Food for thought: autophagic vacuolar myopathies

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Arch. Dis. Child. 2009;94;567-569
doi:10.1136/adc.2008.155010

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Most paediatricians are familiar with the terms lysosomal storage disorders (LSDs) and neuromuscular disorders (NMDs), and these disease entities seem to have little in common. Here, I am giving a synopsis of autophagic vacuolar myopathies (AVMs), a relatively new group of diseases at the interface between LSDs and NMDs.[1 2] AVMs (lysosomal myopathies) are characterised by the accumulation of abnormal lysosomes in muscle fibres resulting in specific clinical signs and symptoms.

The term "autophagy" is derived from the Greek words "autos" and "phago" meaning "self-eating". Autophagy takes place in the lysosomes and refers to the process of breaking down cellular components and expelling the products back into the cytoplasm. Over the last few years this phenomenon has increasingly attracted the attention of scientists and doctors as it also has implications for ageing, cancer, infection, cardiovascular disease and neurodegenerative disease.[3]

LYSOSOMAL STORAGE DISORDERS

LSDs are a group of approximately 50 predominantly autosomal recessive diseases caused by a malfunctioning lysosomal protein. The defective protein can be a lysosomal enzyme, a lysosomal transporter or a membrane protein, which leads to accumulation of biological substrates and cell dysfunction. Central nervous system, skeleton, liver and spleen are frequently involved. The combined prevalence of LSDs is 1 in 7700.[4 5] Enzyme assays are currently the gold standard for diagnostic testing. Several successful strategies have been developed to treat patients with specific lysosomal storage diseases. Allogeneic bone marrow transplantation has been used, among others, in mucopolysaccharidosis (MPS) types I and II. Intravenous enzyme replacement therapy (ERT) is available for MPS I and IV, Gaucher disease type I (GD I), Fabry disease and Pompe disease. More recently, substrate inhibition therapy with miglustat has been approved for GD I and Niemann-Pick disease type C (NPC). LSDs with central nervous system involvement pose a particular challenge due to difficulties in transporting the deficient enzyme into brain cells.[6]

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NEUROMUSCULAR DISORDERS

Inherited NMDs, on the other hand, affect 1 in 3500 of the world population, and their mode of transmission can be recessive, dominant or X-linked.[7] Duchenne muscular dystrophy (DMD) has the highest incidence among genetically transmitted muscle diseases, followed by myotonic dystrophy and facioscapulohumeral muscular dystrophy. NMDs in children usually present with muscle weakness and wasting, and gross motor delay. A diagnosis can often be made through a blood test and identification of the causative gene mutation. If there is any doubt, a muscle biopsy should be performed. Treatment is mainly supportive, but oral steroids and angiotensin-converting enzyme (ACE) inhibitors have proven to be beneficial in patients with DMD. A new treatment strategy is the attempt to change the Duchenne phenotype into the milder Becker phenotype through morpholino-mediated exon skipping.[8] The Duchenne phenotype is caused by out-of-frame mutations in the dystrophin gene resulting in the absence of the muscle protein dystrophin. In Becker muscular dystrophy the amount of dystrophin is reduced due to in-frame mutations. Morpholino molecules (antisense oligonucleotides (AONs)) consist of approximately 25 bases attached to six-membered morpholino rings. AONs can block a mutated exon in the dystrophin gene thus enabling the remaining intact exons to produce some functioning protein.

ROLE OF LYSOSOME

Lysosomes originate from the rough endoplasmic reticulum and the *trans*-Golgi network, and act as a recycling system for eukaryotic cells. They contain hydrolytic enzymes and have an acidic pH which is maintained by a vacuolar H^+-ATPase (V-ATPase). Most of these hydrolases (except β-glucosidase) carry a mannose-6-phosphate (M6P) marker which targets them to the lysosomes during their assembly. Macromolecules such as carbohydrates, proteins, lipids and DNA are degraded in the lysosomal vacuoles and transported back into the cytoplasm for recycling. There are three main types of cellular digestion, and lysosomes are central to each of them. Phagocytosis refers to the process of surrounding large food particles or bacteria with a segment of the cell membrane resulting in a phagosome (fig 1). Pinocytosis is a mechanism similar to phagocytosis and involves cellular ingestion of solute material. During autophagy, vacuoles derived from the endoplasmic reticulum are formed around cellular organelles, for example, aged mitochondria. These vacuoles have been named autophagosomes or autophagic vacuoles. Autophagy can be divided into macroautophagy and microautophagy (fig 1), where waste products are taken up by the lysosome from the cytoplasm directly. The third mechanism is called receptor-mediated endocytosis, whereby an area of the cell membrane containing receptors and ligands is absorbed into the cell resulting in an endosome (fig 1). When phagosome, autophagosome and endosome fuse with a lysosome, the catalytic process can begin.[9 10]

LYSOSOMES AND MYOPATHY

Lysosomes are barely visible in healthy muscle fibres on electron microscopy but become more prominent in patients with LSDs. So far, two true LSDs associated with myopathy have been identified.

Acid maltase deficiency is a glycogen storage disease (GSD II) characterised by hypotonia, progressive muscle weakness and cardiomyopathy. It can be divided into a severe infantile type (Pompe disease), an intermediate and an adult type. Pompe disease was first described in 1932.[11] On light microscopy large glycogen-filled vacuoles (autophagosomes) can be identified in muscle fibres of affected patients.[12] Initial reports of intravenous treatment with the missing enzyme alpha-glucosidase are promising.[13] However, exogenous enzyme substitution can induce the production of neutralising antibodies, and additional treatment with immunosuppressant drugs may be required.[14]

In 1981, Danon *et al* described two young, unrelated males with muscle weakness, hypertrophic cardiomyopathy and learning difficulties; they both died at the age of 17 years.[15] Muscle biopsy in these patients was reminiscent of GSD II

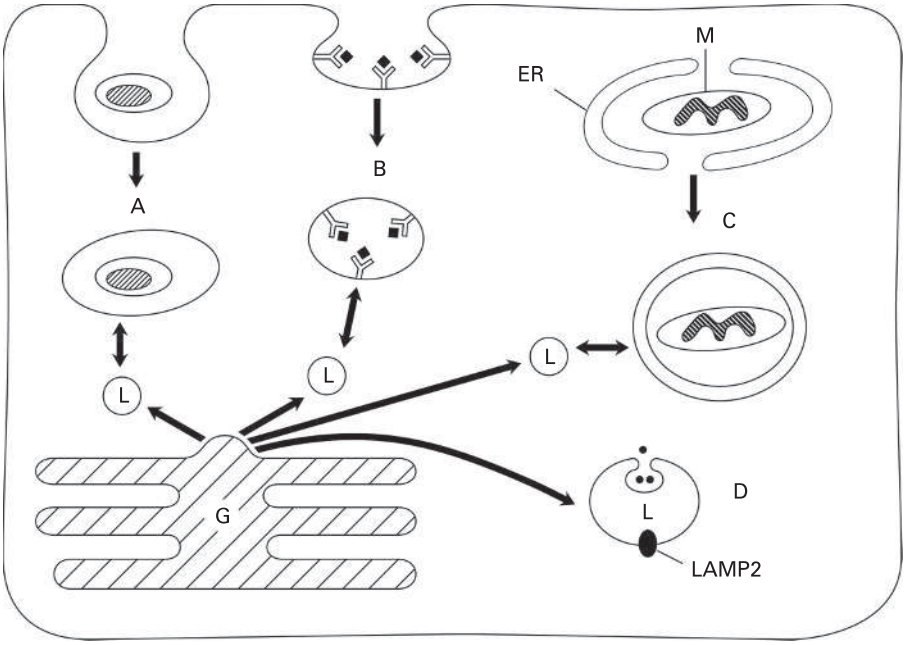

Figure 1 Diagram of the cellular digestion and recycling system. Precursors of lysosomal enzymes are recognised by mannose-6-phosphate (M6P) receptors in the *trans*-Golgi network. The hydrolytic enzymes are transported in clathrin-coated vesicles (not shown) to primary or secondary lysosomes which then fuse with autophagosomes, phagosomes or endosomes. Arrows represent flow. A, phagosome; B, endosome; C, macroautophagy; D, microautophagy; ER, endoplasmic reticulum; G, Golgi apparatus; L, lysosome; LAMP2, lysosome-associated membrane protein 2; M, mitochondrion.[25]

but the autophagosomes were smaller and denser. Alpha-glucosidase activity was within the normal range. Further studies revealed that Danon disease (OMIM 300257) is caused by mutations in the *LAMP2* gene on Xq24 which encodes the lysosome-associated membrane proteins 2a, 2b and 2c.[16] They have multiple functions which are not fully understood.[17] Manifesting female carriers of this X-linked condition are affected to a milder degree. One patient with Danon disease from Italy has received a heart transplant, which is currently the only treatment available, and survived more than 5 years.[18]

MEAX AND MAVIO

X-linked myopathy with excessive autophagy (MEAX, OMIM 310440) shows a similar histological picture as Danon disease (autophagic vacuoles with sarcolemmal features) but LAMP2 is present in the lysosomal membrane. Furthermore, the complement membrane attack complex C5b-C9 can be detected in affected muscle fibres. Patients with MEAX have a slowly progressive muscle weakness but no cardiac involvement. The gene locus for this disorder has been assigned to Xq28.[19] MAVIO (infantile onset autophagic vacuolar myopathy, OMIM 609500) has been reported in two infants with abnormal muscle glycogen storage and severe cardiomyopathy. Histochemical analysis suggested that these cases were on the same spectrum as MEAX.[20] In both conditions the underlying protein defect is unknown.

MYOPATHIES WITH RIMMED VACUOLES

The last, rather heterogeneous group to be considered, are the myopathies associated with rimmed vacuoles. Rimmed vacuoles are aggregates of autophagosomes found predominantly in atrophic muscle fibres. They contain β-amyloid and various other proteins, for example, cathepsins. The focus here will be on Nonaka myopathy or distal myopathy with rimmed vacuoles (DMRV, OMIM 605820), and on autosomal recessive inclusion body myopathy (IBM2, OMIM 600737). DMRV is a relatively fast progressing muscular dystrophy that starts in early adulthood and affects the distal muscles. The disease is more common in Japan and has an autosomal recessive mode of inheritance.[21] IBM2 was first described in Jewish families living in the Middle East. It is characterised by onset in the third decade of life and rapidly advancing proximal and distal muscle weakness. Interestingly, there is sparing of the quadriceps muscle.[22] Both disorders are caused by mutations in the *GNE* gene on chromosome 9p12-9p11 (OMIM 603824). *GNE* encodes uridine diphosphate N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase, which is the rate-limiting enzyme in the biosynthesis of sialic acid (N-acetylneuraminic acid, NeuAc). *GNE* mutations are also the cause of sialuria (OMIM 269921) where they lead to cytoplasmic accumulation and urinary excretion of free NeuAc. Patients with DMRV and IBM2 do not excrete excessive amounts of sialic acids and, conversely, patients with sialuria do not show myopathy and rimmed vacuoles. A possible explanation for this observation is the existence of two specific isoforms of the *GNE* gene. Sialic acids are negatively charged amino sugars that are found as components of oligosaccharides, polysaccharides, glycoproteins and gangliosides. They occur in low concentrations in many organisms and have a variety of functions, for example, preventing the aggregation of blood cells and increasing the viscosity of glycoproteins in mucous.[23] Recent studies have demonstrated the importance of hyposialylation for the pathogenesis of Nonaka myopathy and inclusion body myopathy.[24]

CONCLUSION

Lysosomal myopathies are a small but important group of inherited myopathies which add to our understanding of autophagic processes occurring in the cell and lysosomes. It is likely that further subtypes of autophagic vacuolar myopathies will be delineated in the near future. Gene therapy for these conditions is under investigation.

Competing interests: None.

Accepted 27 January 2009

Arch Dis Child 2009;**94**:567–569.
doi:10.1136/adc.2008.155010

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