other CNS features*	*Psychiatric/ behavioral changes*	*Storage features*	*Opthalmological features*	*Other features*
Metachromatic leukodystrophy	Yes (I, J)	Yes (I, A)	Yes (I, J)	Psycho-motor regression (I, J), Seizures (I, J), Brain stem dysfunction (I, J)	Psychoses, dementia (A), Behavior changes (J, A)	No	Optic atrophy	
Farber	Yes	No	Yes	Irritability, Hoarse cry	No	HSM	CRS	Painful swollen joint nodules, Nephropathy, Hydrops fetalis.
Niemann-Pick A	Yes	Yes	Yes	Seizures	No	HSM	CRS (50%)	Short stature, FTT, anemia
Niemann-Pick B	No	No	No	No	No	HSM	CRS (rare)	Short stature, FTT, anemia, pulmonary infections

Emphasis is placed on neurological features, but for completeness, some other non-neurological symptoms are listed. Further details can be found at http://www.ncbi.nlm.nih.gov/omim. Appendix 1 gives a glossary of medical terms.
[a]Abbreviations used in the Table are: HSM, Hepatosplenomegaly; A, adult; CNS, central nervous system; CRS, Cherry Red spots; DM, Dysosotosis multiplex; FTT, Failure to thrive; I, infantile; J, juvenile; L, late; PM regression, psycho-motor regression.

with very similar symptoms, and it is difficult to clinically differentiate chronic adult Tay-Sachs patients from chronic adult Sandhoff patients because of the overlapping clinical picture.

Fabry disease

Hemizygous males usually experience multisystem involvement while heterozygous females have a more variable expression with severe symptoms [13]. Over time, renal failure and vascular disease of the heart and brain are responsible for the early death of the patients [8]. Recently, children were also diagnosed with Fabry disease for the first time [14].

Metachromatic leukodystrophy

There are three forms, infantile, juvenile, and adult. The infantile form is the most common and often begins with abnormal gait. Children with this form of MLD usually die in the first decade of life. In juvenile forms, gait disturbance and mental regression are the earliest signs of disease which develops with a slower progression. Adults are sometimes misdiagnosed as psychiatric patients due to behavioral disturbances and dementia [15].

Krabbe disease (Globoid cell leukodystrophy)

This disease also occurs in two forms. The infantile form is characterized by progressive delay, irritability, seizures and death during the first 2 years of life. The late onset form (both juvenile and adult) have a slower progression [16,17]. Clinical diagnosis of the late onset form can be extremely difficult and is characterized by progressive deterioration of the central and peripheral nervous system.

Niemann-Pick disease A and B

Although this is a sphingolipid storage disease, rather than a GSD, it is considered here since GSLs accumulate as secondary storage products (Table 1). Type A is the most common with patients presenting with hepatosplenomegaly, neurological regression and seizures [18]. Most type A patients die in their first years of life. Type B patients do not normally display neurological symptoms.

In summary, the lack of specificity of the neurological symptoms to the GSDs in general, and to each specific disease, does not easily allow disease diagnosis for the clinician not familiar with these disorders. Moreover, the resemblance of many of the clinical symptoms between individual diseases (Table 2) may suggest common mechanisms of disease pathology, but in themselves do not shed any light on the mechanistic connection between GSL accumulation and disease progression. For instance, ataxia is common to many diseases, such as that observed in the GM1 and GM2 gangliosidoses. What is the cause of ataxia in these patients? Is it similar to the cause of ataxia in other diseases such as Niemann-Pick A where Purkinje cell degeneration is observed [19]? What is the cause of mental retardation? Why do patients suffer from seizures? These kind of issues lead to mechanistic and biochemical questions concerning how GSL accumulation causes these symptoms, and begs the question of whether neuronal cell dysfunction and/or neuronal cell death in specific brain regions are the cause of any of the clinical symptoms? For instance, are the inflammatory responses [20,21] observed in some GSDs common to all diseases, and if so, what is the trigger of inflammation? Answers to these and similar mechanistic questions are for the most part lacking and at present are the missing link in our understanding of disease progression and pathology, and in the development of possible new therapies. The latter point is particularly important since no therapeutic avenues exist at present to treat any of the neurological symptoms of the GSDs. Thus, enzyme replacement therapy, while useful for non neuronopathic Gaucher disease patients and Fabry disease patients [22], cannot be used for patients with central nervous system pathology since the recombinant enzymes do not cross the blood brain barrier.

In the next section we address the issue of whether neuronal cell death occurs in the GSDs and focus on whether apoptotic cell death might be one of the underlying causes of GSD pathology. In addition, we discuss recent data suggesting a role for inflammatory responses in GSD pathology.

Apoptosis and inflammation in GSDs

Apoptosis is a regulated physiological process that leads to the elimination of unwanted cells during development or during renewal of cell populations in mature tissues [23]. The rationale behind thinking that apoptosis might be a mechanism of neuronal cell death and/or dysfunction in the GSDs is based on the essential roles that GSLs lipids play in development, and their roles in various biological processes, including apoptosis [3]. Moreover, the building block of SLs and GSLs, ceramide, regulates apoptosis [24], suggesting that alterations in metabolic pathways leading to or from ceramide formation could have disastrous consequences for neuronal function [25]. Thus, early studies in our laboratory demonstrated that ceramide could cause apoptotic neuronal cell death in cultured hippocampal neurons [26].

However, there is little evidence that ceramide levels change in the GSDs. In contrast, ceramide levels do change in the SLSDs, Niemann-Pick A/B disease and Farber disease, which are caused by defective sphingomyelinase and ceramidase activities, respectively. However, there are no studies unambiguously demonstrating apoptotic cell death in neurons from either human brain tissue or in animal models of these two diseases although cell death has been observed in non-neuronal tissues [27]. Conversely, apoptosis has been observed in many of the GSDs, both in tissues of neuronal origin (Table 3) and in other tissues [27]. For instance, DNA fragmentation was observed in the cerebral cortex, brain stem, cerebellum and spinal cord in an animal model of Sandhoff disease (the Hexb−/− mouse, [28]), but was not seen in a mouse model of Tay-Sachs disease

Table 3. Evidence for apoptosis in GSDs (For further details, see text)

	Human brain tissue		*Brains of animal disease models*	
Disease	*Morphological changes consistent with apoptosis*	*Molecular* & *biochemical changes*	*Morphological changes consistent with apoptosis*	*Molecular* & biochemical *changes*
Gaucher	Yes [30]		Yes [32]	Bcl-2 down-regulation [32]
Tay-Sachs	Yes [29]	Elevation of heat shock proteins, death-associated protein 6 (DAXX) [34]	Not observed [29]	Not observed [29]
Sandhoff	Yes [29]	Elevation of heat shock proteins, death-associated protein 6 (DAXX) [34]	Yes [20,21,29]	DNA laddering detected [29]
Krabbe	Yes [33]			
Sphingolipid activator protein deficiency			Yes [45]	

(the Hexa−/− mouse, [28,29]). In a type 2 Gaucher disease patient, apoptosis of neuronal cells from the anterior horn and brainstem was detected [30], and in an animal model of Gaucher disease (the Gba mouse [31]), decreased expression of the bcl-2 gene in the brain stem and cerebellum but not in the cortex was observed by DNA microarray analysis [32]. Nuclear DNA fragmentation, nuclear localization of p53 and positive annexin-V immunostaining have been observed in the brain of a human Krabbe patient brain [33].

Thus, although apoptosis has been detected in some of the GSDs, a definitive relationship cannot be derived linking apoptotic cell death to the clinical symptoms discussed above. One of the reasons for this is probably the paucity of post mortem human brain tissue from GSD patients, although more systematic research examining apoptosis in specific brain regions of all of the mouse models of each disease is required. In conclusion, although apoptosis may be involved in some of the pathological pathways, more research is required before evoking a central role for apoptosis in the GSDs.

Another mechanism which has received considerable attention over the past few years is inflammation [21]. Inflammation is a local response to cellular insult, and markers indicating inflammatory responses have been detected in a number of GSDs. It is not known at present whether inflammation is a direct response to intracellular GSL accumulation, or a response to the appearance of apoptotic or injured cells [20]. Irrespective of what leads to what, the over-expression of genes associated with activated macrophages/microglia and astrocytes has been detected in Sandhoff and Tay-Sachs diseases mouse models [21,34]. In addition, in Sandhoff and Tay-Sachs diseases mouse models, and in a mouse model of GM1 gangliosidosis, progressive CNS inflammation was observed that correlates with the disease severity [20].

Despite the apoptotic and inflammatory responses observed in the GSDs, a description of the initial molecular trigger that causes these responses is lacking. In the next section, we will discuss recent data, obtained for the most part in our laboratory, which has begun to address this issue, and may eventually provide a temporal description of the events leading from GSL accumulation to pathology as manifested by the clinical symptoms discussed earlier.

Biochemical pathways involved in neuronal cell dysfunction and death

Five years ago, we observed that neurons that had accumulated glucosylceramide (GlcCer), the GSL that accumulates in Gaucher disease (Table 1), released more calcium from intracellular stores in response to caffeine-treatment than their untreated counterparts [35], which led to enhanced sensitivity to agents that induced neuronal cell death [35,36]. Since calcium release was evoked by caffeine, a known agonist of the ryanodine receptor, a calcium release channel, we proposed that GlcCer accumulation in neurons somehow enhanced agonist-induced calcium-release via the ryanodine receptor, which is located in the endoplasmic reticulum. Moreover, subsequent analysis of calcium-release *in vitro* from isolated microsomes revealed that GlcCer was the only GSL or SL tested that enhanced agonist-induced calcium-release via the ryanodine receptor [37]. Further studies demonstrated that the lyso-GSL, glucosylsphingosine also stimulated calcium-release, but via a different mechanism to GlcCer [38]. Finally, we demonstrated a correlation between levels of GlcCer accumulation in human brain tissue obtained post mortem from neuronopathic Gaucher disease patients (type 2) and levels of calcium-release (Pelled *et al.*, submitted for publication). Together, these findings suggested that defective calcium homeostasis might be one of the mechanisms responsible for at least some of the neuropathophysiology in acute neuronopathic (type 2) Gaucher disease (Table 4).

We also obtained evidence that calcium-homeostasis is affected in Sandhoff disease. In a Sandhoff disease animal model,

Table 4. Changes in biochemical and morphological parameters in neuronal models of GSDs (For further details, see text)

Disease model	*Neuronal development and growth*	*Phospholipid metabolism*	*Calcium homeostasis*
Gaucher neurons (type 2/3) (animal models)	Increased axonal growth rates [41,46] and sensitivity to agents that induce neuronal cell death [36]	Increased [41] phosphatidylcholine synthesis [41]	Increased Ca^{2+} release via ryanodine receptor [37,38]
Gaucher human brain tissue			Increased Ca^{2+} release from ER via ryanodine receptor (submitted for publication)
Sandhoff brain (mouse model)	Decreased axonal growth rates [43]	Decreased phospholipid synthesis [42]	Decreased Ca^{2+} uptake via sarco/endoplasmic reticulum ATPase [39]

the rate of calcium-uptake into the endoplasmic reticulum, via the sarco/endoplasmic reticulum ATPase (SERCA), was significantly reduced [39]. Thus, microsomes from Hexb−/− brains showed a significant reduction in the rate of calcium-uptake via SERCA, which was reversed by feeding Hexb−/− mice with *N*-butyldeoxynojirimycin (NB-DNJ), an inhibitor of glycolipid synthesis that reduces GM2 storage [40], and correlated with the increased life span of these animals. This study suggested a mechanistic link between GM2 accumulation, reduced SERCA activity, neuronal cell death, and the prolonged survival of these animals (Table 4).

In another series of experiments we also observed an unexpected connection between GSL accumulation in GSDs and phospholipid metabolism. We demonstrated that the synthesis of phosphatidylcholine (PC), the major structural lipid of animal cell membranes, was significantly elevated in either a chemically-induced model of neuronopathic Gaucher disease, or in a mouse model of Gaucher disease, the Gba mouse [31]. Moreover, we went on to demonstrate that PC synthesis was stimulated due to direct activation of CTP:phosphocholine cytidylyltransferase (CCT), the rate-limiting enzyme in the pathway of PC biosynthesis, by GlcCer [41]. Similar to the effect observed in agonist-induced calcium-release stimulated by GlcCer, the only SL that activated CCT was GlcCer. Thus, we suggest that GlcCer accumulation in models of Gaucher disease results in activation of CCT and as a result, enhanced PC synthesis, which might contribute towards increased rates of axonal growth. In contrast, we observed decreased levels of phospholipid synthesis in brain tissue from the Hexb−/− mouse [42], which correlated with decreased rates of axonal growth in neurons cultured from these mice [43].

In summary, we suggest that GSLs upon their accumulation in GSDs may escape from lysosomes [44] in order to interact with, and modulate down-stream pathways.

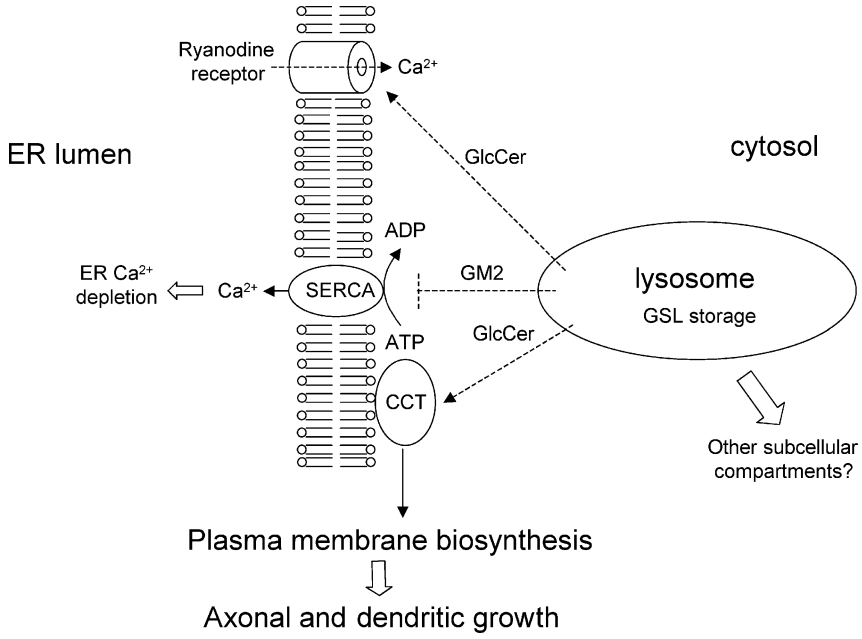

Figure 1. The relationship between GSL storage and some down-stream biochemical pathways.

Interactions with enzymes of phospholipid synthesis and two proteins involved in calcium homeostasis (Figure 1) may be responsible for some of the changes in cellular physiology that might be involved in triggering apoptotic and/or inflammatory responses. For instance, GM2 which accumulates in the GM2 gangliosidoses (Table 1), blocks calcium uptake via the sarco/endoplasmic reticulum ATPase, leading to a reduction in lumenal calcium levels. Since GM2 accumulates in a number of other LSDs as a secondary storage product (Table 1), this implies that it may be involved in pathological mechanisms in some of these other LSDs. As a narrow range of calcium levels is optimal for neuronal growth, changes in cytosolic calcium concentrations may lead to changes in the rate of axonal and dendritic growth. In contrast, the activation of CCT by GlcCer, and the stimulation of agonist-induced calcium-release via the ryanodine receptor may together lead to stimulation of neurite growth (Figure 1). Further delineation of the sequence of these and of other biochemical pathways should eventually provide a comprehensive mechanistic understanding of GSD pathology.

Perspectives

In this short review, we have attempted to highlight the many of the open questions that remain unresolved concerning the etiology and pathology of the GSDs. Thus, work is beginning to emerge that defines how GSL accumulation in the GSDs affects down-stream biochemical pathways. Furthermore, although apoptosis and inflammation have been shown to occur in the GSDs, the biochemical triggers are largely unknown. Finally, no explanations have yet been proposed to link any of these biochemical events to the known neurological symptoms, which remains the most challenging task for future research in this area.

Appendix 1: Glossary

Further details can be found at http://www.albany.net.

Anemia: An abnormally low number of red blood cells
Ataxia: An unsteady wide-based gait. Affected patients cannot accurately perform rapid alternating movements.
Dementia: A progressive loss of intellectual function, with personality change.
Dysarthria: Abnormal speech (slurring, inappropriate phrasing, modulation in speech volume).
Dysphagia: Difficulty in swallowing either solids, liquids, or both.
Dystonia: Involuntary movements (twisting, repetitive movements, abnormal posture).
Hepatomegaly: Enlargement of the liver.
Hyperacusis: Increased sensitivity to sound.
Hypertrichosis: Excessive growth of hair on the body.
Hypotonia: Muscles offer less resistance to passive displacement.
Inguinal hernia: Protrusion of an organ or part of an organ through the wall of the inguinal cavity.
Left ventricular hypertrophy: Enlargment of the left cardiac ventricle.
Optic atrophy: Degeneration of the optic nerve.
Paraparesis: A weakness but not a total paralysis of the lower extremities (legs).
Paresthesias: Abnormal sensations such as burning, prickling.
Splenomegaly: Enlargement of the spleen.
Thrombocytopenia: an abnormally low number of thrombocytes (platelets).

References

1 Hopwood JJ, Brooks DA, An introduction to the basic science and biology of the lysosome and storage diseases. In *Organelle Disease*, edited by Applegarth DA, Dimmick JE, Hall J (Chapman and Hall Medical, 1997), pp. 7–36.
2 Futerman A, van Meer G, The cell biology of lysosomal storage disorders, *Nature Reviews Molecular Cell Biology* **5**, 554–65 (2004).
3 Bektas M, Spiegel S, Glycosphingolipids and cell death, *Glycoconj J* **20**, 39–47 (2004).
4 Meikle PJ, Hopwood JJ, Clague AE, Carey WF, Prevalence of lysosomal storage disorders, *J Am Med Assoc* **281**, 249–54 (1999).
5 Pinto R, Caseiro C, Lemos M, Lopes L, Fontes A, Ribeiro H, Pinto E, Silva E, Rocha S, Marcao A, Ribeiro I, Lacerda L, Ribeiro G, Amaral O, Sa Miranda MC, Prevalence of lysosomal storage diseases in Portugal, *Eur J Hum Genet* **12**, 87–92 (2004).
6 Ledeen RW, Yu G, Ganglioside function in the neuron, *Trends Glycosci Glycotechnol* **4**, 174–87 (1992).
7 Walkley SU, Secondary accumulation of gangliosides in lysosomal storage disorders, *Semin Cell Dev Biol* **15**, 433–44 (2004).
8 Desnick RJ, Ioannou YA, Eng CM, α-Galactosidase A deficiency: Fabry disease. In *The Metabolic and Molecular Bases of Inherited Disease*, edited by Scriver CR, Sly WS, Childs B, Beaudet AL, Valle D, Kinzler KW, Vogelstein B (McGraw-Hill Inc., 2001), pp. 3733–74.
9 Beutler E, Grabowski GA, Gaucher disease. In *The Metabolic and Molecular Bases of Inherited Disease*, Vol. II, edited by Scriver CR, Sly WS, Childs B, Beaudet AL, Valle D, Kinzler KW, Vogelstein B (McGraw-Hill Inc., 2001), pp. 3635–68.
10 Erikson A, Bembi B, Schiffmann R, Neuronopathic forms of Gaucher's disease, *Baillieres Clin Haematol* **10**, 711–23 (1997).
11 Suzuki Y, Oshima A, E. N. β-galactosidase deficiency (β-galactosidosis): GM1 gangliosidosis and Morquio B disease. In *The Metabolic and Molecular Bases of Inherited Disease*, edited by Scriver CR, Sly WS, Childs B, Beaudet AL, Valle D, Kinzler KW, Vogelstein B (McGraw-Hill Inc., 2001), pp. 3775–807.
12 Gravel RA, Kaback MM, Proia R, Sandhoff K, Suzuki K, Suzuki K. The GM2 gangliosidosis. In *The Metabolic and Molecular Bases of Inherited Disease*, edited by Scriver CR, Sly WS, Childs B, Beaudet AL, Valle D, Kinzler KW, Vogelstein B (McGraw-Hill Inc., 2001) pp. 3827–76.

13 Desnick RJ, Brady R, Barranger J, Collins AJ, Germain DP, Goldman M, Grabowski G, Packman S, Wilcox WR, Fabry disease, an under-recognized multisystemic disorder: Expert recommendations for diagnosis, management, and enzyme replacement therapy, *Ann Intern Med* **138**, 338–46 (2003).
14 Desnick RJ, Brady RO, Fabry disease in childhood, *J Pediatr* **144**, S20–6 (2004).
15 von Figura K, Gieselmann V, Jaeken J. Metachromatic leukodystrophy. In *The Metabolic and Molecular Bases of Inherited Disease*, edited by Scriver CR, Sly WS, Childs B, Beaudet AL, Valle D, Kinzler KW, Vogelstein B (McGraw-Hill Inc., 2001), pp. 3695–724.
16 Wegner DA, Suzuki K, Suzuki Y, Suzuki K, Galactosylceramide lipidosis: Globoid cell leukodystrophy (Krabbe disease). In *The Metabolic and Molecular Bases of Inherited Disease*, Vol. II, edited by Scriver CR, Sly WS, Childs B, Beaudet AL, Valle D, Kinzler KW, Vogelstein B (McGraw-Hill Inc., 2001) pp. 3669–94.
17 Wenger DA, Coppola S, Liu SL, Insights into the diagnosis and treatment of lysosomal storage diseases, *Arch Neurol* **60**, 322–8 (2003).
18 Schuchman EH, Desnick RJ, Niemann-Pick Disease Types A and B: Acid Sphingomyelinase Deficiences. *In The Metabolic and Molecular Bases of Inherited Disease*, Vol. II, edited by Scriver CR, Sly WS, Childs B, Beaudet AL, Valle D, Kinzler KW, Vogelstein B (McGraw-Hill Inc., 2001), pp. 3589–605.
19 Sarna JR, Larouche M, Marzban H, Sillitoe RV, Rancourt DE, Hawkes R, Patterned Purkinje cell degeneration in mouse models of Niemann-Pick type C disease, *J Comp Neurol* **456**, 279–91 (2003).
20 Jeyakumar M, Thomas R, Elliot-Smith E, Smith DA, van der Spoel AC, d'Azzo A, Perry VH, Butters TD, Dwek RA, Platt FM, Central nervous system inflammation is a hallmark of pathogenesis in mouse models of GM1 and GM2 gangliosidosis, *Brain* **126**, 974–87 (2003).
21 Wada R, Tifft CJ, Proia RL, Microglial activation precedes acute neurodegeneration in Sandhoff disease and is suppressed by bone marrow transplantation, *Proc Natl Acad Sci USA* **97**, 10954–9 (2000).
22 Futerman AH, Sussman JL, Horowitz M, Silman I, Zimran A, New directions in the treatment of Gaucher disease, *Trends Pharmacol Sci* **25**, 147–51 (2004).
23 Vaux DL, Strasser A, The molecular biology of apoptosis, *Proc Natl Acad Sci USA* **93**, 2239–44 (1996).
24 Hannun YA, Obeid LM, The ceramide-centric universe of lipid-mediated cell regulation: Stress encounters of the lipid kind, *J Biol Chem* **277**, 25847–50 (2002).
25 Buccoliero R, Futerman AH, The roles of ceramide and complex sphingolipids in neuronal cell function, *Pharmacol Res* **47**, 409–19 (2003).
26 Schwarz A, Futerman AH, Distinct roles for ceramide and glucosylceramide at different stages of neuronal growth, *J Neurosci* **17**, 2929–38 (1997).
27 Tardy C, Andrieu-Abadie N, Salvayre R, Levade T, Lysosomal storage diseases: Is impaired apoptosis a pathogenic mechanism? *Neurochem Res* **29**, 871–80 (2004).
28 Sango K, Yamanaka S, Hoffmann A, Okuda Y, Grinberg A, Westphal H, McDonald MP, Crawley JN, Sandhoff K, Suzuki K, Proia RL, Mouse models of Tay-Sachs and Sandhoff diseases differ in neurologic phenotype and ganglioside metabolism, *Nature Genetics* **11**, 170–6 (1995).
29 Huang JQ, Trasler JM, Igdoura S, Michaud J, Hanal N, Gravel RA, Apoptotic cell death in mouse models of GM2 gangliosidosis and observations on human Tay-Sachs and Sandhoff diseases, *Hum Mol Genet* **6**, 1879–85 (1997).
30 Finn LS, Zhang M, Chen SH, Scott CR, Severe type II Gaucher disease with ichthyosis, arthrogryposis and neuronal apoptosis: Molecular and pathological analyses, *Am J Med Genet* **91**, 222–6 (2000).
31 Tybulewicz VLJ, Tremblay ML, LaMrca ME, Willemsen R, Stubblefield BK, Winfield S, Zablocka B, Sidransky E, Martin BM, Huang SP, Mintzer KA, Westphal H, Mulligan RC, Ginns EI, Animal model of Gaucher's disease from targeted disruption of the mouse glucocerebrosidase gene, *Nature* **357**, 407–10 (1992).
32 Hong YB, Kim EY, Jung SC, Down-regulation of Bcl-2 in the fetal brain of the Gaucher disease mouse model: A possible role in the neuronal loss, *J Hum Genet* **49**, 349–54 (2004).
33 Jatana M, Giri S, Singh A, Apoptotic positive cells in Krabbe brain and induction of apoptosis in rat C6 glial cells by psychosine, *Neurosci Lett* **330**, 183–7 (2002).
34 Myerowitz R, Lawson D, Mizukami H, Mi Y, Tifft CJ, Proia RL, Molecular pathophysiology in Tay-Sachs and Sandhoff diseases as revealed by gene expression profiling, *Hum Mol Genet* **11**, 1343–50 (2002).
35 Korkotian E, Schwarz A, Pelled D, Schwarzmann G, Segal M, Futerman AH, Elevation of intracellular glucosylceramide levels results in an increase in endoplasmic reticulum density and in functional calcium stores in cultured neurons, *J Biol Chem* **274**, 21673–8 (1999).
36 Pelled D, Shogomori H, Futerman AH, The increased sensitivity of neurons with elevated glucocerebroside to neurotoxic agents can be reversed by Imiglucerase, *J Inherit Metab Dis* **23**, 175–84 (2000).
37 Lloyd-Evans E, Pelled D, Riebeling C, Bodennec J, de-Morgan A, Waller H, Schiffmann R, Futerman AH, Glucosylceramide and glucosylsphingosine modulate calcium mobilization from brain microsomes via different mechanisms, *J Biol Chem* **278**, 23594–9 (2003).
38 Lloyd-Evans E, Pelled D, Riebeling C, Futerman AH, Lysoglycosphingolipids mobilize calcium from brain microsomes via multiple mechanisms, *Biochem J* **375**, 561–5 (2003).
39 Pelled D, Lloyd-Evans E, Riebeling C, Jeyakumar M, Platt FM, Futerman AH, Inhibition of calcium uptake via the sarco/endoplasmic reticulum $Ca2^{+}$-ATPase in a mouse model of Sandhoff disease and prevention by treatment with *N*-butyldeoxynojirimycin, *J Biol Chem* **278**, 29496–501 (2003).
40 Lachmann RH, Miglustat. Oxford GlycoSciences/Actelion, *Curr Opin Investig Drugs* **4**, 472–9 (2003).
41 Bodennec J, Pelled D, Riebeling C, Trajkovic S, Futerman AH, Phosphatidylcholine synthesis is elevated in neuronal models of Gaucher disease due to direct activation of CTP:phosphocholine cytidylyltransferase by glucosylceramide, *Faseb J* **16**, 1814–6 (2002).
42 Buccoliero R, Bodennec J, van Echten-Deckert G, Sandhoff K, A.H. F, Phospholipid synthesis is decreased in neuronal tissue in a mouse model of Sandhoff disease, *J Neurochem* **90**, 80–8 (2004).
43 Pelled D, Riebeling C, van Echten-Deckert G, Sandhoff K, Futerman AH, Reduced rates of axonal and dendritic growth in

embryonic hippocampal neurones cultured from a mouse model of Sandhoff disease, *Neuropathol Appl Neurobiol* **29**, 341–9 (2003).
44 Ginzburg L, Kacher Y, Futerman AH, The pathogenesis of glycosphingolipid storage disorders, *Semin Cell Dev Biol* **15**, 417–31 (2004).
45 Tohyama J, Oya Y, Ezoe T, Vanier MT, Nakayasu H, Fujita N, Suzuki K, Ceramide accumulation is associated with increased apoptotic cell death in cultured fibroblasts of sphingolipid activator protein-deficient mouse but not in fibroblasts of patients with Farber disease, *J Inherit Metab Dis* **22**, 649–62 (1999).
46 Schwarz A, Rapaport E, Hirschberg K, Futerman AH, A regulatory role for sphingolipids in neuronal growth. Inhibition of sphingolipid synthesis and degradation have opposite effects on axonal branching, *J Biol Chem* **270**, 10990–8 (1995).