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# Biochemistry of glycosphingolipid storage disorders: implications for therapeutic intervention

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The physiological importance of the degradative processes in lysosomes is revealed by the existence of at least 40 distinct inherited diseases, the so-called lysosomal storage disorders. Most of these diseases are caused by a deficiency in a single lysosomal enzyme, or essential cofactor, and result in the lysosomal accumulation of one, or sometimes several, natural compounds. The most prevalent subgroup of the lysosomal storage disorders is formed by the sphingolipidoses, inherited disorders that are characterized by excessive accumulation of one or multiple (glyco)sphingolipids. The biology of glycosphingolipids has been extensively discussed in other contributions during this symposium. This review will therefore focus in depth on (type 1) Gaucher disease, a prototypical glycosphingolipidosis. The elucidation of the primary genetic defect, being a deficiency in the lysosomal glucocerebrosidase, is described. Characterization of glucocerebrosidase at protein and gene level has subsequently opened avenues for therapeutic intervention. The development of successful enzyme replacement therapy for type 1 Gaucher disease is discussed. Attention is also paid to the alternative approach of substrate modulation using orally administered inhibitors of glucosylceramide synthesis. Novel developments about the monitoring of age of onset, progression and correction of disease are described. The remaining challenges about pathophysiology of glycosphingolipidoses are discussed in view of further improvements in therapy for these debilitating disorders.

**Keywords:** glycosphingolipid; lysosomal storage disorder; Gaucher disease

## 1. INTRODUCTION

A characteristic feature of the long-lived eukaryotic cell is the continuous recycling of macromolecular components. Mammalian cells are equipped for this purpose with single membrane-enclosed compartments in which a variety of biological macromolecules can be safely and efficiently degraded. These acid organelles, named lysosomes (De Duve *et al.* 1955), receive substrates for degradation by several routes. Endogenous and exogenous macromolecules are generally imported into lysosomes by membrane flow processes such as endocytosis, pinocytosis, phagocytosis and autophagocytosis. In addition, direct chaperon-mediated import of specific proteins from the cytoplasm has been reported (Holzmann 1989; Dice *et al.* 1990). Lysosomes contain a relatively small set of approximately 60 acid hydrolases and a dozen accessory proteins that allow sequential degradation of almost all macromolecules, including lipids, glycosaminoglycans, oligosaccharides, proteins and nucleic acids (Sandhoff & Kolter 2003). Specific carriers in the lysosomal membrane mediate the export of the products of intralysosomal catabolism to the cytoplasm where they can be reused. The presence of transmembrane proteins with large, highly glycosylated intralysosomal domains protects the lysosomal membrane against self-digestion (Peters & von Figura 1994). The highly lytic action of the lysosomal hydrolases is normally contained in the lysosomal/endosomal compartments by several mechanisms. First, many lysosomal hydrolases show a truly acidic pH optimum, being hardly enzymatically active at near-neutral pH. Second, some lysosomal hydrolases are intralysosomally activated by proteolytic modification or by association with additional cofactors that are required for optimal catalytic activity (Sandhoff & Kolter 2003). Third, newly synthesized or extracellular lysosomal hydrolases are very efficiently delivered into lysosomes. The lectin-based mechanism that governs the selective routing of newly formed acid hydrolases to lysosomes was elucidated two decades ago (Kornfeld & Mellman 1989). Upon co-translational translocation of lysosomal enzymes into the lumen of the endoplasmic reticulum their signal peptide is removed, and specific asparagine residues are glycosylated by transfer of a preformed oligosaccharide from a dolichol phosphate lipid carrier. The glycoproteins are folded, assembled in correct multimeric structures and terminal glucose moieties are removed from their glycans, an important checkpoint in the quality control of protein folding (Helenius 1994). Next, the glycoproteins are exported to the Golgi apparatus where, exclusively, some of their oligosaccharide chains obtain mannose-6-phosphate moieties by a two-step process. The phosphomannosyl moieties act as a specific recognition signal (Gieselmann *et al.* 2003). Selective binding of a major fraction of most lysosomal enzymes to cation-dependent or cation-independent MPRs, allows their segregation from the secretory proteins in the trans-Golgi network. In

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endosomal compartments dissociation of MPRs and lysosomal protein ligands occurs because of local acidity. After uncoupling, the receptor recycles to the Golgi apparatus and the newly formed hydrolases are delivered into lysosomes. The cation-independent MPR is also involved in the delivery to lysosomes of extracellular soluble acid hydrolases containing mannose-6-phosphate residues. Other lectin-type receptors are thought to play additional important roles in uptake of extracellular acid hydrolases, such as the asialo-glycoprotein receptor and mannose receptor in the case of hepatocytes and macrophages, respectively. In sharp contrast, targeting of integral lysosomal membrane proteins is not mediated by phosphomannosyl or other saccharide moieties but by specific peptide motifs in their cytoplasmic domains. Further alternative targeting mechanisms to lysosomes have to exist. Some membrane-associated lysosomal enzymes such as glucocerebrosidase do not acquire any phosphomannosyl moieties in their glycans but are nevertheless efficiently targeted to lysosomes by still unknown mechanisms (Aerts *et al.* 1988). The lysosomal targeting of lysozyme and chitotriosidase in macrophages is also independent of lectin receptors because these enzymes completely lack N-linked glycans (Renkema *et al.* 1997). Moreover, investigations on patients suffering from I-cell disease, in which formation of phosphomannosyl moieties is impaired, have indicated that in hepatocytes and lymphocytes very efficient intracellular sorting of newly formed soluble acid hydrolases can also occur independently of MPRs (Owada & Neufeld 1982). The precise mechanism of the mannose-6-phosphate independent targeting of soluble acid hydrolases is unknown, but it has been suggested that it involves a transient membrane-association in the Golgi apparatus (Rijnboutt *et al.* 1991).

The physiological importance of lysosomes is revealed by the existence of at least 40 distinct inherited diseases, the so-called lysosomal storage disorders (Neufeld 1991). Most of these diseases are due to a deficiency in a single lysosomal enzyme or essential cofactor and result in the lysosomal accumulation of one or sometimes several natural compounds. According to the prevailing stored compound, the lysosomal storage diseases are grouped as mucopolysaccharidoses, sphingolipidoses, mucolipidoses, lipidoses, glycoproteinoses, glycogenoses, ceroid lipofuscinoses and mucopolysaccharidoses. Some of the lysosomal storage diseases are not single enzymopathies but are based on defects in activator proteins, insufficient transport of hydrolytic products across the lysosomal membrane, deficiencies in non-lysosomal proteins involved in lysosome biogenesis or post-translational modification of lysosomal enzymes or inherited abnormalities in intracellular membrane flow.

All lysosomal storage diseases are relatively rare, with an overall birth incidence for the whole group of 1 : 5000–1 : 10 000. The individual incidence of the more prominent lysosomal diseases is estimated to be between 1 : 20 000 and 1 : 100 000 in most populations (Meikle *et al.* 1999; Poorthuis *et al.* 1999). Genetic drift and founder effects have led to unusually high incidences of specific lysosomal storage diseases in some populations. The best examples of this are Gaucher and Tay–Sachs disease among Ashkenazim, and aspartylglucosaminuria, Salla disease and infantile neuronal ceroid lipofuscinosis in Finland (Peltonen 1997). In general, the clinical manifestation of lysosomal storage disorders is remarkably heterogeneous, contributing to the limited awareness of these diseases. The age of onset and progression of disease vary considerably for almost each individual storage disorder. This remarkable phenotypic variability is usually linked to the extent of the deficiency that is determined by the exact nature of the underlying genetic defect. In some lysosomal enzymopathies a strict correlation between residual enzyme activity and severity of disease manifestation exists. A common feature of lysosomal storage disorders is that storage material only accumulates in lysosomes of particular cell types. The nature and residual capacity of the defective metabolic pathway, in combination with the actual flux through this pathway in various cell types, determine the particular cell types that are affected. This phenomenon explains why in some lysosomal storage disorders external genetic or environmental factors that influence the flux through the defective pathway also have a major impact on disease manifestation. The genotype–phenotype relation is therefore not strict in many lysosomal storage disorders.

The most prevalent subgroup of the lysosomal storage disorders is formed by the sphingolipidoses, inherited disorders that are characterized by excessive accumulation of one or multiple (glyco)sphingolipids. Table 1 summarizes the nature and prevalence of this group of diseases. The biology of glycosphingolipids has been extensively discussed in other contributions during this symposium. The remainder of this review will therefore focus in depth on (type 1) Gaucher disease, a prototypical glycosphingolipidosis. Attention will be paid to current insights into the biochemical aspects of the disorder and the progress that has been made in therapeutic correction of the deficiency.

## 2. GAUCHER DISEASE (GLUCOSYLCERAMIDOSIS): A MACROPHAGE DISORDER

Gaucher disease is the most frequently encountered lysosomal storage disorder in man (Barranger & Ginns 1989; Beutler & Grabowski 1995). The French medical student Philippe C. E. Gaucher first described, in 1882, the clinical features of the disease, reporting the presence of large unusual cells in a 32-year-old female with an enlarged spleen. At the beginning of the twentieth century, it was already suggested that the disease was a familial disorder. In 1934, the primary storage material in Gaucher disease was finally identified as glucocerebroside (glucosylceramide). This glycosphingolipid is the common intermediate in the synthesis and degradation of gangliosides and globosides. In 1965, Patrick and Brady *et al.* showed independently that the primary defect in Gaucher disease is a marked deficiency in activity of the lysosomal enzyme glucocerebrosidase (EC. 3.2.1.45) (Brady *et al.* 1966; Patrick 1965).

Inherited deficiencies in glucocerebrosidase result in an accumulation of its lipid substrate in the lysosomal compartment of macrophages throughout the body. Three different phenotypes are recognized, which are differentiated on the basis of the presence or absence of neurological symptoms. More recently, additional phenotypes of Gaucher disease have been identified. For example, complete deficiency in glucocerebrosidase activity results in

Table 1. Birth prevalence (per 100 000) of common sphingolipidoses in The Netherlands (Poorthuis *et al.* 1999).

| disease | enzyme deficiency | prevalence |
|---|---|---|
| Fabry | α-galactosidase A | 0.21 |
| Gaucher | glucocerebrosidase | 1.16 |
| Niemann–Pick type A and B | acid sphingomyelinase | 0.53 |
| Niemann–Pick type C | NPC1/NPC2 | 0.35 |
| Krabbe | galactosylceramidase | 1.35 |
| Sandhoff | β-hexosaminidase A and B | 0.34 |
| Tay–Sachs | β-hexosaminidase A | 0.41 |
| GM1-gangliosidosis | β-galactosidase | 0.41 |
| metachromatic leukodystrophy | arylsulphatase A | 1.42 |

major skin permeability abnormalities with lethal consequences, either prenatally or shortly after birth. The most prevalent variant of the disease is the non-neuronopathic form, named type 1 Gaucher disease. The age of onset and clinical manifestations of type 1 Gaucher disease are highly variable. The most common symptoms include splenomegaly with anaemia and thrombocytopenia, mostly due to hypersplenism, hepatomegaly and bone disease. Anaemia may contribute to chronic fatigue. Thrombocytopenia and prolonged clotting times may lead to an increase in bleeding tendency. Atypical bone pain, pathological fractures, avascular necrosis and extremely painful bone crises may also have a great impact on the quality of life. Type 1 Gaucher disease is relatively common in all ethnic groups. It is prevalent among Ashkenazim with a carrier frequency as high as approximately 1 in 10 and an incidence of approximately 1 in 5000. The most common mutation in the glucocerebrosidase gene of Caucasians, including Ashkenazim, encodes the amino acid substitution N370S. The heteroallelic presence of the N370S mutation is always associated with a non-neuronopathic course (Jonsson *et al.* 1987). It has been demonstrated that the N370S glucocerebrosidase is normally produced and present in lysosomes. Its catalytic activity is only severely impaired at pH values above 5.0, illustrating the subtle nature of the mutation (Van Weely *et al.* 1993*a*). This may contribute to the fact that most, but not all, homozygotes for the N370S mutation do not develop significant clinical symptoms. Twin studies and the poor predictive power of phenotype–genotype investigations in Gaucher disease have clearly indicated that epigenetic factors also play a key role in Gaucher disease manifestation (Aerts *et al.* 1993; Cox & Schofield 1997).

Although glucocerebrosidase is present in lysosomes of all cell types, type 1 Gaucher disease patients solely develop storage of glucocerebroside in cells of the mononuclear phagocyte system. Macrophages participate in the degradation of invading microbes, the natural turnover of blood cells and in tissue modelling. Because of this, it is not surprising that in many of the lysosomal storage disorders accumulation of storage material also takes a prominent place in tissue macrophages. The type 1 variant of Gaucher disease is unique because lysosomal storage occurs exclusively in macrophages. It is believed that the storage material stems from the breakdown of exogeneous lipids derived from the turnover of blood cells. Pseudo-Gaucher cells may be observed in non-Gaucher patients during conditions with markedly increased blood cell turnover. This illustrates that, at least in man, the capacity of glucocerebrosidase in macrophages to cope with glucosylceramide degradation is relatively limited.

The glucocerebroside-loaded cells in Gaucher patients show a characteristic morphology with a 'wrinkled paper'-like appearance of their cytoplasm, which contains lysosomal inclusion bodies; these cells are referred to as Gaucher cells (figure 1). In recent decades, it has become apparent that Gaucher cells are not inert containers of storage material but viable, chronically activated macrophages that contribute to the diverse clinical manifestations of Gaucher disease. Increased circulating levels of several pro-inflammatory cytokines (TNF-α, IL-1 β, IL-6 and IL-8), the anti-inflammatory cytokine IL-10, and M-CSF have been reported (Aerts & Hollak 1997; Cox 2001). It has been suggested that cytokine abnormalities may play a crucial role in the development of common clinical abnormalities in Gaucher patients such as osteopenia, activation of coagulation, hypermetabolism, gammopathies and multiple myeloma and hypolipoproteinaemias. More recently, examination of gene expression profiles by suppressive subtraction hybridization analysis of Gaucher and control spleens has led to the identification of overexpression by Gaucher cells of transcripts for cathepsins B, K and S (Moran *et al.* 2000). It is interesting to note that osteoclast-derived cathepsin K is prominently involved in osseous type 1 collagen destruction. Local release of this cathepsin may therefore contribute to the osteolysis in Gaucher disease. It has to be stressed that it is still unclear to what extent Gaucher cells themselves, or rather their precursors, secrete for themselves the various harmful factors. There is, however, overwhelming evidence that the local presence of Gaucher cells is a prerequisite for initiation of pathological processes.

## 3. THERAPY OF TYPE 1 GAUCHER DISEASE

One of the most attractive candidates among the inherited lysosomal storage disorders for developing effective therapeutic interventions is type 1 Gaucher disease. The molecular basis of the underlying genetic defect has already been established in detail at gene and protein level. Most importantly, just a single cell type, the tissue macrophage, is primarily implicated in the pathophysiology of the disorder. The rationale for therapeutic intervention of type 1 Gaucher disease is therefore relatively simple: correction (or prevention of ongoing formation) of Gaucher cells. This could be accomplished by: (i) supplementation of macrophages with the enzyme glucocerebrosidase (enzyme replacement therapy; Brady (2003)); (ii)

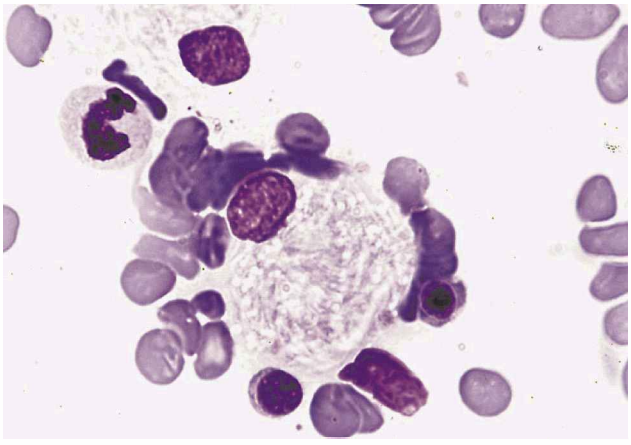


Figure 1. Gaucher cell in bone marrow aspirate of type 1 Gaucher patient.

reduction of glycolipid synthesis with specific inhibitors (substrate deprivation or substrate balancing therapy; Butters *et al.* (2003); Platt *et al.* (2003)); or (iii) introduction of glucocerebrosidase cDNA in haemopoietic progenitors of macrophages (gene therapy; Gieselmann *et al.* (2003)). Other more speculative avenues may be the stabilization of specific mutant forms of the enzyme (chemical chaperons or inhibitors of proteolytic degradation) and the manipulation of intralysosomal pH.

### (a) *Enzyme therapy*

The pioneering work of Brady, Barranger and co-workers at the National Institutes of Health (Bethesda, USA), as well as valuable contributions by many others, has led to a highly effective treatment of type 1 Gaucher disease that is based on chronic intravenous administration of human glucocerebrosidase (Brady 1997; Barranger & O'Rourke 2001). The pioneering attempts to treat type 1 Gaucher disease by infusions with glucocerebrosidase isolated from human placenta had already begun in the early 1970s at the National Institutes of Health. Unfortunately, these did not result in an effective therapy for two compelling reasons. First, too little and insufficiently pure glucocerebrosidase could be generated with the existing technology. Second, most of the administered enzyme was not delivered to macrophages but to other cell types such as hepatocytes. The final development of an effective enzyme replacement therapy for type 1 Gaucher disease relied on a fortunate intersection of scientific disciplines: the discovery of receptors for glycoproteins and the complete purification of glucocerebrosidase. Purification of the protein to homogeneity was achieved in 1977 and subsequently isolation procedures were markedly improved (Murray *et al.* 1985; Aerts *et al.* 1986). In 1974, the first mammalian cell lectin, the asialoglycoprotein receptor was described, followed by identification of a mannose-specific lectin on Kupffer cells in the liver (Ashwell & Morell 1974). The mannose receptor was shown to interact avidly with mannose-terminal glycoconjugates and mediate their delivery into lysosomes (Stahl *et al.* 1978). Barranger, Brady and co-workers realized that this receptor-mediated uptake mechanism could be exploited for therapy of Gaucher disease. Analysis of the carbohydrate composition of placental glucocerebrosidase showed the presence of three complex-type glycans and a single high-mannose-type glycan per molecule (Takasaki *et al.* 1984). The presence of terminal galactose moieties in the glycans of placental glucocerebrosidase provided an explanation for the undesired preferential targeting to hepatocytes. To increase the amount of terminal mannose moieties in placental glucocerebrosidase an *in vitro* method based on sequential enzymatic removal of N-acetylneuraminic acid, galactose and N-acetylglucosamine moieties with exoglycosidases was developed (Furbish *et al.* 1981). The modified 'mannose-terminated' glucocerebrosidase remained fully enzymatically active. It was later demonstrated that a similar mannose-terminated form of the enzyme also occurs naturally in lysosomes of human fibroblasts (Van Weely *et al.* 1990). It is generated by sequential action of lysosomal exoglycosidases during maturation of endogenous glucocerebrosidase. Animal studies with the mannose-terminated placental glucocerebrosidase revealed that the enzyme was delivered differentially to Kupffer cells compared with hepatocytes (Furbish *et al.* 1981). Upon treating a 5-year-old Ashkenazi Jewish boy with the modified placental enzyme, Barranger and co-workers noted promising clinical improvements. In subsequent years, the involvement of industry (Genzyme Corporation, Boston, USA) was required to produce sufficient enzyme for further clinical studies with mannose-terminated placental glucocerebrosidase (Ceredase). In a study in 1990 with 12 type 1 Gaucher patients, Barton and co-workers finally demonstrated unequivocally that two-weekly intravenous administration of Ceredase (130 IU $kg^{-1}$ $month^{-1}$) resulted in a marked improvement in organomegaly and corrections of haematological abnormalities (Barton *et al.* 1991). The spectacular clinical response to enzyme replacement therapy has led to a rapid application worldwide. At present, approximately 3000 type 1 Gaucher patients benefit from therapeutic intervention with Cerezyme, the recombinant form of glucocerebrosidase that has superseded the placenta-derived Ceredase (Grabowski *et al.* 1995).

The introduction of Ceredase was associated with considerable controversy about optimal dosing regimens, further stimulated by concerns about the safety of the incompletely pure placental enzyme preparation and the extreme costs for treatment of adult patients ($50 000 to $500 000 per patient per year). The availability of pure recombinant glucocerebrosidase and clinical investigations on optimal individualized dosing regimens resolved most of the debate (Hollak *et al.* 1995). However, at present little is known about the optimal dosing regimens during maintenance therapy. In many type 1 Gaucher patients enzyme therapy initially results in a fast removal of a proportion of Gaucher cells; however, complete removal is difficult to accomplish. Apparently, a fraction of storage cells is difficult to correct, even upon therapy with a very high dose of enzyme. The true efficacy of targeting of mannose-terminated glucocerebrosidase to macrophages or Gaucher cells is unclear. Investigations in rats have revealed that a major fraction of Ceredase is actually not delivered to macrophages but rather endocytosed by liver endothelial cells (Bijsterbosch *et al.* 1996). This finding is not unexpected, because it has been demonstrated that the mannose receptor is also expressed on these cells (see Linehan *et al.* 1999). Despite elegant studies with radiolabelled enzyme in volunteers, it remains an unanswered

question, to which cells precisely mannose-terminated glucocerebrosidase is delivered in Gaucher patients (Mistry *et al.* 1996). Further research on potential improvements in enzyme delivery to Gaucher cells in various body locations is required.

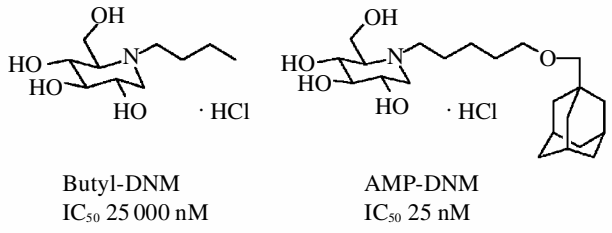
Systemically administered glucocerebrosidase, a glycoprotein of *ca.* 60 kDa, is unable to pass through the blood–brain barrier. The outcome of enzyme replacement therapy for acute neuronopathic (type 2) and severe forms of chronic neuronopathic (type 3) Gaucher disease is disappointing (Erikson 2001). Several clinical investigations have revealed that in the severe neuronopathic Gaucher patients, the effects of enzyme replacement therapy on visceral and haematological symptoms are good, but the fatal neurological deterioration continues. Accumulation of glucocerebroside and its metabolite glucosylsphingosine inside the brain underlies the severe neuropathology of these patients. Importantly, milder forms of type 3 Gaucher disease, where the chronic neuronopathic disease is primarily caused by perivascular storage cells, respond well to enzyme replacement therapy, and treatment with a high dose enzyme regimen is recommended by the European Working Group on Gaucher Disease (Vellodi *et al.* 2001). Perivascular macrophages in the brain are known to express mannose receptor (Linehan *et al.* 1999).

### (b) *Substrate deprivation therapy*

An alternative approach for therapeutic intervention of type 1 Gaucher and other glycosphingolipidoses is substrate deprivation (also termed substrate reduction) therapy. This challenging concept was first formulated by Radin and co-workers (for a review, see Radin (1996)). The approach aims to reduce the rate of glycosphingolipid biosynthesis to levels that match the impaired catabolism. The concept is that patients who have a significant residual lysosomal enzyme activity could gradually clear lysosomal storage material and therefore should profit most from reduction of substrate biosynthesis.

Two main classes of inhibitor of glycosphingolipid biosynthesis have already been described, both of which inhibit the ceramide-specific glucosyltransferase (also termed glucosylceramide synthase; GlcT-1; UDP-glucose: *N*-acylsphingosine D-glucosyl-transferase, EC 2.4.1.80). The enzyme catalyses the transfer of glucose to ceramide, the first step of the biosynthesis of glucosphingolipids. The first class of inhibitor is formed by analogues of ceramide. The prototype inhibitor is PDMP (D, L-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol). More specific and potent analogues have subsequently been developed based on substituting the morpholino group for a pyrrolidino function and by substitutions at the phenyl group: 4-hydroxy-1-phenyl-2-palmitoylamino-3-pyrrolidono-1-propanol (p-OH-P4) and ethylenedioxy-1-phenyl-palmitoylamino-3-pyrrolidino-1-propanol (EtDo-P4) (Lee *et al.* 1999). Studies in a knockout mouse model for Fabry disease have shown that oral administration of the compounds can result in a marked reduction of the accumulating glycosphingolipid globotriaosylceramide (Abe *et al.* 2000).

The second class of inhibitor of glucosylceramide synthase is formed by N-alkylated iminosugars (see figure 2). Such types of compounds were already in common use as inhibitors of N-glycan-processing enzymes and the potential application of NB-DNJ as an HIV inhibitor had been studied in AIDS patients. Platt and Butters, at the Glycobiology Institute in Oxford, were the first to recognize the ability of NB-DNJ to inhibit glycosylceramide synthesis at low micromolar concentrations (Platt *et al.* 1994). The same researchers demonstrated significant reductions in glycosphingolipid storage in the brain in knockout mouse models of Tay–Sachs disease and Sandhoff disease (Jeyakumar *et al.* 1999). Preclinical studies in animals and the previous clinical trial in AIDS patients, have indicated (transient) adverse effects in the gastrointestinal tract, probably related to the ability of NB-DNJ to inhibit disaccharidases on the intestinal brush border. Animal studies have shown that the galactose analogue N-butyldeoxygalactonojirimycin may have the same therapeutic efficacy as NB-DNJ, but does not cause gastrointestinal side effects (Andersson *et al.* 2000). Overkleeft and co-workers, in their search for inhibitors of glucosidases, have serendipitously developed a more potent inhibitor of glucosylceramide synthase. Adamantane-pentyl-deoxynojirimycin was found to inhibit glycosphingolipid biosynthesis at low nanomolar concentrations (Overkleeft *et al.* 1998) and was able to prevent globotriaosylceramide accumulation in a Fabry knockout mouse model without overt side effects (D. Copeland, personal communication).




Butyl-DNM
$IC_{50}$ 25 000 nM

AMP-DNM
$IC_{50}$ 25 nM

Figure 2. Structures of deoxynojirimycin-type inhibitors of glycosylceramide synthase. Butyl-DNM: butyldeoxynojirimycin; AMP-DNM: adamantane-pentyl-deoxynojirimycin.

The first clinical study of the use of NB-DNJ to treat a glycosphingolipid storage disorder has been reported recently (Cox *et al.* 2000). In an open-label phase I/II trial 28 adult type 1 Gaucher patients received 100 mg of NB-DNJ three times daily (OGT 918; Oxford GlycoSciences). Improvements in visceromegaly and haematological abnormalities as well as corrections in plasma levels of glucosylceramide and biomarkers of Gaucher disease activity have been described, although the extent of the response is less spectacular than generally observed with enzyme replacement therapy. As expected, a dose–response relationship is demonstrable for NB-DNJ in type 1 Gaucher patients. It has been reported that administration of 50 mg of NB-DNJ three times daily is far less effective (Heitner *et al.* 2002). Very recently, the European Medicines Evaluation Agency (EMEA, the European equivalent of the US Food and Drugs Administration) has granted marketing authorization of NB-DNJ ('Zavesca', miglustat, Oxford GlycoSciences) for treatment of type 1 Gaucher patients who are unsuitable to receive enzyme replacement therapy. Despite the imminent registration of the drug in Europe, important insights will still have to be gained about clinical efficacy and safety. Provided iminosugars or other inhibitors of glucosylceramide synthase prove to be safe in the long term, they should have

an important role to play in the management of glycosphingolipid storage disorders, including Gaucher disease.

### (c) *Gene therapy*

Because tissue macrophages are derived from the bone marrow, it is logical that curative bone marrow transplantations have been reported for some patients with Gaucher disease (Ringden *et al.* 1995). The risks of allogeneic transplantation, however, do not justify this approach in patients with milder forms of the disease. The observed efficacy of enzyme replacement therapy and bone marrow transplantation has stimulated the pursuit of gene therapy for Gaucher disease. Three independent studies of gene transfer to the haemopoietic cells of Gaucher patients have been done but none produced encouraging results (Richter & Karlsson 2001). Low transduction efficiencies of CD34 cells and no sustained expression of glucocerebrosidase in white blood cells have contributed to this. The development of gene therapy strategies to correct haematological and genetic disorders has been hampered by the low levels of gene transfer into human stem cells using vectors derived from oncoretroviruses. Much interest has recently been focused on vectors derived from lentiviruses that have been shown to transduce a variety of non-dividing cells, including haemopoietic cells (Richter & Karlsson 2001). The use of such vectors and new developments for macrophage-specific gene targeting may open novel possibilities for effective gene therapy of Gaucher disease in the future.

### (d) *Monitoring of therapeutic correction*

Considerable attention has been paid in type 1 Gaucher disease to treatment goals and the monitoring of response to therapeutic interventions (Cox 2001; Hollak & Aerts 2001). The definition of treatment goals has to depend on clinical endpoints or surrogate endpoints that can predict clinical benefit based on epidemiological, pathophysiological or other scientific evidence. Because of the burden imposed by chronic intravenous infusions and the high costs associated with enzyme therapy, as well as the uncertainty about dose-dependent, long-term adverse effects of iminosugar therapy, it seems wise to establish for the individual Gaucher patient the minimal dose of drug required for effective intervention. In severely affected patients, the initial response to therapy can be accurately assessed by determination of spleen and liver volumes, haemoglobulin level and platelet count. During maintenance therapy these clinical parameters are, however, of little value. Monitoring of the effect of therapy on bone disease is complicated and has usually been restricted to documentation of the occurrence of bone crises, pathological fractures or the need for surgical intervention. More recently, quantitative chemical shift imaging has been applied to study the triglyceride content of lumbar bone marrow (Hollak & Aerts 2001). The fat fraction of the bone marrow is variably reduced in Gaucher disease owing to the displacement of normal triglyceride-rich adipocytes by Gaucher cells. It has been noted that a marked reduction in bone marrow fat fraction is predictive for the occurrence of bone complications. A marked correction in bone marrow fat content after therapy can be therefore defined as a treatment goal (Hollak *et al.* 2002).

A search for plasma abnormalities in Gaucher disease has led to the discovery of a marked elevation in chitotriosidase, a hitherto unknown human chitinase (Hollak *et al.* 1994). In symptomatic Gaucher patients plasma chitotriosidase levels were found to be approximately 1000-fold higher than in normal individuals. It has been shown, subsequently, that Gaucher cells are the source of this hydrolase in plasma and that the elevated levels are an indicator of the burden of storage cells in a patient. Chitotriosidase is synthesized in the pathological macrophages, and its elevated activity correlates with tissue glucosylceramide storage as well as clinical parameters of disease severity. Enzyme replacement therapy, substrate deprivation therapy or bone marrow transplantation rapidly reduces the plasma chitotriosidase activity, (see figure 3). To assess the utility of chitotriosidase activity measurements as a biomarker for treatment efficacy, the relationship and clinical parameters have been studied (Hollak & Aerts 2001). Based on this investigation, it has been proposed that in patients in whom initiation of treatment is questionable, based solely on clinical parameters, a chitotriosidase activity above 15 000 nmol $ml^{-1}$ $h^{-1}$ may serve as an indicator of a high Gaucher cell burden and an indication for the initiation of treatment. A reduction of less than 15% after 1 year of treatment should be a reason to consider a dose increase. Furthermore, a sustained increase in chitotriosidase at any point during treatment should alert the physician to the possibility of clinical deterioration and the need for dose adjustment. The assay of chitotriosidase activity is complicated by the existence of apparent substrate inhibition due to transglycosidase activity (J. M. Aerts, in preparation). Another pitfall results from the complete absence of the enzymatic activity in *ca.* 6% of all individuals. This results from homozygosity for a null allele of the chitotriosidase gene (Boot *et al.* 1998). Plasma chitotriosidase levels in heterozygotes for this mutation (*ca.* 35% of all individuals) underestimate the actual presence of Gaucher cells in patients. Determination of chitotriosidase genotype in Gaucher patients is therefore recommended.

Chitotriosidase has been characterized in detail at the gene and protein level (Boot *et al.* 1995, 1998; Renkema *et al.* 1995, 1997). The enzyme mimics lysozyme in several aspects. It is also selectively expressed in phagocytes, particularly in chronically activated macrophages, and likewise is a compact globular endoglucosaminidase lacking N-linked glycans. The physiological role of chitotriosidase also seems to be found in innate immunity. It has been observed in studies with *Candida albicans* and *Aspergillus fumigatus* that the enzyme exerts a potent fungistatic effect by selective lysis of the growth tip of hyphae. The molecular basis for the massive overexpression of chitotriosidase in Gaucher cells and in related foam cells observed in arteriosclerosis, sarcoidosis, Wolman disease and Niemann–Pick disease is still unknown and the subject of ongoing investigation.

### (e) *Prospects for therapy of type 1 Gaucher disease*

Enormous progress has been made in the therapy of type 1 Gaucher disease, a severely debilitating disorder characterized by intralysosomal storage of glucocerebroside in tissue macrophages. A highly effective therapy based on chronic intravenous administration of mannose-

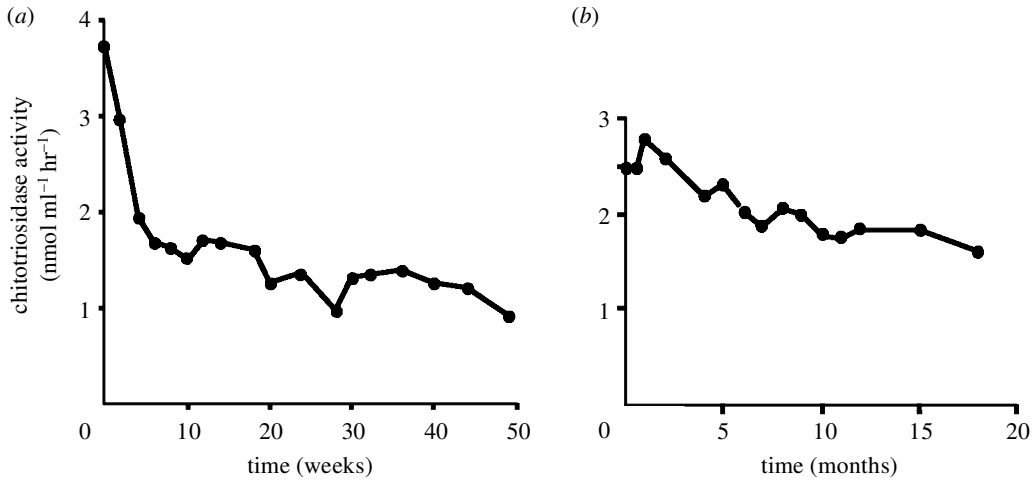



Figure 3. Corrections in elevated plasma chitotriosidase after therapeutic intervention. (*a*) The response of the first type 1 Gaucher patient treated in continental Europe by intravenous administration of Ceredase (48 IU kg$^{-1}$ month$^{-1}$). (*b*) The response of first type 1 Gaucher disease patient treated in continental Europe by oral administration of butyldeoxynojirimycin (300 mg day$^{-1}$).

terminated recombinant human glucocerebrosidase is available. During the past decade, this therapy has been applied in several thousands of patients without serious adverse effect. Moreover, for the same orphan disease promising clinical responses have been observed upon oral administration of an iminosugar inhibitor of glucosylceramide synthesis. Provided long-term treatment with such inhibitors is without adverse effects, substrate deprivation therapy (in conjunction with enzyme replacement therapy) may play an important role in the future clinical management of patients suffering from glycosphingolipid storage disorders. Progress in vector technology and selective expression of the transgene in macrophages seem to be essential requirements before gene therapy can fulfil its promise as cure for type 1 Gaucher disease.

Despite the success of the present enzyme replacement therapy with Cerezyme, the question should be raised whether the enzyme supplementation treatment can be further improved to be more economic and widely available. It is unclear, for example, what percentage of the mannose-terminated Cerezyme is actually endocytosed by tissue macrophages and storage cells and what percentage is 'wasted' in other cell types such as liver endothelial cells. The occurrence and consequences of binding of the therapeutic enzyme to receptors other than the mannose-receptor, to soluble receptor fragments like sMR or to serum mannose-binding lectins still warrants further examination. Little attention has so far been paid to the expression of the mannose receptor on macrophages and other cells types of Gaucher patients. Increased knowledge of this matter may give valuable clues for further improvement of the current enzyme replacement therapy. Similar considerations can be made for substrate deprivation therapy. In type 1 Gaucher disease one would prefer to selectively inhibit the synthesis of glucosylceramide in blood cells. More selective targeting of drugs to blood cells might therefore result in major improvement of efficacy and reduce the risk of side effects. The design of more potent and selective inhibitors of glycosphingolipid synthesis is another interesting avenue.

## 4. NOVEL CHALLENGES

The molecular basis of most inherited glycosphingolipidoses has been elucidated in recent decades. Rational approaches for therapeutic intervention have evolved and in type 1 Gaucher disease effective intervention has become available. By analogy, enzyme replacement therapy with phosphomannose-terminated α-galactosidase A is now applied for Fabry disease. Despite the fact that Fabry disease is a true orphan disease, two different enzyme preparations have recently been simultaneously registered in Europe (Replagal (TransKaryotic Therapies) and Fabrazyme (Genzyme)). Thorough comparative clinical investigations must now reveal whether one of the enzyme preparations is superior in clinical benefit and cost efficacy (Blom *et al.* 2003).

A major limitation in developing and improving therapy remains the lack of mechanistic insight into the pathophysiological processes underlying the various manifestations of glycosphingolipidoses. It is still not understood why, and how, chronic accumulation of glycosphingolipids in lysosomes affects various cell types and triggers eventually pathology. Further studies on inter- and intracellular signalling events are highly desirable. In this connection, the non-lysosomal glyucosylceramidase activity may be particularly relevant. Our research group discovered the occurrence of non-lysosomal glycosylceramide catabolism several years ago (Van Weely *et al.* 1993*b*). The enzyme activity is not affected in Gaucher patients and shows a different inhibitor specificity, all pointing to a product from a distinct gene (Overkleeft *et al.* 1998). It remains unclear to what extent the non-lysosomal glucosylceramidase plays a role in the pathophysiology of Gaucher cells. Its activity might be compensatory to a deficiency in lysosomal catabolism. However, it is also conceivable that excessive extra-lysosomal glucosylceramide catabolism results in excessive production of cytosolic ceramide that may act as a signalling molecule and cause abnormal cell behaviour (see figure 4). It is of interest to note that the hydrophobic deoxynojirimycins are also potent inhibitors

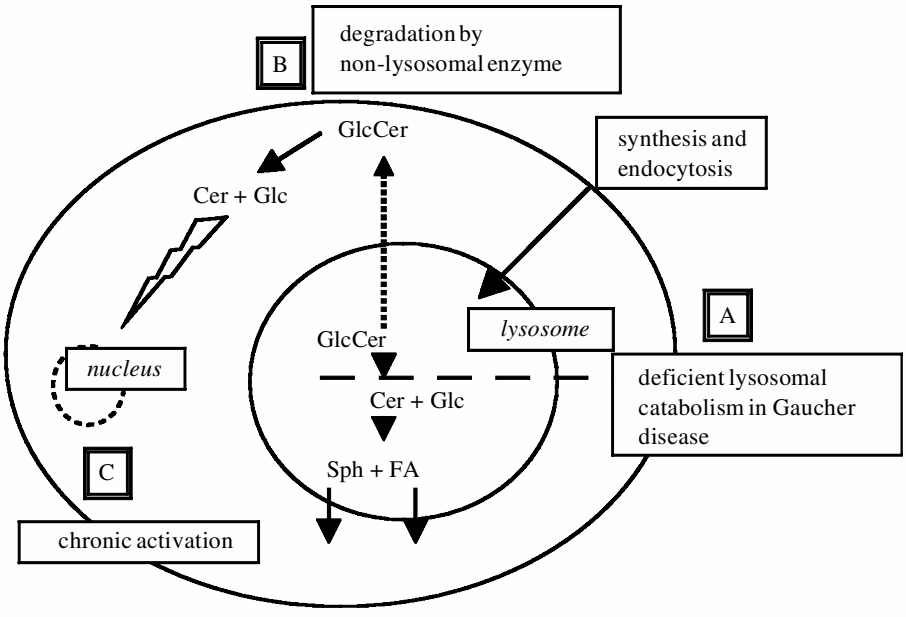



Figure 4. Hypothetical role for the non-lysosomal glucocerebrosidase in Gaucher cell pathology. Normal lysosomal degradation of glucosylceramide does not result in generation of ceramide. The excessive extra-lysosomal glucosylceramide catabolism in Gaucher macrophages results in excessive production of cytosolic ceramide that may act as a signalling molecule and cause abnormal cell behaviour.

of the non-lysosomal glucosylceramidase (Overkleeft *et al.* 1998). Current approaches of substrate deprivation are consequently also likely to inhibit the non-lysosomal catabolism of glucosylceramide.

In recent years, our research group has employed conventional proteomics technologies (two-dimensional gel electrophoresis and mass finger printing, LC-MS/MS) to gain additional insights into the molecular actors in the pathophysiology of Gaucher disease. More recently, a comparative analysis was made of Gaucher spleen and serum samples using SELDI-technology (Protein Biomarker, Ciphergen). This type of investigation has led to the identification of a series of novel protein abnormalities associated with symptomatic Gaucher disease. Most striking is the dramatic production by Gaucher cells of a chemokine, resulting in several 10- to 100-fold elevated serum levels (R. Boot and T. M. Cox, unpublished data). Recent histochemical studies indicate that true Gaucher cells should probably not be viewed as highly inflammatory cells, but rather as alternatively activated macrophages (ongoing collaborative study with J. Laman and S. Gordon and their co-workers). The elevated serum levels of pro-inflammatory cytokines in patients seem most probably due to excessive production by macrophages surrounding mature storage cells.

A very distinct but equally important future challenge forms the identification of the adaptations that occur in patients suffering from glycosphingolipidoses in response to gradually developing, chronic abnormalities in particular cell types. It can be envisioned that such research lines may provide valuable information on mechanisms that prevent or limit disease manifestation. Novel insights in this area may be applicable for other disease conditions.

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## GLOSSARY

MPR: mannose-6-phosphate receptor
NB-DNJ: *N*-butyldeoxynojirimycin