GAUCHER DISEASE

A HETEROGENEOUS CLINICAL COMPLEX

WHERE

EFFECTIVE ENZYME REPLACEMENT HAS COME OF AGE

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Clinical Cases

Case # 1: N.S.S.: Mrs. S. at age 69 was brought to the emergency room following a motor vehicle accident where she had been thrown against the dashboard. Survey films revealed lytic changes in her right hip and a possible linear fracture.

Over the previous five years she had noted aching discomfort in her knees and hips, primarily in the early morning hours. Radiologic evaluations of knees, hips and shoulders were said to reveal only changes compatible with osteoarthritis by her family physician. She had been treated during that time with a variety of non-steroidal anti-inflammatory agents with little response.

Her past history included two previous pregnancies without complications and a cholecystectomy at age 38 for gallstones.

Her mother and father had died in their late seventies of arteriosclerotic heart disease. She had no siblings.

Physical examination was reported as within normal limits, except for limitation of motion in her right hip because of pain.

Because of the question of a pathologic fracture, the patient was taken to the operating room where a biopsy was performed; a tissue diagnosis of Gaucher disease was made. A hip replacement was carried out when the frozen section data was reported.

The patient was referred to us with the above data. The only additional features included a spleen palpable 3 centimeters beneath the left costal margin.

Her peripheral hematologic values and a chemical survey were normal. Her serum acid phosphatase was 2.8.

Case # 2:R.E.P.: R.E.P.is a fifty-four year old woman who was well until age 13 when her family physician noted splenic enlargement. She was referred to Dr. Charles Doan at Ohio State University. Dr. Doan noted the spleen extended approximately 2 centimeters beneath the left costal margin. A sternal bone marrow aspiration identified 5.5% Gaucher's cells and a diagnosis of Gaucher disease was made. Since she had two siblings, one 8-1/2 and the other 7 years old, Dr. Doan evaluated them as well. Each had a palpable spleen tip, and each on bone marrow aspiration demonstrated Gaucher cells, the youngest (7 years old) had 2% Gaucher cells and the elder (8-1/2 years old) had 3% Gaucher cells. Dr. Doan then did sternal marrow aspirations on the parents; and, they were within normal limits.

Mrs.R.E.P. was asymptomatic until the delivery of her second child in 1965. During the early part of her pregnancy her obstetrician noted that her spleen was palpable 3 centimeters beneath the left costal margin. Immediately after the delivery, she was noted to have significant splenic enlargement; it was palpable 12 centimeters beneath the left costal margin. Her platelet count was 1,000/μl. Immediately post-partum she was begun on steroids with a slight increment in platelet numbers. Her spleen size decreased slightly, but remained significantly larger than prior to her pregnancy. She also developed anemia (hemoglobin of 9 grams). Shortly thereafter a splenectomy (and elective cholecystectomy) was performed; and, her platelets increased to the

range of 100,000/ μl and her hemoglobin to 11 grams. These have remained in that range.

In 1983 the patient was admitted to the hospital with excruciating pain and local tenderness over her entire left tibia. Her ESR was 110 and she had a WBC of 22,000/ μl; the remainder of her laboratory findings were unchanged. The severity of the pain required continuous infusion narcotics. The clinical picture was not incompatible with osteomyelitis, however; with her known history and the failure to have an alteration in radiographs the clinical condition appeared quite classic for "Gaucher's crisis". Slow resolution over two weeks occurred. She has not had further difficulty with that tibia. She did have a similar episode approximately two years later on the right that lasted about five days.

Within three years of her splenectomy, she began to have significant difficulty with pain in her lower extremities, primarily her hips. Bilateral osteonecrosis was identified along with multi-cystic changes in both femurs. She has been followed by Dr. Henry Mankin at the Massachusetts General Hospital and she was managed primarily with supportive procedures. A variety of stress fractures developed over the next several years. However, on a program of physical therapy and occasional cane support, she was able to function quite well. In 1987 progressive symptoms in the right hip region led to a total hip arthroplasty.

Although she has similar remarkable changes in her left hip, she has been able to function normally with only an intermittent requirement for a cane.

It is of some interest that each of the three children have developed congestive splenomegaly with evident hypersplenic features at around age 30, requiring splenectomy. In addition each have had at least one hip replacement in their late 40s. Each however is highly functional and they are active and prominent citizens within their respective communities.

Case # 3: A.W.: A.W. is a 44 year old Hispanic woman from Mexico. She became symptomatic at age 5 with episodes of severe distal femur pain, swelling and erythema that persisted for days to weeks. These episodes were treated as osteomyelitis with antibiotics. At age 11 a diagnosis of anemia was made (her hematocrit was 27). She was treated with a variety of hematemics without effect. At age 14 she had a pathologic fracture of her left proximal femur and was incapacitated for several months. At age 16, osteotomies of the left tibia and fibula were performed in an attempt to correct a developing genu valgum deformity. Following this surgical procedure she developed clinical findings compatible with osteomyelitis of the tibia, and she was treated with antibiotics over a nine year period. When she was 18, her anemia was noted to be more severe (hematocrit of 15). Hepatosplenomegaly was recognized. A bone marrow examination resulted in a diagnosis of Gaucher's disease. Elective splenectomy was performed at that time. She continued to have difficulties with both hips and at age 27 a right hip osteotomy was carried out. At age 30 while pregnant she moved to Dallas. She was given two transfusions during the pregnancy, and had an uneventful parturition. Shortly thereafter her hip symptoms increased, and at age 31 bilateral hip replacements were done. She developed an infection of the right hip and had a repeat replacement. She became transfusion-dependent following that repair. At age 33 she developed the clinical picture of a conus medullaris syndrome that was secondary to bleeding, progressive thrombocytopenia identified during the previous two years. Her platelet counts were in the range of 20,000. At age 43 she had a mechanical small bowel obstruction. A liver biopsy was done and a diagnosis of hemochromatosis was made.

Her family history is not clear. She is from a very large family with no history of consanguinity. She has ten siblings; one brother is known to have Gaucher disease and died at age 31 of an unknown mechanism. Six other siblings died in infancy and no data is available.

Over the past several years she has had a progressive transfusion requirement with decreased functional capacity and clinical evidence of a transfusional iron overload. Iron chelation has been proposed, but she has refused. She has had recurrent mucosal bleeding secondary to her thrombocytopenia (platelets 5-10,000) and clear evidence of left hip instability. Because of her remarkable transfusion requirement and poor tissue turgor, the orthopaedic team did not feel the risk of repeat left hip surgery merited.

Her previous treatment has included biphosphonates, a prolonged trial of erythropoietin, and recently, therapy with Ceredase.

SELECTED ISSUES IN GAUCHER DISEASE

1. What is the nature of the lesion?

2. Can the defect explain the clinical manifestations?

    - Clinical diversity?

    - Do the sites of glucocerebroside correlate with expected sites of storage cells?

    - Are some findings epiphenomena?

        - energy deficient

        - infections

        - bleeding

        - abnormal laboratory studies

3. Can the recent molecular biologic characterizations of the gene(s) explain the clinical heterogeneity?

4. How should the clinical diagnosis be made?

5. Who should have a splenectomy?

6. Who should be treated with enzyme replacement?

SELECTED ISSUES IN GAUCHER DISEASE

I. What is the nature of the lesion?

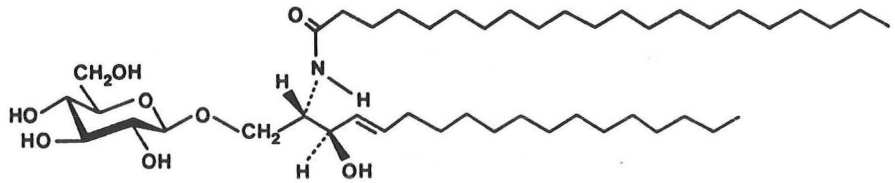
The eponymic designation of defective glucosylceramide catabolism is the result of the description by Gaucher in 1882 (1) of a 32 year old woman with a large spleen that he described as an epithelioma.

GLUCOSYLCERAMIDE LIPIDOSES

GAUCHER DISEASE

1882	**Gaucher:**	**Doctoral thesis, "Splenic epithelioma"**
1900	**Bovaird:**	**Familial pattern**
1918	**Mandelbaum:**	**Cells were reticulum cells**
1924	**Lieb:**	**It's a cerebroside**
1965	**Brady and Patrick:**	**Lesion shown to be glucocerebrosidase deficiency** (2, 3, 4)

In 1965 Roscoe Brady's group (2) and Patrick (3) demonstrated a deficiency of the lysosomal enzyme glucocerebrosidase in the tissues of patients with Gaucher disease which resulted in defective hydrolysis of glucosylceramide (to glucose and ceramide).The major source of glucocerebroside is the degradation of non-neuronal cell membranes.



Glucocerebroside
(D-glucosylceramide)

from (4)

Since this catabolic step occurs in the macrophage-monocyte system, it is not surprising that the "organs of the reticuloendothelial system" (liver, spleen, bone marrow) are the primary sites of the lipid deposition.

Gaucher's disease has commonly been divided into 3 subtypes (4,5):

Type I: Nonneuronopathic type: This is the most prevalent form. It is seen in adults and lacks nervous system involvement.

Type 2: Acute neuronopathic type.

Type 3: Subacute neuronopathic type.

Type I, the common adult form, is the topic at issue in the current review. However, Barringer et al. (4) have provided an excellent comparative table of the characteristics of each of the types:

Subtypes of Gaucher Disease

	Type 1 *Nonneuronopathic Chronic*	*Type 2* *Acute Neuronopathic*	*Type 3* *Subacute Neuronopathic*
Clinical characteristics	1. Heterogeneous presentation 2. Marked differences in age of symptoms (from birth to 80 yrs) 3. Marked differences in rate of progression of signs and symptoms 4. Marked differences in number of organ systems involved and rate of progression in organ systems 5. No neurologic involvement 6. Common signs: Hepatosplenomegaly; osseous lesions: osteopenia, lytic lesions, osteonecrosis, failed remolding 7. Rare signs: Pulmonary infiltration; pulmonary hypertension; cyanosis; clubbing; renal involvement; cirrhosis and liver failure; pericarditis	1. Stereotypic presentation 2. Onset of clinical signs at 3 mo 3. Death before 2 yrs 4. Common signs: hepatosplenomegaly, hypertonic posture, strabismus, trismus, brain stem signs, seizures	1. Heterogeneous presentation 2. Variable age of onset of systemic signs; variable progression 3. Onset of neurologic signs in childhood or adolescence 4. Common signs: hepatosplenomegaly; osseous lesions: osteopenia, lytic lesions, osteonecrosis, failed remolding; slowly progressive dementia; myoclonus; supranuclear ophthalmoplegia
Pathology	1. Gaucher cells and variable degree of fibrosis in all organs 2. Perivascular Gaucher cells in brain 3. Storage in reticuloendothelial cells causing eccentric nucleus and expanded fibrillar cytoplasm	1. Gaucher cells in all organs including both perivascular and parenchyma of brain 2. Areas of mild gliosis, cell death neuronophagia especially occipital cortex	1. Gaucher cells in all organs but without marked changes in brain
Biochemistry	1. Deficiency of glucocerebrosidase 2. Accumulation of glucosylceramide in all organs except brain 3. Cross reactive material (CRM) to normal enzyme present	1. Deficiency of glucocerebrosidase 2. Accumulation of glucosylceramide in all tissues including brain 3. CRM present, but altered	1. Deficiency of glucocerebrosidase 2. Accumulation of glucosylceramide in all tissue including brain, but to a lesser extent than in type 2 3. CRM present, but altered
Genetics	1. Autosomal recessive inheritance 2. Incidence among Ashkenazim 1/600–1/2500 3. Incidence among general population: rare 4. Suspect many allelic mutations different from types 2, 3	1. Autosomal recessive inheritance 2. Incidence: rare 3. No ethnic predilection 4. Suspect allelic mutations different from types 1, 3	1. Autosomal recessive inheritance 2. Incidence: rare 3. Panethnic with large Norrbottnian subgroup 4. Suspect allelic mutations, different from types 1, 2

II. Can the metabolic defect explain the clinical manifestations?

A. Issues of clinical diversity:

1. Age at onset of clinical symptoms and the severity of the illness is very variable.

2. Great variability in the (number) of organ systems involved.

3. Variability in the rate of progression of the organ involvement.

B. Sites of glucocerebroside deposition:

1. Spleen:

Splenomegaly with associated:
- Thrombocytopenia
- Leukopenia
- Anemia

is the most common initial presentation (6).

2. Liver:

The degree and rate of hepatic enlargement is significantly more variable than that of spleen. Since the major mass of the liver, the hepatocytes, do not store glucocerebroside, this is not too surprising. Nevertheless, the degree of storage in liver macrophages (Kupffer cells) can be enormous.

An interesting albeit not common event is that of a clinical presentation consistent with the diagnosis of cirrhosis (7) or the identification of hepatic cirrhosis at biopsy (8). This finding is of considerable interest since potential pathophysiologic mechanisms for the fibrosis are not evident and because some patients have a significant transfusion (e.g. case #3) history, thereby raising the consideration of transfusional hemosideroses as the basis of any observed changes.

3. Bones and Bone Marrow:

The classical observation in Type I Gaucher disease is that the dominant clinical feature is that of bone involvement (3,9) although asymptomatic splenic enlargement is numerically more frequent. Roentgenographic changes in the femur have been identified even in the absence of focal symptoms. Indeed, many patients (as case # 1 above) have had no identification of their true underlying disease until an injury led to radiographic studies (or alternatively someone felt the spleen in a careful examination).

It is of historical interest that it was almost 50 years after the original clinical

description that the first complete roentgenographic studies were reported by Junghagen (10,ll). He described:

l) Osteoporosis: that was generalized.
2) Porous and trabeculated spongiosum.
3) Worm-eaten defects resulting from destruction of the spongiosa.
4) Cortical thinning.
5) Widening of the long bones with compression of the heads of the diseased bones (especially of the femoral heads).

The bone manifestations of Gaucher disease have commonly been divided into those that define the clinical lesions (12) and those that produce symptoms (i.e., pain) (13).

A. Bone Manifestations of Gaucher Disease(12)

l. Failure of remodeling:

- Seen in 80% of patients in the distal femur and proximal tibia.
- Such failure results in bones with cortical thinning with a very wide metaphyseo-diaphyseal region: often termed "Erlenmeyer flask" deformity.
- Not a pathognomonic finding
- Finding is always bilateral

2. Diffuse and Localized Bone Loss

- Pattern of osteopenia with no specific features, except that if the entire skeleton is examined other features of Gaucher disease are seen.

- Lesions can be confused with those of myeloma or metastatic carcinoma.

However, a noteworthy feature is the absence of skull lesions and a rarity of pelvic lesions.

3. Osteosclerotic Lesions

- Areas of increased density in the medullary cavities, primarily in long bones; sometimes in pelvis.

- Can be diffuse or patchy.

- These appear to be the "end stage" of diffuse or localized medullary osteonecrosis.

- Variable pattern on bone scan; although often positive, it may actually be normal.

4. Corticomedullary Osteonecrosis:

- Corticomedullary osteonecrosis of femoral (or humeral) head is common.

- This is the major cause of symptoms and debility in Gaucher disease.
- Common in young patients.
- No correlation with the percentage of Gaucher's cells in the marrow.
- Cause is unknown; presumed cause is vascular occlusive event.
- Corticomedullary necrosis can occur along a segment of long bone.

5. "Gaucher's Crisis"

- Painful, disabling process
- Appears to be a bone infarct

B. Patterns of Bone Pain (13)

Four patterns of bone pain have been seen in patients with Gaucher disease.

I. Non-specific bone pain:

- Transient, lasting 1-2 days
- Migratory
- Of unknown origin

2. Pain secondary to pathologic fractures

3. Pain secondary to degenerative joint disease

4. Bone crisis: Gaucher's crisis

- Often termed pseudo-osteomyelitis or aseptic osteomyelitis
- Most common in early decades of life
- Severe, acute pain unrelieved by narcotics lasting 2-3 weeks
- Local redness, swelling and increased heat
- Fever
- Polymorphonuclear leukocytosis: WBC in 10-30,000/ μl
- Elevated erythrocyte sedimentation rate

- Especially seen in distal femur or proximal tibia
- Initial radiographic studies are normal
- Early bone scan may show reduced activity over this site (of infarction)
- At 2-5 weeks bone scans become positive
- 4 to 6 weeks later there is evidence of periosteal elevation at the involved site
- 3 to 6 months later there is evidence of osteonecrosis.
- Restoration of normal laboratory studies may take several months.

The prominent and remarkable involvement of bone has focused interest on several interesting issues:

? Since the bone changes are the presumed result of the medullary canal (i.e., the marrow space where the macrophages are found) being packed with Gaucher's cells, why is the pattern of bone involvement not similar to that seen when the marrow space is expended in such lesions as hemoglobinopathies, congenital hemolytic anemias, etc. In these latter circumstances the skull and vertebrae are commonly affected (14, 15, 16). This is not the case in Gaucher disease. Sites where marrow is uncommon (such as the distal tibia) are involved.

? Does splenectomy make the bones worse. For many years there appeared to be correlation between splenectomy and the "development of symptomatic bony lesions (11, 17, 18, 19). The view appeared to be based on bedside observations with the rationale that the removal of the "garbage can" function (of the spleen) for glycolipid allowed the deposits to be made at physiologically more critical sites such as bone, where symptoms would more readily be expressed. At the present time, whether splenectomy is associated with acceleration and/or progression of disease at other sites is not certain (12, 20).

? Can current technology alter our diagnostic approach and/or affect our understanding of Gaucher disease. Recent use of magnetic resonance imaging and application of the modified Dixon Quantitative Chemical Shift Imaging (QCSI) has identified some new features of Gaucher disease (21). These studies show that as Gaucher's cells infiltrate the marrow and displace normal marrow elements a shift in weighted (T1) images occurs providing a sensitive measure of the extent of cellular infiltration in the tissue (marrow). In addition, the Gaucher's cell images as a low fat cell. This is apparently explained by the remarkable expansion of tubular like structures (lysosomes) that contain the aggregated glucocerebroside molecules which self-associate into densely packed, twisted membrane bilayers that are 60 Å (6 nm) thick (21, 22). These observations help explain the effect of these cells on marrow sinusoids and bone trabeculae.

4. Other Clinical Features and Findings:

A variety of other clinical findings have been seen in patients with Gaucher disease. Some, like those of spleen, liver and bone, are at expected sites of glycolipid macrophage residence; others have less evident relationships:

A. Pigmented Pingueculae:

- evident as slight elevation at either side of the limbus (of the eye). These appear to have an increased incidence in patients with Gaucher disease; however, although these lesions are fat deposits, they are not deposits of Gaucher cells.

B. Increased Resting Energy Expenditure:

- in an interesting study of resting energy expenditure, Grabowski and colleagues (23) showed a significant increased caloric requirement. This findings has been explained by the concept that although individual Gaucher cells are hypometabolic, the great mass that accumulates results in increased resting energy expenditure.

C. Abnormalities of Lipoprotein Metabolism:

- Patients with Type I Gaucher disease have been shown to have reduced levels of total, low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol (24). Plasma apolipoprotein E, known to be synthesized in macrophages, is increased. The hypocholesterolemia has been shown to be the result of increased fractional catabolism of LDL and HDL. Since this increased catabolism (and the increased apo E production) are products of macrophage activity, it appears that the Gaucher cell has a biologic activity beyond that of a simple glycolipid degradative repository.

D. Uncommon Organ Sites of Involvement:

- Largely in the form of case reports other organs have been involved to a level where clinical dysfunction has been identified. These include the kidneys (where the Gaucher cell infiltration was in the mesangial region) (25), the lungs (where, as one would expect, the cells were in the interstitial area) (26), and the myocardium (27).

E. Other Clinical Correlates of Indeterminate Mechanism(s)

I. Immunoglobulin abnormalities: Diffuse hyperglobulinemia (28), monoclonal gemmopathies (29, 30), and amyloidosis (31) have been described with increased incidence in Gaucher disease. The mechanisms for these changes are not clear, but the most attractive one is that they result from chronic antigenic stimulation. Shoenfeld, et al, (3) have identified three potential antigens in Gaucher disease:

- glucocerebroside
- glucocerebrosidase
- acid phosphatase

Nevertheless, no known pathophysiologic sequence has yet been defined.

2. Malignant lymphoproliferative lesions: Coexistence with multiple myeloma has been seen (32, 33, 34). Coexistence with chronic lymphocytic leukemia also, albeit it less commonly, has been recorded (35), as has Hodgkins disease (36).

3. Increased incidence of infections: This primarily has been correlated with bone lesions and the mechanism commonly involved is that of defective RE function. Recently, abnormal chemotaxis has been noted in Gaucher and it may be very important (37).

4. Bleeding diathesis: Although thrombocytopenia , as mentioned, is common, increased bleeding has not been evident except with surgical procedures. These differences have been thought to be circumstances where hemostasis has had a compound defect (i.e., the presence of a coagulation defect in addition to the thrombocytopenia). Abnormal Factor X function has been proposed, as has interference due to increased glucosylceramide in plasma (38), but definitive characterization does not exist.

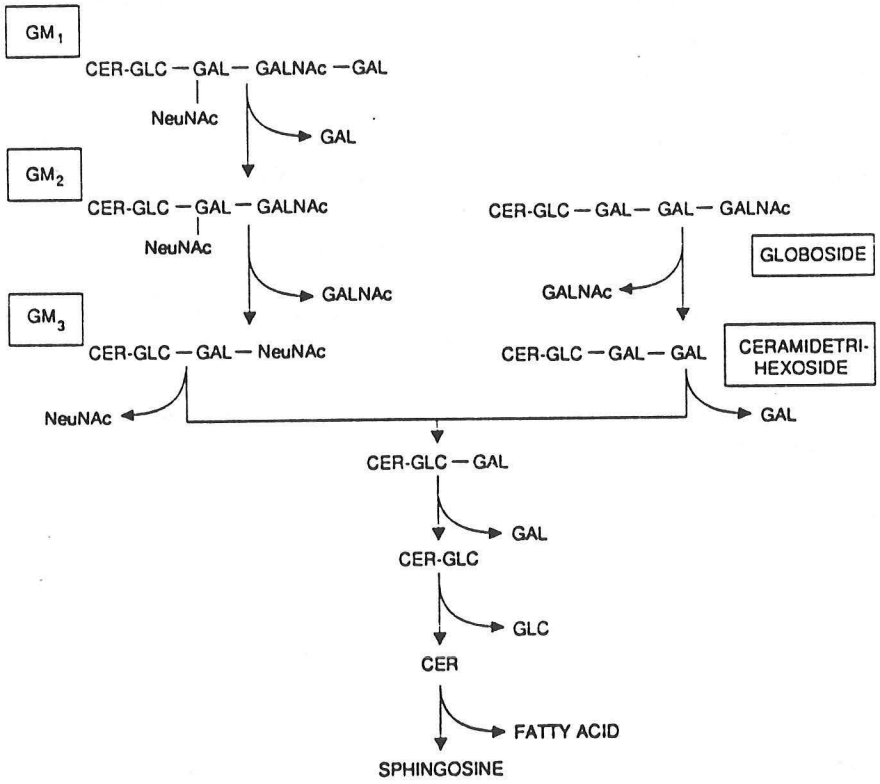
5. Laboratory abnormalities: A variety of abnormal laboratory findings have been identified in patients with Gaucher disease, only some of which have a reasonable mechanism:

Abnormality	Proposed Mechanism
Elevated serum acid phosphatase (39, 40, 41)	Derived from endoplasmic reticulum from dying Gaucher cells
Elevation of serum angiotensin converting enzyme (42)	? perhaps from "stimulated" RE cells
Increased levels of transcobalamin II (43)	? presumed from RE cells
Iron deficient erythropoiesis (44, 45)	Iron sequestration in Gaucher cells
Elevated serum ferritin (46)	? mechanism

III. Can the biochemical and molecular observations explain the clinical lesions and the clinical heterogeneity?

The biochemical delineation of Gaucher disease has well characterized this lysosomal storage disease, where an accumulation of glucosylceramide is the result of defective activity of the lysosomal hydrolase, acid β-glucosidase (although one variant case had a deficiency of the protein activator of the enzyme) (4, 18, 47). Glucosylceramide, the major natural substrate for acid β-glucosidase, is widely distributed in cell membranes and is an intermediate in the degradative pathway of most complex glycosphingolipids.

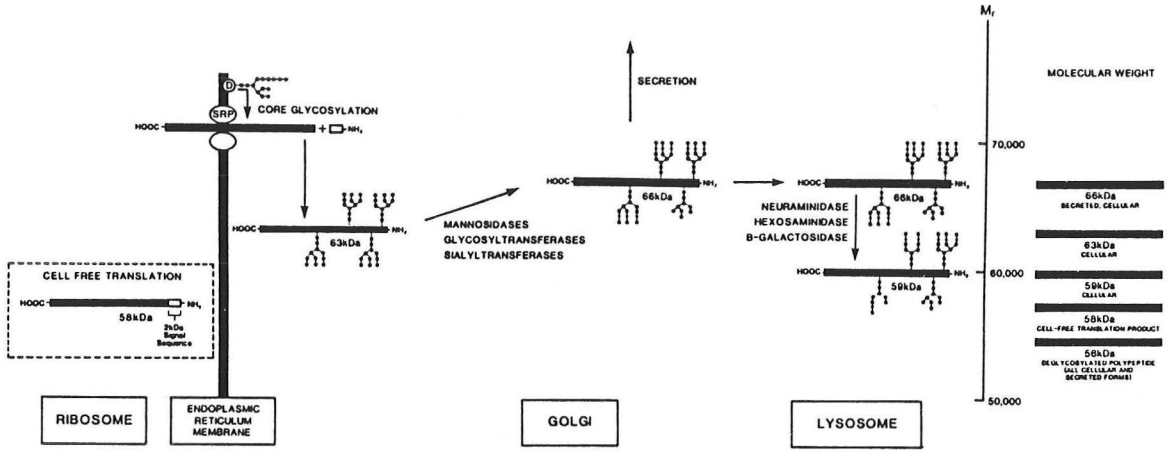
The clinical phenotypic variation in Gaucher disease was largely focused on characterization of functional properties of the enzyme β-glucosidase (4, 47, 48, 49). The purification properties of the enzyme from patients with Type I Gaucher disease were the same as normal enzyme (47). As the extensive heterogeneity of the molecular lesions were identified, kinetics and immunologic properties of the mutant (i.e., disease-associated) enzymes have been pursued (50,51), this has been more difficult than expected because the enzyme in the native state requires detergents and organic solvents for extraction and analyses. In addition, complex inhibitors and activators of the enzyme are known (4, 47) thereby making it very difficult to determine the exact architecture and function of the enzyme within the lysosome. A further difficult problem in the analysis of enzyme function is the evidence that lysosomal enzymes undergo extensive post-translational processing which include the removal of a signal sequence and glycosylation (47, 48, 52).



Sequential degradation of G_{M1}-ganglioside and globoside by lysosomal enzymes; Glucosylceramide (CER-GLC) is the common intermediate.

(Ref. 47)

Thus, Grabowski and colleagues (52) were able to show that three forms of the enzyme were derived from the same polypeptide chain by differential post-translational oligosaccharide remodeling. Even the trafficing of acid β-glucosidase to the lysosome appears to have special complexity,. Usually an exposed mannose-6-phosphate in the oligosaccharide side chain of the glycoprotein is important for the targeting to the lysosome (via the cation independent receptor) (47). The mechanism for acid β-glucosidase lysosome targeting appears different; but, the precise mechanism and the true biologic half-life of the enzyme have not yet been clarified.



Schematic representation of the details of the biosynthesis of glucocerebrosidase. Glucocerebrosidase is synthesized by mechanisms similar to other translocated glycoproteins.[310–322] In a cell free translation system (as indicated by the box on the lower left), a polypeptide of 58 kDa is synthesized on ribosomes. This polypeptide contains a signal sequence which facilitates its translocation to the lumen of the endoplasmic reticulum (ER). The translocation is mediated by the interaction of the signal sequence with a signal recognition particle (SRP) permitting the polypeptide to slide through the ER membrane as it is peeling off the ribosome while its translation is ongoing. As the first part of the polypeptide chain enters the ER, but before its translation is complete, two events occur. The signal sequence is cleaved from the polypeptide, and the growing chain is core glycosylated. Glycosylation occurs by the en bloc transfer of a 14-member oligosaccharide unit from a lipid in the ER membrane called dolichol (D). The "high mannose" precursor of 63 kDa is posttranslationally processed in the ER and Golgi apparatus without further proteolytic cleavage to form a 66-kDa intermediate which is also secreted in small amounts. This intermediate contains "complex-type" oligosaccharide chains (see Fig. 67-10) and is further processed in the lysosome to the mature form with a molecular weight of 59 kDa. The right side of the figure shows the molecular weight of the various forms.

(Ref. 4)

Summary features of these studies include:

I.) Three steady state forms of the enzyme (M_r = 63,000 and 56,000) are found in normal and Type I Gaucher patients.

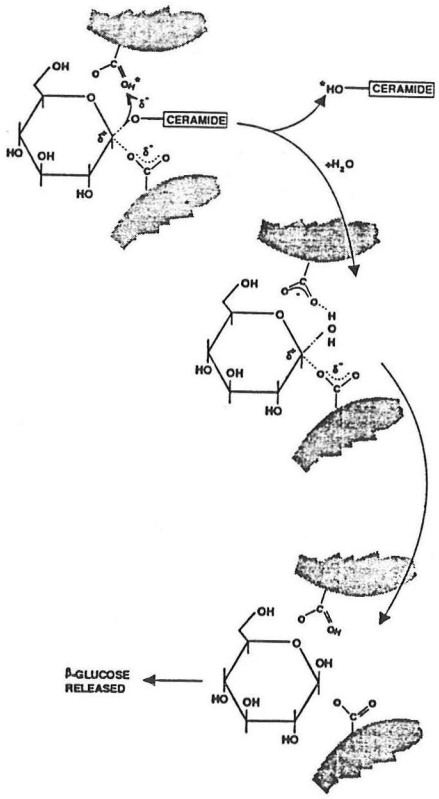
2.) The patterns of molecular weight forms of the enzyme in these patients are normal.

3.) Normal processing patterns and sequence and nearly normal time courses for processing have been measured (47).

There are several specific abnormalities of the residual enzyme:

1) Altered Stability (49)
2) Altered Specific Activity (53)
3) Altered Kinetic Properties (47)

The reaction mechanism for acid β-glucosidase substrate hydrolysis is still incompletely defined. A working schema has been proposed by Grabowski (47):



Proposed reaction scheme for the hydrolysis of glucosylceramide by acid β-glucosidase. Glucosylceramide binds to the active site and partial bonds are formed between the anomeric *O*-glycosidic linkage and a proton donor and nucleophile on the enzyme. Ceramide is released and water is added to the active site-glucosyl complex. This complex is stabilized either by ion pair formation or by a covalent linkage to Asp^{443}, the nucleophile for catalysis (second panel). Glucose then is released with retention of anomeric configuration.

(Ref. 47)

The human acid β-glucosidase structural gene (7604 bp) was cloned (54) and completely sequenced (55) by Ernie Beutler and his colleagues (18, 56). The gene maps to chromosome I (q 21 → 31). The analysis of mutations is complicated by the presence of a pseudogene 16 kilobases downstream from the glucosidase gene (57).

Both human sequences are contained within a single 32 kb fragment of genomic DNA. The pseudogene is about 95% homologous to the functional gene. It is transcribed but it cannot be translated into the glucosidase presumably due to numerous deletions of coding sequences (18). There are four large deletions in the pseudogene which represent *Alu* sequences flanked by direct repeats within the structural gene. It has been suggested that since *Alu* sequences are believed to be mobile elements that may be inserted into DNA, perhaps the pseudogene is the ancestral glucosidase gene and the recognized structural gene is one that has been altered by the insertion of these sequences.

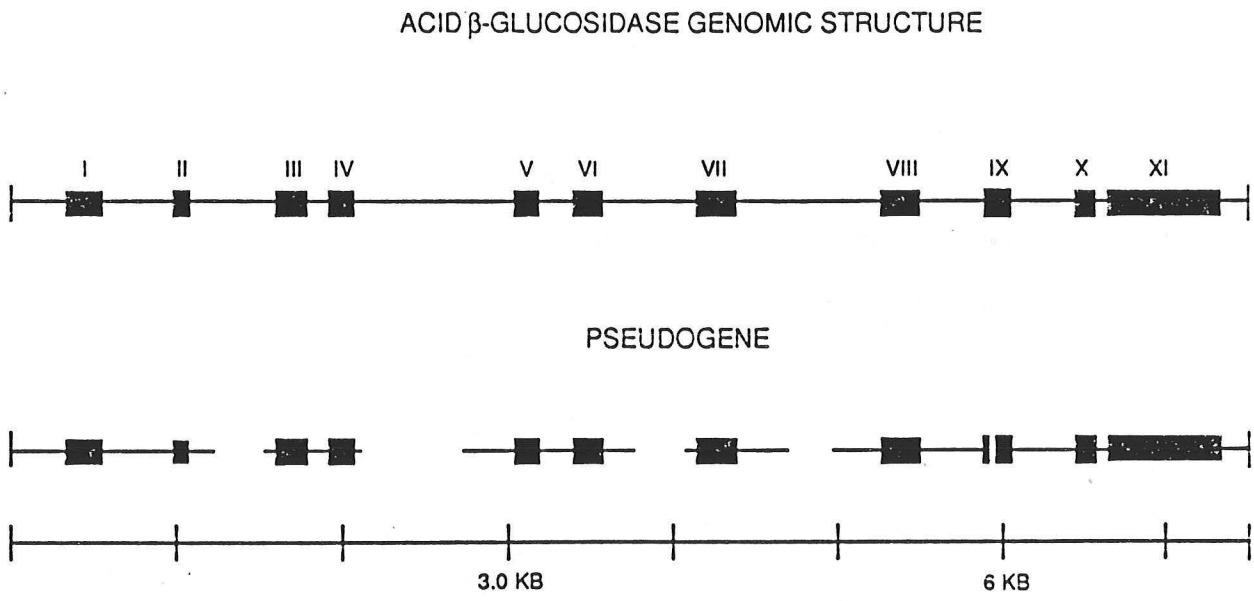
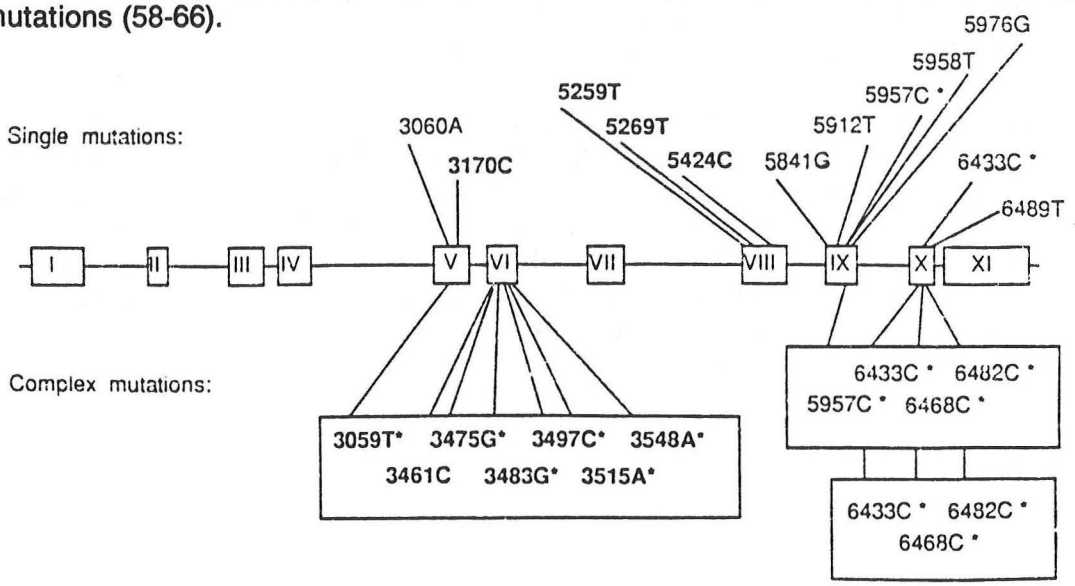


Diagram of the acid β-glucosidase structural gene (top) and pseudogene (bottom). The corresponding "exons" in each sequence are aligned and the intronic deletions are indicated. A 55 bp deletion in exon 9 of the pseudogene is also shown. Additional point mutation differences between the two sequences are not shown.

(Ref. 47)

Intensive study has been done to try and identify genetic markers and correlate these with the clinical phenotypic heterogeneity. The point mutations identified to date have been recognized by sequencing across exons in genomic clones or sequencing complete cDNAs from libraries from Gaucher patients (47). These studies have further demonstrated the heterogeneity of the mutations (58-66).



Schematic diagram depicting positions of the mutations identified in the β-Glc genes of Gaucher disease patients

(Ref. 66)

Mutations of the Acid β-Glucosidase Gene in Gaucher Disease

Disease phenotype	Nucleotide number		Base change	Designation	Exon	Amino acid change	Enzyme abbreviation
	cDNA	Genomic					
Frequent alleles							
1	1226	5841	A → G	5841G	9	Asn^{370} → Ser	β-$Glc^{Asn370→Ser}$
2 and 3	1448	6433	T → C	6433C	10	Leu^{444} → Pro	β-$Glc^{Leu444→Pro}$
Rare alleles							
1	476	3060	G → A	3060A	5	Arg^{120}–Cln	β-$Glc^{Arg120→Gln}$
1	535[a]	3119	G → C	3119C	5	Asp^{140}–His	β-$Glc^{Asp140→His}$
	1093[a]	5309	G → A	1093A	8	Glu^{328}–Lys	β-$Glc^{Glu328→Lys}$
1	580	3164	A → C	3164C	5	Lys^{155}–Gly	β-$Glc^{Lys155→Gly}$
1	764	4113	T → A	4113A	7	Phe^{216}–Tyr	β-$Glc^{Phe216→Tyr}$
2	1090	5306	G → A	5306A	8	Gly^{325}–Arg	β-$Glc^{Gly325→Arg}$
2	1141	5357	T → G	5357G	8	Cys^{342}–Gly	β-$Glc^{Cys342→Gly}$
1 and 3	1297	5912	G → T	5912T	9	Val^{394}–Leu	β-$Glc^{Val394→Leu}$
1	1342	5957	G → C	5957C	9	Asp^{409}–His	β-$Glc^{Asp409→His}$
3	1343	5958	A → T	5958T	9	Asp^{409}–Val	β-$Glc^{Asp409→Val}$
2	1361	5976	C → G	5976G	9	Pro^{415}–Arg	β-$Glc^{Pro415→Arg}$
1 and 2	1505				10	Arg^{463}–Cys	β-$Glc^{Arg463→Cys}$
Complex alleles							
1 and 2	1448	6433	T → C	6433C	10	Leu^{444}–Pro	
	1483	6468	G → C	6468C	10	Ala^{456}–Pro	β-$Glc^{COMPLEX A}$
	1497	6482	G → C	6482C	10	Val^{460}–Val	
3	1141	5957	G → C	5957C	9	Asp^{409}–His	
	1448	6433	T → C	6433C	10	Leu^{444}–Pro	
	1483	6468	G → C	6468C	10	Ala^{456}–Pro	β-$Glc^{COMPLEX B}$
	1497	6482	G → C	6482C	10	Val^{460}–Val	

[a] Both mutations found on the same allele. The other allele in this patient contained only the 3164C mutation.

(Ref. 47)

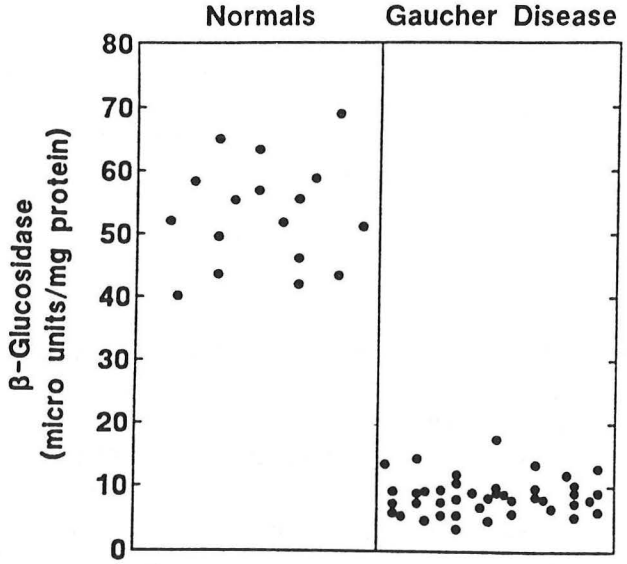
Although the primary goal was to link a mutation to a clinical disease pattern, this has not been possible. Certain combinations of alleles have been shown to correlate well with the severity of the disease, but the recorded mutations (to date) fail to provide a clear explanation of the clinical heterogeneity of the disease, the prognosis or applicability to therapeutic intervention (66). Thus, the clinical heterogeneity has not yet been clarified by the evident molecular heterogeneity.

IV. How should the clinical diagnosis be made?

A. Clinical diagnostic approach in suspect patients:

Groen in 1948 (67) described the use of the sternal (actually he used the manubrium) puncture as a diagnostic method. He showed easy access of tissue likely to contain Gaucher's cells to be used then in clinical circumstances that suggested this diagnosis. This has become a simple and popular route for a tissue diagnosis. However, in 1966 Albrecht (68) described these same abnormal storage cells in chronic myelogenous leukemia. These were termed "pseudo Gaucher" cells and they have now been identified in: acute and chronic myelogenous leukemia, aplastic anemia, myeloma, Hodgkins disease, chronic lymphocytic leukemia, rheumatoid arthritis, thalassemia major, and gangliosidosis (68a,b). The occurrence of cells histologically compatible with those seen in patients with Gaucher disease in these diverse other clinical circumstances coupled with an available WBC enzyme assay has led to gentle chiding against the use of the marrow examination; i.e., "misuse of marrow examination in the diagnosis of Gaucher disease" (69).

The introduction of a simple enzyme assay for β-glucosidase activity in leukocytes by Beutler in 1970 (70), has in fact provided a non-invasive and inexpensive method to identify the deficient state (71). Attractive as this approach is, it must be stressed that the leukocyte enzyme is unstable making shipping to laboratories that are distant a difficult problem.



Leukocyte β-glucosidase activity of the lymphocyte/monocyte layer obtained after Ficoll-Hypaque separation of the white blood cells from 42 patients with Gaucher disease and 17 normal controls. In each case the diagnosis could have been established by leukocyte β-glucosidase assay.

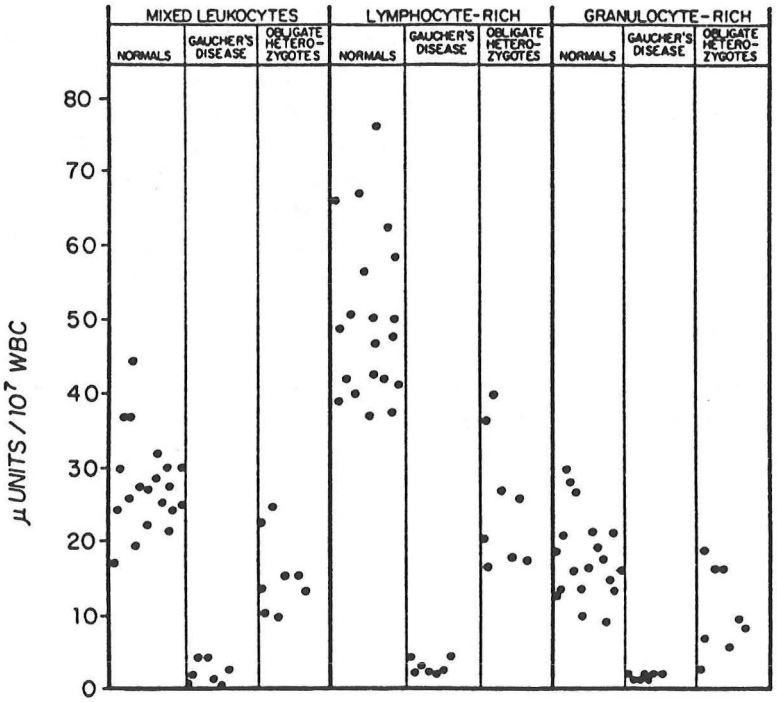
(Ref. 68)

B. Prenatal diagnosis:

It is now established that affected fetuses can be diagnosed by β-glucosidase assay in chorionic villi or by cultured aminocytes (4).

C. Carrier Detection and Population Genetics:

Gaucher disease is inherited as an autosomal recessive disease. It is the most common form of lysosomal storage disease. The availability of the leukocyte β-glucosidase enzyme assay led to extensive attempts to identify the carrier state. Unfortunately, regardless of the assay method used there is overlap in the leukocyte β-glucosidase activity of (putative) normal individuals and obligate heterozygotes (4,69,70).



(Ref. 68)

The application of molecular observations on mutant enzyme forms has provided an alternative to the identification and characterization of the carrier state in selected populations. An increased incidence of Type I Gaucher disease has been recognized in Ashkenazi Jews. The disease incidence is approximately 1 in 835 and the heterozygote frequency is estimated at 9% (18, 64). Beutler, et al. (63) have shown that about 75% of the disease- causing alleles contain a characteristic mutation at cDNA nucleotide 1226 (amino acid 370 of the mature protein). This A → G mutation has been termed 1226G mutation. Further studies have identified a second, less common, mutation at cDNA 1448 (a T → C mutation). The 1448C mutation accounts for 2% of Jewish disease-producing alleles (and 40% of the alleles in non-Jewish patients). They (65) have

just reported another mutation common in the Jewish population. It is the insertion of a single nucleotide, a second guanine at cDNA nt 84 (the 84 GG mutation). This produces early chain termination and no transcript is found. It's estimated to represent approximately 20% of the mutant alleles.

Determining the presence of these 3 alleles (1228G, 84GG and 1448C) appears capable of identifying over 97% of the mutations among Ashkenazi Jewish patients (72). Genetic risk has been estimated to be one chance in a million that a Jewish couple lacking 1226G, 84GG or 1448C alleles would be at risk for bearing a child with Gaucher disease. By contrast where only one partner has been found to have one of the mutations, the risk would be about 1 in 1000 (73). This model can be used to do carrier screening and genetic review and planning.

V. Who should have a splenectomy?

Splenectomy has been the classical approach in patients with Gaucher disease who develop:

- Congestive splenomegaly with associated symptomatic peripheral cytopenias (19).
- Severe mechanical pressure (especially where weight loss due to caloric deficit occurs).
- Presence of severe dilutional anemia (due to expanded plasma volume) (74).
- Splenic pain and/or infarction.
- Clinical evidence of portal hypertension (75).

Because of the increased risk of infection and because of the oft repeated clinical suggestion that bone lesions (and perhaps liver function) worsen after the spleen is removed, partial splenectomy has been utilized with increasing frequency since 1980 (11, 76, 77). Grabowski, et al. (19) have longitudinal follow up on 48 patients. Twenty-three percent of those with total splenectomy had accelerated bone lesions (mean follow up 96 months); none of the partial splenectomized patients developed progressive bone disease (mean follow up however was only 25 months).

Partial splenectomy does appear to have distinct advantages in Gaucher disease patients. One word of caution is appropriate in that partial splenectomy may not be long-lasting. Progressive splenomegaly and hypersplenism can recur, even to the level of requiring re-operation.

VI. What other contemporary therapeutic options are available:

A. Erythropoietin Therapy:

As noted, cytopenias, especially thrombocytopenia and anemia, are commonly seen in patients with Gaucher disease. A small subset of such patients even become transfusion-dependent (see Case # 3). As cytokine therapy became available, a trial of recombinant erythropoietin was proposed. We have participated in this NIH study. As we had actually

anticipated, most such patients have elevated levels of endogenous erythropoietin and they fail to respond to erythropoietin therapy (78).

B. Enzyme Replacement Therapy:

Enzyme replacement as a therapeutic strategy was tried by many groups in the 1970s (79, 80, 81). Modest responses were recorded with insignificant toxicity. Although the clinical results were trivial, feasibility of such treatment was established and the problems attendant to such therapy were identified; i.e., need for large scale production of large quantities, alteration in the carrier molecule so that the enzyme was delivered to the macrophages rather than the hepatocytes, and development of a stable enzyme product.

All of these hurdles have now been overcome and the product alglucerase (Ceredase ^{R} Genzyme Corp.; Boston, MA) is capable of providing functional enzyme to the Gaucher cell *in vivo*.

This was achieved by:

- development of large scale production and purification capacity from human placenta glucocerebroside (82). Parenthetically, it requires approximately 9 metric tons of placenta to make enough enzyme to treat one patient for one year.

- development of a macrophage-targeted enzyme preparation was achieved by a technique of sequential deglycosylation popularized by John Barranger (4). He had shown that when the native enzyme (a glycoprotein containing 7% carbohydrate in complex oligosaccharide units) was subjected to sequential deglycosylation a mannose-terminated enzyme resulted which is specifically bound by a lectin on the plasma membrane of macrophages. The bound enzyme is internalized and traffics to the lysosomal compartment where it has been shown to be a functional enzyme (83, 84).

- the dose popularized by the NIH team of Barton and Brady (83, 84, 85) has been 60μ/kg I.V. over 1-2 hours given every 2 weeks.

Presently Ceredase has a cost of $3.50/ unit. Therefore, using the NIH criteria, treatment of a patient weighing 60 kg uses 3600 units and cost $12,600 per therapy, or approximately $327,600 per year. It is now evident from Beutler's experience (86) that more frequent infusions given over a longer time period (for better and more effective access to the Gaucher cells) allows a significantly lesser amount of enzyme to be used. Nevertheless, current costs for such patients are still close to $100,000 per year.

- Current FDA approval for its use includes:

anemia
thrombocytopenia
bone disease
spleen and/or liver enlargement

In light of the significant costs, clearly more stringent criteria for use are needed and these are slowly developing.

However, as an example of its effectiveness, Case # 3, is now no longer transfusion-dependent, and is actually in a phlebotomy program to reduce her iron overload state.

Still unsettled issues in Ceredase replacement therapy are the best route and dosage schedule, the duration treatment is needed, the value of subsequent maintenance therapy and evaluation of long term side effects. All of these are currently being investigated. In addition, recombinant enzyme production has begun by Genzyme and this product should, at least, relieve the concerns generated by the human placental source for Ceredase.

C. Bone Marrow Transplantation:

In 1982, the first 2 patients with severe Gaucher disease underwent allogeneic bone marrow transplantation. The first patient died without improvement, but the second has now been followed for over 6 years (87) with clear evidence of effect. Approximately 18 patients have been transplanted to date. As can be expected it has been used in severe disease. It requires an HLA-identical match with normal β-glucosidase activity. Although Joel Rappeport has tried to accumulate longitudinal data on these patients, no registry exists to provide such information (88, 89).

D. Gene Replacement Therapy:

Gaucher disease appears to be an excellent candidate disease for gene replacement therapy. We already know that when functional enzyme is delivered it reverses most and perhaps all (if given early enough) of the clinical findings and sequelae; and that allogeneic hematopoietic stem cells correct the disease. Intensive efforts at such an approach are now well under way (90, 91, 92). Effectiveness will require enzymatically functional cells. Also since the genetically corrected cells do not have a selective advantage over the defective (endogenous) cells, some method of altering (or destroying) those endogenous cells may be required (73).

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