Chapter 5

Gaucher Disease

Enzymology, Genetics, and Treatment

Gregory A. Grabowski

*Division of Human Genetics
Children's Hospital Medical Center
Cincinnati, Ohio 45229-2899*

INTRODUCTION

The eponym “Gaucher disease” designates the heterogeneous sets of signs and symptoms in patients with severely decreased intracellular hydrolysis of glucosylceramide (Fig. 1) and related glucosphingolipids. These autosomal recessively inherited disorders result almost exclusively from mutations in the gene encoding the lysosomal hydrolase, acid β -glucosidase or glucocerebrosidase (EC 3.2.1.45). Rare examples of mutations at the prosaposin or “activator” locus also lead to severe Gaucher disease-like phenotypes with glucosylceramide accumulation. At the clinical, biochemical, and molecular levels, marked heterogeneity has been delineated since the first description by Phillippe C. E. Gaucher in 1882 (Gaucher, 1882). Since this original description, massive splenomegaly and unusual, large storage cells in the spleen and bone marrow have become the hallmarks of Gaucher disease. In addition, this disease was the first lysosomal storage disease described (Gaucher, 1882), the second to have its enzymatic defect delineated (Brady *et al.*, 1965; Patrick, 1965), and the first to be successfully treated by enzyme therapy (Barton *et al.*, 1991; Beutler *et al.*, 1991b; Fallet *et al.*, 1992). The disease is the most frequent lysosomal storage disease and the most prevalent Jewish genetic disease ($q \sim 0.04$ – 0.05). This high frequency, very good genotype/phenotype

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Fig. 1. Diagram of the active site of acid β -glucosidase containing glucosylceramide. Aspartates 358 and 443 are located near or in the active site and aspartate 443 has been identified as a binding site for Br-conduritol B epoxide, an active site-directed covalent inhibitor. The negatively charged residue near D443 and the functional group near D358 are the proposed locations of the nucleophile and proton donor necessary for catalysis. Asn 370 has been localized to the active-site region by site-directed mutagenesis and an allele encoding N370S is the most common Gaucher disease allele. The three "sites" and the hydrophilic region have been proposed by kinetic analyses to contain residues important for the interaction with substrate substituents. Continuity of the amino acid sequence is not implied in the figure.

correlations in the Ashkenazi Jewish population, and the occurrence of many asymptomatic to very mildly affected individuals make Gaucher disease a prototype for genetic susceptibility testing, with the attendant counseling and social concerns.

CLINICAL CLASSIFICATION

Three major types of Gaucher disease have been delineated based on the absence (type 1) or presence and severity (types 2 and 3) of primary central

TABLE I. Gaucher Disease: Clinical Types

Clinical feature	Type 1	Type 2	Type 3
Onset	Childhood/adulthood	Infancy	Childhood
Hepatosplenomegaly	+	+	+
Hypersplenism	+	+	+
Bone crises/fractures	+	—	+
Neurodegenerative course	—	+++	++
Survival	6–80+ years	<2 years	Second to fourth decade
Ethnic predilection	Ashkenazi Jewish	Panethnic	Swedish Norrbottnian
Frequency	~1/40,000–1/60,000, 1/400–1/600 (Ashkenazi Jewish)	<1/100,000	~1/50,000

nervous system involvement (Table I). To avoid the nonspecific descriptors “adult,” “infantile,” and “juvenile,” the following nosology has been adopted: type 1—nonneuronopathic; type—acute neuronopathic; type 3—subacute neuronopathic (Frederickson and Sloan, 1978). Within each type, however, even within the same ethnic and/or demographic groups, the phenotypes and genotypes can be markedly heterogeneous.

Gaucher Disease Type 1

The most common form of the disease is type 1, which is characterized by the lack of neuronopathic involvement. Estimates of the disease frequency range between 1/40,000 and 1/60,000 in the general population. These estimates reach 1/400 to 1/600 in the Ashkenazi population (Zimran *et al.*, 1991; Beutler and Grabowski, 1993). The clinical manifestations of Gaucher disease type 1 result from hypertrophy and hyperplasia of macrophages in various organs (Desnick, 1982; Parkin and Brunning, 1982). This process leads to dysfunction of the liver and spleen, displacement of bone marrow elements by storage cells, and bony involvement leading to infarctions and fractures. Other organs of the monocyte/macrophage system also can be involved, including the lungs (pulmonary macrophages), Peyer’s patches, and lymph nodes. The heart and kidneys are rarely, if ever, involved by the disease process (Beutler and Grabowski, 1993).

The phenotypic variability of Gaucher disease type 1 is striking. Onset of clinical manifestations or age at diagnosis can occur from childhood to adulthood. In 165 unrelated symptomatic patients, the mean age at onset was 21.1 years

(range = 2–73 years) with a median age of 16 years (Sibille *et al.*, 1993). Also, asymptomatic or mildly affected, enzyme-deficient, “patients” have been diagnosed in the eighth and ninth decades of life (Berrebi *et al.*, 1984; Medoff and Bayrd, 1954; Beutler, 1977). Many of these individuals have been ascertained in the course of family studies or population surveys (Beutler, 1977; Zimran *et al.*, 1991). In comparison, affected children have been described with massive hepatosplenomegaly as well as liver function abnormalities, pancytopenias, and extensive skeletal abnormalities. The complications of Gaucher disease in the first or second decade of life may lead to demise in such severely involved patients.

In addition to the variation of the age at onset, there is great variability in the organ manifestations and degree of organ involvement among patients. Nearly all symptomatic patients present with splenomegaly. Some patients with severe visceral (liver and spleen) enlargement are largely spared skeletal involvement and vice versa. In other patients visceral involvement and skeletal involvement are approximately equal in severity.

Gaucher Disease Type 2

This variant is rare, with estimated frequencies of less than 1/100,000 live births. There is a moderate degree of heterogeneity in the onset and course of the acute neuronopathic form of the disease. Early and late onset varieties have been delineated (Kolodny *et al.*, 1982), but the overall clinical course is similar. The principal difference is in the time of onset, which varies between birth and 4–6 months. Indeed, Gaucher disease has been reported to manifest as hydrops fetalis (Sun *et al.*, 1984) and the author knows of additional cases where this has been the outcome. Also, severe neonatal ichthyosis (“collodion babies”) has been described and resembles the skin abnormalities in the “knock-out” mouse model (Tybulewicz *et al.*, 1992).

Extensive visceral involvement with hepatosplenomegaly is the rule. Oculomotor abnormalities are often the first manifestations with the appearance of bilateral fixed strabismus (Kolodny *et al.*, 1982) or of oculomotor apraxia. This may result in the appearance of rapid head thrusts as an attempt to compensate when following a moving object (Conradi *et al.*, 1991). Hypertonia of the neck muscles with extreme fixed retroflexion of the neck (opisthotonos), bulbar signs, limb rigidity, seizures, and, sometimes, choreoathetoid movements occur, although the latter are more common in type 3 disease. Most infants with type 2 disease die within the first 2 years of life. An exhaustive review of the clinical manifestations in type 2 disease is available (Frederickson and Sloan, 1978).

Gaucher Disease Type 3

In the general population Gaucher disease type 3 has an estimated frequency of 1/50,000 to 1/100,000 live births. The severity of type 3 disease is intermediate between that of types 1 and 2. Massive visceral involvement is usually present, although exceptions have been reported (Wenger *et al.*, 1983). Neurologic symptoms similar to those observed in type 2 disease are present but with a later onset and relatively lesser severity. The prototype of type 3 disease is the Norrbottnian form. The clinical manifestations have been described in detail (Tibblin *et al.*, 1982; Kyllerman *et al.*, 1990; Dreborg *et al.*, 1980; A. Erikson, 1986). The median age of onset of symptoms is 1 year, with a range in 22 patients of 1.4–14.2 years. The first symptoms are usually the result of visceral involvement, with neurologic findings developing in about half of the children during the first decade of life. As in type 2 disease, disorders of eye movement (Sidransky *et al.*, 1992) are the usual first symptoms with the subsequent development of other neurologic manifestations such as ataxia. Even in the Norrbottnian population with a homogeneous genetic mutation at the acid β -glucosidase locus, there is extensive phenotypic heterogeneity (A. Erikson *et al.*, 1987; A. Erikson and Wahlberg, 1985; Dreborg *et al.*, 1980). The variation in genetically heterogeneous populations would be expected to be greater.

SPECIFIC ORGAN SYSTEM INVOLVEMENT

Spleen

Although normal or nearly normal size spleens can be present in asymptomatic patients, splenomegaly is present in all symptomatic patients by physical examination or scanning procedures (Hill *et al.*, 1986). The degree of splenic enlargement is highly variable. Spleen sizes measured at autopsy (R. E. Lee, 1982) or surgery (Aufses and Salky, 1982), by computed tomography (Fallet *et al.*, 1992), or by magnetic imaging scans (Barton *et al.*, 1992; Beutler *et al.*, 1991b) range from about 5 to over 75 times normal size when adjusted for body weight [$\sim 0.2\%$ of body weight (Ludwig, 1979)]. The absolute sizes range from a few hundred grams to about 10 kg and can account for 15–25% of body weight. In a series of 72 patients (age 2–69 years) the mean splenic size as determined by quantitative CT scan was 17.9-fold greater than predicted (range 1.8–75, median 14-fold) (Sibille *et al.*, 1992). In proportion to body weight, the largest spleens were present in the younger severely affected patients with Gaucher disease types

1 and 3 (R. E. Lee, 1982; Stevens *et al.*, 1987; Sibille *et al.*, 1993). Exceptions have been observed with patients 40–70 years old having spleens 25- to 50-fold increased in weight (Fallet *et al.*, 1992). Although not systematically studied, the spleen appears to enlarge most rapidly in children. Acceleration of splenic enlargement in adults should prompt evaluations for other causes of blood dyscrasias: e.g., malignancies, immune-mediated thrombocytopenia (Lester *et al.*, 1984), and autoimmune hemolytic anemia (Haratz *et al.*, 1990) have been documented in patients with Gaucher disease.

Nodules on the surface of the spleen result from regions of extramedullary hematopoiesis, collections of Gaucher cells, or resolving fibrotic infarcts (R. E. Lee, 1982). Evidence of old splenic infarcts can be found in nearly all spleens that are 20-fold larger than normal. Most infarcts have been asymptomatic, but sub-capsular infarcts can present as localized abdominal pain. Usually point tenderness can be elicited directly over the involved area of the spleen and the pain resolves within a few days to 1 week. The spleen is usually very hard. Ultrastructural studies have demonstrated active erythrophagocytosis by Gaucher cells (Elleder and Jirásek, 1981) as well as the extrusion of stored glucosylceramide tubular structures into the extracellular spaces in the spleen (Hibbs *et al.*, 1970).

Liver

Hepatomegaly occurs in over 50% of patients with Gaucher disease type 1 (James *et al.*, 1982). In a series of 88 patients, liver volumes ranged from 0.74 to 8.7 times the predicted normal (2.5% of body weight) with a mean value of 1.84 and a median value of 1.75 (Sibille *et al.*, 1983). Massively enlarged livers are usually hard and can have irregular surfaces. Frank hepatic failure and/or cirrhosis with portal hypertension and ascites are uncommon (R. E. Lee, 1982; James *et al.*, 1982), but do occur in a small percentage of patients, and bleeding from varices (Fellows *et al.*, 1975; Henderson *et al.*, 1991) may cause death. Minor abnormalities of liver function tests (increases in plasma transaminase and gamma-glutamyl transferase) are commonly present, even in mildly affected patients. Jaundice attributable to Gaucher disease is a very poor prognostic sign.

Hepatic involvement by Gaucher disease is always evident on biopsy by the presence of Gaucher cells, i.e., abnormal Kupffer cells, in the liver sinusoids. Importantly, even in the presence of extensive replacement of liver tissue by these Gaucher cells, the hepatocytes do not manifest grossly apparent glucosylceramide storage (R. E. Lee, 1982; James *et al.*, 1981). This sparing of the hepatocytes probably accounts for the low incidence of severe hepatic failure. Consistent with

the monocyte/macrophage origin of Kupffer (Gaucher) cells was the recurrence of severe hepatic involvement following transplantation of a liver from a normal donor into a patient with Gaucher disease (Carlson *et al.*, 1990).

Hematologic Manifestations

Bleeding can occur from thrombocytopenia. Deficiencies of factor XI are prevalent, but may be coincidental because a deficiency of this clotting factor is common in Ashkenazi Jews (Seligsohn *et al.*, 1976; Berrebi *et al.*, 1992), the population that is most frequently affected by Gaucher disease.

Abnormalities of blood-formed elements are frequent, and thrombocytopenia is the most common in nonsplenectomized patients (Medoff and Bayrd, 1954). Early in the course of the disease it is usually due to splenic sequestration of platelets and responds to splenectomy (A. Zimran, unpublished). Later in the course of the disease, particularly following splenectomy, replacement of the marrow by Gaucher cells may lead to decreased production of megakaryocytes and other bone marrow elements. Anemia is usually mild to moderate, although severe anemias do occur. Leukopenia also occurs in some patients usually due to a combination of increased splenic sequestration, when the spleen is present, and decreased production because of replacement of the marrow by Gaucher cells. Isolated leukopenia is likely due to intercurrent illness and requires extensive evaluation. Bone marrow failure and myelofibrosis can occur in a small number of patients.

Bones

The skeletal manifestations of Gaucher disease can be totally debilitating (Stowens *et al.*, 1985). By radiographic (Stowens *et al.*, 1985; Hermann *et al.*, 1986; Beighton *et al.*, 1982; Goldblatt *et al.*, 1988; Myers *et al.*, 1975), scintigraphic, computed tomographic, or magnetic imaging scans (Hermann *et al.*, 1986; Rosenthal *et al.*, 1986; Cremin *et al.*, 1990) nearly all affected patients have bony lesions. The “Erlenmeyer flask deformity” of the distal femur is a common (~30%) radiographic finding, but is not universally present. Generalized bone loss has been documented (Hermann *et al.*, 1984, 1986).

Many patients with radiographically significant to severe Gaucher disease of bone have few or no bony symptoms. Episodic, excruciatingly painful “bone crises” occur in about 20–40% of patients (Goldblatt and Beighton, 1982). Fever occurs in patients with Gaucher disease, often (Amstutz, 1973; Draznin and

Singer, 1948), but not always (Billings *et al.*, 1973) in connection with bone crises. These are much more frequent in children and adolescents, but can occur for the first time in the third to seventh decades. Based on observations in several hundred type 1 and type 3 patients, the majority of severe bony lesions seem to appear during childhood and adolescence. This aggressive phase of bony involvement is followed in adults by a more slowly progressive process or a cessation of active bony destruction as assessed by current radiographic and MRI techniques. These observations provide a rationale for the institution of corrective therapy early in the course of the disease.

As summarized in Table II, marked variations have been reported in the degree and type of bone involvement (Stowens *et al.*, 1985; Hermann *et al.*, 1986; Cremin *et al.*, 1990). The first abnormality is the loss of calcification of trabecular bone or osteoporosis (Stowens *et al.*, 1985; Beighton *et al.*, 1982). Almost all bones have been involved, including, in approximate decreasing frequency, the femoral necks and heads, femoral shaft, humeri, vertebral bodies, tibias, ribs, pelvis, bones of the feet, calvarium, and mandible (Bildman *et al.*, 1972) [see Frederickson and Sloan (1978) for review]. Complete loss of bone marrow perfusion has been observed (Hermann *et al.*, 1986). The initiating event and pathophysiology of Gaucher disease of the bones remain poorly understood.

Lungs

Although it is uncommon, pulmonary involvement in Gaucher disease has a very severe prognosis. It may occur as a result of frank infiltration of lung by

TABLE II. Radiologic Stages of Skeletal Lesions in Gaucher Disease Type 1^a

Stage	Type of lesion/site involved	Radiographic appearance
1	Diffuse osteoporosis/tubular bones and vertebrae	Course trabecular pattern of osteoporosis
2	Medullary expansion/femora, long bones, ribs	Loss of normal concavity above femoral condyles; "Erlenmeyer flask" deformity
3	Localized destruction (osteolysis)/long bones	Small erosions (well-defined or moth-eaten); cortex rarefied and endosteal notching; ground-glass veiling
4	Ischemic necrosis, sclerosis, osteitis/long bones	Patch densities and erosions; serpiginous sclerotic streaks; layered periostitis; sequestra
5	Diffuse destruction; epiphyseal collapse; osteoarthritis/hips, shoulders, vertebrae, sacroiliac joints	Flattening or irregular destruction of femoral or humeral heads; mixed lytic and sclerotic foci; larger "soap-bubble" pattern

^aAdapted from Hermann *et al.* (1986) and Beighton *et al.* (1982).

Gaucher cells, particularly in children (Schneider *et al.*, 1977), but severe pulmonary disease also occurs in patients without demonstrable Gaucher cells in the lungs. In such patients right-to-left intrapulmonary shunting, probably secondary to liver disease, seems to be the cause.

Nervous System

The absence of primary central nervous system involvement defines Gaucher disease as type 1. However, occasionally central nervous symptoms can be observed as a secondary manifestation. Thus, massive systemic fat emboli involving the brain and lung have been reported (Melamed *et al.*, 1975). Compression of the spinal cord secondary to vertebral collapse has occurred (Markin and Skultety, 1984; Hermann *et al.*, 1989). In the author's experience with six cases and in the few autopsy reports (R. E. Lee, 1982) on type 1 patients, periadventitial accumulation of Gaucher cells has been the only consistent abnormality in the nervous system.

Microscopic abnormalities have been found in all patients dying of type 2 Gaucher disease (R. E. Lee, 1982; Banker *et al.*, 1962; Norman *et al.*, 1956; Espinas and Faris, 1969; Lacey and Terplan, 1984; Adachi *et al.*, 1967; Kaye *et al.*, 1986). Neuropathologic studies of Norrbottnian (Conradi *et al.*, 1988; Conradi *et al.*, 1984) and non-Swedish type 3 (R. E. Lee, 1982; Kaye *et al.*, 1986) patients have shown less severe involvement of the central nervous system. In contrast to other lipidoses, massive storage of material does not occur in the types 2 or 3 Gaucher disease brains. The most striking feature is the presence of "Gaucher cells" in the perivascular Virchow–Robin spaces in the cortex and deep white matter (Banker *et al.*, 1962; Adachi *et al.*, 1967; Norman *et al.*, 1956). Not infrequently these periadventitial cells also are found in the gray matter of the thalamus and subependymal tissues of the pons and medulla (Banker *et al.*, 1962; Adachi *et al.*, 1967). Neuronal loss is widespread in the brains of type 2 patients. Most consistently, large losses of neurons occur in the basal ganglia, nuclei of the midbrain, pons, and medulla and hypothalamus (Adachi *et al.*, 1967; Espinas and Faris, 1969; Kaye *et al.*, 1986). The dentate nucleus of the cerebellum always is severely involved. These findings are summarized in Table III. Severe neuronal loss in the cochlear nuclei and the superior olivary complex has been demonstrated by physiologic (BAER) studies and histologic correlation in the same patients (Lacey and Terplan, 1984). Free Gaucher cells are found within brain parenchyma of the cerebral cortex, with larger numbers in the occipital lobes (R. E. Lee, 1982; Kaye *et al.*, 1986; Banker *et al.*, 1962). The numbers of these cells in cerebral cortex appear to be greater in the more severe patients with type 2 disease and absent in type 3 patients (R. E. Lee, 1982; Conradi *et al.*, 1984). In either case,

TABLE III. Central Nervous System Abnormalities in Type 2 Gaucher Disease^a

Region	Neuronal loss	Gliosis	Periadventitial Gaucher cells	Neuronophagia	Free Gaucher cells
Cerebral cortex					
Frontal	3+	2+	3+	2+	2→4+
Temporal	1→3+	2+	3+	0	3+
Parietal	4+	2+	3+	1+	2+
Occipital	1+	3+	3+	0	4+
White matter	4+	1+	4+	0	0
Thalamus	2→4+	2+	1+	4+	1+
Caudate nucleus	4+	2+	0	2→4+	1+
Clastrum	4+	2+	0	2+	2+
Putamen	3+	2+	0	1+	2+
Globus pallidus	4+	2+	0→1+	4+	0
Midbrain	2+4+	2+	1+	4+	0
Pons	3+	1+	1+	4+	1+
Medulla	3+	1+	1+	4+	0
Cerebellum					
Dentate nucleus	4→5+	0→1+	1→3+	4+	0
Purkinje layer	2+	0	0	0	0

^aThe severity of the abnormalities ranges from normal (0) to very extensive changes (5+).

they are few in number. The neuropathology has been reviewed in detail (Beutler and Grabowski, 1993).

Gaucher Cells

The presence in visceral tissues of lipid-engorged cells with a characteristic appearance (Gaucher, 1882), termed Gaucher cells, is the hallmark of this disease. These cells are derived from the monocyte/macrophage system through pre-Gaucher monocytoïd cells in peripheral blood and bone marrow of affected patients. These cells contain small numbers of characteristic tubular cytoplasmic inclusions (Parkin and Brunning, 1982). This monocyte/macrophage origin of Gaucher cells and the evolution of monocytoïd cells into end-stage Gaucher macrophages have had major implications for the understanding of the pathophysiology of Gaucher disease and toward the development of targeted enzyme augmentation therapy as well as gene and cellular replacement therapeutic strategies (see Therapeutic Prospects).

The Gaucher cell has a nearly pathognomonic appearance by light microscopy. In smears and imprints of affected tissues, Gaucher cells are about 20–100 µm in diameter (Parkin and Brunning, 1982), although larger cells can be isolated

from affected tissues. These cells contain one or more nuclei and cytoplasm with a striated, fibrillary, or tubular pattern which has been likened to “wrinkled tissue paper” or “crumpled silk” (Parkin and Brunning, 1982). On Romanowsky-stained preparations these cells manifest a characteristic pale blue to gray cytoplasm. In comparison, the cells from patients with Niemann–Pick disease and other lipidoses are foam cells that contain discrete membrane-bound white or empty-appearing lipid inclusions.

By electron microscopy, the cytoplasm of pre-Gaucher cells and end-stage Gaucher cells has dilated membrane-bound vesicles (0.6–4 μm) containing characteristic twisted structures (R. E. Lee, 1968). These tubules are about 90% glucosylceramide and about 0.3% protein in composition (R. E. Lee, 1968). It has been suggested that the tubules result from the polymerization of glucosylceramide in a polar head-to-apolar tail manner. In addition to their distribution, the origin of the stored glucosylceramides in Gaucher cells has important implications for the pathophysiology and therapeutic interventions in Gaucher disease (Frederickson and Sloan, 1978). Evidence indicates that phagocytosis of blood-formed elements is the primary source of the accumulated intracellular lipid in the viscera. Erythrocyte fragments are present in Gaucher cells from spleen (Pennelli *et al.*, 1969; Hibbs *et al.*, 1970), suggesting the erythrocyte membrane as a source of stored glycolipid. Gaucher cells also are formed in culture by macrophage phagocytosis of exogenously supplied glucosylceramide (Christianson, 1941). The accumulation of β -galactose, N-acetylglucosamine, fucose, and sialic acid-containing glycopeptides and glycans has been demonstrated in delipidated Gaucher cells from splenic red pulp, liver, and the alveolar, perivascular, and interstitial areas of the lungs (Banker *et al.*, 1962; DeGasperi *et al.*, 1990). Since these glycoconjugates are not substrates for acid β -glucosidase, they probably represent digestive remnants of phagocytosed cells or cell membranes. From turnover calculations, leukocyte membranes have been suggested as the major source of stored glucosylceramide in visceral Gaucher cells (Kattlove *et al.*, 1969). The clear demonstration that previously stored intracellular glucosylceramide is extruded from lysing Gaucher cells into the extracellular spaces of the spleen (Pennelli *et al.*, 1969) indicates a source of the elevated plasma levels of this lipid.

PATHOPHYSIOLOGY

The Storage Substance: Glucosylceramide

The major natural substrate for acid β -glucosidase is N-acyl-sphingosyl-1-O- β -D-glucoside (Fig. 1). This compound also has been called glucosylceramide,

ceramide β -glucoside, or glucocerebroside and the enzyme has been referred to as glucocerebrosidase, glucosylceramidase, ceramide β -glucosidase, or acid β -glucosidase (EC 3.2.1.45). Although the chain length does vary, the usual sphingosyl moiety is (2S,3R,4E)-2-amino-4-octadecene-1,3-diol (Mislow, 1953; Carter and Fujino, 1956). Glucosylceramide is synthesized from ceramide and UDP-glucose by glucosylceramide synthase (Basu *et al.*, 1968, 1973; Coste *et al.*, 1985, 1986). Glucosylceramide is widely distributed in many mammalian tissues in small quantities as a metabolic intermediate in the synthesis and degradation of complex glycosphingolipids, such as the gangliosides or globoside. It is localized primarily in cellular membranes. Glucosylceramide is the penultimate intermediate in the degradation pathway of most complex glycosphingolipids (Fig. 2). Further degradation by the acid β -glucosidase produces ceramide, the latter being further

Fig. 2. Degradation pathway of lower-order gangliosides and neutral glycosphingolipids through the common pathway of glucosylceramide (CER-GLC).

degraded by acid ceramidase (Gatt, 1966; Yavin and Gatt, 1969) to sphingosine and fatty acid.

Plasma levels of glucosylceramide are elevated 2- to 20-fold in affected patients, but the levels do not correlate with the type of Gaucher disease nor the degree of involvement within a type (Dawson and Oh, 1977; Strasberg *et al.*, 1983; Nilsson *et al.*, 1982). Extensive lipid analyses of livers, spleens, and brains of Gaucher disease patients have shown large (20- to 100-fold) increases in glucosylceramide (Conradi *et al.*, 1991; Kaye *et al.*, 1986; Nilsson and Svennerholm, 1982a,b; Nilsson *et al.*, 1985, 1982; Kennaway and Woolf, 1968; French *et al.*, 1969). The fatty acid and sphingosyl base compositions of the accumulated glucosylceramides in liver and spleen are typical of visceral organs (Nilsson *et al.*, 1985; Nilsson and Svennerholm, 1982a). There is a predominance of C₂₀ to C₂₄ fatty acids and shorter-chain sphingosyl bases in all Gaucher disease variants (Nilsson *et al.*, 1985). Substantial concentrations of glucosylsphingosine are also present (Nilsson and Svennerholm, 1982b). Increased levels of glucosylceramide are found in brains from several disease variants. However, the distribution, amount, and type of this accumulated lipid vary with the Gaucher disease variant. The fatty acid and sphingosyl base compositions of the accumulated lipid in brain from one classical type 1 patient were consistent with the extraneural location and visceral origin of this lipid (Nilsson *et al.*, 1985). In comparison, composition analyses of glucosylceramide from brains of nonsplenectomized type 2 and type 3 patients were consistent with a neural origin and location (Conradi *et al.*, 1991; Nilsson and Svennerholm, 1982b; Nilsson *et al.*, 1985). The levels of glucosylsphingosine accumulation also were severalfold increased over that found in the classical type I brain (Nilsson and Svennerholm, 1982b; Nilsson *et al.*, 1985). In splenectomized type 3 patients much greater accumulations of glucosylceramide were found in liver and brain compared to their nonsplenectomized cohorts (Nilsson and Svennerholm, 1982b; Nilsson *et al.*, 1982).

Pathogenesis

The earliest histologic abnormalities observed in several tissues are reticulum fibers surrounding Gaucher cells (R. E. Lee, 1982). Thus, visceral organ fibrosis and brain gliosis appear to occur prior to or coincident with secondary tissue events resulting in advanced disease. In visceral organs and bone, secondary disease findings include infarction, necrosis, and scarring. In brain of severe type 2 patients neuronal loss is the most apparent pathologic finding. It is possible that the regenerative capacities of visceral tissues could obscure the role of cell death as an early and/or unifying pathogenic event in these tissues. In addition, the

tissue variation in pathology results from tissue-specific characteristics: i.e., the solid containment of bone marrow, the specialized extracellular environment of osteoclasts, and the sinusoidal structures of the liver and spleen. As a consequence, fibrosis can be considered the earliest pathologic event from which a unified pathophysiology of this disease should be developed. In addition, unlike most other lysosomal storage diseases, the storage material in Gaucher cells of visceral tissues derives from phagocytosis of components which are external to the cell. Neurons of the brain may be the only cell type in which endogenous synthesis plays an important pathogenic role in the neuronopathic Gaucher disease variants. These observations suggest the presence of cytotoxic substances, i.e., glucosylceramide, its lysosphingolipid derivative, glucosylsphingosine, or other glucolipids. In visceral organs from patients with type 1, 2, or 3 disease glucosylsphingosine, if present, occurs only at exceedingly low concentrations, whereas in the CNS of type 2 and 3 patients significant amounts are present. The toxic role of this lysosphingolipid is consistent with the proposal of Hannun and Bell (1987), but experimental support in the lysosomal storage diseases is lacking. Glucosylceramide has been shown to have growth-promoting potential in animal models (Datta and Radin, 1988). In addition, normal macrophages release cytokines when exposed to solvent-solubilized glucosylceramide (Gery *et al.*, 1981), although the levels of several interleukins and tumor necrosis factor α in plasma from Gaucher patients are not elevated (G. A. Grabowski and L. Meier, unpublished). As with most of the lysosomal storage diseases, the pathogenic mechanisms leading to the Gaucher disease phenotypes remain to be elucidated.

BIOCHEMISTRY

Acid β -Glucosidase

Acid β -glucosidase is a tightly associated peripheral membrane protein (Imai, 1985). Purification has been achieved from a variety of species, but most efforts have focused on the human enzyme (Pentchev *et al.*, 1973; Furbish *et al.*, 1977; Strasberg *et al.*, 1982; Dinur *et al.*, 1986; Grabowski and Dagan, 1984; Osiecki-Newman *et al.*, 1986). The specific activities of the homogeneous acid β -glucosidase preparations varied by an order of magnitude ($\sim 5 \times 10^5$ to 5×10^6 nmole/hr per protein), primarily due to different assay conditions. Based on active site quantitation the human placental enzyme had a catalytic rate constant, k_{cat} of about 2350 min^{-1} for a variety of glucosylceramide and 4-alkylumbelliferyl- β -glucoside substrates (Grabowski *et al.*, 1986). The human enzyme is a homomeric

glycoprotein, and partial (Osiecki-Newman *et al.*, 1986; Dinur *et al.*, 1986) and complete (Tsuji *et al.*, 1986) amino acid sequences have been determined chemically and deduced from the nucleotide sequences of the cDNAs (Tsuji *et al.*, 1986; Sorge *et al.*, 1985; Graves *et al.*, 1988; Reiner *et al.*, 1987). The mature human polypeptide contains 497 amino acids with a calculated molecular weight M_r of 55,575. The pure glycosylated enzyme from placenta has a $M_r \sim 65,000$. Aerts *et al.* (1987) described a high-molecular-weight form of acid β -glucosidase in human cells and urine, termed glucocerebrosidase II, which was an enzyme/saposin C aggregate. Because acid β -glucosidase requires detergent solubilization, the native state of the enzyme in cells and tissues has not been resolved. Molecular weights, estimated by sedimentation and molecular exclusion chromatography, have varied between about 60,000 and 450,000 (Pentchev *et al.*, 1973; Prencz *et al.*, 1985; Garrett *et al.*, 1985). In comparison, estimates of the native molecular weight in tissues by *in situ* radioinactivation of the enzymatic activity were consistent with either a monomeric or dimeric structure (Dawson and Ellory, 1985; Maret *et al.*, 1984, 1983). About 13% of the residues are basic (lysine, arginine, or histidine) and the calculated pI value is 7.2, which is consistent with experimental values ($pI = 7.3-7.8$) for placental enzyme preparations containing only the neutral mannosyl oligosaccharide core (Furbish *et al.*, 1981). The protein has about 11% leucine residues and 45% nonpolar amino acids, but transmembrane domains are not present in the mature polypeptide. Proteolytic digestion studies of acid β -glucosidase, obtained by *in vitro* translation in the presence of microsomal membranes, indicated the absence of a large cytoplasmic domain (A. H. Erickson *et al.*, 1985). No obvious basis for its tight membrane association is evident from the primary sequence.

The human and murine sequences have conserved placement of the five N-glycosylation sequons, but only those at the third and fifth positions were identical (O'Neill *et al.*, 1989). Takasaki *et al.* (1984) have shown typical bi- and tri-antennary complex N-linked oligosaccharides on the human placental acid β -glucosidase. The less common NeuAc $\alpha 2 \rightarrow 3$ Gal $\beta 1$ linkages were found (Takasaki *et al.*, 1984). Endoglycosidase F digestion studies of human fibroblast acid β -glucosidase indicated that only four of the potential glycosylation sites are used (A. H. Erickson *et al.*, 1985). Expression of the human cDNA in prokaryotes indicated that glycosylation is essential for the development of a catalytically active enzyme (Grace *et al.*, 1992; Berg *et al.*, 1993), but it was not required to maintain activity in cells (Grace and Grabowski, 1990). Using site-directed mutagenesis, the first four glycosylation sequons were shown to be normally occupied and occupancy of the first sequon was needed to develop an active conformer (Berg *et al.*, 1993) (Fig. 3).

Posttranslational Processing of Normal Acid β -Glucosidase

Biosynthesis of normal acid β -glucosidase has been characterized in cultured porcine kidney cells and human skin fibroblasts (Jonsson *et al.*, 1987; Beutler and Kuhl, 1986; A. H. Erickson *et al.*, 1985; Bergmann and Grabowski, 1989). Three molecular-weight forms of acid β -glucosidase were observed by immunoelectroblotting of human skin fibroblast (Ginns *et al.*, 1982; Jonsson *et al.*, 1987; Fabbro *et al.*, 1987) and brain extracts (Ginns *et al.*, 1982). Total removal of N-linked oligosaccharides from acid β -glucosidase resulted in a single immunoreactive form ($M_r = 56,000$) of acid β -glucosidase (Fabbro *et al.*, 1987). These findings demonstrated that the three forms of the enzyme were derived by differential posttranslational oligosaccharide remodeling (Fabbro *et al.*, 1987; Bergmann and Grabowski, 1989). Use of ^{35}S -methionine metabolic labeling showed the first detectable forms of the human (Jonsson *et al.*, 1987; Bergmann and Grabowski, 1989; A. H. Erickson *et al.*, 1985) and porcine (A. H. Erickson *et al.*, 1985) enzymes to contain high mannosyl chains (Jonsson *et al.*, 1987; A. H. Erickson *et al.*, 1985) which indicated residence in the endoplasmic reticulum. These initial forms were quantitatively remodeled by 24 hr into higher-molecular-weight forms with complex oligosaccharide chains (Jonsson *et al.*, 1987; A. H. Erickson *et al.*, 1985). Over about 72 hr a final glycosylated form ($M_r = 59,000$) was produced in the lysosomes. Acid β -glucosidase is not trafficked to the lysosome via the mannose-6-phosphate-mediated pathway (Kaplan *et al.*, 1977; Varki and Kornfeld, 1981). Following signal peptide clipping, transport of the enzyme from the Golgi apparatus to the lysosomes was not associated with detectable proteolytic processing (A. H. Erickson *et al.*, 1985) as had been observed with several other lysosomal hydrolases (Hasilik and Neufeld, 1984). This was confirmed by amino acid sequence analysis of the entire placental protein (Tsuji *et al.*, 1986). The rate of transport of acid β -glucosidase from the endoplasmic reticulum to the Golgi apparatus is about 3 hr, which is long compared to that for other lysosomal enzymes (Bergmann and Grabowski, 1989). The half-life of acid β -glucosidase in cultured skin fibroblasts is estimated to be about 60 hr, which is short for lysosomal enzymes (Hasilik and Neufeld, 1984).

Posttranslational Processing of Acid β -Glucosidase in Cells from Gaucher Disease Patients

Ginns *et al.* (1982) suggested a differential processing of the mutant residual acid β -glucosidase in fibroblasts from variant patients. By immunoelectroblotting, the three normal steady-state forms of enzyme were found to be present in

cultured skin fibroblasts from type 1 patients (Ginns *et al.*, 1982; Fabbro *et al.*, 1987). Remarkable heterogeneity of processing patterns was demonstrated (Fabbro *et al.*, 1987; Jonsson *et al.*, 1987) in ethnically heterogeneous populations of Gaucher disease patients. In retrospect, this would have been expected from the extensive genetic heterogeneity in this disease (Fig. 4) and (Table IV). The presence of two different mutant acid β -glucosidase alleles in cells from many patients makes assignment of differential stability or processing properties to a mutation difficult. However, in cells homozygous for the N370S or L444P alleles, normal or decreased stability, respectively, of the residual enzyme proteins has been observed (Bergmann and Grabowski, 1989; Jonsson *et al.*, 1987). By immunoelectronmicroscopic localization some mutations seem to preclude localization of the abnormal acid β -glucosidases to the lysosomes, particularly in type 2 disease (Willemsen *et al.*, 1988). Studies with protease inhibitors (Jonsson *et al.*, 1987; Tager *et al.*, 1986) and *in situ* hydrolysis of exogenously supplied glucosylceramide (Agmon *et al.*, 1993) indicate that some of the mutant enzymes do reach this compartment in most variants of Gaucher disease, so the block is not absolute. Recreation of the individual mutations and expression of each in heterologous systems has begun to indicate regions on the protein which modulate some of these properties (Grace and Grabowski, 1993; Grace *et al.*, 1990; 1992, 1993; Ohashi *et al.*, 1991) (Fig. 3) (see Molecular Enzymology).

Saposins C and A

In 1971, Ho and O'Brien (1971) characterized a factor from Gaucher disease spleen which enhanced acid β -glucosidase hydrolysis of 4-methylumbelliferyl- β -D-glucopyranoside (4MU-Glc). Subsequently, this "factor" has been designated heat-stable factor, co- β -glucosidase, saposin C, and SAP-2 (sphingolipid activator protein 2). Saposin C is now the term in common usage. The physiologic importance of saposin C was demonstrated by glucosylceramide storage and a Gaucher disease-like phenotype in two patients with a deficiency of saposin C cross-reacting immunological material and normal *in vitro* acid β -glucosidase activity (Christomanou *et al.*, 1987, 1989). Recently, a second protein activator of *in vitro* acid β -glucosidase activity has been described, saposin A (Morimoto *et al.*, 1989). Both of these activators derive from a single gene product by extensive proteolytic and glycosidic posttranslational processing (see Molecular Biology of the Activator Proteins).

The complete amino acid sequences of human (Kleinschmidt *et al.*, 1987) and guinea pig (Sano *et al.*, 1988) saposin C have been chemically determined and those from human (O'Brien *et al.*, 1988; Nakano *et al.*, 1989; Gavrieli-Rorman and Grabowski, 1989; Reiner *et al.*, 1989) and rat (Collard *et al.*, 1988) saposin C

Fig. 4. Schematic representation of the distribution of Gaucher disease alleles in the acid β-glucosidase gene. The mutations below the schematic of the gene are point mutations and those above are nonsense or more complex alleles. The base substitutions in the cDNA and gene are presented in Table IV. Alleles marked with asterisks mimic base changes in the pseudogene (see Fig. 6). The alleles represented by N370S, 84GG, L444P, IVS2, and R463C are common alleles, whereas the other alleles are private or rare. The 84GG, Δ55, and IVS2 are, respectively, a 55-bp deletion, an insertion, a 55-bp deletion, and an exon2/intron2 splice junction mutation which are functionally null. The alleles containing multiple missense mutations (in boxes) probably arise from genetic rearrangements with the pseudogene.

TABLE IV. Gaucher Disease Alleles

cDNA #	Amino acid #	Genomic #	Base substitution	Amino acid substitution	Phenotypic effect	Frequency ^a	Enzymatic effect		Ref.
							Catalytic	Stability	
A. Missense mutations that cause Gaucher disease									
476	120	3060	G→A	Arg→Gln	? Severe	Rare	Inactive	Stable	Graves <i>et al.</i> (1988)
481	122	3065	C→T	Pro→Ser	Mild	Rare	—	—	Beutler <i>et al.</i> (1992a)
535 ^b	140	3119	G→C	Asp→His	?	Rare	—	—	Eyal <i>et al.</i> (1991)
1093 ^b	326	5309	G→A	Glu→Lys	?				
586	157	3170	A→C	Lys→Gln	Severe	Rare	Inactive	Stable	Eyal <i>et al.</i> (1991), Latham <i>et al.</i> (1991)
751	212	3545	T→C	Tyr→His	?Mild	Rare	—	—	Beutler <i>et al.</i> (1992a)
754	213	3548	T→A ^c	Phe→Ile	Severe	Uncommon	↓↓ Activity	Unstable	Kawame and Eto (1991), He <i>et al.</i> (1993)
764	216	4113	T→A	Phe→Tyr	?Mild	Rare	↓↓ Activity	Unstable	Beutler and Gelbart (1990)
983	289	4332	C→T	Pro→Leu	?Mild	Rare	↓↓ Activity	Unstable	He <i>et al.</i> (1993)
1043	309	5259	C→T	Ala→Val	Mild	Rare	—	—	Latham <i>et al.</i> (1991)
1053	312	5269	G→T	Trp→Cys	?Mild	Rare	—	—	Latham <i>et al.</i> (1991)
1085	323	5301	A→C	Thr→Ile	Severe	Rare	↓ Activity	Stable	He <i>et al.</i> (1993)
1090	325	5306	G→A ^c	Gly→Arg	Severe	Rare	—	—	Eyal <i>et al.</i> (1990)
1141	342	5357	T→G	Cys→Gly	Severe	Rare	—	—	Eyal <i>et al.</i> (1990)
1208	364	5424	G→C	Ser→Thr	Mild	Rare	↓ Activity	Stable	Latham <i>et al.</i> (1991)
1226	370	5841	A→G	Asn→Ser	Mild	Common	↓ Activity	Stable	Tsuji <i>et al.</i> (1988a)
1297	394	5912	G→T	Val→Leu	Severe	Uncommon	↓ Activity	Stable	Theophilus <i>et al.</i> (1989b)
1342	409	5957	G→C ^c	Asp→His	Severe	Uncommon	↓↓ Activity	Unstable	Theophilus <i>et al.</i> (1989b), Eyal <i>et al.</i> (1990)
1343	409	5958	A→T	Asp→Val	Severe	Rare	↓↓ Activity	Stable	Theophilus <i>et al.</i> (1989b)
1361	415	5976	C→G	Pro→Arg	Severe	Rare	Inactive	Stable	Wigderson <i>et al.</i> (1989)
1448	444	6433	T→C ^c	Leu→Pro	Severe	Common	↓↓ Activity	Unstable	Tsuji <i>et al.</i> (1987)
1504	463	6489	C→T	Arg→Cys	?Severe	Uncommon	↓ Activity	Stable	Hong <i>et al.</i> (1990)
1604	496	6683	G→A	Arg→His	Mild	Uncommon	—	—	Beutler <i>et al.</i> (1992a)

B. Nonsense mutations that cause Gaucher disease							
84	1035	G→GG	Frameshift	Severe	Common	Null	Beutler <i>et al.</i> (1991a)
IVS2+1	1067	G→A	Aberrant splicing	Severe	Uncommon	Null	Beutler <i>et al.</i> (1992a), He and Grabowski (1992)
1263–1317	5879–5933 ^c	Del	—	?Severe	Rare	Null	Beutler <i>et al.</i> (1992a)
C. Genetic rearrangements causal to Gaucher disease							
Nucleotides involved		Location of crossover events(s) ^d		Phenotypic effect		Frequency	Ref.
>1343 <1388	>5957 <6272 ^a		Severe		Uncommon		Zimran <i>et al.</i> (1990)
>455 <475 ^f	>3039 <5957		?Severe		Rare		Latham <i>et al.</i> (1990)
>754	>3548						
>1317 <1343	>5932 <5957		Severe		Uncommon		Eyal <i>et al.</i> (1990), Latham <i>et al.</i> (1990)
>1343 <1388	>5957 <6272		Severe		Uncommon		Latham <i>et al.</i> (1990), Hong <i>et al.</i> (1990)
>1225 <1263	>5588 <5878		Severe		Rare		Eyal <i>et al.</i> , 1991, 1990

^aCommon = high frequency in at least one population; uncommon = found in a number of unrelated patients; rare = found in only one or two individuals.

^bBoth mutations are found in a single allele.

^cSequence mimicking that in the pseudogene.

^dOnly approximate ranges can be given, since the pseudogene and structural gene contain long identical sequences.

^ePhysical fusion with loss of intergenic segment.

^fThe first range represents crossover from gene to pseudogene; the second from pseudogene to functional gene. This region contains seven mutations; only six are identical with the corresponding pseudogene sequences. At genomic nt 3474, the nucleotide corresponds to that in the structural gene. Adapted from Beutler and Grabowski (1993)

have been deduced from their cDNA sequences. The 80-amino acid ($M_r = 8950$) human saposin C is a very acidic (calculated $pI \sim 4.2$) glycoprotein (San *et al.*, 1988), which is consistent with the presence of 20% acid (Asp and Glu) and 10% basic (Lys and His) amino acids. The single N-linked glycosylation sequon is occupied. The composition of the guinea pig saposin C oligosaccharides has been reported (Sano *et al.*, 1988; Kleinschmidt *et al.*, 1987). Similar to saposin C, saposin A is small ($M_r = 9148$), is very acidic (calculated $pI = 4.14$), and has six cysteine residues which align with those in saposin C (see below, Fig. 8A). Saposin A has two N-glycosylation sequons and both are occupied (Morimoto *et al.*, 1989). The human and murine proteins are nearly identical (Fig. 5).

Biosynthesis and Processing of Saposins C and A

Fujibayashi and Wenger (1986a), using rabbit anti-human saposin C antibodies in metabolic labeling studies, demonstrated that saposin C had extensive proteolytic and glycosidic processing in fibroblasts. In chase experiments following a short pulse (15 min), the major product was a 68-kilodalton (kD) glycosylated species which over 1 hr was converted to a 73 kD form. Subsequently, the total amount of these forms decreased coincident with the appearance of diffuse species with $M_r \sim 12,000$ and 9,000. By 5 hr of chase, the 12-kD species had nearly disappeared, while the 9-kD species was predominant. Endoglycosidase F digestion studies of ^{35}S -methionine-labeled saposin C immunoprecipitates demonstrated that the 73- and 68-kD or the 12- and 9-kD species were glycosylated species with polypeptides of $M_r = 50,000$ or 7600, respectively. Very similar results were reported for the biosynthesis of saposin B (Fujibayashi and Wenger, 1986b), which would now be expected since saposin C and saposin B are encoded by the same gene (see Molecular Biology of the Activator Proteins). Neither the

Fig. 5. Schematic diagram of the saposin A, B, C, and D regions of the rat and the human prosaposins. The amino acid identity between the rat and human sequences is shown above each region. The 90-base in-frame insertion occurs in the rat, but not the human, sequence. The alternative splice site (see text) has been demonstrated only in the human sequence.

sequence or events involved in proteolytic processing of the “prosaposin” precursor nor their potential tissue specificity has been elucidated. The highly homologous precursor produced in the rat analogue (SGP-1) (Collard *et al.*, 1988) is not proteolytically processed prior to its secretion into the culture medium of Sertoli cells. Human prosaposin is found in serum, milk, and brain (Kishimoto *et al.*, 1992), but its function is unknown.

Enzymology of Acid β -Glucosidase

Kinetic studies of acid β -glucosidase have been complicated by its membrane association and its requirements for detergents, delipidation for solubilization, and specific amphiphiles for reconstitution of enzymatic activity. The enzyme's natural substrates, glucosylceramides and its deacylated analogue (glucosylsphingosine), have been identified by their accumulation in the tissues of patients with the Gaucher disease variants (Nilsson and Svennerholm, 1982b; Nilsson *et al.*, 1985). In the absence of detergents or negatively charged lipids, delipidated human (Mueller and Rosenberg, 1979; Dale *et al.*, 1976; Basu and Glew, 1984; Glew *et al.*, 1982), bovine (Legler and Liedtke, 1985), and murine (Basu and Glew, 1984) acid β -glucosidases have little or no hydrolytic activity toward natural lipid or more water-soluble synthetic substrates.

Because of the *in vivo* membrane association of acid β -glucosidase, investigators reasoned that specific membrane-derived lipids might be required for enzymatic activity. Dale *et al.* (1976) and Berent and Radin (1981) demonstrated that acid β -glucosidase activity toward 4MU-Glc was enhanced by negatively charged phospholipids in the series phosphatidylserine > phosphatidylinositol > phosphatidic acid, whereas phosphatidyl-ethanolamine or -choline had no effect on enzymatic activity. These results have been reproduced by numerous investigators in human, bovine, and murine sources (e.g., Mueller and Rosenberg, 1979; Osiecki-Newman *et al.*, 1986; Legler and Liedtke, 1985; Grabowski *et al.*, 1984; Glew *et al.*, 1982), albeit with some variation in the composition of the assay mixtures and in the degree of activation. In artificial membrane systems, optimal activity also required the presence of negatively charged phospholipids to which acid β -glucosidase directly binds (Vaccaro *et al.*, 1989). In a series of publications, Glew and co-workers delineated some structure/activity relationships between acid β -glucosidase and negatively charged phospholipids or glycosphingolipids (Basu and Glew, 1984, 1985; Basu *et al.*, 1984, 1985, 1986; Prencz *et al.*, 1986a; Gonzales *et al.*, 1988). Intriguingly, bis-(monoacylglyceryl)phosphate activated the enzyme. This lipid is present in liver lysosomal membranes, which suggest

that the lipid milieu of β -glucosidase in the intact lysosome may play a role in modulating its activity in different tissues (Prence *et al.*, 1986b). Characterization of the natural lipids associated with acid β -glucosidase from different tissues could provide an interesting insight into the enzyme's *in vivo* functional environment.

Saposin C has been shown to enhance acid β -glucosidase *in vitro* activity in the presence of negatively charged phospholipids (Brady *et al.*, 1965; Berent and Radin, 1981; Grabowski *et al.*, 1984; Prence *et al.*, 1985). The direct interaction of saposin C with acid β -glucosidase has been extensively studied by many investigators (Ho and O'Brien, 1971; Berent and Radin, 1981) and it does not interact with glucosylceramide under equilibrium or nonequilibrium conditions (Berent and Radin, 1981). Consequently, saposin C enhances the hydrolysis of both glucosylceramide and 4MU-Glc substrates (Berent and Radin, 1981; Grabowski *et al.*, 1984; Osiecki-Newman *et al.*, 1987) in the presence of a variety of negatively charged lipids. Berent and Radin (1981) suggested that at low concentrations phosphatidylserine interacts with an anionic site on acid β -glucosidase, whereas at high concentrations it decreases the activation effects of saposin C by competing for this site on the enzyme. These effects are thought to be mediated by conformational changes in acid β -glucosidase so that the interaction of phosphatidylserine conforms the enzyme into a "poised" form for the interaction of saposin C (Berent and Radin, 1981). The use of inhibitory monoclonal antibodies has provided direct support for saposin C effecting a conformational change in acid β -glucosidase which enhances catalytic activity (Fabbro and Grabowski, 1991). It is not clear if saposins C and A bind to the same, overlapping (Van Weely *et al.*, 1990), or different sites (Fabbro and Grabowski, 1991). No mutations in Gaucher disease have been shown to directly affect saposin C or A binding.

Using their results (Grabowski *et al.*, 1984; Osiecki-Newman *et al.*, 1987, 1988; Graves *et al.*, 1986; Greenberg *et al.*, 1990; Gatt *et al.*, 1985; Grace *et al.*, 1990) and previous work (J. S. Erickson and Radin, 1973; Hyun *et al.*, 1975) on substrate specificity and inhibitor specificity, Grabowski and co-workers proposed a kinetic model of the active site of acid β -glucosidase which includes binding sites for the glycon head group, the sphingosyl moiety, and the fatty acid acyl chain of glucosylceramide (Fig. 1). Interaction at the glycon binding site requires a specific bonding with each hydroxyl group (Osiecki-Newman *et al.*, 1988) on glucose, while the sphingosyl and fatty acid acyl binding sites are thought to accommodate hydrophobic chains up to about 16 carbon atoms in length. From the results of linear free-energy analyses, these latter sites have been proposed to be composed of multiple subsites which accommodate single methylene groups of substrates and inhibitors (Osiecki-Newman *et al.*, 1987; Grabowski *et al.*, 1984,

1986). The sphingosyl and fatty acid acyl chain lengths affect K_m and V_{max} , respectively. This model is similar to those proposed for the binding of globotriaosylceramide to saposin B (Wynn, 1986) and phospholipids to phospholipid transfer protein (Van Paridon *et al.*, 1987). A nearly identical model has been proposed for a human neutral β -glucosidase (Glew *et al.*, 1988). Although additional supporting structural data will be required for these models, they may serve as prototypes for binding sites on lipid transfer proteins and partial or complete active sites of glycosphingolipid hydrolases.

The specificity of glycon binding has been assessed by apparent binding constants for a series of epimers and substituted glucose derivatives (Osiecki-Newman *et al.*, 1988). The enzyme binds β -glucose poorly, but has high affinity for 5-amino derivatives, i.e., the nojirimycins (Osiecki-Newman *et al.*, 1988). Only the gluco derivatives of nojirimycin bound to the enzyme: i.e., the galactodeoxynojirimycin and deoxymannojirimycin did not inhibit the enzyme. These results and the lack of inhibition by galactosylsphingosine (Grabowski *et al.*, 1984) indicated the importance of the C2 and C4 hydroxyl configuration of the hexose. Alkyl-glycons were extremely powerful inhibitors of acid β -glucosidase (Grabowski *et al.*, 1984; Osiecki-Newman *et al.*, 1987, 1988). In particular, the N-alkyl-deoxynojirimycins and N-alkyl- β -glucosylamines had K_i values in the nanomolar and picomolar ranges, respectively (Osiecki-Newman *et al.*, 1987; Greenberg *et al.*, 1990). Since the additional free energies of binding were additive for the alkyl chain and the glycon head group, the existence of individual subsites for each methylene group up to about 16 was inferred to be within the active site (Osiecki-Newman *et al.*, 1987). In addition, the N-alkyl- β -glucosylamines were slow-tight binding inhibitors (Morrison and Walsh, 1988) which induced a conformational change of the enzyme after formation of an initial binary complex (Greenberg *et al.*, 1990). These results were consistent with the N-alkyl-glucosylamines being reaction intermediate analogues and with the catalytic mechanism requiring a conformational change in the enzyme during substrate hydrolysis. The catalytic mechanism is thought to involve the cleavage of the β -glucosidic linkage, the release of ceramide, donation of water to a glucose-enzyme complex, and the release of β -glucose (Grabowski *et al.*, 1990). Recent use of site-directed mutagenesis and heterologous expression has provided some initial data to begin localization of portions of the enzyme which are responsible for catalytic activity (Fig. 3). Based on these studies and the degree of structural homology to the murine enzyme, the residues involved in catalysis and possibly saposin C binding appear to be located in the carboxy-terminal 40% of the amino acid sequence (Grabowski *et al.*, 1990; Grace *et al.*, 1990, 1991, 1993; O'Neill *et al.*, 1989).

Molecular Enzymology of Acid β -Glucosidase

The existence of several different mutant alleles with different effects on enzymatic function was indicated by the phenomenological responses to the various modifiers of acid β -glucosidase activity (Osiecki-Newman *et al.*, 1986, 1987; Basu and Glew, 1984; Glew *et al.*, 1982; Grabowski *et al.*, 1985, 1986; Basu *et al.*, 1985; Wenger and Roth, 1982; Morimoto *et al.*, 1990) or differential stability of the protein (Bergmann and Grabowski, 1989) in cells from affected patients. These enzymes were separated into two general categories: (a) residual activities having blunted responses to negatively charged lipids and normal interaction with active site-directed inhibitors; (b) residual activities having enhanced response to negatively charged lipids and very abnormal interaction with active site-directed inhibitors (Osiecki-Newman *et al.*, 1987; Paulson *et al.*, 1989; Glew *et al.*, 1982; Grabowski *et al.*, 1984, 1985). Enzymes in the former category have normal or abnormal stability and processing, whereas those in the latter group have normal processing (Bergmann and Grabowski, 1989). Curiously, no mutant enzymes have been found which alter the K_M values for any substrate. This suggests a need to understand this parameter in its more fundamental aspects for this enzyme. With the extensive molecular heterogeneity (Table IV) of Gaucher disease, in retrospect, there was a remarkable consistency in the properties of the residual enzymes. Characterization of protein expressed from mutagenized cDNAs has begun to provide a functional map of the enzyme. As shown in Table V, several mutations lead to severely diminished catalytic rate constants. As expected, "disruptive" mutations (Bordo and Argos, 1991), e.g., D409V, lead to catalytic rate constants greater than 100-fold less than normal. However, some expected conservative substitutions, e.g., V394L or S364T, lead to very compromised enzymes and, in one case, D358E, an inactive enzyme (Grace *et al.*, 1990). Such mutations help define critical residues to catalytic activity. Figure 3 depicts the results from extensive analyses by Grace and co-workers (Grace and Grabowski, 1993; Berg *et al.*, 1993; Grace *et al.*, 1990, 1991, 1992, 1993) as a schematic of an initial function map. Using the transition-state analogues N-alkyl- β -glucosylamines, kinetic analyses of the N370S protein demonstrated no alterations in the required conformational change for catalysis and that the abnormal active function was due to diminished binding affinity (Greenberg *et al.*, 1990; Fabbro and Grabowski, 1991). Similarly, bromo-conduritol B epoxide (Br-CBE) was thought to be a mechanism-based covalent inhibitor whose attachment site to the enzyme defined the nucleophile in catalysis (Legler, 1977; Lalegerie *et al.*, 1982). Recent studies by Withers and co-workers (Gebler *et al.*, 1992; Trimbur *et al.*, 1992) cast doubt on the mechanistic generality of CBE's reaction with glycosidases. Mutagenesis studies of Asp⁴⁴³ of the human enzyme were conducted

TABLE V. Specific Activity of Human Acid β -Glucosidases Expressed in Sf9 Cells

cDNA	Glucosylceramide substrate nmole/hr per mg protein	1/CRIM SA ^a	Protein
Normal	2400	1	Stable
T138N	11–18	>100	Transient
F216Y	2.5–6.5	NA	Unstable
P289L	0.2–1.3	NA	Unstable
T3231	210	15	Stable
N370S	621	4	Stable
D358E	0.02	NA	Stable
S364T	306	~3	Unstable
V394L	450	5	Stable
D409H	4–5	NA	Unstable
D409V	0.3–10	>100	Stable
P415R	0.15–0.3	NA	Unstable
L444P	115	~20	Unstable
A456P	0.3–4	~100	Unstable
N462Q	13	>100	Stable
R463C	860	10	Stable
AcNPV ^b	0.02	NA	NA

^aRelative CRIM specific activity referenced to the mature peptide.

^bSf9 cells infected with wild-type baculovirus.

to provide direct data on its role in catalysis. In addition, Asp⁴⁴⁵ was mutagenized to explore its potential role as an “opportunistic” nucleophile. All possible pairwise combinations of D, S, G, and E were substituted, expressed, and characterized. As shown in Figure 3, these results indicate the importance of aspartates 443 and 445 in catalytic function, but clearly demonstrate that neither is the catalytic nucleophile. These residues and a CBE binding site are within or near the active site. Clearly, additional analyses are required to elucidate further structure/function relationships.

MOLECULAR BIOLOGY

Genomic Organization

The complete sequences of the human acid β -glucosidase structural gene [7604 base pairs (bp)] and its nonprocessed pseudogene (5769 bp) have been reported (Horowitz *et al.*, 1989). These sequences are contained within a 32-kilo-base (kb) genomic DNA fragment with the pseudogene being about 16 kb 3' to the

structural gene. Both sequences map to human chromosome 1(q21→31) (Devine *et al.*, 1982; Barneveld *et al.*, 1983; Ginns *et al.*, 1985) and each contains 11 exons (Fig. 6). In the regions present in both sequences, 96% nucleotide identity was found (Horowitz *et al.*, 1989). The pseudogene is smaller than the structural gene due to large deletions in introns 2 (313 bp), 4 (626 bp), 6 (320 bp), and 7 (277 bp) as well as two small exonic deletions (exon 9, 55 bp; and exon 4, 5 bp) (Fig. 6). The four large deletions in the pseudogene represent *Alu* sequences flanked by direct repeats within the structural gene. The structural gene also contains an inverted *Alu* sequence in intron 2 not found in the pseudogene (Horowitz *et al.*, 1989). In addition, the pseudogene contains numerous point substitutions and deletions in introns and exons scattered throughout the sequence. These differences and homologies have implications for the identification and characterization of mutations which result in Gaucher disease as well as the diagnostic accuracy of DNA-based tests for the disease variants (see Diagnosis by DNA Analysis).

Since *Alu* sequences are thought to be mobile elements (Hess *et al.*, 1983; Schmid and Jelinek, 1982) that may insert into DNA, the pseudogene may more closely resemble an ancestral acid β -glucosidase gene, with the current structural gene having been modified by insertion of *Alu* sequences. The proposed mechanism of insertion of *Alu* sequences (Jagadeeswaran *et al.*, 1981; Van Arsdell *et al.*, 1981) results in the duplication of single-copy sequences to form the direct repeats that flank the *Alu* sequences. This relationship is present in the appropriate corresponding regions of the pseudogene and structural gene. The mouse has only the structural gene (O'Neill *et al.*, 1989). In a study of 172 acid β -glucosidase alleles, eight intronic or 5' flanking region polymorphisms were identified (Table VI). These base changes segregated with an ancient *PvuII* polymorphic restriction site. Two of the eight intronic changes duplicate the sequence found in the pseudogene [nucleotides (nt) 2834 and 3297]. These appear to be localized alterations which do not involve large exchanges of structural gene and pseudogene material (Beutler *et al.*, 1992b). The base change at nucleotide 6144 marks the 5' extent of a large 3' exchange of structure and pseudogene material which includes the corresponding exons 10 and 11 (Beutler *et al.*, 1992b). Several disease alleles have also arisen by apparent rearrangements between the structural gene and pseudogene (Fig. 4). Only a single pseudogene sequence has been determined, however, and other forms may exist which might account for some rare alleles in isolated populations.

Analyses of the 5' genomic sequences of the structural gene and the pseudogene indicated the presence of promoter elements (Reiner *et al.*, 1988a,b). The promoter of the structural gene contained two TATA boxes that lie between nucleotides (-23) to (-27) and (-33) to (-38) and two possible CAAT-like boxes between nucleotides (-90) to (-94) and (-96) to (-99) in relation to

Fig. 6. Schematic diagram of the acid β -glucosidase pseudogene (top) and structural gene (bottom) and the depiction of the IVS2⁺1 mutation leading to aberrant splicing. The homologous "exons" in the pseudogene and structural gene are indicated, as are the intronic deletions in the pseudogene. The pseudogene exon 9 contains a 55-bp deletion which corresponds to a rare disease allele designated Δ 55. The G→A transition at the exon2/intron2 junction leads to the cDNA products shown at the bottom. The other allele in the patient encoded the N370S mutation.

TABLE VI. Nucleotides at 12 Positions in the Four Acid β -Glucosidase Haplotypes

Designation ^a	Frequency	nt	Acid β -glucosidase haplotype									
			-802	-725	-614	2128	2834	3297	3747	3854	<i>Pvu</i> II	3931
1 <i>Pvu</i> II ⁺	Common	a	c	c	c	a	c	g	g	t	g	del
2 <i>Pvu</i> II ⁻	Common	g	t	t	t	g	g	a	g	c	a	a
3 African	Common African	g	t	t	t	g	a	a	g	c	a	a
4 Uncommon	Rare	a	c	c	c	a	c	g	a	t	g	del
												g

^a*Pvu*II⁺ and *Pvu*II⁻ refer to the absence or presence, respectively, of a polymorphic *Pvu*II restriction endonuclease site at nucleotide 3931 of the reported genomic sequence (Horowitz *et al.*, 1989). Corrections to that sequence are reported in Beutler *et al.* (1992b).
^b5' nucleotide of a structural gene/pseudogene rearrangement which includes exons 10 and 11 of both sequences. This was found only in two Gaucher disease patients and represents a disease allele, associated with the *Pvu*II⁺ polymorphism. Adapted from Beutler *et al.* (1992b).

the most 5' in-frame AUG of the mRNA (Horowitz *et al.*, 1989; Reiner *et al.*, 1988a,b). No Sp1 binding site, i.e., GGC GGG motif, was found. These results are interesting since the promoter elements that contain TATA and CAAT boxes have been identified most commonly in highly regulated genes (Dyan, 1986). The promoter activity was monitored using a plasmid pSVOCAT construct containing the appropriate acid β -glucosidase 5' sequences and the bacterial CAT gene (Horowitz *et al.*, 1989; Reiner *et al.*, 1988a,b). Following transfection of these constructs into different human cell lines, expression of CAT activity indicated that the promoter sequences from the structural gene were at least eight times more potent than the corresponding pseudogene sequences. The pseudogene contains several termination codons within its exons (Horowitz *et al.*, 1989). Although the pseudogene has a very weak promoter (Dyan, 1986), transcription has been reported (Sorge *et al.*, 1990).

No other primate or mammalian acid β -glucosidase sequence have been published, except that from the mouse (O'Neill *et al.*, 1989). The mouse acid β -glucosidase gene has been mapped to mouse chromosome 3 and its genomic organization determined (O'Neill *et al.*, 1989). The exons of the human and mouse genes have 84% nucleotide identity in the protein coding, 54% in the 5' noncoding, and 78% in the 3' noncoding regions. The positions of the 20 intron/exon junctions in the structural genes from each species are precisely conserved. Compared to the human sequence: (1) 64% of the nucleotide substitutions resulted from third position changes, (2) a single amino acid deletion, His²⁷³, was predicted in the mouse sequence, (3) 100% of the cysteines, 91% of the prolines, and 89% of the glycines were preserved, and (4) the greatest variation in the predicted amino acid sequences occurred in the N-terminal 60%. All six uncommon amino acid substitutions as well as the His²⁷³ deletion occurred in this region of the predicted mature protein sequence. If analyzed on the basis of percentage of the mature protein sequence per exon compared to the percent variation per exon, the expected ratio of these parameters for random divergence would be about 1. When calculated from the published murine and human cDNA sequences, the 65 predicted nonconservative amino acid differences gave a ratio of about 1–1.5 for all exons except exons 8 and 9, where the ratio was 0.2 and 0.4, respectively. This result and the carboxy-terminal conservation suggested conserved functions for selected portions of these genes.

mRNA and cDNA Structure, Organization, and Expression

Northern analyses of poly (A)⁺ RNAs from HeLa (epithelioid cells) and cultured skin fibroblasts from normal individuals and Gaucher disease patients

identified three acid β -glucosidase-specific mRNAs of ~ 5.6 , 2.5 , and 2.0 kb in length (Reiner *et al.*, 1988a; Graves *et al.*, 1986). The longest mRNA represented an unspliced or partially spliced nuclear transcript. S1 nuclease analyses demonstrated that the shorter mRNAs arose from alternate transcription initiation and alternative polyadenylation (Graves *et al.*, 1986). Use of alternative polyadenylation signals about 450 bp apart was demonstrated by sequence analyses (Reiner *et al.*, 1988b). The full-length cDNA extends at least 237 bp 5' of the most 5' ATG (Horowitz *et al.*, 1989). Thus, the major full-length mRNA is about 2.5 – 2.6 kb in length and has about 250 kb of 5' and 550 bp of 3' untranslated sequence as well as a 100-bp poly (A) tail (Reiner *et al.*, 1988a; Wigderson *et al.*, 1989). Acid β -glucosidase is expressed constitutively in all tissues. The level of mRNA may be increased, however, by about 2–3 fold in cultured fibroblasts and lymphoid lines from Gaucher disease patients (Reiner *et al.*, 1988a; Wigderson *et al.*, 1989). Also, epithelial cells expressed high levels of acid β -glucosidase mRNA, while skin fibroblasts and promyelocytes had intermediate steady-state levels (Reiner *et al.*, 1987). In comparison, macrophages had low and B cells had barely detectable steady-state levels of acid β -glucosidase mRNA. These results were consistent with the parallel levels of CAT activity expressed from constructs containing the acid β -glucosidase promoter in the corresponding tissues (Reiner *et al.*, 1987). These results suggest the involvement of a feedback regulation of acid β -glucosidase expression.

An unusual feature of the human acid β -glucosidase cDNA was the presence of two 5' in-frame ATGs as possible translation initiation codons (Reiner *et al.*, 1987; Graves *et al.*, 1988; Wigderson *et al.*, 1989; Sorge *et al.*, 1985). A similar observation was made with acid sphingomyelinase (Schuchman *et al.*, 1991). These two ATGs begin 57 and 118 nucleotides 5' to the first nucleotide of the codons for the mature polypeptide. Although both can be used (Sorge *et al.*, 1987; Choudary *et al.*, 1986), the immediate translation products encoded by sequences downstream from these ATGs differed markedly. If translation is initiated from the most 5' ATG, the initial sequence of 19 amino acids is hydrophilic and charged. In comparison, a typical hydrophobic signal sequence of 20 amino acids in length would begin at the second ATG. The downstream ATG is preceded by a Kozak consensus sequence for translation initiation. The relative physiologic relevance, if any, of the most 5' ATG is unknown. Expression of functional acid β -glucosidase from the cDNAs in mammalian or insect cells (Grabowski *et al.*, 1988, 1989; Martin *et al.*, 1988) demonstrated their authenticity. However, the two originally reported cDNAs (Sorge *et al.*, 1985; Tsuji *et al.*, 1986) contain cloning artifacts when compared to genomic DNA sequences or to the chemically determined amino acid sequences. The reported Leu²⁵⁹ was a cloning artifact which leads to an

inactive enzyme and Phe²⁵⁹ is the correct amino acid (Tsuji *et al.*, 1986; Choudary *et al.*, 1986). The His⁴⁹⁵ for Arg⁴⁹⁵ (Sorge *et al.*, 1985) also was a cloning artifact, but no functional abnormalities resulting from this substitution have been observed (G. A. Grabowski, unpublished).

Mutations in the Acid β -Glucosidase Gene Resulting in Gaucher Disease

Interest in the genetic mutations causal to Gaucher disease relates to the need to explain the marked heterogeneity of phenotypes within and among the variants. Also, the need for genetic markers of disease severity (Theophilus *et al.*, 1989a; Zimran *et al.*, 1989) has had increasing practical (i.e., prognostic) and basic (structure/function) import. Numerous disease alleles have been identified (Fig. 4 and Table IV). Several different nomenclatures used to designate the mutations are shown in Table IV. The genomic sequence numbering refers to the original report (Horowitz *et al.*, 1989), which has been corrected and extended (Beutler *et al.*, 1992b). The relationships of these mutant alleles to the disease have been established by family, population, and expression studies (see Disease Allele Frequencies and Genotype/Phenotype Correlations). A significant complicating problem for DNA diagnosis is the presence of the highly homologous pseudogene and the presence of disease alleles bearing multiple authentic, nonpolymorphic, point mutations. Since polymerase chain reaction (PCR) amplification of genomic sequences has supplanted the use of unamplified DNA, methods have exploited sequence differences to selectively amplify structural gene sequences (Theophilus *et al.*, 1989a; Zimran *et al.*, 1989, 1990; Tsuji *et al.*, 1987, 1988b, 1990; Firon *et al.*, 1990). This is particularly important since several missense and nonsense mutations arise from introduction of "pseudogene" base changes into the structural gene (Table IV, Fig. 4). These substitutions can be single (i.e., 1448C, 754A) or multiple. The latter mutant alleles probably represent rearrangements between the homologous sequences by crossover events (Firon *et al.*, 1990) or other poorly defined genetic events. These types of alleles have been referred to as complex alleles (Latham *et al.*, 1990), a "pseudo" pattern (Hong *et al.*, 1990; Ohashi *et al.*, 1991), or a "rec" (recombinant) (Eyal *et al.*, 1990). In addition, several disease mutations in the same codon have occurred directly following a pseudogene difference (Theophilus *et al.*, 1989a,b; Graves *et al.*, 1986), suggesting the possibility of repair difficulties. The apparent imperfect exchange of exons 5 and 6 (Latham *et al.*, 1990) and other disease alleles with multiple exon substitutions or deletions indicate the participation of the pseudogene in the pathogenesis of this disease. The only splice junction abnormality mimics a pseudogene sequence at

the exon2/intron2 splice donor site. This mutation was limited to a single base change and leads to the production of several alternatively spliced mRNA forms due to activation of a cryptic splice site (Fig. 6). There are several additional exonic differences between the structural gene and pseudogene, which are likely to be found in disease alleles in the future.

From studies on large populations of Gaucher disease patients, two mutations in exon 9 (N370S) and exon 10 (L444P) account for about 60% of all alleles present in patients with Gaucher disease. Table VII provides a compilation of frequency data on these two alleles in each of the three clinical variants. With the possible exception of one patient (Sidransky *et al.*, 1992), no patient with neuronopathic disease has had even a single copy of an N370S allele. In addition, with the exception of one Japanese patient (Masuno *et al.*, 1990), L444P homozygosity is associated with the neuronopathic variants or lethal disease early in childhood. Although these studies will require continuous updating, sufficient families have been studied to indicate the presence and prognosis of nonneuronopathic Gaucher disease from genotype analysis.

A puzzling finding among the neuronopathic phenotypes was apparent L444P homozygosity in both the very acute neuronopathic, type 2, and the subacute neuronopathic, type 3, patients (Theophilus *et al.*, 1989a; Tsuji *et al.*, 1987; Firon *et al.*, 1990). Complete sequencing of the cDNAs representing both alleles from such type 2 patients revealed the presence of multiply mutated alleles which contained the L444P missense error (Latham *et al.*, 1990). Consequently, these type 2 patients had two different alleles. The generality of this finding is not known.

TABLE VII. Frequency of Mutant Alleles among the Gaucher Disease Variants^a

Variant	Mutant allele		
	N370S	L444P	Other
Type 1	439/704 (62%)	79/635 (12.4%)	107/352 (30%)
Type 2	0/30 (0%)	55/52 (48%)	12/18 (67%)
Type 3 ^b	0/69 (0%)	55/80 (68.8%)	8/24 (33%)

^aAdapted from Tsuji *et al.* (1987, 1988b), Sidransky *et al.* (1992), Theophilus *et al.* (1989a,b), Latham *et al.* (1990, 1991), Zimran *et al.* (1989), Firon *et al.* (1990), Choy *et al.* (1991), Sibille *et al.* (1993), Grace *et al.* (1991).

^bDoes not include the Norrbottnian type 3 population.

Disease Allele Frequencies and Genotype/Phenotype Correlations

Of the numerous disease mutations only four occur with substantial frequencies in Gaucher disease populations. The N370S, 84GG, L444P, and IVS2⁺¹ alleles account for about 60–70, 10, 5, and 2%, respectively, of the identified Gaucher disease alleles (He and Grabowski, 1992; Sibille *et al.*, 1993; Beutler *et al.*, 1992a) (Table VIII). The R463C and multiply mutated complex alleles also have been reported independently by several groups (see Table IV). In the Gaucher disease type 3 population from the Norrbottnian region of Sweden the L444P allele is found almost exclusively (Dahl *et al.*, 1990; Theophilus *et al.*, 1989a). The Ashkenazi Jewish Gaucher disease population has been the only other intensively studied population (Zimran *et al.*, 1991). In studies of over 300 symptomatic Jewish patients with type 1 disease the N370S, 84GG, L444P, and other (including the IVS2⁺¹ and rare) alleles account for 71.2, 11.6, 4.44, and 2.12%, respectively, of the disease alleles in that population (He and Grabowski, 1992; Beutler *et al.*, 1992a). The largest discrepancies in allele frequencies are with the N370S allele, which varied by almost 10% in the studies by Beutler (1992a) (77.16%) and our analyses [68.7% (He and Grabowski, 1992)]. Thus, the allele identification rate in the Ashkenazi Jewish population using the above four alleles would vary between 89.34 and 95.9%. Our studies were strictly limited to symptomatic, unrelated patients and asymptomatic “patients” were excluded (Sibille *et al.*, 1993). This is a potential source of the frequency differences, since significant numbers of asymptomatic or very mildly affected family members and random Ashkenazi Jews are homozygous for the N370S mutation. Using molecular analyses, the true carrier frequencies for Gaucher disease alleles have been estimated by random screening in the healthy Ashkenazi Jewish population to be about 1/10 to 1/12

TABLE VIII. Frequency of Gaucher Disease Mutations in 258 Jewish Patients

Mutation	Number of alleles	Percent
N370S	369	71.2
84GG	60	11.58
L444P and XOVR ^a	23	4.44
IVS2 ⁺¹	11	2.12
V394L	5	0.9

^aXOVR is a fusion gene. Adapted from Beutler *et al.* (1992a) and Sibille *et al.* (1993).

individuals (Zimran *et al.*, 1991; Beutler *et al.*, 1992a). These results indicate a disease frequency of about 1/400 to 1/600. This is consistent with a large number of asymptomatic/mildly affected individuals in this population. In the author's experience with over 500 patients with documented acid β -glucosidase deficiency, only N370S homozygotes have been asymptomatic.

For effective delivery of medical care to patients affected with Gaucher disease, prognostic tests are required which predict the eventual degree of disease involvement in an individual or fetus. The simplest hypothesis is that various combinations of mutant and null alleles would lead to different phenotypic expressions of the disease. Genotype/phenotype correlations in 161 Ashkenazi Jewish patients demonstrate clear symptomatic prognostic import (Table IX). Symptomatic patients with N370S homozygosity had later onset and much less severe visceral and bony involvement than patients with the other genotypes. Such information has immediate genetic counseling and therapeutic impact.

Implicit in these data is the hypothesis that various thresholds of residual acid β -glucosidase activity levels exist in humans, as has been proposed in a more general manner by Conzelmann and Sandhoff (1983). One threshold prevents the development of symptomatic accumulation of acid β -glucosidase's major substrate, glucosylceramide. Obviously, this level is found in completely normal

TABLE IX. Genotype/Phenotype Correlations in Jewish Gaucher Disease

Genotype	Age of onset (years)	Bone (scale = 0– 5)	Liver enlargement ^a	Splenic enlargement ^a	Age at splenectomy (range) (years)
N370S/N370S	34.68 (3–73) <i>n</i> = 59 SD = 19.1	2.03 (1–4) <i>n</i> = 37 SD = 0.763	1.22 (0.79–2.14) <i>n</i> = 35 SD = 0.304	8.58 (1.8–25.1) <i>n</i> = 33 SD = 15.5	56 (26–69) <i>n</i> = 6 SD = 15.5
N370S/84GG	9.09 (2–28) <i>n</i> = 37 SD = 7.57	3.53 (1–5) <i>n</i> = 26 SD = 1.14	2.67 (1.0–4.12) <i>n</i> = 15 SD = 0.93	33.7 (10.2–62) <i>n</i> = 9 SD = 13.4	21.18 (5–57) <i>n</i> = 17 SD = 13.4
N370S/L444P	16.9 (2–35) <i>n</i> = 17 SD = 11.3	3.11 (1–5) <i>n</i> = 9 SD = 1.23	2.11 (1.7–2.69) <i>n</i> = 10 SD = 0.33	22.23 (6.89–43.3) <i>n</i> = 9 SD = 12.1	25 (2–44) <i>n</i> = 4 SD = 19.1
N370S/IVS 2 or ? and ??	14.28 (1–45) <i>n</i> = 48 SD = 11.38	3.03 (1–5) <i>n</i> = 38 SD = 1.19	2.08 (0.74–3.48) <i>n</i> = 28 SD = 0.68	23.83 (7.4–75) <i>n</i> = 7 SD = 18.06	26.1 (5–69) <i>n</i> = 23 SD = 18.24

^aFold increase over normal.

individuals and carriers for Gaucher disease. The next level is in enzyme-deficient patients who are asymptomatic or mildly affected (e.g., N370S homozygotes). Another threshold of acid β -glucosidase activity is that which leads to the development of the severe and/or neuronopathic Gaucher disease phenotypes. This threshold may be bounded by type 1 patients with the N370S/"inactive allele" (e.g., 84GG, IVS2⁺¹) genotype and type 3 patients with homozygosity for L444P. The relative ethnic homogeneity of the Norrbottnian population of type 3 Gaucher disease patients provides a genetic background to assess the variation of the type 3 phenotype due to L444P homozygosity. Characterization of this population of patients indicated that all develop mental retardation, have severe visceral and/or bony disease, and have shortened life spans (Svennerholm *et al.*, 1982). The severity and age of onset vary significantly among Norrbottnian type 3 patients (Dreborg *et al.*, 1980). This may explain reports in the literature that homozygosity for L444P has been associated with very severely affected patients who were apparently free of neuronopathic manifestations (Theophilus *et al.*, 1989a; Masuno *et al.*, 1990). Potentially, if these young patients had survived longer, neurological manifestations could have developed. However, greater insight into the molecular determinants of this threshold should be provided by thorough characterization of the mutant acid β -glucosidase genes from other type 3 patients with horizontal gaze palsy and myoclonic seizures in the juvenile age group (Paterson *et al.*, 1991; Yu *et al.*, 1990), as well as from adult patients who develop the onset of mental deterioration due to Gaucher disease late in life or apparently nonneuronopathic teenage patients (Winkelman *et al.*, 1983).

The distinction between types 2 and 3 is much clearer than those above and may be absolute, since type 2 disease has the onset of severe neuronopathic involvement in the first year of life and death occurs by about 2 years of age. In this regard the distinction between these two forms of Gaucher disease is similar to that between the infantile and the later onset forms of Tay–Sachs disease, Krabbe disease, and metachromatic leukodystrophy. For example, in Tay–Sachs disease, the infantile form has no residual β -hexosaminidase A activity (Meyerowitz and Proia, 1988), whereas the mutation in the later onset form of G_{M2}-gangliosidosis results in low levels (2–5% of normal) of this enzymatic activity (d'Azzo *et al.*, 1984).

MOLECULAR BIOLOGY OF THE ACTIVATOR PROTEINS

Previous pulse-chasing processing studies of SAP-1 and SAP-2 indicated extensive posttranslational proteolytic processing of each from a large-molecular-weight precursor (Fujibayashi and Wenger, 1986a,b) and that the genes for both

map to the same portion of chromosome 10 (Inui *et al.*, 1985; Fujibayashi *et al.*, 1985). The recent cloning of cDNAs encoding these activators has demonstrated that these two “activators” as well as two additional “activators” of sphingolipid hydrolases are encoded by a single gene in humans (Gavrieli-Rorman and Grabowski, 1989; O’Brien *et al.*, 1988; Nakano *et al.*, 1989; Reiner *et al.*, 1989) and rats (Collard *et al.*, 1988). As shown schematically in Fig. 7, the full-length human “proactivator” cDNA (Gavrieli-Rorman and Grabowski, 1989; Reiner *et al.*, 1989) contains regions of high amino acid similarity (>80%). The existence of these activators has been demonstrated by their partial or complete amino acid sequence determined from proteins isolated from human (Morimoto *et al.*, 1988, 1989; Kleinschmidt *et al.*, 1988; Dewji *et al.*, 1987) or guinea pig (Sano *et al.*, 1988) sources. The only major difference in the amino acid sequences predicted by the full-length cDNAs (Gavrieli-Rorman and Grabowski, 1989; Nakano *et al.*, 1989) was the presence of an in-frame 9-bp insertion (three amino acids) in the saposin B coding sequence of cDNAs isolated by Nakano *et al.* (1989) from lung and skin fibroblast libraries. Furthermore, the complete chemically determined sequence of saposin B did not contain the predicted three-amino acid insertion (Kleinschmidt *et al.*, 1988). These results suggest a potential for tissue-specific

Fig. 7. Diagram of the human prosaposin genomic organization and the relationship to the cDNA. The 5' end of the gene has not been fully characterized, but is probably composed of several small exons and larger introns. No large contiguous regions of high homology are present in the intronic sequences. The exons are not numbered since the 5' organization is not complete. The shown structure accounts for all but about 100 bp of exonic sequence.

alternative splicing of the “proactivator” RNA in the saposin B region (Holt-schmidt *et al.*, 1991b). A mutation resulting in aberrant splicing of the prosaposin mRNA leads to a 33-bp insertion in the saposin B region (Zhang *et al.*, 1991) surrounding the 9-bp insertion described by Nakano *et al.* (1989). Similar to the acid β -glucosidase mRNA, two species of human proactivator mRNA arise from alternative polyadenylation (Holt-schmidt *et al.*, 1991a) and the levels of mRNA expression parallel those of acid β -glucosidase, i.e., the levels of proactivator mRNA were higher in normal skin fibroblasts than in B cells and the mRNA levels are higher in the corresponding cells from Gaucher disease patients. The genomic organization (Holt-schmidt *et al.*, 1991a; Rorman *et al.*, 1992) and sequence (Rorman *et al.*, 1992) have been determined for all but the most 5' region. The available sequence is about 21 kb long, which accounts for all but about 100 bp of cDNA sequence. The high homology of the various saposin regions suggested a duplication scheme for evolution. However, there were no significant intronic similarities to suggest regions of duplication prior to intron insertion. Based on the placement sites of intron insertion, Rorman *et al.* (1992) (Fig. 8) suggested a duplication and recombination model for the evolution of this gene from an ancestral saposin B gene. Several mutations in the prosaposin gene have led to clinical sphingolipid diseases mimicking isolated enzyme deficiencies, including: (1) saposin B mutations (Kretz *et al.*, 1990; Rafi *et al.*, 1990; Zhang *et al.*, 1990, 1991) leading to metachromatic leukodystrophy-like diseases, (2) a saposin C mutation (Schnabel *et al.*, 1991) leading to a Gaucher-like disease, and (3) a mutation in the prosaposin initiation codon leading to deficiencies of both saposin B and C (Schnabel *et al.*, 1992).

THERAPEUTIC PROSPECTS IN GAUCHER DISEASE

Gaucher disease has been considered a prime candidate for therapeutic interventions since it is the most prevalent lysosomal storage disease, its major forms are nonneuronopathic, and its visceral manifestations result from bone marrow-derived reticuloendothelial cells. Indeed, the monocyte/macrophage system involvement has led to the use of bone marrow transplantation for effective treatment of the nonneuronopathic variants (Rappeport and Ginns, 1984; Starer *et al.*, 1987; Tsai *et al.*, 1992). The morbidity and mortality of nonautologous bone marrow transplantation restricts its therapeutic usefulness to the most severely involved nonneuronopathic patients. Recent studies have focused on the use of large doses of enzyme which has been specifically modified to expose terminal α -mannose residues on its oligosaccharide for targeting to the reticuloendothelial

Fig. 8. (A) The alignment of the placement of the introns (double-ended arrows) in the saposin A, B, C, and D amino acid sequences. The stippled regions correspond to the mature saposin regions. (B) An evolutionary model from an ancestral saposin B (I) gene to account for the preservation of the intronic placement. In II a tandem duplicated version of I is shown with introns being inserted following the duplication of I. In III and IV are shown a proposed double crossover to lead to the intronic alignment shown in (A) and in IV. This mechanism required a tandem duplication of the structure in II.

organs (Barton *et al.*, 1991). Based on these initial results of bone marrow transplantation, enzyme replacement, and the recent successes in long-term expression of foreign genes in murine stem cells (Fink *et al.*, 1990; Nolte *et al.*, 1992; Correl *et al.*, 1992), the future development of genetic therapeutic approaches or more available enzyme replacement strategies portends a bright future for affected patients with Gaucher disease.

The available supportive therapies for Gaucher disease have been reviewed recently (Beutler and Grabowski, 1993) (Table X). Although many of these will

TABLE X. Available Intervention for Gaucher Disease

Hematologic care	Bone marrow transplantation
Iron and vitamin supplementation	Curative
Supportive intervention	Matched sibling donor
Total or partial splenectomy	High risk for nonlethal diseases
Androgen therapy	Prenatal diagnosis
Erythropoietin (not useful)	Heterozygote and homozygote detection
Bone crisis	Enzyme replacement
Pain management	
Core decompression	

continue to have supplementary roles in the total care of affected patients with type 1 disease, most have been supplanted by efficacious enzyme therapy.

Enzyme Therapy

In comprehensive reviews Desnick *et al.* (1976) and Desnick and Grabowski (1981) emphasized requisites for enzyme therapy:

1. The availability of large amounts of stable enzyme suitable for administration.
2. Delivery of the enzyme to the major cellular and organellar sites of pathology.
3. Development of practical serial evaluation procedures to monitor efficacy.

Early attempts at enzyme therapy for Gaucher disease (Brady *et al.*, 1974, 1975; Pentchev, 1977; Beutler *et al.*, 1977, 1980; Beutler and Dale, 1982) reemphasized these requisites. The macrophage and its tissue-specific counterparts (Kupffer cells, alveolar macrophages) have long been recognized as the principal cells involved in Gaucher disease type 1. The discovery of specific receptors for α -mannosyl-terminated oligosaccharides on macrophages provided the impetus to develop a modified acid β -glucosidase for targeted delivery (Achord *et al.*, 1978; Stahl *et al.*, 1978). Industrial-scale production of the human placental acid β -glucosidase with α -mannosyl residues exposed (Ceredase®; al-glucerase) made possible the clinical and biochemical evaluation of enzyme therapy. The evaluation of this preparation in one child suggested clinical efficacy (Barranger *et al.*, 1989; Barton *et al.*, 1990). Larger clinical trials demonstrated (Barton *et al.*, 1990; Beutler *et al.*, 1991b; Fallet *et al.*, 1992; Figueroa *et al.*, 1992; Pastores *et al.*, 1993) clinical and biochemical efficacy in about 60 reported

patients. Summaries of the visceral organ results are presented in Fig. 9A and 9B. In anemic patients the hemoglobin concentration of blood increases by about 1.5 gm% in the first 4–6 months of enzyme therapy. An additional 1 gm% increase is observed in the subsequent 9–18 months in persistently anemic patients (Pastores *et al.*, 1993). Platelet count increases are much slower and require at least 1 year to double. The most dramatic hemoglobin and platelet responses have been in splenectomized patients with pulmonary involvement, but in general the response to

Fig. 9. (A) Hepatic and (B) splenic volume decreases following enzyme therapy (60–30 IU/kg q 2 weeks initial doses) with macrophage-targeted acid β -glucosidase (alglucerase). In (A) several different doses of alglucerase were used with an overall response of $\sim 25\%$ decrease in hepatic volume by 6 months. The squares represent patients who had or decreased to hepatic volumes of $\leq 150\%$ of predicted normal by 6 months of therapy and demonstrate a nearly asymptotic approach to normal volumes. The presence or absence of a spleen did not influence the response to therapy. In (B) several different doses of enzyme demonstrated no overall trends in the effect of differing initial doses. The transitions to different types of lines for any patient indicate a decrease in dose by 50%. The patients with dosage reductions are indicated by filled triangles. Overall, no consistent differences in rates of response were observed with diminishing doses from 60–30 IU/kg to 20–10 IU/kg q 2 weeks.

Fig. 9. (Continued)

enzyme therapy is the same in patients with or without intact spleens (Pastores *et al.*, 1993). Bone pain gradually decreases, but the X-ray abnormalities of bone appear to respond very slowly. Magnetic resonance imaging shows a decrease in bone marrow involvement (N. Barton, personal communication). About 10–15% (36/272) of patients treated develop antibodies against the enzyme protein (Richards *et al.*, 1993; Pastores *et al.*, 1993). A few of these develop pruritus with enzyme infusion, but it has been possible to continue therapy in such patients without the occurrence of serious reactions (Pastores *et al.*, 1993). One patient has had an anaphylactoid reaction with complement activation (G. A. Grabowski, unpublished observation). All antibodies reported to date have been IgG, predominantly IgG1 subclass (Richards *et al.*, 1993). The processing of the placental extract destroys known viruses, including the human immunodeficiency virus. Recombinant enzyme is not yet commercially available, but initial clinical trials have indicated efficacy (Grabowski *et al.*, 1993).

To monitor responses to therapy, some investigators have expressed the

changes in visceral organs as a percentage decrease in excess liver or spleen volume (Figuroa *et al.*, 1992). However, this method greatly exaggerates small decreases in livers that are only slightly enlarged. For example, a liver which is calculated to be 150% of normal size has an excess volume of 50%. If this decreases to 120% of normal, the percent decrease in excess liver is 60% (i.e., $0.3/0.5 \times 100\%$), whereas the absolute change was 20% ($30\%/150\% \times 100\%$). If an additional 20% decrease is observed in the same patient, the liver is now normal in volume, so the absolute decrease of 17% corresponds to a 100% decrease in percent excess liver volume. This and the finding that the error of repeated measurement in volume determinations is from 4 to 12% indicate the inappropriateness of this calculation. In addition, nearly all patients receiving alglucerase gain substantial weight. Consequently, body weight normalization of hepatic volume calculations could suggest decreases when little or no absolute change actually occurs. This error coupled with the calculation of percent excess liver decrease could greatly exaggerate changes in visceral organ response. For adults, it is better to calculate the absolute percentage change referenced to an average body weight. In growing children this calculation is more problematic. The need to standardize the hepatic and splenic volumes to body weight is not trivial. For the liver volume, the response to alglucerase becomes less pronounced as this volume approaches normal (Pastores *et al.*, 1993). At volumes less than about 150% of normal there is an asymptotic approach to the normal range. Thus, no or small changes could suggest a lack of therapeutic effect. If this occurred following a dose reduction, a misinterpretation would indicate a diminished response. Indeed, no difference in response rate was found with diminishing doses in patients with livers greater than 150% of normal and efficacy was demonstrated at lower doses. Currently, trials of decreasing doses of enzyme with differing schedules of administration (q 2 weeks or several times per week) are attempting to determine optimal doses and schedules of administration. This has not been established. To date, all doses and schedules have shown efficacy.

Very little data on the tissue, cellular, and subcellular distribution or the half-lives of the administered enzyme have been published. Only the single case report of enzyme analyses in tissues of a treated patient suggests persistence of exogenous enzyme for a significant period in particular organs (Fallet *et al.*, 1992). The lack of such data is a severe limitation to optimizing doses and dosing schedules and acquiring such data is more relevant than current discussions limited to overall clinical responses. Indeed, ongoing clinical trials are being conducted without benefit of such formal tissue pharmacokinetic data.

In addition, several substantive and scientifically difficult issues need to be resolved in an effort to promote optimal use of this highly efficacious therapy or

the development of a new generation of recombinant drugs (Grabowski *et al.*, 1993). Our zeal to decrease doses of alglucerase has been primarily focused on monitoring diminishing sizes of visceral organs and good hematologic responses. In focusing on these easily measured responses, are the effects on bone or lungs being more poorly documented? Is there a tissue-specific dose requirement which will determine overall efficacy of alglucerase therapy? Indeed, pulmonary involvement can be the most lethal and bony disease the most debilitating aspects of this disease. Is there a diminution in dose requirement for continued improvement as the mass of Gaucher cells and accumulated glucosylceramide decreases (i.e., how important is the distribution space of the enzyme to therapeutic response and what is that space?)? What is the minimal effective dose and at what degree of involvement? What is the maximal effective dose and who should receive it? To date, there is no clear relationship between the rate of improvement and initial doses between 30 and 120 IU/kg every month. Most importantly, is it more efficacious to prevent the disease manifestations than to attempt to return a very sick individual, with potentially irreversible tissue damage, to health? If very small amounts of enzyme are required to maintain health, then long-term alglucerase may be very affordable. In addition, the efficacy or lack thereof of enzyme therapy for the neuronopathic variants remains to be evaluated.

In view of the high cost of enzyme therapy with all current infusion protocols, the timing of the initiation of therapy must be carefully evaluated. In highly symptomatic patients therapy should begin immediately. In addition, other patients with major hematologic and visceral organ involvement should receive high priority. Although bone disease appears to be the slowest to respond, patients with substantial bone involvement are candidates for treatment to recover bone integrity and to prevent irreversibility of some bony damage. More difficult questions are posed by presymptomatic patients whose susceptibility to major disease manifestations can be anticipated by genotype analysis. Clearly, the issue of the timing and use of an apparently universally effective therapy must be carefully considered and the potential for preventive intervention should be evaluated. Although the drug appears safe, all risks of treatment may not, as yet, be appreciated.

Bone Marrow Transplantation

Since the manifestations of Gaucher disease type 1 are due to abnormalities of the macrophage, replacement of hematopoietic stem cells via allogeneic marrow transplantation should be curative. A number of patients with type 1 disease (Rapaport and Ginns, 1984; Starer *et al.*, 1987; Tsai *et al.*, 1992) have undergone bone marrow transplantation with therapeutic responses. There have been deaths

secondary to the transplantation procedure. In patients with subacute neuronopathic (type 3) disease, bone marrow transplantation has reversed the visceral disease and there are suggestions that the progression of neurologic disease may be arrested (Tsai *et al.*, 1992; Ringdén *et al.*, 1988; A. Erikson *et al.*, 1990). In one well-documented case, enzyme reconstitution to expected levels was achieved in visceral organs, but no increase in brain enzyme levels was found after 2½ years of full engraftment from an enzymatically normal sibling (Tsai *et al.*, 1992) (Table XI). The kinetics of cellular replacement were documented in liver and bone marrow of this patient. About 9 months was required to normalize the Kupffer cell population, whereas over 18 months was required for bone marrow normalization. This patient was uneventfully and fully engrafted for over 2 years. At autopsy of this patient, who died of pneumococcal sepsis, a few pockets of Gaucher cells were found in bone marrow, liver, lung, and lymph nodes. These results indicate a prolonged life span of tissue Gaucher cells.

The risks of the procedure impacts on the decision for bone marrow transplantation therapy. When successful, however, it provides the potential for permanent cure. In the short term, transplantation costs are less than those of enzyme augmentation, but the chronic care costs of complications may be very high. Recommendation of transplantation in type 1 patients is difficult due to the high (10%) mortality of the procedure, from long-term adverse effects (Sullivan and Reid, 1991). Indeed, the procedural risk is even higher in patients who most need treatment, i.e., those with advanced organ dysfunction. With the availability of safe enzyme therapy, there is likely little, if any, role for bone marrow transplanta-

TABLE XI. Tissue Levels of Acid β -Glucosidase in Normals and Gaucher Disease Patients^a

Source	Liver	Lung	Lymph node	Brain
BMT Patient				
(4MUGlc)	35	14	19	1
(GC)	30	31	22	9
Normal (n = 11)				
(4MUGlc)	84 $\pm$ 25	23 $\pm$ 9	31 $\pm$ 11	61 $\pm$ 21
(GC)	71 $\pm$ 18	56 $\pm$ 12	37 $\pm$ 9	69 $\pm$ 25
Gaucher disease (n = 5)				
(4MUGlc)	12 $\pm$ 5	0.6 $\pm$ 0.5	4 $\pm$ 2	2 $\pm$ 1
(GC)	14 $\pm$ 7	6 $\pm$ 4	3 $\pm$ 2	11 $\pm$ 7

^aTissue samples were obtained at autopsy of normals between 2 and 24 hr of death. 4MUGlc and GC refer to the synthetic and glucosylceramide substrates, respectively. Levels are given as nmole/hr per mg protein. From Tsai *et al.* (1992).

tion in the treatment of Gaucher disease type 1. This modality should be systematically evaluated in type 3 disease, particularly since it is unknown if the neurologic disease could be prevented or arrested by the intravenous administration of an enzyme that is not known to cross the blood-brain barrier.

Gene Transfer

Since Gaucher disease type 1 can be cured by bone marrow transplantation, correction of the molecular defect in the patient's own hematopoietic stem cells by gene transfer should also prove effective. For such gene therapy to be effective, however, several special features of Gaucher disease must be considered. Acid β -glucosidase is an intracellular membrane protein which is not normally present or functional in the extracellular space. As such, cross correction between cells is not observed. Thus, genetic reconstitution would be required in 100%, or at least a large percentage, of bone marrow stem cells to avoid pathologic cellular mosaicism in tissues. Gaucher cells were demonstrated in liver, bone marrow, lungs, and lymph nodes of a patient receiving bone marrow transplantation (Tsai *et al.*, 1992). This patient was fully engrafted with her homozygous normal brother's cells for 2½ years. Consequently, cellular replacement via turnover is the mechanism of therapy. These results also suggest that if only a portion of stem cells are corrected, Gaucher cells would develop in tissues at some level of macrophage chimerism and could have pathophysiologic effects. In addition, the persistence of Gaucher cells in the presence of a normal plasma glucosylceramide level for over 2 years in a bone marrow-transplanted patient indicates that efforts to directly lower plasma substrate levels will likely not decrease intracellular storage by a "diffusion" mechanism. Also, stem cells that produce active acid β -glucosidase do not have any obvious proliferative advantage over those that do not. Thus, therapeutic effects would be expected only if the patient's untransformed cells were ablated by chemotherapy or irradiation. Rational strategy for the treatment of Gaucher disease would be marrow ablation followed by autologous transplantation with transformed hematopoietic stem cells, and considerable effort has been expended to develop the required efficient gene transfer technology (Fink *et al.*, 1990; Nolte *et al.*, 1992; Correl *et al.*, 1992; Ohashi *et al.*, 1992).

The transfer of stable, functional acid β -glucosidase into cultured fibroblasts and transformed lymphoblasts using a retroviral vector was readily accomplished (Sorge *et al.*, 1987), but transfer into hematopoietic stem cells has been difficult (Fink *et al.*, 1990; Nolte *et al.*, 1992; Correl *et al.*, 1992). Recently, high-efficiency transfer of the human acid β -glucosidase cDNA into murine and human hematopoietic stem cells or progenitors has been accomplished (Kohn *et al.*, 1991; Nolte

et al., 1992; Fink *et al.*, 1990; Ohashi *et al.*, 1992) with evidence of sustained gene expression. As gene therapy is developed for Gaucher disease and as the use of enzyme therapy is optimized, difficult decisions of “cure” with unknown risks and “treatment” will confront the treating physicians.

DIAGNOSIS

Morphological Diagnosis

The diagnosis of Gaucher disease has been most frequently suspected by finding Gaucher cells in tissues. Similar cells, pseudo-Gaucher cells, have been described in a variety of other disorders, including chronic granulocytic leukemia (Kattlove *et al.*, 1969; Keyserlingk *et al.*, 1972; Dosik *et al.*, 1972), thalassemia (Parkin and Brunning, 1982), multiple myeloma (Scullin *et al.*, 1979), Hodgkin disease (Zidar *et al.*, 1987; K. S. Lee *et al.*, 1982), plasmacytoid lymphomas (Parkin and Brunning, 1982), and in the acquired immunodeficiency syndrome (AIDS) in patients with *Mycobacterium avium* infections (Solis *et al.*, 1986). These “pseudo-Gaucher” cells do not contain the typical tubular structures of authentic Gaucher cells (R. E. Lee, 1982; Parkin and Brunning, 1982). Because of the need for an invasive procedure and its nonspecificity, histologic diagnosis of Gaucher disease may have selective use in monitoring the efficacy and progress in certain patients undergoing therapy for Gaucher disease.

Enzymatic Diagnosis

Numerous assay procedures have been developed to establish the diagnosis of Gaucher disease. Determinations of acid β -glucosidase activity levels in peripheral blood leukocytes, cultured skin fibroblasts, amniotic fluid cells, and chorionic villae are readily accomplished using the glucosylceramide or 4-methylumbelliferyl- β -D-glucopyranoside substrates. The absolute level of enzyme activity varies with the assay conditions and the type of substrate. A finding of <15% of mean normal activity is diagnostic of the disease [see Grabowski *et al.* (1990) for review]. It has been problematic to develop an *in vitro* assay system which mimics the native environment of the enzyme in the cell. Consequently, the determined *in vitro* acid β -glucosidase activity does not accurately reflect the available hydrolytic capacity toward glucosylceramide. As a result, the *in vitro* level of enzyme activity has not consistently differentiated the neuronopathic (types 2 and 3) and nonneuronopathic (type 1) variants.

In heterozygotes peripheral blood leukocytes and cultured skin fibroblasts

have about one-half normal β -glucosidase activity. Most screening assays have used peripheral blood leukocyte components as the enzyme source. There is considerable overlap (up to 20%), however, between the normal and heterozygote ranges. This results in a large number of false positive and false negative results. Although many methods have been used to improve the accuracy of discrimination [see Grabowski *et al.* (1990) for review], a substantial overlap range remains. To establish 95% confidence ranges for inclusion of all obligate heterozygotes, about 25–30% of normal controls will be in the heterozygote range. Thus, enzyme assays for heterozygote detection are not suitable for mass screening and should be used with caution in at-risk families where there is an *a priori* probability of carrying a Gaucher disease gene.

Diagnosis by DNA Analysis

DNA-based technology has significant advantages over enzymatic diagnosis of Gaucher disease since the results are qualitative and the samples are very stable. Many Gaucher disease alleles have been characterized (Fig. 4, Table IV), but others have yet to be identified. As with all genetically heterogeneous traits, inability to detect a mutant allele does not exclude its presence. In the Ashkenazi Jewish population, the mutations at cDNA N370S, 84GG, L444P, and IVS2⁺¹ account for 89–95% of the Gaucher disease alleles. Thus, Gaucher disease can be confirmed by DNA analysis in a high percentage of Jewish patients and heterozygote screening in this population is feasible by DNA analysis. Among non-Jewish patients, the mutations L444P and N370S account for 60–75% of the disease-producing alleles. In the non-Jewish populations the presence of many rare or private alleles decreases the utility of DNA testing for diagnosis and heterozygote screening. A variety of PCR-based techniques for facile detection of mutations have been described (Theophilus *et al.*, 1989a,b; Beutler *et al.*, 1990, 1991a; Eyal *et al.*, 1990; Firon *et al.*, 1990) and the amplification refractory mutation system (ARMS) has also been successfully employed (Mistry *et al.*, 1992).

CLINICAL GENETICS AND PREVENTION

Prenatal Diagnosis

Acid β -glucosidase activity is readily measured in amniotic fluid cells and chorionic villus samples and thus prenatal diagnosis is routine for all types of Gaucher disease (Schneider *et al.*, 1972; Svennerholm *et al.*, 1981; Heilbronner

et al., 1981). More recently, DNA-based technology has provided for identification of the genotype of the fetus where disease alleles are known. With the use of PCR, an ultrasensitive technique, one must be particularly concerned about contamination of fetal DNA with maternal DNA. However, in the absence of preferential amplification, maternal contamination, if small, should not present major difficulties.

Genetic Counseling

When a couple already has a child with Gaucher disease and both are heterozygotes, the recurrence risk, as in other autosomal recessive disorders, is 1 in 4. Because homozygotes for the N370S mutation often have very mild disease, there are many instances in which one of the parents is a homozygote. Indeed, this happens sufficiently frequently that Gaucher disease was once regarded as being transmitted as an autosomal dominant disorder (Greon, 1948). When one of the parents is a homozygote and the other heterozygous, the recurrence risk is 1 in 2.

Prediction of disease severity in an at-risk family is more straightforward if there is already an affected individual. There is a considerable concordance of disease involvement between sibs, but occasionally substantial differences can exist. When a child is at risk for L444P homozygosity the prognosis is very grave. The likelihood of eventual, if not early, neurologic involvement is high and in any case severe visceral and skeletal disease is to be expected. Counseling may be more difficult when one of the less common alleles is present.

In Gaucher disease type 1, genetic counseling and provision of medical care to affected patients has been particularly difficult due to the marked interfamilial phenotypic variation of the disease types (Beutler and Grabowski, 1993). With the advent of effective therapies, the genetic counseling and provision of care to families with Gaucher disease has become even more time consuming and difficult. In an effort to develop prognostic indicators, five objective parameters of Gaucher disease involvement (age of onset, bony severity, liver and splenic enlargement, and age at splenectomy) were determined and correlated with the genotypes in 161 symptomatic type 1 patients (Sibille *et al.*, 1993) (Table IX). These results demonstrate that symptomatic patients with genotypes other than N370S homozygosity will develop much more severe disease of their bone, liver, and spleen at a much earlier age. Based on these results in Gaucher disease type 1, genetic counseling and provision of medical care to at-risk families must now include informed discussions of fetal involvement and damage as well as the probability of future involvement by the disease process and the potential of early intervention therapy.

In spite of these clear differences in the clinical expression of different genotypes, there is tremendous intragroup variation. Some patients with N370S homozygosity manifest moderately severe disease and some patients with the N370S/“other mutant allele” genotypes have moderate disease (Beutler *et al.*, 1992a; Sidransky *et al.*, 1992; Sibille *et al.*, 1993). There are currently insufficient data to be certain how much of this variation is interfamilial, how much is intra-familial, and how much may be due to modifier genes or environmental factors.

Clearly, the above discussion is predicated on the delivery of informed genetic counseling and genetic care to patients affected with Gaucher disease. The use of genotype/phenotype correlation data cannot be in a vacuum. They must be presented to patients and affected families in a highly controlled, carefully designed infrastructure, in centers staffed by knowledgeable, experienced medical genetic personnel. The lack of appropriate genetic infrastructure and treatment facilities is a disservice to the patients. In addition, knowledgeable physicians in the area of Gaucher disease must be available since broad variation in phenotypic manifestations, present even within the genotypes, must be addressed when providing care and counseling to affected patients and their families. Furthermore, as the base of well-studied patients expands, exceptions to the above genotype/phenotype correlations are likely to be detected. The rate of such exceptions will have significant impact on the information provided to at-risk and affected families. As a prototype for genetic susceptibility counseling, the complex personal and psychosocial issues will need to be addressed and require a restructuring of current genetic care delivery to accommodate such. The implications for the expanding list of other life-threatening or chronic, debilitating inherited diseases, e.g., cystic fibrosis, for which gene-based therapies and genotype/phenotype correlations will be developed necessitates the expansion and reeducation of genetic care providers.

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