Lysosomal Storage Disease: Revealing Lysosomal Function and Physiology

The discovery over five decades ago of the lysosome, as a degradative organelle and its dysfunction in lysosomal storage disorder patients, was both insightful and simple in concept. Here, we review some of the history and pathophysiology of lysosomal storage disorders to show how they have impacted on our knowledge of lysosomal biology. Although a significant amount of information has been accrued on the molecular genetics and biochemistry of lysosomal storage disorders, we still do not fully understand the mechanistic link between the storage material and disease pathogenesis. However, the accumulation of undegraded substrate(s) can disrupt other lysosomal degradation processes, vesicular traffic, and lysosomal biogenesis to evoke the diverse pathophysiology that is evident in this complex set of disorders.

The lysosome was first described by De Duve et al. in 1955 and is an acidic organelle containing an array of lysosomal hydrolases (31). Macromolecules are delivered toward lysosomes for degradation via either endocytic pathways from the extracellular environment or by routes from the cytosol (35, 68, 101). Specialist endosomes and lysosomes have many important functions within cells, including antigen presentation, innate immunity, autophagy, signal transduction, cell division, and neurotransmission. Each component of the endosome-lysosome system is a potential target for dysfunction leading to a diseased state (FIGURE 1).

Lysosomal storage disorders comprise a group of more than 50 different genetic diseases (124). These disorders mostly involve the dysfunction of lysosomal hydrolases, which result in impaired substrate degradation. However, proteins involved in vesicular traffic and the biogenesis of lysosomes have also been shown to cause storage disorder phenotypes. Any disruption of lysosomal function can lead to the accumulation of undegraded substrate(s) in endosomes and lysosomes, eventually compromising cellular function (55).

Although lysosomal proteins are ubiquitously distributed, the accumulation of undegraded substrate(s) in lysosomal storage disorder patients is normally restricted to those cells, tissues, and organs in which substrate turnover is high. The accumulation of the primary storage material can cause a chain of secondary disruptions to other biochemical and cellular functions, which leads to the severe pathology in lysosomal storage disorders. This review will discuss the pathophysiology of selected lysosomal storage disorders and how these diseases have informed our knowledge on lysosomal cell biology and function (FIGURE 2). We have discussed the following disorders: Pompe disease because of its pivotal role in defining the concept of enzyme deficiency and lysosomal storage disorder biology; the mucopolysaccharidoses and the lipidoses, which are themselves major groups of disorders that provide early clinical descriptions, reveal important biological pathways, and highlight interconnecting pathological cascades; 1-cell and multiple sulphatase deficiencies, which are representative of protein targeting/processing defects; and the albinism disorders, which reveal a new class of vesicular trafficking, lysosomal-related disorders.

Pompe Disease

In 1932, Pompe made the critical observation of extensive glycogen accumulation, within membrane-bound vesicles in the heart muscle, of a 7-mo-old patient who had died from cardiac complications (96). The metabolic basis of Pompe disease was determined later by delineating the glycogen metabolism pathway (27) and defining a new cellular organelle, the lysosome (31). Based on these findings, Hers and coworkers deduced the link between the deposition of glycogen in Pompe patients and the inherited deficiency of the lysosomal enzyme α-D-glucosidase (50). The involvement of the lysosome in glycogen degradation gave rise to the concept that other lysosomal storage disorders could also be explained by specific enzyme deficiencies.

Pompe patients present within a spectrum of clinical phenotypes that can be defined by the age of onset, rate of disease progression, and degree of...
cardiac and/or skeletal muscle involvement (Table 1) (52). In the infantile form of Pompe disease, patients present with rapidly progressive cardiac and skeletal myopathy (52). Death usually occurs in the first year of life, primarily due to cardiorespiratory failure. Pathophysiology studies have shown the progressive enlargement of glycogen storage vesicles, with evidence of autophagic storage vacuoles (FIGURE 3D) in type II, but not type I, muscle fibers (39, 40, 100). In addition, acidification defects in populations of endosomes and lysosomes and decreased vesicular mobility have been reported in Pompe cells (40), suggesting a secondary defect in vesicular traffic.

Enzyme replacement therapy has been developed for Pompe patients and utilizes the extracellular uptake of functional enzyme by cell surface mannose-6-phosphate receptors. This therapy can clear glycogen in cardiac muscle but has been less effective in skeletal muscle, particularly for the autophagic glycogen in type II fibers (39, 98). This failure of enzyme replacement therapy to clear glycogen from skeletal muscle may be due to the inability of endocytozed enzyme to reach storage compartments either due to the limited number of mannose-6-phosphate receptors on these muscle cells or due to disrupted vesicular traffic in type II fibers. The fact

FIGURE 1. Stylized cell showing the functions that can be disrupted in lysosomal storage disorders

Pompe disease involves a lysosomal hydrolase defect that results in the inability to degrade autophagocytosed vesicular glycogen. The mucopolysaccharidoses (MPS) involve different defects in either a processing enzyme, glycosidase, or sulphatase and impact on glycosaminoglycan degradation within the lysosome. The sphingolipidoses (lipidoses) involve different hydrolase defects that alter the lysosomal catabolism of lipids. I-cell disease involves a Golgi processing enzyme defect, altering the traffic of soluble lysosomal hydrolases to the lysosome and causing the secretion of these enzymes. Multiple sulphatase deficiency (MSD) prevents the active site processing of sulphatases in the endoplasmic reticulum, generating catalytically inactive sulphatase proteins. The hereditary albinism syndromes Chediak-Higashi (CHS), Hermansky-Pudlak (HPS), and Griscelli (GS) each impact on different aspects of vesicular traffic, altering the biogenesis of lysosomal-related organelles (LRO). Because of the dynamic interrelationship of the endosome lysosome system, these primary defects can have a secondary impact on other lysosomal function.
that only type II skeletal muscle fibers display disorganization of the intracellular microtubule trafficking network, in areas of autophagic accumulation (40, 97, 99), is consistent with the disruption of vesicular traffic. The combination of altered vesicular traffic and autophagic accumulation of glycogen is clearly central in the development of the pathophysiology in Pompe disease.

The autophagic pathway has been implicated in the pathogenesis of other lysosomal storage disorders including multiple sulphatase deficiency, mucopolysaccharidosis IIIA, mucolipidosis IV, Batten disease, and Niemann-Pick disease (62, 64, 66, 106). Activation of autophagy may represent a common response mechanism by affected cells trying to engulf and clear undegraded substrate and nonfunctional organelles.

The Mucopolysaccharidoses

Hurler (α-L-iduronidase deficiency) and Hunter (iduronate-2-sulphatase deficiency) syndromes were the first of the mucopolysaccharidoses to be described (FIGURE 2) (60). However, the term mucopolysaccharidoses was not suggested until 1952, after the identification of excessive amounts of glycosaminoglycan in samples from patients with Hurler syndrome (18, 36). Observations of different relative amounts of heparan, dermatan, keratan, and chondroitin sulphates in patient urines confirmed that there were multiple syndromes (46, 77, 81, 103).

The mucopolysaccharidoses were originally thought to result from the overproduction of glycosaminoglycans, but van Hoof and Hers proposed that these disorders were analogous to Pompe disease and therefore due to specific lysosomal hydrolase deficiencies. This was consistent with the detection of enlarged lysosomes in Hurler hepatocytes (113) and 35SO4 uptake studies in Hurler fibroblasts, demonstrating impaired glycosaminoglycan degradation (37). The classification of the mucopolysaccharidoses (85) now recognizes 10 different diseases (types I, II, IIIA, IIIB, IIIC, IID, IVA, IVB, VI, and VII), each caused by a deficiency in the activity of a specific lysosomal hydrolase involved in the sequential degradation of one or more glycosaminoglycans. Patients can present within a spectrum of clinical phenotypes and have symptoms that include somatic, skeletal, and neural dysfunction (Table 1) (92). The studies on the metabolic basis of the mucopolysaccharidoses have increased our knowledge of the complex degradative pathways associated with the lysosome.

Although the primary defect in the mucopolysaccharidoses leads to glycosaminoglycan storage, the secondary accumulation of gangliosides in...
the brains of affected patients (21) has been postulated to be a causal factor in neuropathology (63). For example, the accumulation of ganglioside (GM2) has been associated with ectopic dendritogenesis, which alters the synaptic connectivity and plasticity of neurons (84). In addition, the secondary role of lysosomal enzymes in the degradation of cytoplasmic components, such as lipids, proteins, and carbohydrates, is crucial in maintaining cellular homeostasis. This is exemplified by the lysosomal storage disorders, which are a group of inherited metabolic disorders characterized by the accumulation of undegraded substrates due to deficiencies in lysosomal enzyme activity. The pathophysiology of these disorders is complex and involves disruptions in cellular functions, such as autophagy and endocytosis.

Table 1. Lysosomal storage disorders: patterns of storage, pathophysiology, and implications for cell biology

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Storage Pattern</th>
<th>Major Clinical Symptoms</th>
<th>Pathophysiology Common to Other Disorders</th>
<th>Cell Biology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pompe disease GSD II, AMD</td>
<td>Electron dense inclusions Autophagic accumulation in type II muscle fibres Cell debris, lipofuscin, ubiquitinated proteins</td>
<td>Cardiomegaly and cardio-respiratory failure Hepatomegaly and macrogliaosis Progressive muscle weakness</td>
<td>Organomegaly Cardio-respiratory symptoms Autophagic vacuoles</td>
<td>Lysosomal glycogen hydrolysis Manno-6-phosphate targeting Autophagy in skeletal muscle Dysfunctional fusion of vesicles</td>
</tr>
<tr>
<td>Mucopolysaccharidoses MPS</td>
<td>Electron luscent vacuoles Electron dense inclusions Lamellar and zebra bodies Ubiquitin positive bodies</td>
<td>Growth restriction Organomegaly Dysostosis multiplex Cardio-respiratory symptoms Mental retardation</td>
<td>Organomegaly Growth restriction Lipid accumulation Neurological dysfunction (some MPS)</td>
<td>Hydrolase degradation pathways Secondary storage Microglial activation</td>
</tr>
<tr>
<td>Gaucher disease</td>
<td>Glucocerebroside in tubular bilaminar structures Macrophages with “wrinkled paper” appearance</td>
<td>Hepatosplenomegaly Osteonecrosis (types 1 and 3) Acute (type 2) and subacute (type 3) neurodegeneration</td>
<td>Organomegaly Neurological dysfunction (type 2)</td>
<td>Lipid degradation in macrophages Alternatively activated macrophages Macrophage manno-8-phosphate receptor targeting</td>
</tr>
<tr>
<td>Fabry disease</td>
<td>Electron dense inclusions Granular or lamellar lipids Zebra bodies</td>
<td>Renal and Cardiac symptoms Gastrointestinal symptoms Angiokeratoma Growth restriction Cerebrovascular symptoms</td>
<td>Growth restriction Cardio-respiratory problems Lipid accumulation</td>
<td>Glycosphingolipid hydrolysis Disruption of caveolae function Inflammation &amp; reactive oxygen species (ROS) signalling</td>
</tr>
<tr>
<td>Niemann-Pick disease (Types A, B, and C)</td>
<td>Electron dense inclusions Lamellar lipids Zebra bodies</td>
<td>Hepatosplenomegaly “Cherry-red” spot in the eye Respiratory failure, pneumonia Purkinje cell apoptosis Neurological symptoms</td>
<td>Organomegaly Lipid raft signalling defects Respiratory Failure Neurological dysfunction</td>
<td>Cholesterol transport Disruption of lipid-rafts Ceramide related apoptosis Phospholipid processing Neuronal synaptic vesicle docking</td>
</tr>
<tr>
<td>GM2 Gangliosidoses (Tay-Sachs, Sandhoff)</td>
<td>Electron dense inclusions Lamellar lipids Zebra bodies</td>
<td>Hepatosplenomegaly (Sandhoff) Neurological symptoms Bronchopneumonia “Cherry-red” spot in the eye</td>
<td>Organomegaly Neurological dysfunction Lipid accumulation Lipid-raft signalling defects</td>
<td>Lysosomal sphingolipid hydrolysis Glial cell expansion and inflammation Neuronal dendritogenesis</td>
</tr>
<tr>
<td>I-cell disease ML II, ML III</td>
<td>Electron dense inclusions Lamellar lipids Zebra bodies</td>
<td>Severe growth restriction Organomegaly Dysostosis multiplex Cardio-respiratory symptoms Mental retardation</td>
<td>Organomegaly Dysostosis multiplex Lipid accumulation Neurological dysfunction</td>
<td>Increased enzyme secretion Recognition of manno-6-phosphate lysosomal targeting</td>
</tr>
</tbody>
</table>
The transport of neuronal growth factors is critical for neuronal survival and function, and alterations in vesicular traffic have been implicated in mucopolysaccharidosis neuropathology (19, 114, 118). Impaired autophagy also appears to be involved in the brain pathogenesis observed in the mucopolysaccharidoses (106). The presence of activated microglia, elevated levels of inflammatory cytokines, and oxidative stress all support a substantial role for inflammation in the neuropathology associated with the mucopolysaccharidoses (19, 48, 94). Animal models [e.g., mucopolysaccharidosis types I (26), IIIA (11, 29), IIIB (74)] are currently being used to investigate the mechanisms that link lysosomal dysfunction and neuropathology. Clearly, the neuropathology in the mucopolysaccharidoses involves the interplay of an array of complex mechanisms, which reflects some of the specialist functions that are now attributed to the lysosome. Thus autophagocytosis, apoptosis, inflammation, and impaired neurotransmission have all been implicated in the devastating pathology associated with the mucopolysaccharidoses.

Critical cross-correction studies using Hunter and Hurler fibroblasts (38) were a key step in recognizing lysosomal enzyme secretion and uptake mechanisms (FIGURE 2) and pivotal in develop-
oping enzyme replacement therapy as a treatment for lysosomal storage disorder patients. Although intravenous enzyme replacement therapy has been successful for the amelioration of somatic pathology, it has proven less effective for skeletal pathology and had minimal impact on neuropathology. This is most likely due to the inability of enzyme to access sites of pathology distant from the circulation and to cross the blood-brain barrier.

Sphingolipidoses

Gaucher, Niemann-Pick, Tay-Sachs, and Fabry diseases were among the first lysosomal storage disorders to be described (FIGURE 2). Initially, Gaucher and Niemann-Pick diseases were termed xanthomatoses until Ludwig Pick proposed the term lipidoses, based on the abnormal amounts of lipid in patient serum samples. A shared histological feature of these disorders was the presence of highly vacuolated cells with a foamy appearance. Gaucher, Niemann-Pick, Tay-Sachs, and Fabry diseases are now all recognized as lipid storage disorders and collectively referred to as the sphingolipidoses. This classification was substantiated by the identification of the specific storage material in these disorders, including glucocerebroside in Gaucher disease, sphingomyelin in Niemann-Pick disease, N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosylglucosylceramide (GM2) in Tay-Sachs disease, and globotriaosylceramide in Fabry disease (67).

Each of the sphingolipidoses is associated with a specific lysosomal hydrolase deficiency: β-glucocerebrosidase in Gaucher disease, sphingomyelinase in Niemann-Pick disease, β-hexosaminidase A and B in Tay-Sachs disease, and α-galactosidase in Fabry disease (67). The localization of these activities to the lysosome was an important step in understanding the pathophysiology of the lipidoses. Moreover, similar disturbances in lipid metabolism are evident in other lysosomal storage disorders. For example, studies on Niemann-Pick disease have elucidated roles for the traffic of cholesterol (and possibly other cargo) out of the lysosome/late endosome (93, 120), and cholesterol accumulation appears to be a common feature for many lysosomal storage disorders.

Gaucher disease

Phillippe Gaucher was the first to describe the clinical features and the lipid-laden macrophages that are hallmarks of this disorder (42). Later, it was recognized that this storage material involved the accumulation of glucocerebroside (2) in lysosomal organelles (121). In 1965, Brady and coworkers demonstrated that Gaucher disease was caused by a defect in the lysosomal hydrolase β-glucocerebrosidase (16, 95). Although the defect in β-glucocerebrosidase is systemic, glucocerebroside storage is restricted mainly to cells of the macrophage lineage. Clinically, this results in hepatosplenomegaly, skeletal pathology, pancytopenia, and, in variant forms of the disorder, neuropathology (Table 1).

β-Glucocerebrosidase acts to catabolize the degradation of glucocerebroside into glucose and ceramide, the latter of which is a known second messenger for activating the apoptotic cascade. Glucocerebrosidase is a common intermediate in the degradation of plasma membrane gangliosides and globosides. These substrates are believed to be primarily derived from the membranes of apoptotic red and white blood cells, which are normally phagocytosed by macrophages for recycling. Gaucher macrophages appear as enlarged cells

FIGURE 3. Morphology of storage compartments commonly observed in lysosomal storage disorder cells
A: floccular-granular storage. B: lipid whorls. C: zebra bodies. D: autophagic vacuoles. For example, in the mucopolysaccharidoses, A is typical of the primary storage vacuoles observed early in the disease course, whereas other storage compartments appear later in the disease process due to autophagy (D) and the secondary disruption of lipid metabolism (B and C).
with a characteristic wrinkled tissue paper appearance due to the accumulation of glucocerebrosidase in tubular, bilaminar membrane structures (71, 72). These macrophages develop an eccentric nucleus as the accumulation of storage material in endosomes and lysosomes begins to exclude the cytoplasm. Histochemical analysis of Gaucher spleens has shown that glucocerebroside laden macrophages are characteristically surrounded by activated macrophages (15). The influx of activated macrophages to the liver and spleen of Gaucher patients and the resulting inflammation presumably contribute to the hepatosplenomegaly. The infiltration of macrophages into the bone marrow is believed to displace hematopoietic stem cells, leading to reduced numbers of red and white blood cells in peripheral blood (pancytopenia).

There are links between the pathophysiology in Gaucher disease and altered macrophage function. Gaucher patients display impaired innate immunity, with attenuated antibacterial function (80). There have been reports of chronic low-grade inflammation in patients with Gaucher disease, consistent with increased levels of pro-inflammatory and anti-inflammatory cytokines and chemokines (1, 14). In fact, Gaucher macrophages remain metabolically active despite the accumulated glycolipid, resembling alternately activated macrophages (15). Cells isolated from Gaucher patients have been reported to have an upregulation of major histocompatibility complex class II molecules, which may result from increased endocytosis and recycling to the plasma membrane (5). In addition, serum chitotriosidase (53), CCL18 (14), and soluble CD163 (87) are elevated in Gaucher disease, which are indicators of alternately activated macrophages and the inflammatory condition. Macrophage-derived cells appear to also have an important role in the pathophysiology of other lysosomal storage disorders, including glial cells in mucopolysaccharidosis type III (119).

The development of enzyme replacement therapy for Gaucher disease involved the identification of the cell surface mannose receptor as a targeting system in macrophages. The internalization of the glucocerebrosidase enzyme by macrophage mannose receptors was distinct from the mannose-6-phosphate targeting utilized for enzyme uptake and treatment in other lysosomal storage disorders. Enzyme replacement therapy has proven to be an enormous success for patients with type 1 Gaucher disease, reducing the storage load, decreasing the size of the liver and spleen, and restricting the progression of bone pathology. Moreover, it has also been shown to restore the bactericidal function of macrophages in Gaucher patients (80). The success of enzyme replacement therapy in Gaucher disease reflects the high accessibility of enzyme to visceral tissues and to macrophages in the circulatory system (23).

**Niemann-Pick disease**

Discovery of the underlying cause of Niemann-Pick pathology, lysosomal accumulation of cholesterol, was greatly aided by mouse models of types A, B, C1, and C2. Niemann-Pick disease can be caused by three gene products (ASM, NPC1, and NPC2) acting on two pathways: sphingomyelin hydrolysis and cholesterol transport. The complex interrelationship between the key components involved in each of these pathways may explain the similarities in the pathological features displayed in patients with lipid storage disorders (Table 1). Sphingomyelin and its hydrolytic byproduct ceramide are intimately associated with cholesterol, showing either affinity in the case of sphingomyelin (45) or exclusion properties in the case of ceramide (110). These lipids are key components for the formation and function of detergent-resistant membrane microdomains or “lipid-rafts.” Lipid-rafts are postulated to act as a platform on the plasma membrane, recruiting molecules to coordinate and amplify signaling responses. Thus research into the mechanism of Neimann-Pick pathology has highlighted the role of sphingomyelin, ceramide, and cholesterol in lipid-raft formation and function.

The loss of sphingomyelinase activity in Neimann-Pick disease has a significant impact at the plasma membrane through its effect on ceramide production and lipid raft formation (54). A loss of sphingomyelinase activity may alter apoptotic signaling, which has been associated with Purkinje cell loss in the cerebellum and may account for some of the neurological features in Niemann-Pick disease (78). Moreover, sphingomyelin can accumulate at inappropriate sites in neurons of Niemann-Pick patients, resulting in defective neuronal survival, synaptic vesicle docking, and potentially synaptic function (22). This further highlights the link between disturbed lipid metabolism, altered lysosomal function, and neuropathology in the lysosomal storage disorders.

Due to the affinity of cholesterol for sphingomyelin and other lipids (GM2, GM3, sphingosine, and the lysosome specific phospholipid LBPA), perturbations in the localization of cholesterol may alter the distribution of other lipids. This has been shown to cause impaired trafficking and disrupted recovery of mannose-6-phosphate receptors and other tubulovesicular structures from late endosomes (65, 125). Interestingly, this phenotype has been modulated in vitro by over expression of the small GTPase Rab9, which is part of the vesicular trafficking machinery, and this reduced the amount of cholesterol accumulation (25) and lipid storage (91). The loss of NPC1/NPC2 results in Purkinje cell depletion from the cerebellum (76).
and is likely to disrupt cellular signaling and trafficking events in other neurons.

Abnormal lipid distribution in Niemann-Pick disease affects macrophage function, causing increased macrophage tissue infiltration and visceral pathology. Pulmonary pathology has been related to excessive macrophage recruitment and the overproduction of inflammatory chemo-attractants, which in turn impact on lung surfactant regulation (61). Macrophage infiltration has also been observed in the liver and spleen of Niemann-Pick patients and, along with defective apoptotic signaling, is likely to contribute to the pathology in these organs (108). The macrophage-related pathophysiology that is common between the lipidoses, may be important in other lysosomal storage disorders, particularly where phagocytosis is involved in clearing damaged cells and undegraded substrate.

**Fabry disease**

Fabry disease was first described as a skin disorder (angiokeratoma) before Anderson and Fabry recognized that it was a systemic disease. Further investigation into angiokeratomas revealed the underlying accumulation of glycosphingolipids in endothelial cells within small capillaries of the skin (56). The gastrointestinal (6), retinal (30), renal (3), cardiac (112), and neuronal (88) complications in Fabry disease (Table 1) can be partially linked to the malfunction of endothelial cells in the vasculature of these organs. Kidney pathology also appears to be mediated by additional mechanisms, including podocyte and tubulointerstitial injury. Fabry patients can present with cardiac complications, including hypertrophic cardiomyopathy, arrhythmias, and valve defects (112). Although the vascular endothelial cells of the Fabry heart store lipid, resulting in hypertrophy, this cannot alone account for the grossly enlarged size of the heart. It has been proposed that cardiomyopathy may be caused by over-proliferation of vascular smooth muscle cells induced by a yet-to-be-identified factor in circulation (7). The globotriaosylceramide storage in Fabry patients also alters signaling responses, causing reactive oxygen species production, oxidative stress, persistent vasodilatation, and inflammation (104), all of which contribute to the pathogenesis.

**GM2 gangliosidosis**

Pathology in the GM2 gangliosidoses is primarily restricted to the brain, but hepatosplenomegaly is also observed in Sandhoff disease (Table 1) (79). Ganglioside storage in the brain of Sandhoff/Tay-Sachs patients can lead to meganeurite formation (uncontrolled dendriteogenesis) and impact on neuronal cell function (79). Neuronal pathology is also believed to result from abnormal glial cell expansion and neuronal apoptosis (58, 111). The neuronal damage correlates with activation of microglia (117) and can further potentiate apoptotic signaling (12). In addition, the impaired phospholipid synthesis can contribute to axonal growth defects and abnormal neuronal signaling (20). This may also be a factor in the neurodegeneration that has been observed in other lysosomal storage disorders.

**I-Cell Disease**

In 1967, Leroy and DeMars used the term “I-cell” (FIGURE 2) to describe patient fibroblasts that contained characteristic inclusion bodies (73). I-cells were shown to have reduced amounts of intracellular acid hydrolase activities but hyperactivity of these hydrolases in the culture medium. I-cell patients also have high levels of circulating acid hydrolases in body fluids (123), suggesting excessive secretion of lysosomal enzymes. Similar inclusion bodies were observed in cells from patients with another condition, Pseudo-Hurler polydystrophy. These patients had later onset and slower disease progression than the patients with I-cell disease (Table 1). By 1975, there was strong appreciation that I-cell disease (73), mucolipidosis type II (109), and mucolipidosis III were all different forms of the one disease (82).

The hyper-secretion of multiple lysosomal enzymes from I-cells suggested a common defect in processing and targeting for these hydrolases (116), which is now known to involve a deficiency in the carbohydrate processing enzyme, N-acetylglucosamine-1-phosphotransferase (47). This cis-Golgi localized enzyme is responsible for the modification of N-linked high mannose oligosaccharides on newly synthesized lysosomal hydrolases. This processing event generates the key mannose-6-phosphate targeting motifs that are involved in the intracellular delivery of certain soluble enzymes to lysosomes. Failure to generate this targeting motif results in the constitutive secretion of many soluble lysosomal hydrolases, reducing the amount of intracellular hydrolase activity and resulting in reduced substrate degradation.

The N-acetylglucosamine-1-phosphotransferase is synthesized from two different gene products, which give rise to an α2β2γ2 multi-subunit enzyme. The attenuated form of I-cell disease (mucolipidosis III) has been attributed to molecular lesions in the γ-subunit, whereas classical I-cell disease (mucolipidosis II) appears to be mainly due to mutations affecting the α- and β-subunits. From early studies, it was evident that N-acetylglucosamine-1-phosphotransferase was deficient in all tissues, but the pattern of storage did not match the pathogenesis (82, 83). It was recognized that
mesenchymal cells exhibited extensive storage, whereas the enzyme deficiency in non-mesenchymal cells did not exhibit storage. This gave rise to theories that there may be other targeting mechanisms for lysosomal enzyme delivery (83).

Lysosomal enzyme and protein analysis in I-cells have demonstrated 1) reduced amounts of α-mannosidase, β-hexosaminidase, 4-sulphatase, iduronate-2-sulphatase, α-L-iduronidase, α-galactosidase, α-D-glucosidase, and α-fucosidase; 2) no change in acid phosphatase; and 3) variable effects on arylsulphatase A and β-galactosidase. These variable effects on hydrolases indicated which enzymes are reliant on the mannose-6-phosphate targeting system for traffic to the lysosome. In addition, the increased amount of lysosomal membrane protein LAMP-1 in lysosomes and mannose-6-phosphate receptors retained in the trans-Golgi of I-cells demonstrated that there are changes to lysosomal cell biology that ensue from the primary defect.

The characteristic histological feature for I-cell disease is the abundant membrane-bound vacuolar structures in mesenchymal cells, which have either electron-lucent or fibrillogranular contents (FIGURE 3). In older patients, lamellar lipid-like material (FIGURE 3, B AND C) has been observed within cytoplasmic inclusions. The vesicular structures in I-cell disease vary, with fibroblasts having more pleomorphic and non-electron-lucent contents (82, 83) and endothelial cells having contents with a concentric ring appearance (4, 75). This combination of vesicular structures in fibroblasts and endothelial cells appears to be unique to I-cell disease and of diagnostic significance. The characteristic pattern of storage in I-cell neurons does not correlate with the increased psychomotor retardation observed in classical, compared with attenuated, I-cell patients (83). Neurons of the cerebral and cerebellar cortex from patients with classical I-cell disease appear to exhibit only minimal storage, whereas anterior horn neurons (83) and spinal ganglia neurons (90) exhibit numerous zebra body inclusions (FIGURE 3C). This diversity in patterns of substrate storage is presumed to reflect different degrees of substrate turnover in different tissues, together with the different biology of the substrates. In I-cell disease, the mis-targeting of multiple lysosomal hydrolases results in a very rapid onset disorder, with clinical symptoms that can be evident in other lysosomal storage disorder patients (Table 1).

**Multiple Sulphatase Deficiency**

The early history of multiple sulphatase deficiency (FIGURE 2) is not clearly delineated, partly because of its interrelationship with the metachromatic leukodystrophy disorders, but also because it represents a protein processing defect that was difficult to discern biochemically. Multiple sulphatase deficiency patients display biochemical and clinical symptoms (Table 1) that are consistent with a combination of different individual sulphatase deficiency disorders (89). The net result of the deficiency in a range of sulphatases is the storage of glycosaminoglycans, sulphatides, and gangliosides. In 1979, it was postulated that a common sulphatase posttranslational processing modification was the most likely explanation for the deficiency (9, 57). The identification of the conserved active site cysteine residue in sulphatases was a key step in unraveling the molecular basis of multiple sulphatase deficiency. This crucial amino acid is posttranslationally modified in sulphatases to generate unique Cα-formylglycine residues (105). The sulphatase modifying factor gene (SUMF1) was identified and characterized (28, 34, 70, 126), leading to an understanding of the molecular basis for this disorder.

The finding that multiple sulphatase deficiency patients express structurally stable but catalytically inactive sulphatase enzymes guided subsequent studies on sulphatase cell biology. Multiple sulphatase deficiency provided the pathophysiological evidence for one of the key aspects of sulphatase biology, the posttranslational protein processing of sulphatase active site residues in the endoplasmic reticulum. This disorder also aided in shaping the concept of the structural similarity between sulphatases, which was further substantiated by the resolution of the crystal structure for several of the sulphatases (13).

**Hypomelanization/Albinism Syndromes**

Hereditary albinism syndromes were initially recognized due to a dysfunction in the lysosome-related organelles (FIGURE 2), melanosomes. In 1943, Beguez Cesar recognized Chediak-Higashi syndrome (10). Chédik linked the albinism in these patients with anomalies in leukocytes and recurring infections (24), whereas Higashi made the seminal observation of giant peroxidase-granules in patient blood cells (51). In 1959, Herman-sky-Pudlak syndrome (49) was described, with patients displaying symptoms similar to Chediak-Higashi syndrome. Tissues from these albinism patients showed evidence of storage material in endosomes and lysosomes, but unlike traditional lysosomal storage diseases this defect was attributed to altered organelle biogenesis and traffic rather than to the loss of a specific lysosomal hydrolase activity. Griscelli syndrome is also a member of this albinism group of disorders (59). Clinical
symptoms in these disorders include hypopigmentation of the skin, eye, and hair (hyperpigmentation of melanocytes and hypopigmentation of keratinocytes), prolonged bleeding (platelet dense granule deficiency), neurodegenerative traits, immunodeficiency, and early death (Table 1) (59).

**Hermansky-Pudlak syndrome**

Hermansky-Pudlak syndrome is classified as a disease involving miss-sorting of a large cohort of cargo proteins, resulting from the abnormal processing of intracellular vesicles (32). This protein miss-sorting typically results in protein and lipid not reaching correct destinations and causes substrate storage in endosomal compartments. For example, in the BLOC-1 and BLOC-2 protein complex deficiencies, the tyrosinase-related protein-1 and copper transporter either accumulate in early endosomes or, if delivered to melanosomes, are retained due to defective exocytosis (32, 107). Hermansky-Pudlak syndrome can also be caused by a defect in the AP-3 adaptor protein, which also affects intracellular sorting of tyrosinase-related proteins to melanosomes (59). The pathognomonic histological features of Hermansky-Pudlak syndrome include storage of ceroid-lipofuscin-like pigment in lymphocytes, circulating blood monocytes, and bone marrow macrophages. Enlarged lipid-containing vacuoles contain particulate debris resulting from incomplete digestion of erythrocytes, which are a major substrate for reticular macrophages (59, 122). Lung lamellar bodies are affected in Hermansky-Pudlak syndrome and contain increased amounts of surfactant phospholipid and hydrophobic proteins due to defects in secretion (44). This results in lung inflammation and reduced survival due to emphysema. In a mouse model of Hermansky-Pudlak syndrome, the miss-sorting of proteins from early endosomes to melanosomes has been shown to increase the cell surface presentation of some lysosome-related proteins, presumably due to the default cell surface trafficking pathway (33).

**Chediak-Higashi syndrome**

Chediak-Higashi syndrome can be characterized by multiple defects in specialized endosomes and lysosomes. A current model for the regulation of vesicular fusion favors a role for the Chediak-Higashi/LYST protein in limiting either the homotypic or heterotypic fusion of early endosomes and lysosomes (69). In Chediak-Higashi syndrome, uncontrolled fusion or fission in affected cells results in giant dysfunctional secretory lysosomes and other organelles, which in turn impair cargo delivery (59). For example, peroxidase appears to accumulate in giant granules instead of being delivered to phagosomes (102). In immune cells from Chediak-Higashi syndrome patients, the impaired delivery of major histocompatibility complex class II molecules to the plasma membrane leads to altered antigen presentation (59). The disruption to vesicular traffic in Chediak-Higashi cells impacts on both innate and adaptive immunity, causing symptoms of immune-deficiency. T cells accumulate the cytotoxic T-lymphocyte antigen 4 (CTLA4) in enlarged cytoplasmic vesicles instead of at the plasma membrane, preventing the inhibition of T-cell activation (8). This leads to the uncontrolled infiltration of T-lymphocytes and macrophages into several organs (8). Secretion defects are responsible for the accumulation of enlarged glucuronidase-containing lysosomes in the kidney of Chediak-Higashi/Beige mouse mutants, and this correlates with lower amounts of enzyme secretion into the urine (17).

**Griscelli syndrome**

Griscelli syndrome has been associated with altered vesicular trafficking due to its affect on the assembly of actin-based vesicular machinery, involving the GTPase Rab27a, melanophilin, and myosin Va (59). Loss of any component of this complex leads to melanosome clustering in the perinuclear region of affected cells, rather than delivery to keratinocytes, resulting in coat color mutants in mice and partial albinism in humans (86). Despite being caused by a different mechanism, Griscelli syndrome shares some pathophysiological features with other hypopigmentation syndromes. This includes altered plasma membrane delivery, impaired secretion, and immune deficiency (86).

The common aetiology in the albinism group of syndromes relates directly to similarities in their impact on the biogenesis and traffic of endosome and lysosome organelles. Disruptions to the integrity of the endosome-lysosome vesicular trafficking network can be at different points in this system (FIGURE 1) but still manifest with common functional outcomes. The altered vesicular traffic observed in albinism syndromes has some similarity with other lysosomal storage disorders, but in the latter this is caused by secondary effects on lysosomal biogenesis that ensue from the primary enzyme deficiency and substrate storage.

**Conclusions**

Pivotal studies in the early to mid-1900s established the existence of the lysosome and led to the concept of lysosomal storage disorders (FIGURE 2) (31, 50). The subsequent investigation of the genetic, biochemical, and physiological aspects of
lysosomal storage disorders provided an understanding of many of the intricate cellular processes that are now attributed to a functional endosome-lysosome system. Here, we have discussed a select group of lysosomal disorders to show how the field has progressed and how it has revealed important concepts on enzyme deficiency, biochemical pathways, pathological cascades, lysosomal protein processing, organelle biogenesis, and vesicular traffic.

A comparison of the clinical phenotypes observed for lysosomal storage disorders has revealed a number of key concepts: 1) within each disorder, there is usually a clinical spectrum that reflects the impact of different mutations on the function of the lysosomal compartment; 2) different disorders have remarkable similarities in clinical phenotype, especially where the stored substrates are similar (as is the case with some of the mucopolysaccharidoses and the sphingolipidoses), or where similar cells and tissues are involved (e.g., organomegaly, bone pathology, and neuropathology); 3) there are also some characteristic clinical symptoms that can be used to distinguish disorders from each other, with for example Pompe, Fabry, and the albinism disorders. Thus there are some similarities in the clinical phenotype for different lysosomal storage disorders, but no two disorders have identical pathophysiology (Table 1).

Over time, clinical findings have been related to aspects of endosome-lysosome biology (Figure 2), including the type of substrate and its biological function, the amount of substrate utilisation in particular tissues, the rate of substrate turnover, and the balance between residual enzyme activity and substrate catalysis. Each of these factors will have an impact on the specific tissue pathogenesis and the rate of disease progression. The accumulation of the primary storage material can also have a functional impact on the cell, including the inhibition of other enzymatic processes, causing the accumulation of secondary undegraded substrates and the disruption of lysosomal biogenesis.

The lysosome is no longer viewed as just an end-point degradative compartment but rather as part of a very complex and interactive set of intracellular organelles that have a wide array of specialist functions. Lysosomes are integrally involved in phagocytosis, autophagy, exocytosis, receptor recycling and regulation, intracellular signalling, immunity, pigmentation, bone biology, and neurotransmission. There is an appreciation that defects in any one of these processes can be associated with lysosomal disease.

Lysosomal diseases are typically characterized by enlarged lysosomes that contain partially degraded material as a result of J) defects in either glycosaminoglycan, lipid, or protein degradation; 2) transport across the lysosomal membrane; or 3) endosome-lysosome trafficking. We are beginning to understand how substrate storage impacts on cellular function to cause disease pathogenesis. As suggested by Walkley and coworkers, lysosomal diseases may be better viewed as conditions of molecular deprivation that result in specific pathogenic cascades (118, 119).

Intracellular traffic between different organelle compartments is mediated through the formation and movement of vesicles and is controlled by specific targeting signals and trafficking machinery. By determining the function of proteins in lysosomal diseases such as the albinism disorders, we should increase our understanding of the normal trafficking mechanisms in cells. To this end, genetic studies including gene ablation of orthologs, in organisms such as Danio rerio, Drosophila melanogaster, and Caenorhabditis elegans are likely to uncover new components of vesicular machinery that are critical for normal cell physiology (41). Vesicular traffic is central to the function of endosomes and lysosomes, and the impact of undegraded substrates on this important function is only just beginning to be appreciated.

The ability to gain a complete understanding of the pathogenesis in lysosomal disease will, in effect, involve a dissection of the functionality and interrelationship of the entire endosome-lysosome system. This knowledge will be critical if we are to recognize the underlying molecular basis of storage disorder pathogenesis and how to utilize this information to develop novel therapeutic strategies for these devastating disorders.

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