Storage problems in lysosomal diseases

Jean Michel Heard1, Julie Bruyère, Elise Roy, Stéphanie Bigou, Jérôme Ausseil and Sandrine Vitry
Rétrovirus and Genetic Transfer Unit, Department of Neuroscience, Institut Pasteur, 28 rue du Dr Roux, 75015 Paris, France, and INSERM U622, Institut Pasteur, Paris, France

Abstract
Biochemical disorders in lysosomal storage diseases consist of the interruption of metabolic pathways involved in the recycling of the degradation products of one or several types of macromolecules. The progressive accumulation of these primary storage products is the direct consequence of the genetic defect and represents the initial pathogenic event. Downstream consequences for the affected cells include the accumulation of secondary storage products and the formation of histological storage lesions, which appear as intracellular vacuoles that represent the pathological hallmark of lysosomal storage diseases. Relationships between storage products and storage lesions are not simple and are still largely not understood. Primary storage products induce malfunction of the organelles where they accumulate, these being primarily, but not only, lysosomes. Consequences for cell metabolism and intracellular trafficking combine the effects of primary storage product toxicity and the compensatory mechanisms activated to protect the cell. Induced disorders extend far beyond the primarily interrupted metabolic pathway.

Introduction
LSDs (lysosomal storage diseases) form a group of rare inherited genetic defects of the metabolism, which is at the same time well defined and extremely heterogeneous. More than 50 disorders are recognized as LSDs. This number is constantly increasing, and it is expected that more diseases, on which knowledge is currently limited, will be considered to be LSDs when better documented. Beyond genetic inheritance and alteration of a metabolic pathway, criteria leading to the identification of a disease as LSD rely on the combination of defined biochemical and histological features.

Biochemical disorders in LSDs consist of the interruption of metabolic pathways involved in the recycling of degradation products of one or several types of macromolecules. As a consequence of inefficient recycling, imperfectly degraded substances accumulate in cells with cascades of deleterious repercussions. Substances accumulating as the direct consequence of the genetic defect are the primary storage products. They represent the initial pathogenic event, which may result in both cell-autonomous defects and environmental reactions, e.g. inflammation [1]. Downstream consequences include the secondary accumulation of other substances, the secondary storage products, which have not always been molecularly identified. The histological hallmark of LSDs is cell vacuolation (Figure 1A). With time, cells in most severely affected tissues become progressively clogged with cytoplasmic inclusions. Since inclusions contain materials that cells are apparently unable to eliminate, and cell vacuolation is clearly related to the storage process, inclusions are often referred to as storage lesions.

Although storage products and storage lesions are the two main characteristics of LSDs, the nature of their relationship is not simple and is still largely not understood. In the present review, we examine their complex links.

A problem of content
Almost every metabolic pathway aimed at recycling substances produced by macromolecule degradation in lysosomes has been involved in LSDs. Indeed, the most convivial classification of LSDs relies on the nature of affected macromolecules [2], and therefore, in most cases, refers to specific storage products. These products may be precisely identified, as, for example, in lipidoses or MPSs (mucopolysaccharidoses), or poorly characterized, as in neuronal ceroid lipofuscinoses.

Despite the fact that the metabolic defect concerns all cells in the organism, depending on the affected pathway and the specific metabolic activity of deficient cells, storage products are preferentially generated in certain tissues, where they predominantly accumulate and cause disorders. The geographies of the accumulation of storage products and that of the installation of storage lesions are largely superimposed. Subsequently, the condition of the most affected organs results in the clinical manifestations that specify the disease type. For example, skeletal muscles are the most diseased organs in Pompe disease, which affects glycogen degradation; bone and joint development is impaired in MPS type VI because of the interruption of dermatan sulfate catabolism; and demyelination in metachromatic leukodystrophy is associated with the accumulation of long-chain fatty acids in the central nervous system.

Key words: autophagy, Golgi matrix protein 130 (GM130), lysosomal storage disease (LSD), lysosome-associated membrane protein 1 (LAMP1), lysosomal biogenesis, Rab GTPase.

Abbreviations used: GM130, Golgi matrix protein 130; LAMP1, lysosome-associated membrane protein 1; LSD, lysosomal storage disease; MPS, mucopolysaccharidosis.

1To whom correspondence should be addressed (email jmheard@pasteur.fr).
Figure 1 | Storage lesions in the MPSIIIB mouse brain

Cortical fragments of a 9-month-old MPSIIIB mouse were processed for electron microscopy (A), immunofluorescence (B), immunogold (C), Western blotting (D) or immunostaining (E). (A) Ultrastructural analysis show polymorphic distended vesicles in neurons (two left-hand columns), microglial cells (third column) and astrocytes (right-hand column). Low (top row) and high (bottom row) magnifications of the same fields are shown. Extreme left- and right-hand panels of the bottom row show zebra bodies. Scale bars, 1 μm. (B) Parasagittal cortical sections immunolabelled for the lysosomal marker LAMP1 (in purple) and the neuronal marker NeuN (left, in green), the microglial marker CD11b (middle, in green) and the astrocyte marker GFAP (glial fibrillary acidic protein) (right, in green) show comparable LAMP1 accumulation, notwithstanding marked differences in the aspect of storage lesions in these three cell types. Images are confocal fluorescence micrographs. Scale bars, 10 μm. (C) Ultrathin cryosection stained with anti-LAMP1 antibodies coupled to 10-nm-diameter gold particles reveal LAMP1 in the limiting membranes of two storage lesions with different aspects (dark vesicle in the upper left corner and clear vesicle in the middle). Scale bar, 0.5 μm. (D) Western blot analysis of brain protein extracts with anti-LAMP1 antibodies show increased signal in the brain of MPSIIIB mice, as compared with wild-type mice. The LAMP1 signal was normalized when the expression of the missing lysosomal enzyme α-N-acetylglucosaminidase was induced through adeno-associated vector-mediated gene therapy directed to the brain (MPSIIIB+AAV-NaGlu). Molecular masses of LAMP1 signals are indicated in kDa on the left. (E) LAMP1 overexpression and correction after gene therapy were similarly observed after immunostaining of MPSIIIB mouse cortical sections. Scale bars, 100 μm.
Coincidence of storage products and storage lesions in the same organs led to the notion that disease expression results from cell disorders that are induced by cytoplasmic inclusions, which are themselves caused by the accumulation of storage products in the lysosomes, the organelles in which these products are generated. This simple view appears to be insufficient with regard to our current knowledge of LSDs. Indeed, the accumulation of the primary storage products is associated with more or less severe malfunctioning of the organelles in which they are produced and stored, inducing pathological consequences beyond the primarily affected metabolic pathway.

**A problem of container**

Examination of storage lesions by electron microscopy confirmed the high heterogeneity of storage lesions, which is already visible on standard pathology sections. Cell inclusions are large structures with different types of content, ranging from clear amorphous material, internal debris, internal vesicles, membrane fragments, dense aggregates and multilamellar structures occasionally forming zebra bodies (Figure 1A). Ultrastructural studies not only showed that storage lesions differed depending on the affected cell type, but also provided evidence that multiple types of inclusions coexisted in the same cell. Thus a given type of primary storage product can be associated with multiple types of storage lesions. Contrasting with their polymorphism in individual disease, inclusions show similar features in different LSDs. For example, zebra bodies were observed in many LSDs. Thus different types of primary storage products can be associated with similar storage lesions.

The relationship between the primary storage products and the storage lesions is therefore not a binary one. It involves additional players, the secondary storage products. Assuming that secondary storage products participate in storage lesions accounts for the observation of different types of lesions in different cell types, since secondary storage products probably differ depending on the cell type. On the other hand, if cascades of events triggered in different diseases converge to common biochemical alterations and to the accumulation of common secondary storage products in different LSDs, it is then understandable that similar lesions are observed in different diseases. According to this view, storage is not solely a problem of content, the accumulating primary products, but also a problem of container, the organelles that become unable to degrade and recycle certain substances as a secondary consequence of primary storage. Malfunctioning of the container leading to the secondary storage of a variety of materials appears a common feature that specifies LSDs.

The most likely problematic container is obviously the lysosome. Consistently, lysosomal markers are associated with storage lesions. Overexpression of the lysosomal marker LAMP1 (lysosome-associated membrane protein 1) is associated with many LSDs [3]. The association of LAMP1 with intracellular inclusions was detected in various cell types, notwithstanding the fact that these inclusions showed very different aspects. For example, in the brain of MPSIIIB mice, both inclusions in microglial cells, which appear as clusters of rounded white drops, and inclusions in neurons and astrocytes, which are frequently dark and spread over the cell surface, were stained with anti-LAMP1 antibodies (Figure 1B). The ultrastructural detection of LAMP1 immunoreactivity in the limiting membranes of intracellular inclusions indicated that storage lesions are related to lysosomes (Figure 1C).

Experimental evidence has actually been provided in some LSDs that lysosomal functions were altered beyond the metabolic pathway directly affected by the missing protein. Early studies of MPSs documented the inhibition of the catalytic activities of several lysosomal enzymes by glycosaminoglycans, the primary storage products in these diseases [4]. Cholesterol accumulation in Niemann–Pick type C and other LSDs causes inhibition of lysosomal sphingomyelinase [5] and lysosomal glucosylceramidase [6]. In mucolipidosis IV, inhibition of the degradation of lysosomal ABC (ATP-binding cassette) transporters increases undegraded substrate overload [7]. Since the fusion of lysosomes with late endosomes, phagosomes or amphisomes is the terminal step of endocytosis, phagocytosis and macro-autophagy respectively, a global deficiency of lysosomal functions affects these pathways. Consistently, alteration of fluid-phase endocytosis was reported in Niemann–Pick type C disease [8], alteration of phagocytosis in monocytes increases susceptibility to infection in patients with Gaucher disease [9] or α-mannosidosis [10], alteration of macro-autophagy was reported in several LSDs [11,12]. Depending on the disease, macro-autophagy appears to be activated, as in Niemann–Pick C disease [13], neuronal ceroid lipofuscinosis [14] or Gb3 gangliosidosis [15], or deficient as in mucolipidosis IV [16] or multiple sulfatase deficiency [17]. In many cases, macro-autophagy is both activated and deficient, as shown in the skeletal and cardiac muscles of mice and patients with Pompe disease [18], and to a lesser extent as a consequence of relative starvation in the liver of the mouse models of MPSI and MPSVII [19]. In contrast, our studies of brain tissues in the mouse models of MPSI, MPSIIIA and MPSIIIB did not find any evidence of increased formation or accumulation of autophagolysosomes, and dynamic studies of macro-autophagy in MPSIIIB cultured neurons indicated that this pathway was normally efficient.

According to the hypothesis of global lysosome malfunctioning, storage lesions in LSDs consist in deficient hybrid vesicles, as documented in the *Caenorhabditis elegans* model of mucolipidosis IV [20]. Hybrid vesicles result from the fusion of lysosomes with late endosomes, amphisomes or autophagosomes that are loaded with materials meant for degradation [21]. In LSDs, these vesicles swell, accumulate and proliferate because they are unable to recycle materials contained in their lumen. Lysosome biogenesis is regulated through a gene network controlled by TFEB (transcription
A problem of delivery
Alteration of macro-autophagy was particularly well documented in Niemann–Pick type C disease, showing overexpression of beclin1, an early inducer of macro-autophagy
lysosomes is tolerated for long periods of time before recycling of macromolecules degradation products in LSDs are progressive diseases in which the inefficient mouse model suggest trafficking defects at a pre-Golgi, or on lysosome biogenesis in addition to lysosome functioning. Intracellular trafficking defects in addition to lysosome disability [31]. These observations support the notion that a number of LSDs are related to defects that have an impact on lysosome biogenesis in addition to lysosome functioning.

Our recent findings in the cortical neurons of the MPSIIIB mouse model suggest trafficking defects at a pre-Golgi, or cis-Golgi, step of lysosome biogenesis. GM130 (Golgi matrix protein 130) is a tethering protein which controls vesicle trafficking in pre- and cis-Golgi compartments. It is also a Golgi matrix component that forms molecular complexes with vesicle coat proteins and cargo transporters, and which plays essential roles in Golgi morphogenesis and nucleation of microtubules at the Golgi surface [32–34]. We detected GM130 in storage lesion membranes, which were also stained for LAMP1 (Figures 2A–2C). These vesicles were immobile, abundant in proximal and distal neurites, and resistant to brefeldin A, a drug that induces Golgi collapse into the endoplasmic reticulum. They were frequently associated with disorganized Golgi ribbon structures (Figure 2D). Our results suggest that storage lesions in MPSIIIB neurons are related to aberrant Golgi saccules, which appear to be unable to interact with adjacent intra-Golgi, downstream or upstream compartments, and form a dead-end pathway that gives rise to accumulating storage vesicles [35].

According to the trafficking defect models, it is not so much the amounts of undigested products to be stored that raise a problem, but rather the toxicity of these products for basic cell biological functions, which results in aberrant delivery systems. Permanent residues left inside inappropriate delivery containers progressively increase the amounts of stored material and form storage lesions.

**Perspectives**

LSDs are progressive diseases in which the inefficient recycling of macromolecules degradation products in lysosomes is tolerated for long periods of time before disorders compromise cell functions and survival. Tolerance to cell disorders is presumably made possible through the activation of compensatory mechanisms. Acute models of LSDs, in which distinction can be made between the consequences of primary storage product toxicity and compensatory mechanisms, will presumably shed new light on the relationship of storage products with storage lesions. Acute models of Niemann–Pick type C [29] or mucolipidosis IV [36] have recently been produced for this purpose in HeLa cells. Observations performed shortly after the induction of lysosomal disorders will probably help to decipher the complexity of the intricate cascades of pathogenic events that characterize LSDs.

**Funding**

This work was supported by the Agence Nationale de la Recherche [grant number ANR-08-MNP-023].

**References**


