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Review

Secondary lipid accumulation in lysosomal disease

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ABSTRACT

Lysosomal diseases are inherited metabolic disorders caused by defects in a wide spectrum of lysosomal and a few non-lysosomal proteins. In most cases a single type of primary storage material is identified, which has been used to name and classify the disorders: hence the terms sphingolipidoses, gangliosidoses, mucopolysaccharidoses, glycoproteinoses, and so forth. In addition to this primary storage, however, a host of secondary storage products can also be identified, more often than not having no direct link to the primary protein defect. Lipids – glycosphingolipids and phospholipids, as well as cholesterol – are the most ubiquitous and best studied of these secondary storage materials. While in the past typically considered nonspecific and nonconsequential features of these diseases, newer studies suggest direct links between secondary storage and disease pathogenesis and support the view that understanding all aspects of this sequestration process will provide important insights into the cell biology and treatment of lysosomal disease.

1. Introduction

Lysosomal diseases represent a complex family of nearly 60 inherited metabolic disorders linked by defects in specific proteins critical for lysosomal function [1]. A majority of these disorders are severe, and many are characterized by a progressive neurodegenerative and mental deterioration. Collectively, their estimated incidence ranges between 1/5000 to 1/9000 live births. Most are due to the deficiency of a specific hydrolytic lysosomal enzyme (or its ancillary "activator" protein) directly responsible for degradation of complex molecules, or of a lysosomal membrane transport protein involved in egress from lysosomes of one of the final products. They can also be the consequence of a multiple deficiency of lysosomal enzymes due to a defect in ER- and Golgi/TGN-associated enzymes involved in a common processing/trafficking step. Finally they can be due to defects in soluble or transmembrane lysosomal proteins of still partially unclear function believed essential for substrate degradation, vesicle fusion, pH regulation, substrate salvage, trafficking/sorting of lipids and proteins and so forth. Indeed, defects in no less than 50 different proteins have been implicated to date as causing lysosomal dysfunction, and new proteins linked to lysosomal disease continue to be identified. Defects in lysosomal function typically lead to lysosomal storage and in the case of single hydrolytic enzyme deficiencies, primary storage materials can usually be readily identified. Yet in reality the storage process affecting cells is far more complex than suggested by this simple one enzyme-one substrate relationship, with multiple substrate storage being typically characteristic. While in some cases this heterogeneity can be explained by a metabolic link, with multiple compounds sensitive to the same enzyme, most examples of secondary storage appear unrelated to this and more likely arise through other, often still poorly defined mechanisms. Importantly, considerable evidence suggests that secondary storage compounds can themselves be actively involved in disease pathogenesis. In order to gain a full understanding of the cell biology and pathogenesis of lysosomal disease, it is essential to understand the basis for these complex metabolic events leading to multiple substrate storage. Various types of compounds may accumulate. Some of them are particularly important in certain diseases, such as the c-subunit of mitochondrial ATP synthase or saposins in ceroid lipofuscinoses, whereas others are more ubiquitous, among which lipids constitute the most common and best studied category and are therefore the focus of this review.

2. Patterns of secondary lipid accumulation

Secondarily accumulating lipids in lysosomal disease can belong to any of the major classes, cholesterol, phospholipids and glycosphingolipids (GSLs), with different patterns in brain and in visceral organs often occurring in the same disease. Most of the changes have been initially described using biochemical methods, first in tissues of patients and then in various animal models. More recently, studies

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using antibodies (especially against GSLs) or cytochemical staining for cholesterol have provided new insights into the cellular site of accumulation. Ancillary studies in living cells have also been useful to pinpoint some secondary metabolic blocks.

2.1. Glycosphingolipids and free sphingoid bases

2.1.1. Glycosphingolipids in brain

A secondary accumulation of GM2 and GM3 gangliosides is a common feature associated with neuropathology in a number of lysosomal storage diseases, principally Niemann-Pick diseases and mucopolysaccharidoses, but also prosaposin deficiency, as well as some glycoproteinoses and ceroid lipofuscinoses. In human brain, GM2 and GM3 are normally very minor components and constitute no more than 1–2% of the total gangliosides. Their proportion is even smaller in normal mouse brain. The largest increase, affecting both gangliosides but more particularly GM2, has been found in autopsy brain of patients with Niemann-Pick disease type A (but not type B) [2–7], and in patients with Niemann-Pick C disease (NPC) [7–13]. Very similar changes have been found in sphingomyelinase-deficient and in NPC1- or NPC2-deficient models in mice or cats [14,15]. For either Niemann-Pick A or C diseases, abnormalities were absent in human brain at the foetal stage [16–18]. However, for both diseases, ganglioside storage occurs early, as shown by studies in mutant mice. In the npc1^{nih} mouse brain, abnormal levels of GM2 were already present in 10-day old animals, with a later increase of GM3 [15; L. Verot, P. Lobel and MT Vanier, unpublished]. Abnormalities were observed for both GM2 and GM3 in the brain of a 3-month old child with NPC [13]. Pronounced abnormalities have also been reported in prosaposin deficiency (a combined defect in the sphingolipid activator proteins saposins A, B, C and D), both in humans and the mouse model [19,20], and very recently in the cathepsin D deficient mouse (model of CLN10) [21]. In Niemann-Pick A and C as well as in prosaposin deficiency, the ganglioside increase is accompanied by a prominent increase in glucosylceramide and lactosylceramide [9,13,18–20]. Clearly abnormal levels of GM3 and GM2, although significantly lower, are further seen in mucopolysaccharidosis (MPS) type III brain, in human, mice and emu bird [22–27], and similar but even milder changes occur in MPS I (Hurler type) brain [22,23] and α-iduronidase deficient cats, dogs and mice [28–31] (Fig. 1). In MPS I and MPS IIIB mice, ganglioside abnormalities appear around 1 month of age, and quantitatively, GM3 appears more affected than GM2. Only few data exist regarding glycoproteinoses but definite abnormalities have been reported for α-mannosidosis in the cat [29]. No increase was found in I-cell disease [32]. In many other lysosomal and some non-lysosomal diseases, a small increase in the proportion of GM2 and GM3 is often seen; however, this is usually more conspicuous by immunocytochemical methods than by biochemical measurement. This can be explained by the fact that biochemistry will take into account the global amount of gangliosides, irrespective of its subcellular localization, while immunocytochemistry can pinpoint even tiny amounts, when present in vesicular structures in cells.

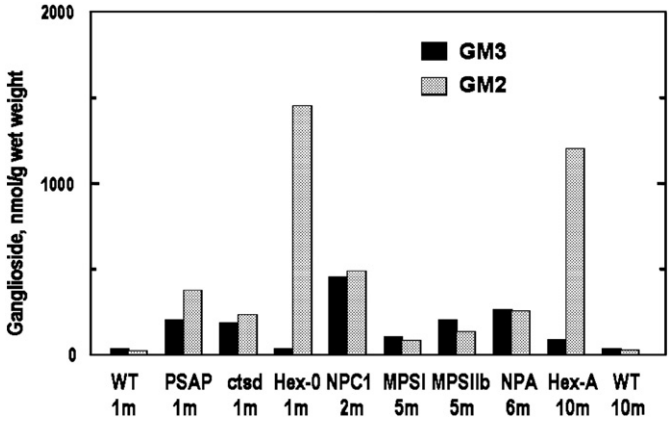

Fig. 1. Gangliosides GM3 and GM2 in brain from mouse models of several lysosomal storage diseases. All mice were studied in the laboratory of one of us (MTV) using the same procedure. Results are expressed in nmol ganglioside/g wet weight of tissue. WT: wild type; PSAP: prosaposin deficiency; ctsd: cathepsin D deficiency (CLN10); Hex-0: Hexosaminidase A + B deficiency (GM2-gangliosidosis, Sandhoff variant); NPC1: NPC1 deficiency (Niemann-Pick C); MPSI: α-iduronidase deficiency; MPSIIIb: α-N-acetylglucosaminidase (NAGLU) deficiency; NPA: acid sphingomyelinase deficiency (Niemann-Pick A); Hex-A: hexosaminidase A deficiency (GM2-gangliosidosis, Tay-Sachs variant). Age at study varied from 1 month to 10 months, as indicated; Note that the GM2 level in the Sandhoff mouse at 1 month of age is already higher than in the Tay-Sachs model at 10 months of age.

The availability of well characterized antibodies to GM2 and GM3 gangliosides, coupled with biochemical data described above indicating only a minimal presence of these two gangliosides in normal brain, has meant that their localization in storage diseases could be identified in individual cell types in brain, and indeed, to individual vesicles within neurons. It has also been found that in some cases probing tissues with antibodies fails to readily reveal individual gangliosides in specific sites (e.g., the plasmalemma). This inaccessibility most likely is due to the blocking action of associated proteins, or simply because the ganglioside molecules do not occur in sufficient abundance to be labeled and identified. This cryptic feature of gangliosides in terms of immunocytochemistry again indicates the importance of combined biochemical and morphological techniques. Immunostaining studies applied to lysosomal diseases undergoing secondary lysosomal accumulation of GM2 and GM3 have consistently shown that these gangliosides are sequestered in vesicles, appearing as punctate, granular structures within the cytoplasm of cells. This labeling has been documented in a wide variety of lysosomal diseases, including Niemann-Pick type A [Walkley, unpublished] and type C (both NPC1 and NPC2 deficiencies) [15,33], MPS diseases including type I, II, IIIA, VI, and VII [34,35], mucolipidosis type IV [36], several of the Batten disorders, including CLN2 [Walkley, unpublished], CLN6 and CLN10 diseases [21], and α-mannosidosis [37,38]. In most such diseases, GM2 and GM3 have been found to accumulate in a variety of neurons and glia, with both gangliosides typically occurring within the same cells. Many other patterns of staining, however, are also evident. For example, in mice with Niemann-Pick C disease Purkinje cells accumulate GM2 ganglioside but not GM3 [15]. In mice lacking cathepsin D (CLN10 disease) it appeared that neurons accumulated GM2 whereas glia primarily harbored GM3 storage [21]. In the glycoproteinosis, α-mannosidosis, all neurons exhibited storage of water soluble oligosaccharides as expected, whereas only scattered numbers of pyramidal and GABAergic neurons in the cerebral cortex also exhibited conspicuous accumulation of GM2 and GM3 gangliosides [37]. As discussed later, this accumulation of gangliosides in α-mannosidosis revealed a close correlation between the abnormal presence of GM2 ganglioside in pyramidal neurons and ectopic dendritogenesis, a phenomenon originally discovered in GM2 gangliosidosis [39] and now known to be unique to lysosomal diseases with GSL storage.

The availability of appropriately fixed tissues from large and small animal models of lysosomal disease, coupled with immunofluorescence and confocal microscopy, has permitted detailed analysis of the subcellular localization of gangliosides. Unexpectedly, GM2 and GM3 gangliosides, while often being sequestered within the same neurons, have been consistently found to reside in separate vesicle populations in those cells [34,40] (Fig. 2). As described below, these labeled vesicles also often occur independently of detectable amounts of unesterified cholesterol visualized by filipin labeling. That GM2 and GM3 are not co-sequestered has been interpreted to mean either that these gangliosides are sequentially processed in separate compartments within the endosomal/lysosomal system, or that these two gangliosides are being generated by separate and independent processes, e.g., in synthetic vs. degradative compartments in cells [40,41].