bjh review

Lysosomal storage disorders

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Summary

Although the first description of a lysosomal storage disorder was that of Tay-Sachs disease in 1881, the lysosome was not discovered until 1955, by Christian De Duve. The first demonstration by Hers in 1963 of a link between an enzyme deficiency and a storage disorder (Pompe's disease) paved the way for a series of seminal discoveries about the intracellular biology of these enzymes and their substrates, culminating in the successful treatment of Gaucher's disease with β-glucosidase in the early 1990s. It is now recognized that these disorders are not simply a consequence of pure storage, but result from perturbation of complex cell signalling mechanisms. These in turn give rise to secondary structural and biochemical changes, which have important implications for therapy. Significant challenges remain, particularly the treatment of central nervous system disease. It is hoped that recent advances in our understanding of lysosomal biology will enable successful therapies to be developed.

Keywords: lysosome, lysosomal storage disorders, therapy, cell biology.

The majority of lysosomal storage disorders (LSDs) result from defective lysosomal acid hydrolysis of endogenous macromolecules and their consequent accumulation. Over 40 disorders have been described. They tend to be multisystemic and are always progressive, although the rate of progression may vary. Involvement of the reticuloendothelial system has particular implications for haematologists as patients can present with hepatosplenomegaly and cytopenia.

Physiological considerations

The endosomal–lysosomal system

The lysosome is just one component of a series of seemingly unconnected intracellular organelles, collectively known as the *endosomal–lysosomal system* or *vacuolar apparatus*, a term first coined by De Duve and Wattiaux (1966) in their historic review and one which has, in its broad sense, stood the test of time. The various components of the system were described over 30 years ago by Novikoff (1973). A confusing plethora of terms have been applied to the various components of this system. However, it is now generally accepted that its principal components are the *early endosome*, situated at the cell periphery, the *late endosome*, which tends to be perinuclear, and the *lysosome*. They form a chain that is responsible for the trafficking and digestion of endocytosed molecules. Until recently, this was considered to be their only function. However, it is now known that endosomes also participate actively in sorting and recycling.

The final compartment of this system is the lysosome. The term 'lysosome' was first coined by De Duve *et al* (1955). It is characterized by the presence of a membrane, a low internal pH, and vesicles containing many hydrolytic enzymes. The membrane contains transport systems that carry particles between lumen and cytosol, and an electrogenic proton pump called the vacuolar proton pump, or V-type H+-ATPase (Arai *et al*, 1993). It also contains several membrane proteins of uncertain function (Eskelinen *et al*, 2003). It is in the lysosome that substrate breakdown occurs. However, it is far from being a 'dead end' compartment. For example, lysosomes are capable of secreting their contents after fusion with the plasma membrane (Luzio *et al*, 2000). *Phagosomes*, on the contrary, are formed by the *phagocytosis* of bacteria and cellular debris; they eventually transform into *phagolysosomes*. For a detailed review see Lloyd (1996).

Lysosomes also have other functions. For example, calcium-regulated exocytosis of lysosomes is important for membrane repair (Reddy *et al*, 2001). However, these are outside the scope of this article and will not be discussed further.

Synthesis and trafficking of lysosomal enzymes (the 'synthetic' pathway)

The various steps involved in the synthesis of lysosomal enzymes are summarized in Fig 1. They are glycoproteins that are synthesized in the rough endoplasmic reticulum (ER). At this early stage they are inactive. They then translocate

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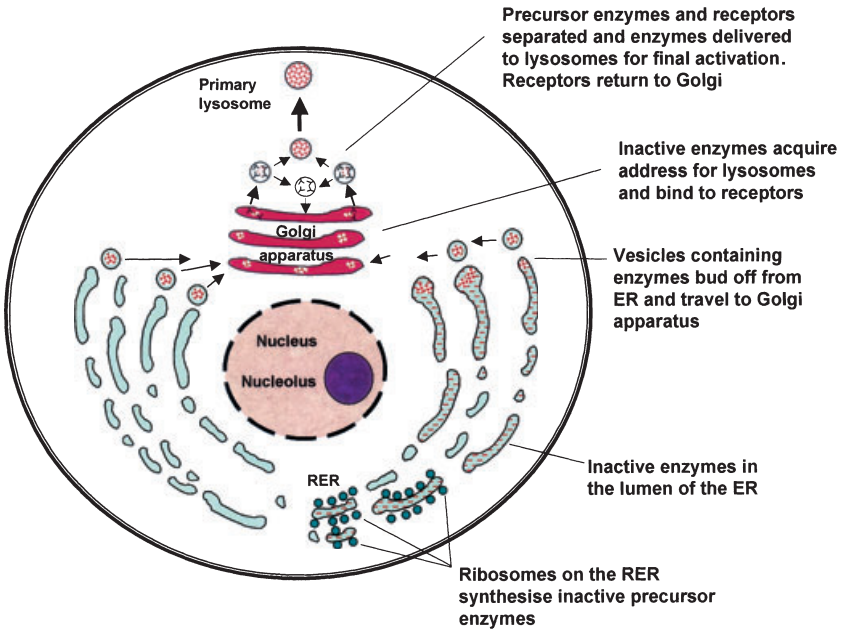

Fig 1. Making the lysosomal enzymes. Published with the permission of B. Winchester, Institute of Child Health, University of London.

through the ER membrane with the help of *N*-terminal signal sequences. Once in the lumen of the ER, they undergo *N*-glycosylation and lose the signal sequence. They then move to the Golgi compartment, and at this stage they acquire a mannose 6-phosphate (M6-P) ligand. This process requires the sequential action of two enzymes, a phosphotransferase (Reitman & Kornfeld, 1981; Waheed *et al*, 1981) and a diesterase (Varki & Kornfeld, 1981; Waheed *et al*, 1981).

It is important to understand these two steps (see Fig 2) because it is the acquisition of the M6-P marker that separates glycoproteins that are destined for the lysosome from secretory glycoproteins. Failure of acquisition of this marker results in mistargeting of lysosomal enzymes; they will not enter the lysosome and substrate breakdown will not occur. This is precisely what happens in two of the mucolipidoses, I-cell disease and mucolipidosis III. These patients lack the enzyme responsible for the first step, i.e. the phosphotransferase. Consequently, all enzymes requiring the M6-P marker fail to enter the lysosome; these patients have very high plasma levels of all such enzymes. In fact, it was this finding that led to the discovery of the M6-P ligand and its receptor (Hickman & Neufeld, 1972).

However, not all lysosomal enzymes require the M6-P ligand. Glucocerebrosidase, which is associated with the lysosomal membrane, does not acquire the M6-P residue, although it does undergo *N*-glycosylation and is targeted. The precise mechanism by which this occurs is unknown.

The receptor–protein complex then moves to the late endosome, where the low pH causes it to dissociate (Gonzalez-Noriega *et al*, 1980). The hydrolase moves on into the lysosome and the receptor then is recycled either to the Golgi to pick up another ligand, or to the plasma membrane.

The final steps in the maturation of the lysosomal enzyme include proteolysis, folding and aggregation.

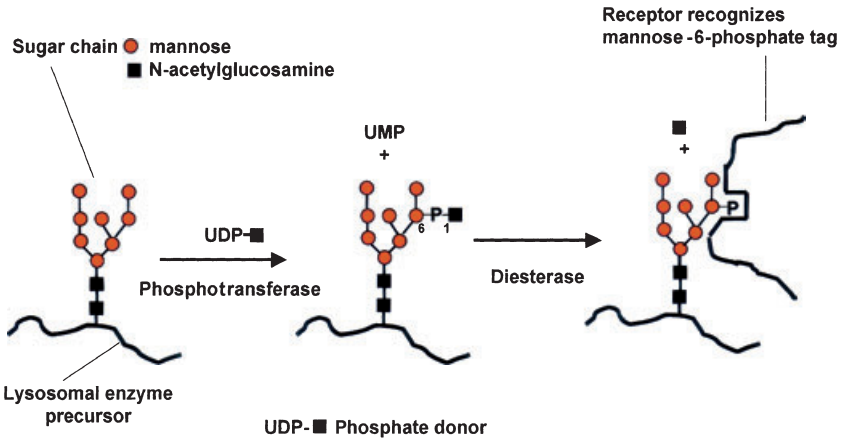

Fig 2. Formation of lysosomal recognition tag or marker, mannose-6-phosphate. Published with the permission of B. Winchester.

Transport of macromolecules to the lysosome (the endocytotic pathway)

The basic steps of the endocytic pathway are summarized in Fig 3. The material to be broken down in lysosomes may be extracellular or intracellular. Extracellular materials enter the cell either by *endocytosis* or *phagocytosis*, depending on the nature of the molecule. *Receptor-mediated endocytosis* is the process by which most biologically important extracellular substances are internalized; this occurs by binding to specific cell surface receptors (Goldstein *et al*, 1985). Ligands are first delivered to early endosomes, and then transported to late endosomes, probably by multivesicular bodies. Finally they are delivered to the lysosomes. *Phagocytosis* is the route of entry into the cell for microorganisms and cellular debris. Such particles are incorporated into *phagosomes*, which fuse with *primary lysosomes* to form *secondary lysosomes*. Finally, intracellular materials undergo *autophagy*. For detailed reviews of these processes see Smythe (1996)and Mortimore *et al* (1996).

Although a small amount of hydrolysis takes place in endosomes, the bulk of it takes place in the lysosome. This is because it is only in the acid milieu of the lysosome that hydrolases are active. The low pH of the lysosome is maintained by the vacuolar proton pump (see above).

Cofactors required for lysosomal enzyme function

A. Activator proteins. The breakdown of certain glycosphingolipids by their respective hydrolases requires the presence of *activator proteins*. As they activate sphingolipid hydrolases these proteins are known as *sphingolipid activator proteins* or *saposins*. Two genes are known to encode saposins. One encodes the GM2 activator protein; its defective function results in the AB variant of GM2 gangliosidosis (Conzelmann & Sandhoff, 1978). The other gene encodes prosaposin (O'Brien *et al*, 1988), which is processed to four homologous saposins (A, B, C and D). Deficiency of a saposin results in a clinical phenotype that may resemble a lysosomal storage disease. For example, mutations in the coding region of Sap B cause a variant form of metachromatic leucodystrophy with sulphatide storage (Wenger *et al*, 1989). Similarly, Sap C deficiency causes a variant form of Gaucher's disease with glucosylceramide storage (Christomanou *et al*, 1989). Deficiency of prosaposin results in a combined saposin deficiency with a very severe phenotype, as might be expected (Bradova *et al*, 1993). However, saposin deficiency often results in features of more than one disorder because each saposin activates more than one enzyme.

B. Cathepsin A. Some lysosomal enzymes may need to coexist in complexes in order to function properly. For example, β-galactosidase and neuraminidase acquire a stable and active conformation through association with protective protein (Hoogeveen *et al*, 1983; Verheijen *et al*, 1985), which has Cathepsin A activity and which is quite separate from its protective effect (Galjart *et al*, 1991).

C. Multiple Sulfatase Deficiency. In this disorder, all 13 of the known sulfatases have reduced activity (Hopwood & Ballabio, 2001). The molecular basis for this has been elucidated. A cysteine residue at the catalytic centre of all the sulfatases fails to convert to a Cα-formylglycine residue (Schmidt *et al*, 1995). This results from a failure of the *Cα-formylglycine generating enzyme* (*FGE*), which in turn results from mutations in the encoding gene, the *sulfatase-modifying factor-I gene (SUMFI)* (Dierks et al, 2003). Although not strictly a cofactor deficiency, it is mentioned here for the sake of convenience.

Secretion–recapture pathway

A significant proportion of newly synthesized enzyme is not bound to the M6-P receptor in the Golgi but instead is secreted and then endocytosed into neighbouring cells via M6-P

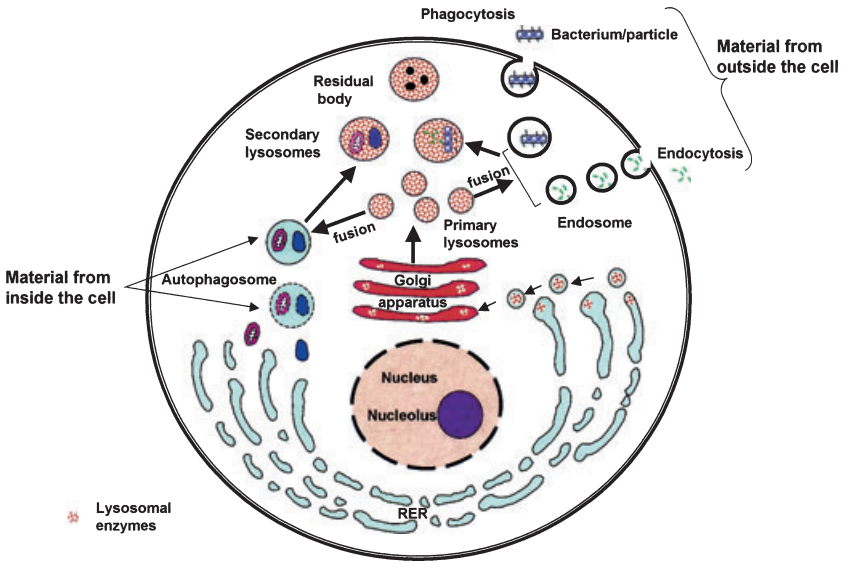

Fig 3. The endocytic pathway. Published with the permission of B. Winchester.

receptors on the plasma membrane (Vladutiu & Rattazzi, 1979). Understanding this *secretion–recapture* pathway was crucial to the understanding of mucolipidoses types II and III. These disorders are characterized by grossly elevated extra-lysosomal (plasma and cytosol) levels of a large number of lysosomal enzymes that require the M6-P recognition marker for receptor-mediated uptake (see above). It was subsequently proven that they were characterized by failure of enzymes to acquire this recognition marker.

The concept of M6-P-based secretion and recapture is of considerable importance when considering therapy. However, there is now evidence that, for some lysosomal enzymes at least, this process is independent of this receptor (Muschol *et al*, 2002).

Plasma membranes and lipid rafts

The plasma membrane is composed of a lipid bilayer. Contrary to earlier theories, the distribution of lipids is not uniform. Sphingolipids and cholesterol form 'platforms' or rafts that float in the liquid phase (Simons & Ehehalt, 2002). These lipid rafts are important in signal transduction processes. Certain key components of signal transduction, including glycosphingolipids, tend to be concentrated on rafts (Simons & Toomre, 2000).

Molecular genetics

All LSDs are single gene disorders and, with three exceptions, they are all autosomal recessive disorders. The exceptions are Hunter's disease [mucopolysaccharidosis (MPS) type II], Fabry's disease and the recently described Danon disease, which is inherited in an x-linked dominant manner (Sugie *et al*, 2003).

The isolation of many of the genes encoding specific lysosomal hydrolases has greatly improved our understanding of these disorders. Studies of the catalytic capacity of mutant enzymes have also been possible. In some disorders, a genotype–phenotype correlation has been established for some genotypes. However, care needs to be taken when interpreting genotype–phenotype relationships, as other factors, such as polymorphisms, within the encoding gene in question should be taken into account (Scott *et al*, 1992).

Sometimes apparently healthy individuals show very low hydrolase activity *in vitro*, indistinguishable from that seen in affected patients. However, activity is normal *in vivo*. This is referred to as *pseudodeficiency*. The mutant proteins responsible for pseudodeficiency are encoded by separate genes. Examples of pseudodeficiency are those of arylsulphatase A (Dubois *et al*, 1975; Gieselmann *et al*, 1989), hexosaminidase A and B (Dreyfus *et al*, 1975) and β-galactosidase (Wenger & Riccardi, 1976).

Lysosomal function and storage in the central nervous system: the role of microglia

The LSD affecting the central nervous system (CNS) pose the greatest challenges in treatment. An understanding of the kinetics of enzyme transfer in the CNS and how this is perturbed in these disorders is therefore essential to successful treatment, and merits separate consideration.

Origin, structure and lysosomal function of microglia. Microglia are the resident macrophage population of the brain. Macrophages form resident populations in many organs (van Furth *et al*, 1972; Naito *et al*, 1996), for example, Kupffer cells of the liver (Gale *et al*, 1978) or alveolar macrophages in the lung (Thomas *et al*, 1976). Microglia have many characteristics of macrophages, including the presence of hydrolases (Ling, 1977). They are derived from circulating blood monocytes that invade the brain early in postnatal life and become amoeboid microglia, which then differentiate into ramified microglia. For a review of these events see Ling and Wong (1993). Postnatally, they are continuously replaced by blood-borne monocytes that cross the blood–brain barrier (BBB) into the brain parenchyma.

As they contain acid hydrolases, it has been postulated that the secretion-uptake machinery applies to microglia and the surrounding neurones. That is to say, a proportion of enzyme is secreted out of microglia and is available for recapture by the surrounding neurones. Indirect evidence for this initially came from studies in cats with α-mannosidosis undergoing bone marrow transplantation (BMT). Correction of storage in diseased neurones accompanied by appearance of enzyme in these cells was observed (Walkley *et al*, 1994). Simultaneously, cells staining strongly positive for α-mannosidase appeared around blood vessels, lending strong support to the haematogenous origin of these cells. Direct experimental evidence of neuronal uptake of enzyme has been shown by the reversal of storage in neural cells surrounding a graft of genetically corrected fibroblasts in the mucopolysaccharidoses (MPS) VII mouse brain (Taylor & Wolfe, 1997).

However, local secretion and uptake is not the only mode of transfer of lysosomal enzymes in the CNS. Axonal transport also occurs (Passini *et al*, 2002) and is probably an important mechanism for transfer to distant sites. Exactly what proportion of secreted lysosomal enzyme undergoes axonal transport is not known. However, it is a potentially important therapeutic route.

Origin of substrate in the CNS. The origin of the substrate may differ inside and outside the CNS. This may explain why some patients within a particular enzyme deficiency have neurological involvement while others do not. Gaucher's disease is a good example of this. There are three broad clinical phenotypes. Patients with type I Gaucher's disease do not have neurological involvement (non-neuronopathic), while patients with types II and III do (neuronopathic). Patients with the neuronopathic forms of Gaucher's disease have increased levels of the substrate glucosylceramide (GCS) in the brain. Glucosylceramide is derived from two sources. Inside the CNS it is derived predominantly from gangliosides, while elsewhere it is derived predominantly from the breakdown of effete blood cells. In type I patients, the degradation of blood cell derived

glucosylceramide is blocked, but there is sufficient enzyme activity in the CNS to break down ganglioside-derived glucosylceramide, thus preventing its accumulation in the brain (Brady *et al*, 1993). In types II and III, however, there is less residual enzyme activity (Brady *et al*, 1966), insufficient to degrade even ganglioside-derived GCS in the CNS (Brady *et al*, 1965a, 1993; Zhao *et al*, 2003).

The blood–brain barrier

The BBB is created by the endothelial cells of the brain capillaries. These cells are linked by tight junctions that form an effective barrier to paracellular aqueous diffusion (Brightman & Reese, 1969; Kniesel & Wolburg, 2000). Such junctions do not exist in the capillary endothelial cells of the peripheral circulation. This difference is thought to be the result of close apposition to both astrocytes and pericytes, both of which are tightly applied to the basement membrane of the cerebral capillaries (Kacem *et al*, 1998). Astrocytes have end feet, which spread in a network around the capillaries. The BBB also forms an electrical barrier in the form of a transendothelial electrical resistance (Begley & Brightman, 2003). It can be readily seen, therefore, that the BBB effectively prevents most polar blood-borne solutes from crossing. Yet monocytes cross the BBB and differentiate into microglia. Migration is significantly inhibited by the addition of blocking antibodies to intercellular adhesion molecule-1, very late antigen-4 integrin, and monocyte chemoattractant protein (CCL-2/MCP-1), or treatment with tissue inhibitor of metalloproteinase (Seguin *et al*, 2003). These results support the concept that monocyte–endothelial cell interactions are somehow responsible for monocyte migration across the BBB.

Pathological considerations

Causes of lysosomal storage disease

Given the many steps in the synthesis and processing of lysosomal hydrolases, it is not surprising that there are many ways in which they can become dysfunctional. Since the identification of the first lysosomal enzyme deficiency, for Pompe's disease (Hers, 1963), over 40 disorders have been described. There may be inherent defects of synthesis or folding, activation defects as in the saposin deficiencies, or targeting defects as in mucolipidoses II and III. In addition, they may be secondary to membrane protein defects, as in cystinosis, Niemann–Pick C disease, infantile sialic acid storage disease and cystinosis. Table I lists the LSDs that have been described to date, classified by the main substrate class.

Table I. Known lysosomal disorders.

Mucopolysaccharidoses (MPS)
MPS I
MPS II
MPS IIIA
MPS IIIB
MPS IIIC
MPS IIID
MPS IVA
MPS IV B
MPS VI
MPS VII
Glycoproteinoses
Aspartylglucosaminuria
Fucosidosis
α-Mannosidosis
β-Mannosidosis
Mucolipidosis I (sialidosis)
Schindler disease
Sphingolipidoses
Fabry's disease
Farber's disease
Gaucher's disease
GM1 gangliosidosis
Tay-Sachs disease
Sandhoff's disease
Krabbe's disease
Metachromatic leucodystrophy
Niemann-Pick disease, types A and B
Other lipidoses
Niemann-Pick disease type C
Wolman's disease
Neuronal ceroid lipofuscinosis
Glycogen storage disease
Glycogen storage disease type II (Pompe's disease)
Multiple enzyme deficiency
Multiple sulphatase deficiency
Galactosialidosis
Mucolipidosis II/III
Mucolipidosis IV
Lysosomal transport defects
Cystinosis
Sialic acid storage disease
Other disorders due to defects in lysosomal proteins
Danon disease
Hyaluronidase deficiency

Relationship to residual enzyme activity

The severity of the phenotype is closely related to the residual enzyme activity. Conzelmann and Sandhoff (1983) proposed that there was a 'critical threshold' of enzyme activity. Above this level, enzyme activity can deal with substrate influx. Below this, it cannot and there is accumulation of substrate. It has been demonstrated that small changes in *residual enzyme activity* can have a profound effect on rate of accumulation of substrate (Conzelmann & Sandhoff, 1983). In general, the lower the residual activity, the earlier the age of onset and the more severe the disease, although there is considerable overlap, for example, in Gaucher's disease. It therefore follows that for those diseases for which enzyme-based therapies are available (see section on *Therapy*, below), residual enzyme activity is of

critical importance in determining response to treatment. The lower the residual activity, the less satisfactory the response.

Effects of lysosomal storage

The buildup of undigested material secondary to lysosomal enzyme dysfunction results in the formation of typical histochemical and ultrastructural changes.

Light microscopy often reveals engorged macrophages with a characteristic appearance, such as that of 'crumpled silk' in Gaucher's disease (Parkin & Brunning, 1982) or 'sea-blue histiocytes' in Niemann–Pick disease (Vanier *et al*, 1988). Characteristic ultrastructural changes have also been described based on the appearance of *residual bodies*. These are vacuoles containing undigested material, and are the hallmark of primary storage in these disorders. The first residual bodies were described in Tay–Sachs disease (Terry & Weiss, 1963). Other disorders in which they have been described include neuronal ceroid lipofuscinosis (Zeman & Donahue, 1963), Gaucher's disease (Lee, 1968) and fucosidosis (Loeb *et al*, 1969; Freitag *et al*, 1971).

How might accumulation of substrate result in disease? There are several potential ways in which accumulated substrate might cause disease. The most obvious is enlargement of the affected cell, resulting in enlargement of the respective organ. For many years it was taken for granted that manifestations such as hepatosplenomegaly, cardiomyopathy etc. were solely the result of accumulation of undegraded substrate. Furthermore, a number of *secondary biochemical* and *structural events* have been reported which appear to be triggered by the primary storage event. All these factors, individually or in combination, appear to play key roles in the pathophysiology of LSD. These are reviewed briefly below.

Secondary lysosomal hypertrophy. It has become clear that simple storage does not satisfactorily explain the organ enlargement that is seen in storage disorders. For example, the cardiomyopathy of Fabry's disease is characterized by cardiac hypertrophy, with weights of approximately 1000 g being recorded (Elleder *et al*, 1990). Less than 0·5% is comprised of the storage product, ceramide trihexoside (Elleder, 2003). The mechanism by which hypertrophy occurs is unclear.

Secondary changes in neurones. Neurones in storage disorders, like cells elsewhere, display storage of primary substrate. However, they also display a variety of other structural changes. These were appreciated even in the earliest papers (Sachs, 1887; Sachs, 1903). However, it was not until 1976 that the abnormal morphology was clearly delineated (Purpura and Suzuki (1976). Two types of morphological changes have now been described: *meganeurites* and *axonal spheroids*. Meganeurites are enlargements of the axon hillock and are of two types, 'spiny' and 'non-spiny' or smooth, depending upon their appearance. They always contain the specific storage bodies found in the neurones. The spiny appearance is conferred by the presence of new dendritic membrane. This process is known as ectopic dendritogenesis, and it exhibits two remarkable phenomena. First, the changes seen appear to be species-specific. Therefore, in certain disorders, both types of meganeurites may be seen, while in others, only smooth meganeurites are encountered. Secondly, they are also cell-specific, so that the changes are confined to certain types of neurones regardless of the underlying disorder. The two phenomena, i.e. organ specificity and cell specificity, are independent of each other. Since ectopic dendritogenesis was first reported in a patient with a gangliosidosis, Purpura and Suzuki (1976) proposed that it was secondary to ganglioside accumulation. However, it soon became clear that it is also seen in disorders in which ganglioside is not the primary substrate. Further studies have established that it is seen only in neurones exhibiting lysosomal storage, and that it is always associated with accumulation of ganglioside, predominantly GM2, irrespective of the disorder. Interestingly, GM2 ganglioside is also seen in the early phases of normal dendritogenesis, disappearing as neurones mature. The precise link between GM2 accumulation and ectopic dendritogenesis remains unclear. Axonal spheroids are focal axonal enlargements and are usually seen in disorders in which ganglioside accumulates, either primarily or secondarily. The relationship is not that close, however, and spheroids do not contain storage bodies. In addition, the morphology of meganeurites is disease specific; that of spheroids is not. However, as with meganeurites, not all neurones display spheroid formation; it tends to be confined to GABA-ergic neurones. For a more detailed review of these processes see Walkley (2004).

Macrophage activation and/or cytokine release. Macrophage activation following storage is seen in many LSD. For example, raised concentrations of cytokines or chemokines have been found in patients with Gaucher's disease (Hollak *et al*, 1997; Barak *et al*, 1999; Boot *et al*, 2004) and have been postulated to play a role in the pathogenesis, especially that of bone disease (Allen *et al*, 1997).

In MPS VI cats, articular chondrocytes were found to have a higher apoptotic rate than normal and this was associated with high nitric oxide (NO) release rates. The high NO release could be directly attributed to the presence of dermatan sulphate. Importantly, co-culture of the MPS VI and normal chondrocytes reduced NO release (Simonaro *et al*, 2001). Interestingly, chronic alteration of the NO pathway has also been implicated in the cerebral blood flow alterations seen in Fabry's disease; and this is reversed by enzyme replacement therapy (ERT; Moore *et al*, 2001).

Macrophage activation has also been reported in the brain in animal models of some storage disorders (Jeyakumar *et al*, 2003) and appears to be a major cause of neuronal death (Wada *et al*, 2000).

Macrophage activation may also affect the BBB. Co-culture of macrophages and brain capillary endothelial cells resulted in augmentation of the *transendothelial electrical resistance* of these cells (Zenker *et al*, 2003). It is possible therefore, that activation of perivascular macrophages results in a functional compromise of the BBB. Physical disruption of the BBB has been demonstrated in animal models, and, in human immunodeficiency virus-associated dementia, weakening of the endothelial tight junctions has been shown to correlate with monocyte infiltration (Boven *et al*, 2000). Interestingly, macrophage activation was demonstrated in all mouse models of GM2 gangliosidoses, BBB disruption was seen only in some (Jeyakumar *et al*, 2003). The link between functional and physical compromise of the BBB has yet to be established. Certainly, disruption of the BBB might permit invasion of the CNS by increasing numbers of macrophages, thereby potentially setting up a vicious cycle. It remains to be seen whether human forms of these diseases exhibit similar pathology.

The relationship between storage and macrophage activation is not clear. However, it may be cytokine-triggered. Wu & Proia (2004) have demonstrated, in an animal model of Sandhoff's disease, that macrophage activation and cellular infiltration is accompanied by increased expression of macrophage-inflammatory protein 1 alpha (MIP-1α), a leucocyte chemokine, in astrocytes. Deletion of MIP-1α expression resulted in reduced cellular infiltration and neuronal apoptosis, an improved neurological status and a prolonged lifespan (Wu & Proia, 2004).

The psychosine hypothesis. This hypothesis has been put forward in an attempt to explain the pathology seen in some glycosphingolipid disorders (GSL) (Suzuki, 1998). It was first proposed in 1972 to explain the pathogenetic mechanisms in globoid cell leucodystrophy (Krabbe's disease) (Miyatake & Suzuki, 1972). In this condition galactosylceramide and its metabolite, galactosylsphingosine or psychosine, accumulate. Studies of the brain had shown that, although there was accumulation of galactosylceramide, it was insufficient to explain the rapid and massive oligodendrocyte loss. It was postulated that this might be caused by psychosine and was subsequently proven to be the case (Igisu & Suzuki, 1984). Psychosine is a lysosphingolipid, i.e. a GSL that does not have the *N*-acylated fatty acid. By extension, therefore, it was proposed that other lyso-GSLs might be pathogenic as well, and certainly there is accumulation of sulphogalactosylsphingosine in metachromatic leucodystrophy (Toda *et al*, 1990), lyso GM2 in GM2 gangliosidosis (Neuenhofer *et al*, 1986), and sphingosylphosphorylcholine in Niemann–Pick A disease (Rodriguez-Lafrasse & Vanier, 1999). There is also some experimental evidence to show that glucosylsphingosine accumulation may play a role in the neurological involvement seen in some patients with Gaucher's disease (Schueler *et al*, 2003) but to what extent remains unclear.

Role of intracellular calcium. Calcium is an important intracellular mediator, and altered calcium homeostasis appears to play an important role in the sphingolipid storage disorders, although the mechanism of action is not the same for all. A detailed description of intracellular calcium flux is beyond the scope of this review, so only the relevant points will be touched upon.

In neurones, calcium is stored mainly in the ER. It is pumped into the lumen of the ER from the cytosol via the action of sarco/endoplasmic reticulum Ca^2-ATPase (SERCA) (Misquitta *et al*, 1999). From the ER it passes into the cytosol through two types of channels, one of which is a ryanodine receptor (Fill & Copello, 2002).

Upon incubation of neurones with conduritol B-epoxide, a specific inhibitor of β-glucosidase, the enzyme deficient in Gaucher's disease, they were found to display increased sensitivity to neurotoxic agents, such as glutamate. Importantly, the neurotoxicity could be completely blocked by preincubation of the neurones with ryanodine (Pelled *et al*, 2000). This demonstrated that intracellular Ca release was responsible for neuronal death in the neuronopathic forms of Gaucher's disease. Further support for this comes from the finding of a 10-fold increase in glucosylceramide, and a concomitant increase in Ca+ release via the ryanodine receptor, in brain microsomes from a patient with type II Gaucher's disease (Lloyd-Evans *et al*, 2003).

Abnormal Ca^2 flux also appears to play a role in the pathogenesis of the GM2 gangliosidoses, although via a different mechanism, this time involving SERCA (Pelled *et al*, 2003).

Extralysosomal accumulation in plasma membranes; perturbation of lipid rafts. There is evidence that extralysosomal accumulation of substrate may take place and have deleterious effects on transmembrane and intracellular signalling. For example, in a mouse model of metachromatic leucodystrophy, accumulation of sulphatide was demonstrated in myelin as well in lysosomes. This may influence the distribution of myelin-associated proteins, such as myelin and lymphocyte protein (MAL). (Gieselmann *et al*, 2003). Interestingly, MAL is associated almost entirely with rafts (see above). These events may in turn disrupt the function of key cell components, such as ion channels and transporters. For a more detailed review see Futerman and van Meer (2004).

There is also the intriguing possibility that lysosomal storage may adversely affect mitochondrial function. There is direct evidence for this in animal models of neuronal ceroid lipofuscinosis (Jolly *et al*, 2002) and in cell lines from patients with Fabry's disease (Lucke *et al*, 2004).

The concept of secondary events has been crucial to our understanding of the pathogenesis of LSD. More than one type of secondary event may well operate in any given LSD. This is particularly crucial when considering therapy. While the primary event, i.e. intralysosomal storage, may be amenable to therapy, the changes brought about by secondary events

may not, and this may well determine the effectiveness of therapy.

Clinical presentation

Table I lists the known LSD. A detailed description of all the known storage disorders is beyond the scope of this review; the reader is referred to several excellent reviews (see Scriver *et al*, 2001).

Nearly every eukaryotic cell, with the exception of the erythrocyte, contains lysosomes. Furthermore, many lysosomal substrates have key roles in cellular structure and function. Consequently, the effects of lysosomal malfunction are widespread.

However, it is useful to separate the various disorders according to the predominant cell type involved, as this has the most important implications for therapy.

Neurological involvement. Most LSD have neurological involvement. Those that have neurological involvement can be further divided into two groups, those in which all patients have neurological involvement, and those in which only the most severely involved patients have it. It is important to make this distinction, as additional mechanisms are probably responsible for the neurological disease in the latter group, particularly as the distinction is usually fairly clear cut, e.g. MPS I-H from the milder MPS I-HS or MPS I-S, or types II and III Gaucher's disease from type I.

Mesenchymal involvement. This group comprises essentially all the mucopolysaccharidoses, in whom mesenchymal involvement is universal; it is responsible for the dysostosis multiplex and spinal cord involvement that is characteristic of this group.

Reticuloendothelial involvement. This group comprises many of the sphingolipidoses, e.g. Gaucher's disease, Fabry's disease, Niemann–Pick disease. Reticuloendothelial cells are usually far more accessible to therapy than mesenchymal cells and neurones. Hence this group of disorders tend to respond the best to therapy, especially in those disorders in which the CNS is not involved.

Other clinical considerations

Hydrops fetalis

Recurrent non-immune hydrops fetalis is an uncommon but important presentation of LSD. Several LSD are known to present in this way, including Farber's disease (Kattner *et al*, 1997), GMI gangliosidosis (Denis *et al*, 1996), galactosialidosis (Haverkamp *et al*, 1996), Niemann–Pick disease type C (Meizner *et al*, 1990), infantile free sialic acid storage disease, mucolipidosis II (I-cell disease) (Appelman *et al*, 1988), type 2 Gaucher's disease (Reissner *et al*, 1998), MPS IVA (Applegarth *et al*, 1987) and VII (Van Dorpe *et al*, 1996) and sialidosis (Ovali *et al*, 1998). Investigation of recurrent non-immune hydrops should include screening for LSD (Piraud *et al*, 1996).

Phenotype delineation

Virtually all known LSD are characterized by considerable phenotypic heterogeneity. This has resulted in considerable difficulty especially when considering treatment, as it is clearly important to target therapy to the right patient (see *Therapy*). Molecular genetics is a potentially useful tool, but in practice it has relatively few clinical applications. (i) The severe form of MPS type I is known as Hurler's disease or MPS I-H. In a study of European patients, the two common nonsense mutations, W402X and Q70X, were identified in 37% and 35% of mutant alleles respectively (Bunge *et al*, 1994). These alleles are not found in patients with the milder phenotypes of MPS I. Their presence in a patient being considered for allogeneic BMT would be useful in confirming phenotypic severity as milder phenotypes are not considered for BMT. (ii) In MPS II (Hunter's disease) all patients with full deletions or gross rearrangements have a severe clinical presentation (Hopwood *et al*, 1993). In some disorders, such as MPS VI (Maroteaux–Lamy disease), there appears to be a good correlation to the residual mutant protein (Litjens *et al*, 1996). Unfortunately, such assays are not routinely available. Therefore the distinction often has to be made clinically, an approach that is prone to error. Until more robust tools are available, it is likely that mistakes will continue to be made.

Therapy

A variety of non-specific therapeutic measures are available for most disorders and will not be dealt with here. Specific therapy can be broadly divided into enzyme-based therapies and non-enzyme based therapies.

The enzyme-based therapies currently available are BMT and ERT. It was observed in a series of seminal experiments that co-culture of cell lines with differing lysosomal enzyme deficiencies resulted in cross-correction of the defects (Fratantoni *et al*, 1968; Wiesmann *et al*, 1971; Kihara *et al*, 1973). This formed the rationale for *endogenous* enzyme replacement, i.e. BMT. Furthermore, the addition of enzymes derived from other sources to cultures of deficient fibroblasts resulted in clearance of the storage products (Di Ferrante *et al*, 1973), thereby providing a rational basis for *exogenous* enzyme replacement, i.e. ERT. The secretion-uptake pathway, referred to earlier, forms the fundamental basis for both forms of therapy.

Bone marrow transplantation. Early attempts at transplantation with amniotic cells (Yeager *et al*, 1985) and fibroblasts (Gibbs *et al*, 1980) yielded disappointing results. The first bone marrow transplants for an LSD were carried out in the early 1980s (Hobbs *et al*, 1981). Since then, BMT has been carried out for at least 20 of the known LSD so far. A detailed

Table II. Lysosomal disorders in which BMT has been carried out.

Disease	Reference
Mucopolysaccharidoses (MPS)	
MPS I	Peters *et al* (1996); Vellodi *et al* (1997); Guffon *et al* (1998); Peters *et al* (1998)
MPS II	Bergstrom *et al* (1994); Coppa *et al* (1995); Vellodi *et al* (1999)
MPS III	Vellodi *et al* (1992); Busca *et al* (1995); Sivakumur & Wraith (1999)
MPS IVA	
MPS VI	Krivit *et al* (1984); Herskhovitz *et al* (1999)
MPS VII	Yamada *et al* (1998)
Glycoproteinoses	
Aspartylglucosaminuria	Autti *et al* (1999); Arvio *et al* (2001)
Fucosidosis	Vellodi *et al* (1995); Miano *et al* (2001)
α-Mannosidosis	Wall *et al* (1998); Albert *et al* (2003)
Sphingolipidoses	
Farber's disease	Yeager *et al* (2000)
Gaucher's disease	Hobbs *et al* (1987); Erikson *et al* (1990); Ringden *et al* (1995)
GM1 gangliosidosis	
Krabbe's disease	Krivit *et al* (1999a,b)
Metachromatic leucodystrophy	Kapaun *et al* (1999); Krivit *et al* (1999a)
Niemann-Pick disease, type A and B	Vellodi *et al* (1987); Bayever *et al* (1992)
Other lipidoses	
Wolman's disease	Krivit *et al* (2000)
Neuronal ceroid lipofuscinosis	Lake *et al* (1997)
Multiple enzyme deficiency	
Mucolipidosis II	Grewal *et al* (2003)
Lysosomal transport defects	
Niemann-Pick disease type C	Hsu *et al* (1999)

description of the results of BMT is beyond the scope of this review (see Table II for further reading). The results have provided proof of principle that donor bone marrow provides a constant and permanent endogenous supply of enzyme-producing cells. It remains the only form of therapy that is capable of doing this.

How does BMT work? As discussed above, monocytes form resident populations of macrophages in different organs. Following engraftment, these are now of donor origin, and therefore capable of providing endogenous enzyme. Transfer of enzyme to surrounding cells takes place and clears accumulated substrate. Importantly, cells of donor origin have been demonstrated in the brain following human BMT (Unger *et al*, 1993). In fact, it is possible that egress across the BBB is increased following BMT, as activated T cells have been shown to enter the CNS more easily (Hickey, 1999). Furthermore, experimental evidence has demonstrated enzyme transfer to surrounding neurones (see *Lysosomal function and storage in the central nervous system*). However, this mechanism is unlikely to operate in, for example, Gaucher's disease, in which the enzyme is membrane-bound.

However, this is probably not the only mechanism of action. As described above, macrophage activation results in neuronal apoptosis in a mouse model of Sandhoff's disease. In this model, BMT was shown to result in suppression of the inflammatory response and neuronal death without any obvious reduction in GM2 storage (Wada *et al*, 2000).

Unfortunately, while BMT results in varying degrees of clinical improvement, it rarely, if ever, results in complete reversal of the clinical phenotype. There are two main reasons for this.

1. Inadequate tissue penetration. Mesenchymal: Skeletal manifestations, particularly those seen in the mucopolysaccharide disorders, respond inadequately to BMT (Field *et al*, 1994; Weisstein *et al*, 2004). The dysostosis that is characteristic of these disorders is largely the result of defects in articular cartilage and endochondral ossification. Articular cartilage is mesenchymal in origin, and therefore any improvement following BMT would have to rely on mesenchymal stem cell (MSC) engraftment. Unfortunately, MSC engraftment following BMT tends to be predominantly host-derived (Simmons *et al*, 1987; Koc *et al*, 1999), although the results are somewhat contradictory (Pereira *et al*, 1998; Almeida-Porada *et al*, 1999).

Neuronal: While BMT does result in enzyme expression and substrate clearance, this is incomplete. There are two possible explanations for this. The first is the slow rate of microglial engraftment. Experiments on transgenic mice suggest that, while there is quick and complete engraftment in bone marrow and spleen, donor microglial engraftment is much slower, reaching only 23% at 6 months and approximately 30% after 1 year (Kennedy & Abkowitz, 1997). Of course, it is unclear what minimum levels of donor microglia are required to reverse or arrest progression. Nevertheless, it can be seen that CNS enzyme levels are unlikely to be satisfactory for several months post-BMT, and progression will not be prevented. It can be readily seen that patients who are symptomatic at the time of BMT, or those with rapidly progressive disorders, are the least likely to respond.

2. Extensive disease prior to commencement of treatment. The most important determinants of this are:

1 The underlying phenotype, which is itself determined by the residual activity. The more severe the phenotype the poorer the response.

2 The interval between onset of symptoms and start of treatment. Clearly the longer the interval, the greater the changes (primary and secondary) and hence the poorer the response.

Enzyme replacement therapy. The very existence of lysosomes suggested the possibility of ERT, a belief reinforced by experimental data demonstrating intracellular correction of storage following addition of exogenous enzyme to fibroblast cultures (O'Brien *et al*, 1973). A crucial observation was that only a very small percentage increase in intracellular activity (1–5%) was required to correct storage. Again, as in the case of BMT, early trials provided proof of principle that ERT could clear substrate. However, there were two main obstacles. First, there were very few good large animal models suitable for preclinical trials. Secondly, purification of enzyme in sufficient quantities was technically challenging. Both hurdles were eventually overcome. There are now good animal models for many storage disorders (Ellinwood *et al*, 2004), which have provided very useful preclinical data. Production of sufficient quantities of enzyme was facilitated by the cloning of the respective cDNAs. It was also discovered that Chinese hamster ovary cells were superior to prokaryotic systems, because the latter are unable to carry out the post-translational modification required for lysosomal stability. Enzyme produced in such systems is also secreted into the medium in large quantities, making them ideal for large-scale production (Ioannou *et al*, 1992).

The first successful treatment of an LSD – Gaucher's disease – by ERT was developed by Brady and colleagues at the National Institutes of Health. β-Glucosidase, the deficient enzyme (Brady *et al*, 1965b), is not taken up by M6-P-receptors, and sequential deglycosylation to expose the mannose group was required for successful uptake (Furbish *et al*, 1981). Using this approach, the group reported substantial improvement in many of the clinical features of Gaucher's disease (Barton *et al*, 1990; Barton *et al*, 1991). Many other investigators have subsequently reported similar results (for review see Weinreb *et al*, 2002).

Clinical trials of ERT in Fabry's disease (Eng *et al*, 2001; Schiffmann *et al*, 2001) and MPS I (Wraith *et al*, 2004) using human recombinant enzyme soon followed, resulting in marketing approval for these two enzyme preparations. Clinical trials are in progress in Pompe's disease (Van den Hout *et al*, 2001, 2004; Winkel *et al*, 2004), MPS II (Muenzer *et al*, 2002) and MPS VI (Harmatz *et al*, 2004). Trials are planned in Niemann–Pick disease type B based on preclinical results (Miranda *et al*, 2000).

The results of ERT vary considerably from disease to disease. Important considerations are the age of onset, rapidity of progression and the presence or absence of neurological involvement. Within each disease, too, there is considerable variation. Mildly affected patients are the most likely to respond. For a detailed review of ERT see Desnick (2004).

However, despite the undoubted benefits, neurological involvement constitutes the greatest challenge. There is no conclusive evidence that ERT crosses the BBB. Indeed there is no reason to suppose that it should. Even intrathecally administered enzyme has no beneficial effect. Although there is experimental data demonstrating that enzyme given directly intracerebrally is taken up by neurones, such an approach is likely to present considerable logistic challenges.

Non-enzyme-based therapy. Substrate reduction therapy: a novel approach to treatment is substrate reduction therapy (SRT). This was first applied to the GSL group of disorders. The imino sugar *N*-butyldeoxynojirimycin (NB-DNJ) has been shown to inhibit ceramide-specific glucosyltransferase, which catalyses the first step in GSL biosynthesis (Platt *et al*, 1994; Fig 4). This results in the inhibition of biosynthesis of all glucosylceramide-based glycosphingolipids (Figs 4 and 5) Preclinical studies in animal models of Tay-Sachs disease (Platt *et al*, 1997) and Sandhoff's disease (Jeyakumar *et al*, 1999) demonstrated reduction in the CNS substrate load and amelioration of the clinical symptoms. By inference, it probably crosses the BBB. These promising results paved the way for a clinical trial in patients with type I (non-neuronopathic Gaucher's disease) who were naïve to ERT. The results showed a response in various disease parameters (Cox *et al*, 2000).

As mentioned above, secondary accumulation of gangliosides is an important factor in the pathophysiology of some LSDs. For example, in Niemann–Pick disease type C, although the primary defect is in intracellular cholesterol trafficking (Pentchev *et al*, 1985; Maziere *et al*, 1987; Roff *et al*, 1992), secondary ganglioside accumulation has been demonstrated in neurones of the mouse model (Walkley *et al*, 2000). So there is a rationale for the use of NB-DNJ in NPD C, although recent evidence (Lachmann *et al*, 2004) suggests that it may have a direct effect on the abnormal cholesterol trafficking itself.

Further studies of NB-DNJ (miglustat, Zavesca®) in Niemann–Pick disease type C, late-onset Tay-Sachs disease and type III Gaucher's disease are in progress. Diarrhoea secondary to glucosidase inhibition appears to be a dose limiting side-effect. In this respect the galactose analogue appears to be better tolerated, at least in preclinical studies (Andersson *et al*, 2004); therefore the potential for dose escalation is greater.

It is unlikely, however, that NB-DNJ will have a role in all the sphingolipid disorders, e.g. Niemann–Pick disease types A and B, metachromatic leucodystrophy and globoid leucodystrophy. Therefore the search is underway to identify other substances. For example, twitcher mice (globoid cell leucodystrophy) treated with L-cycloserine have an amelioration of their disease with significantly longer life span (LeVine *et al*, 2000).

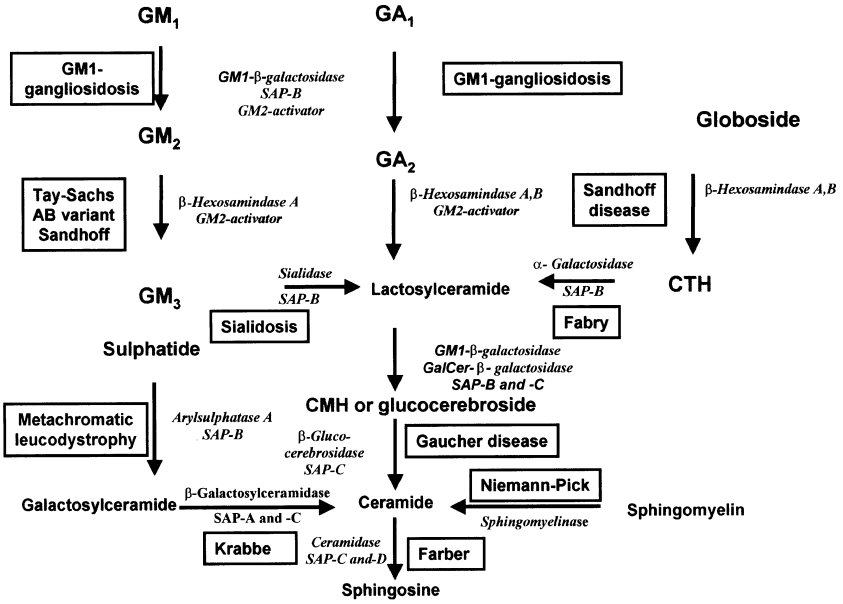

Fig 4. Lysosomal catabolism of some glycosphingolipids. Published with the permission of B Winchester.

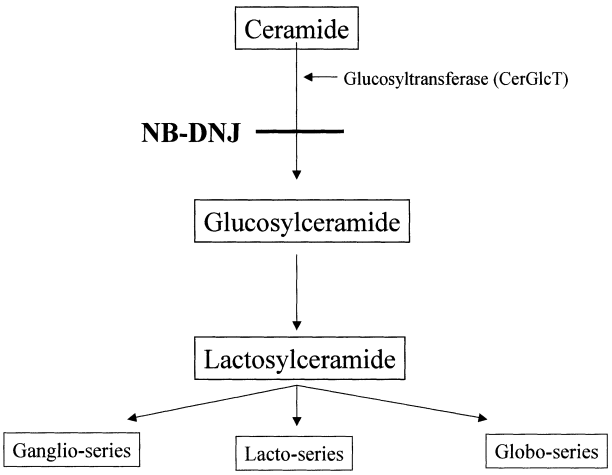

Fig 5. Substrate reduction in the GSL disorders. The first committed step in glucosphingolipid biosynthesis is the transfer of glucose to ceramide by the ceramide-specific glucosyltransferase (CerGlcTT; UDP glucose: *N*-acylsphingosine D-glucosyltransferase). This step is inhibited by NB-DNJ.

Substrate reduction therapy may also have a role in augmenting the effects of other therapies. In the Sandhoff mouse, survival following a combination of BMT and NB-DNJ was significantly greater than that following either therapy on its own (Jeyakumar *et al*, 2001).

Future directions

The two most clinically challenging areas remain the CNS and the mesenchyme. It has been shown that if enzyme can somehow be delivered to the CNS, whether directly (Zirzow *et al*, 1999) or via a transgene (Brooks *et al*, 2002; Fu *et al*, 2002), it is taken up by neurones with clearing of stored material. Traversing the BBB effectively and safely is therefore the Holy Grail of many researchers. Mesenchymal cells are proving far more resistant to therapy (Koc *et al*, 2002).

One potential advantage of gene therapy is the potential for overexpression of enzyme, which may achieve better results than conventional BMT, which is only likely to result in enzyme expression at donor levels. For example, in the mouse model of metachromatic leucodystrophy, stem cell retroviral-based gene therapy did not achieve significant CNS correction (Gieselmann, 2003). However, overexpression of the enzyme, using *ex vivo* lentivirus-mediated transduction and transplantation, achieved superior results in both the central and peripheral nervous system (Biffi *et al*, 2004). The reason for this is not entirely clear, but evidently this avenue deserves further exploration.

Although considerable advances have been made with bone marrow stromal stem cell therapy (Bianco *et al*, 2001) and CNS-directed gene therapy (Hsich *et al*, 2002; Kaye & Sena-Esteves, 2002), these therapies are not yet in the clinical arena.

Chaperone therapy

Lysosomal enzymes may undergo misfolding as a result of mutations in the encoding gene. Misfolded proteins are capable of aggregation and accumulation in cells; this may lead to cell death. They are therefore normally eliminated by the endoplasmic reticulum-associated degradation pathway (ERAD) with the help of naturally occurring molecular 'chaperones', small molecules that ensure their safe degradation via this pathway (Ellgaard & Helenius, 2001; Jarosch *et al*, 2003). In some cases, the active site may fold normally, which means that they are capable of hydrolysis. Such proteins are ideal candidates for salvage by pharmacological 'chaperones'. These are specific, small molecular weight ligands that reversibly bind to such proteins, stabilize them and ensure their correct targeting to the lysosome. An essential prerequis-

ite is that the binding is reversible. That is to say, having safely 'chaperoned' the mutant enzyme to the lysosome, the ligand–protein complex must then dissociate so that the enzyme is free to bind to its substrate. This approach is also known as enzyme enhancement therapy and is attracting considerable interest, especially as it has been shown that chaperones are capable of crossing the BBB and may therefore have therapeutic potential for the CNS.

Such an approach has shown promising preclinical results in at least four enzyme deficiencies: β-glucosidase (Sawkar *et al*, 2002), β-galactosidase (Matsuda *et al*, 2003), α-galactosidase A (Fan *et al*, 1999) and β-hexosaminidase A (Tropak *et al*, 2004). Recently, clinical proof of concept was provided. A patient with a mild form of Fabry's disease (cardiac variant) was treated with intravenous infusions of galactose, a pharmacological chaperone for α-galactose A. There was significant improvement in the cardiac parameters which persisted for more than 3 years (Frustaci *et al*, 2001).

The disadvantage of chaperone-mediated therapy is that it is likely to be effective only in those patients in whom the mutation does not inactivate the catalytic site. However, clearly it merits further study.

Conclusions

Therapy of these disorders has made significant progress in the last two decades. However, significant challenges remain at the cellular level, for example precisely how lysosomal storage causes pathology. There has been an increasing recognition of late that the answers lie within the realm of cell biology rather than molecular genetics, and with researchers beginning to turn their attention to this area, it is hoped that their efforts will reap even richer rewards in the next two decades.

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