25. F. Zorzato *et al.*, *J. Biol. Chem.* **265**, 2244 (1990).
26. K. Otsu *et al.*, *ibid.*, p. 13472.
27. T. Wagenknecht *et al.*, *Nature* **338**, 167 (1989).
28. S. T. Ohnishi, S. Taylor, G. A. Gronert, *FEBS Lett.* **161**, 103 (1983).
29. T. Ohta, S. Ito, A. Ohga, *Eur. J. Pharmacol.* **178**, 11 (1990).
30. E. Rios and G. Brum, *Nature* **325**, 717 (1987).
31. E. Carafoli, *Annu. Rev. Biochem.* **56**, 395 (1987).
32. T. E. Nelson, *Cell Calcium* **9**, 257 (1988).
33. M. Endo *et al.*, *Biomed. Res.* **4**, 83 (1983).
34. P. J. O'Brien, *Can. J. Vet. Res.* **50**, 318 (1986); L. Carrier, M. Villaz, Y. DuPont, *Biochim. Biophys. Acta* **1064**, 175 (1991).
35. M. Fill *et al.*, *Biophys. J.* **50**, 471 (1990).
36. M. Fill, E. Stefani, T. E. Nelson, *ibid.* **59**, 1085 (1991).
37. J. R. Mickelson *et al.*, *J. Biol. Chem.* **263**, 9310 (1988).
38. C. M. Knudson, J. R. Mickelson, C. F. Louis, K. P Campbell, *ibid.* **265**, 2421 (1990).
39. A. E. MacKenzie *et al.*, *Am. J. Hum. Genet.* **46**, 1082 (1990).
40. A. L. Archibald and P. Imlah, *Anim. Blood Groups Biochem. Genet.* **16**, 253 (1985).
41. I. Harbitz *et al.*, *Genomics* **8**, 243 (1990).
42. A. J. Lusis *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 3929 (1986).
43. K. Otsu, M. S. Phillips, V. K. Khanna, S. de Leon and D. H. MacLennan, *Genomics*, in press.
44. G. Eikelenboom and D. Minkema, *Netherlands J. Vet. Sci.* **99**, 421 (1974).
45. E. F. Gillard *et al.*, *Genomics*, in press.
46. A. K W. Brownell, *Br. J. Anaesth.* **660**, 303 (1988).
47. T. D. Heiman-Patterson, H. Rosenberg, J. E. Fletcher and A. J. Tahmoush, *Muscle Nerve* **11**, 453 (1988).
48. J. C. Mulley *et al.*, *Cytogenet. Cell Genet.* **58**, 2022 (1991).
49. J. E. Fletcher *et al.*, *Biochem. Cell Biol.* **68**, 1195 (1990).
50. P. S. Foster, E. Gesini, C. Claudianos, K. C. Hopkinson, M. A. Denborough, *Lancet* i, 124 (1989).
51. A. E. MacKenzie *et al.*, *Anesthesiology* **75**, 4 (1991).
52. J. M. Healy *et al.*, *Brit. Med. J.* **303**, 1225 (1991).
53. M. S. Phillips, J. Fujii, D. H. MacLennan, unpublished observations.
54. G. A. Mignery and T. C. Sudhoff, *EMBO J.* **9**, 3893 (1990).
55. We thank our many colleagues for advice and discussion in the preparation of this review. Research grants supporting original work by D.H.M. were from the Medical Research Council of Canada, the Muscular Dystrophy Association of Canada, and the Heart and Stroke Foundation of Ontario. D.H.M. is a Principal Investigator of the Canadian Genetic Diseases Network of Centers of Excellence. M.S.P. is a Predoctoral Fellow of the Heart and Stroke Foundation of Ontario.

Gaucher Disease: New Molecular Approaches to Diagnosis and Treatment

Ernest Beutler

Gaucher disease is characterized by the accumulation of glucocerebroside, leading to enlargement of the liver and spleen and lesions in the bones. It is caused by an inherited deficiency of the enzyme glucocerebrosidase. Many mutations exist, but four of these account for over 97% of the mutations in Ashkenazi Jews, the population group in which Gaucher disease is the most common. Although there is a strong relation between the mutations and disease manifestations, genetic counseling is made difficult by the fact that within each genotype there is considerable variability in the severity of the disease. Intravenous infusion of glucocerebrosidase is an effective treatment, but the availability of enzyme replacement therapy is limited by its high cost. Marrow transplantation is also effective in treating the disease, but is rarely performed because of the risks involved. In the future gene transfer may become the treatment of choice.

The breakdown of endogenous cellular components and foreign substances by the body is an exquisitely orchestrated function that is largely accomplished within the lysosome. In this organelle highly specific acid hydrolases sequentially separate the building blocks of macromolecules. Deficiencies in any of these enzymes may prevent the breakdown of its substrate and result in its accumulation. The accumulation of these trapped intermediates of the catabolism of complex molecules results in a storage disease, each with its own clinical characteristics. The most common of these disorders is Gaucher disease, which is characterized by a deficiency of glucocerebrosidase and hence accumulation of the glycolipid glucocerebroside. Less common glycolipid storage diseases include Tay-Sachs disease, in which GM_2 ganglioside is stored, Fabry disease, in which ceramide trihexoside accumulates, and Niemann-Pick disease, in which the storage compound is sphingomyelin.

Although glucocerebrosidase deficiency exists in all body cells of patients with Gaucher disease, the disease phenotype is expressed only in the macrophages except in the very rare neuronopathic forms of the disorder. As a consequence, patients with Gaucher disease are burdened by an enlarged liver and spleen and often by painful bone lesions. Macrophages are derived from bone marrow stem cells and are in constant contact with the blood stream. Gaucher disease is therefore ideally suited for the study of a variety of interventional strategies. These have included enzyme replacement, bone marrow transplantation, and gene transfer.

Gaucher disease was first described in 1882. The composition of the storage material was correctly identified in 1934, and the enzyme deficiency was demonstrated in 1965 (*1*). Yet only in the last decade has identification of lesions that cause this disease and implementation of successful therapeutic approaches been possible with the application of modern techniques of cellular and molecular biology. Recent findings have raised a host of biologic, economic, and ethical questions that did not exist previously.

The Enzyme Deficiency

The cause of Gaucher disease is the inability to catabolize glucocerebroside, which is normally hydrolyzed to ceramide and glucose by the β-glucosidase glucocerebrosidase. In the vast majority of cases the disease results from a deficiency of the enzyme itself (*2*). Glucocerebrosidase is a glycoprotein with a molecular size that ranges from 58 to 66 kD, after glycosylation and removal of a leader sequence. Some enzyme protein, generally with decreased catalytic activity, can be detected in the cells of patients with Gaucher disease (*3*). Some of the mutant forms of glucocerebrosidase are unstable (*4*), and kinetic abnormalities of the residual enzyme include abnormalities in activation by saposin C and phospholipids, and in inhibition by the active site inhibitors conduritol B epoxide and glucosphingosine (*5*).

Because glucocerebrosidase is a β-glucosidase, water soluble fluorogenic β-glucosides have been useful as enzyme substrates for both the diagnosis of the disease and purification of the enzyme (*6*). Examination of the bone marrow or other tissues, once the mainstay of diagnosis, has become an anachronism; the modern method of diagnosis is the estimation of the leukocyte acid β-glucosidase activity. Although the average enzyme activity of the leukocytes of heterozygotes is about one-half that of normal individuals, the distribution is so broad that the usefulness of the enzyme assay in the detection of heterozygotes is limited (*7*).

The Glucocerebrosidase Gene

Complementary DNAs (cDNAs) encoding glucocerebrosidase were cloned independently by two groups with the use of expres-

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sion libraries and antibodies to glucocerebrosidase (8). The transcript is about 2 kb and is unusual in that it has two functional AUG start codons (9). As with other lysosomal enzymes, a hydrophobic leader sequence is present. Studies with covalently bound inhibitors suggest that the active site is encoded by exon 10 (*10*). The cDNA is expressed in a variety of eukaryotic cells (*11*).

Located on chromosome 1 (*12*), the glucocerebrosidase gene consists of 11 exons with a primary transcript of 7.25 kb (*13*). A glucocerebrosidase pseudogene has been identified about 16 kb downstream of the functional gene (*13, 14*). The entire sequence of the gene is known, from 1230 bp upstream of the start of transcription to 760 bp downstream from the putative polyadenylation site (*13, 15*). A portion of the pseudogene that extends from 355 bp upstream of the start of transcription extending to 130 bp downstream from the polyadenylation signal has been sequenced (*13*). There is a high degree of sequence similarity between the gene and the pseudogene. The promoter of the pseudogene can drive transcription of a reporter gene (*16*), and pseudogene transcripts can be recovered from normal cultured cell lines (*17*). However, these transcripts cannot be translated, as there are no long open reading frames, and some exons are lost during mRNA processing because of mutations in the splice consensus sequences (*17*). Large portions of introns in the active gene are not present in the pseudogene. These intron segments are Alu sequences that may have been inserted into the functional gene since the duplication that gave rise to the pseudogene (*13*). Understanding of the structure of the pseudogene has been important in the analysis of glucocerebrosidase mutations.

The Genetic Lesions That Cause Gaucher Disease

With the cloning of the glucocerebrosidase gene it became possible to examine the DNA sequence of the gene in patients with Gaucher disease. The alterations that have been discovered thus far include point mutations in exons and in a splice site, a nucleotide insertion, crossovers between the gene and the pseudogene, and gene conversions. On the basis of their deduced functional effects and association with Gaucher disease phenotypes, it is possible to classify these mutations into two groups. The mutations associated with neuronopathic disease are designated as severe, while those that are not associated with neuronopathic disease, even when found in combination with one of the severe mutations, are classified as mild (Table 1). The proximity of and high degree of similarity between the functional gene and the pseudogene have apparently resulted in a large number of recombinational events. This situation is quite similar to that which occurs in the human disease steroid 21-hydroxylase deficiency, which causes congenital adrenal hyperplasia. Here mutations of the *CYP21B* gene often manifest sequences of the closely related *CYP21A* pseudogene (*18*).

The broad range of Gaucher disease

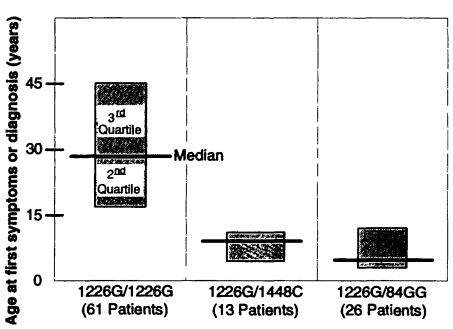

Fig. 1. The median and second and third quartile of the distribution of the age of first symptoms or diagnosis of Gaucher disease in patients with three different genotypes.

Table 1. Gaucher disease mutations.

cDNA #*	Amino acid #	Point mutations that cause Gaucher disease: Genomic #	Nucleotide substitution	Amino acid substitution	Effect	Population frequency†	Ref
IVS2 + 1		1067	G → A‡		? Severe	Uncommon	(*19*)
476	120	3060	G → A	Arg → Gln	? Mild	Rare	(*42*)
481	122	3065	C → T	Pro → Ser	Mild	Rare	(*43*)
535 §	140	3119	G → C	Asp → His	?	Rare	(*44*)
1093 §	326	5309	G → A	Glu → Lys	?		
586	157	3170	A → C	Lys → Gln	Severe	Rare	(*44, 45*)
751	212	3545	T → C	Tyr → His	? Mild	Rare	(*43*)
754	213	3548	T → A‡	Phe → Ile	Severe	Uncommon	(*46*)
764	216	4113	T → A	Phe → Tyr	Mild	Rare	(*47*)
1043	309	5259	C → T	Ala → Val	Mild	Rare	(*45*)
1053	312	5269	G → T	Trp → Cys	Mild	Rare	(*45*)
1090	325	5306	G → A‡	Gly → Arg	Severe	Rare	(*48*)
1141	342	5357	T → G	Cys → Gly	Severe	Rare	(*48*)
1208	364	5424	G → C	Ser → Thr	Mild	Rare	(*45*)
1226	370	5841	A → G	Asn → Ser	Mild	Common	(*49*)
1297	394	5912	G → T	Val → Leu	Severe	Uncommon	(*50*)
1342	409	5957	G → C‡	Asp → His	Severe	Uncommon	(*48, 50*)
1343	409	5958	A → T	Asp → Val	Severe	Rare	(*50*)
1361	415	5976	C → G	Pro → Arg	Severe	Rare	(*51*)
1448	444	6433	T → C‡	Leu → Pro	Severe	Common	(*52*)
1504	463	6489	C → T	Arg → Cys	Mild	Uncommon	(*53*)
1604	496	6683	G → A	Arg → His	Mild	Uncommon	(*43*)

Insertions and deletions (del) that cause Gaucher disease: cDNA #	Genomic #	Nucleotide substitution	Amino acid substitution	Effect	Population frequency†	Ref
84	1035	G→GG		Severe	Common	(*54*)
1263–1317 del	5879–5933‡ del			? Severe	Rare	(*43*)

Recombination events that cause Gaucher disease: Location of crossover event(s)‖ cDNA #	Genomic #	Effect	Population frequency	Ref
>1343 <1388	>5957 <6272¶	Severe	Uncommon	(*14*)
>455 <475 §**	>3039 <3059 §**	? Severe	Rare	(*45*)
>754	>3548			
>1317 <1343	>5932 <5957	Severe	Uncommon	(*48, 55*)
>1343 <1388	>5957 <6272	Severe	Uncommon	(*48, 53, 55, 43*)
>1225 <1263	>5588 <5878	Severe	Rare	(*48*)

?, Insufficient data to be certain of the classification. Ref, reference. *#, Position in the sequence. †Common = high frequency in at least one population, uncommon = found in a number of unrelated patients; rare = found in only one or two individuals. ‡Pseudogene sequence. §Both found in one gene. ‖Only approximate ranges can be given because the pseudogene and functional gene contain long identical sequences. For example, in the first entry the crossover event occurred between nt 1343 and nt 1338. ¶Physical fusion with loss of intergenic segment. **The first range represents crossover from gene to pseudogene, the second from pseudogene to gene. This region contains seven mutations, only six of which are identical with pseudogene sequence. At genomic nt 3474, within the region, the nucleotide conforms to the active gene, not the pseudogene. Thus "conversion" seems imperfect.

phenotypes, ranging from the acute neuronopathic disease (Type II) to the virtually asymptomatic disorder observed in the elderly, defied understanding until molecular analyses of the mutations provided some insight into this variability. In Gaucher disease certain mutations, such as the one at nucleotide (nt) 1448 (Table 1), are regularly associated with neuronopathic disease unless a mild mutation, such as the one at nt 1226, is present. The onset of disease in patients homozygous for the 1226G mutation tends to be late (median 27 years) as compared with the 1226G/84GG (median onset age 5) or 1226G/1448C (median onset age 9 years) genotypes (Fig. 1), and the disease is less severe (*19, 20*). There is, however, variability within each genotype; patients with the ordinarily benign 1226G/1226G genotype can have more serious disease than some patients with genotypes such as 1226G/84GG that are usually associated with severe disease. Such variability in the disease phenotype is seen even between siblings who share the same two glucocerebrosidase mutations, and it is not clear whether unlinked genetic factors or environmental factors are responsible for these differences.

The polymerase chain reaction has made facile mutation detection possible, and because Gaucher disease is relatively common we were able to examine the DNA from 154 unrelated people with this disorder, representing 217 alleles of Jewish and 91 of non-Jewish origin (Fig. 2). The nt 1226G mutation, which results in the substitution of serine for asparagine at amino acid 370 of the processed protein, predominated in the Jewish population. It accounts for ~75% of disease-producing alleles. A second mutation, the insertion of an extra G at nt 84 (84GG), accounts for an additional 13% of the mutations, and a third mutation at the upstream splice junction of intron 2 (IVS2+1) represents about 3% of the Gaucher disease mutations in unrelated Jewish patients. These mutations that predominate in Jewish populations, together with the panethnic mutation at nt 1448, account for about 96% of the mutations in the Ashkenazi Jewish population.

A variety of different mutations accumulates at low frequency in all populations. When mutations are not subjected to positive selection in a population, this background of sporadic mutations predominates. This is what has apparently happened in the case of Gaucher disease in the non-Jewish population, where a large variety of mutations is found (Fig. 2). Even the relatively common nt 1448 mutation occurs in the context of different haplotypes (see below), and therefore probably represents the result of repeated independent mutational events.

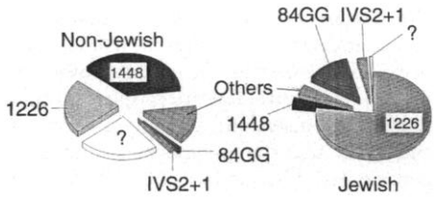

Fig. 2. Mutations that cause Gaucher disease found in 217 Gaucher disease alleles of Jewish origin and 91 alleles of non-Jewish origin. Among the 17 non-Jewish mutant alleles classified as "other," there were six crossovers or gene conversions, one 55-bp deletion, two 764A, one 476A, three 1504T, one 1604A, two 481T (homozygous patient), and one 1342C. The nine Jewish alleles classified as "other" include one crossover, four 1297T, two 1604A, one 1504T, and one 751C.

We have analyzed the DNA samples from more than 1700 healthy persons of Ashkenazi Jewish extraction for the 1226G and the 84GG mutations (*21*). Within this population the frequency of the 1226 mutation was 0.028 and that of the 84GG mutation 0.0028. Among Gaucher disease patients the ratio of the mild 1226G mutation to the severe 84GG mutation is 5.9:1, while in the population of presumably well individuals the ratio is 10.0:1. Because the frequency of the 84GG mutation is quite low this difference does not achieve statistical significance. However we believe the trend observed is probably a result of the fact that those who have the phenotypically mild 1226G/1226G genotype are underrepresented in the patient population; they simply do not have manifestations that cause them to seek medical care. Indeed, we encountered four such persons in our normal population surveys. Moreover it is not unusual for those with the 1226G/1226G genotype to be diagnosed in the sixth or seventh decade of life. Of patients with this genotype, 25% first have symptoms or are diagnosed when they are over 45 years of age. The proportion of 1226G to 84GG mutations in the general Ashkenazi population would correspond to that in the patient population if about 60% of the 1226G homozygotes did not have clinical symptoms of the disease.

In addition to disease-producing mutations, there are at least 12 polymorphic sites in introns of the glucocerebrosidase gene. These sites produce only two major haplotypes (− and +), although their sequential occurrence over time would be predicted to result in 11 different patterns, even if there had been no crossovers (*15*). Either all of these mutations occurred simultaneously or the other theoretic haplotypes have been lost from the population. The existence of these haplotypes has aided us in the understanding of the origin of the common 1226G and 84GG Gaucher disease mutations. The 1226G mutation is always found in the context of the − haplotype; that is, all subjects with the 1226G/1226G genotype that we have examined have the −/− haplotype, and every person who has one 1226G allele has at least one − allele (*22*). In contrast, the 84GG mutation is always found in the + haplotype. While the 84GG/84GG homozygous genotype has never been encountered and is probably lethal, one can draw these conclusions regarding the 1226G and 84GG mutations from the fact that all 26 patients with the 1226G/84GG Gaucher disease phenotype that we studied have the −/+ haplotype. Similarly, in Jewish patients the IVS2+1 mutation seems to be linked to the − haplotype, although we have thus far typed only five Jewish 1226G/IVS2+1 patients. The single IVS2+1 mutation found in a non-Jewish patient was in the + haplotype (*19*).

If the same mutation had occurred repeatedly it would sometimes be in the context of one and sometimes the other haplotype. Even if it had occurred only once in the remote past the mutation would be found in several haplotypes as a result of genetic crossovers. Thus our findings suggest that the two most common Gaucher disease mutations in the Jewish population arose recently and only once, and that each person who now carries these mutations is probably a descendant of the one in whom it originally occurred. Moreover, these findings imply that in the Ashkenazi Jewish population the gene for glucocerebrosidase deficiency either confers some selective advantage or did so at some time in the past. The nature of this advantage is not clear, although it is probably not an accident that two other glycolipid storage diseases, Tay-Sachs disease and Niemann-Pick disease, are also most common in the Ashkenazi Jewish population. Because of the correlation between the geographic distribution of tuberculosis and Tay-Sachs disease, it has been suggested that this advantage might be resistance to this infection, a common cause of death in the medieval ghetto (*23*). However, recent studies of Jewish patients with tuberculosis show no decrease in the frequency of the 1226G allele (*24*).

Treatment of Gaucher Disease

Until recently symptomatic treatment of Gaucher disease, such as the repair or replacement of fractured bones, the removal of an enlarged spleen, and the treatment of intercurrent infections with antibiotics, was all that medical science could offer patients with this disorder. Such treatment has been useful in prolonging and improving the quality of life but is far from satisfactory.

Enzyme replacement. The idea of correcting the intracellular defect in lysosomal diseases by supplying exogenous enzyme is not a new one (25), but difficulties in producing large amounts of enzyme and delivering it to the correct cellular target are only now being overcome. In the 1970s investigators attempted to treat Gaucher disease by infusing enzyme purified from human placenta, either without specific targeting (26), by targeting in resealed erythrocytes coated with immunoglobulin (27), or by targeting with liposomes (28). Although some encouraging results were reported, the clinical improvement of patients was minimal, and ultimate success was dependent on industrial-scale extraction and purification of enzyme and improved understanding of the signals that regulated the trafficking of proteins.

When Achord and Sly (29) discovered the mannose receptor on macrophages they suggested that it might provide a route for enzyme delivery to lysosomes in Gaucher disease patients. Glucocerebrosidase that is modified to increase the number of mannose residues is somewhat more efficiently incorporated by the nonparenchymal cells of murine liver than is unmodified enzyme (30). Human placental glucocerebrosidase was purified on an industrial scale (Genzyme Inc., Boston) and modified by removing outer sugars to expose additional mannose residues. The administration of large amounts of this preparation (aglucerase) has produced clinical improvement in patients with Gaucher disease, with regression of organomegaly, and improvement of blood counts (31, 32). However, treatment is expensive. The quantity of enzyme infused with each dose in the initial studies (31), 60 units per kilogram of body weight, costs $14,700 per infusion for a 70-kg adult. At the recommended frequency of one infusion every 2 weeks the annual cost per patient for the enzyme alone is $382,200 per year, and at the weekly infusions recommended for very ill patients it is $765,000 per year. The *Wall Street Journal* and the *New York Times* have aptly called aglucerase the world's most expensive drug (33).

Because of the high cost of treatment efforts have been made to administer the enzyme preparation in a more efficient manner. Because entry into macrophages is believed to occur via specific mannose receptors, treatment strategies must take into account the number, properties, and distribution of such receptors and the intravascular and intracellular half-life of the enzyme (34). It has been demonstrated that reducing the initial dose from the recommended 60 units/kg to 2.3 units/kg and administering it three times per week rather than every 2 weeks gives comparable results at less than one-fourth the cost (35). However, endothelial cells also contain mannose receptors (36), suggesting that current strategies, even with lower doses of enzyme, result in relatively inefficient delivery to macrophages.

Marrow transplantation. Macrophages are progeny of hematopoietic stem cells. If the Gaucher disease phenotype, at least in the non-neuronopathic form, is expressed entirely by virtue of changes in macrophages, marrow transplantation should be curative. This has proven to be the case (37). However, the risks of transplantation do not justify this procedure in patients with relatively mild disease, and the risk of transplantation increases with the severity of the disease. Thus, especially with the availability of enzyme replacement therapy, it is usually difficult to justify transplantation as a treatment modality. An exception may be patients with neuronopathic disease or those who have a genotype that predicts the development of neurologic disease, although it is not entirely clear to what extent the neurologic disease of such patients is benefitted by transplantation.

Problems and Future Prospects

Progress in science often raises new questions and poses new ethical dilemmas. This has certainly been true of the advances in the understanding of Gaucher disease that have been made in the past year. Until then genetic counseling offered to families was relatively inaccurate, and population-based screening was not feasible. Patients had no alternative to symptomatic management of their disease. Now all of this has changed. Population-based screening is technically possible with accurate identification of most heterozygotes, but in many cases it is difficult to provide useful counseling to couples at risk for producing a child with Gaucher disease because of the marked phenotypic variability. Enzyme therapy is effective but so costly as to be unavailable to all who could benefit.

Population-based screening. Screening for people who are heterozygous for genetic diseases and counseling couples at risk for the disease have contributed to reducing the incidence of several genetic diseases including thalassemia and Tay-Sachs disease (38). The latter, in particular, is relevant to the problem of screening for Gaucher disease because the same population, the Ashkenazi Jewish community, is involved. There are, however, major differences between screening for Tay-Sachs disease and Gaucher disease. When a couple at risk is found to have a fetus with hexosaminidase A deficiency (the enzyme deficient in Tay-Sachs disease) they can be told with a high degree of confidence that their child will have a devastating disease that will lead to blindness, severe neuromotor disability, and almost surely death by the age of 4. There are mutations of the hexosaminidase A gene that do not conform to this gloomy scenario (39), but these are unusual, and the information presented to heterozygotes of the hexosaminidase A deficiency is sound in most cases. The prognosis of patients with Gaucher disease is far less uniform than that of patients with Tay-Sachs disease, and we lack the means to differentiate between the 1226G/1226G homozygote who will have no disease at all, possibly representing more than one-half of the total, and the less fortunate patient with the same genotype who will have serious problems with his disease.

What, then, are we to tell couples when both are heterozygous for Gaucher disease mutations and whose fetus is ordained to develop the disease? Unfortunately there is no clear-cut answer to this question. We do not even know to what extent variability within a given genotype is genetic and to what extent it is environmental in origin. Clearly insights into the marked variation in patients with the same genotype would greatly facilitate genetic counseling. Documentation of the degree of variability between siblings as compared with unrelated patients would help to define the extent to which genetic factors contribute to this variability. Studies of identical twins would also be useful. Discordance in siblings could be a result of genetic factors unlinked to the glucocerebrosidase gene itself, such as, for example, the inducibility of the saposin gene, while discordance among identical twins would implicate environmental influences. Understanding of why some people with the 1226G/1226G genotype have no clinical manifestations while others have moderately severe disease might point to new strategies for treating the disease.

Even in the absence of the ability to clearly predict the course of the disease, the knowledge that a child is fated to develop Gaucher disease can be of great value. Unless there is a family history, many patients ultimately diagnosed with Gaucher disease undergo a large number of diagnostic studies and long delays in achieving the correct diagnosis. Proper and prompt diagnosis becomes especially important when specific treatment in the form of enzyme replacement is available.

The cost of enzyme replacement. The most pressing problem with respect to the treatment of patients today is the high cost of enzyme therapy. Whether or not a patient receives this treatment has become largely a matter of the willingness of insurance companies to pay for the treatment, as few can afford the >$50,000 annual cost. As a result, some patients with mild forms of the

disease are being treated at a cost of over $300,000 per year, while others with much more severe disease are denied access to therapy. Although frequent small infusions of enzyme are more effective than large doses given at more convenient infrequent intervals (35), some patients whose insurance companies cover the cost of treatment select the more convenient treatment course. The problem of delivering this treatment to patients with Gaucher disease is a serious one for society as a whole (40). On the basis of gene frequency estimates and making allowance for the effect of Jewish–non-Jewish marriages, there are probably ~15,000 patients with Gaucher disease, Jewish and non-Jewish, in the United States. If one-third of these have disease sufficiently severe to require treatment and their average weight is only 40 kg, using the dose schedule recommended by the drug label the national cost of treatment for enzyme alone would be over $1 billion per year. Even with the more economical approach of giving the drug three times per week in smaller doses, the drug cost alone would be $250 million per year.

Can society afford to expend such large resources for the treatment of patients with a single disease, no matter how deserving they may be? Part of the reason for the high cost is that the expense of developing the manufacturing facilities and obtaining regulatory approval must be allocated among a small number of patients. But compared to the other storage diseases Gaucher disease is quite common. If treatments are developed for other storage diseases such as the mucopolysaccharoidoses, Fabry disease, and Tay-Sachs disease, the cost per patient might be even higher.

To some extent the problem of drug cost may be a temporary one because technological advances and competitive pressures will surely lower it. Until then further studies to identify better ways to administer the enzyme continue to be of critical importance. However, even implementation of rational administration schedules or decreasing the costs of its production by technological advances will not solve the entire problem as long as developmental and regulatory costs remain high.

Gene transfer. Because the disease can be corrected by transplantation of allogeneic hematopoietic stem cells, introduction of a wild-type form of the glucocerebrosidase gene into autologous hematopoietic stem cells and infusion of these genetically engineered cells into the patient is an attractive prospect for management of Gaucher disease (41). Development of such technology is impeded by the fact that the genetically corrected cells do not enjoy a selective advantage over those that have not received a normally functioning glucocerebrosidase gene. Thus it is not only essential to achieve a high efficiency of transformation of the cells that have been removed from the body, but also to destroy the endogenous cells of the patient.

A less comprehensive implementation of gene transfer therapy might be the transformation of autologous cells such as lymphoblasts with a modified cDNA that allows enzyme to be secreted. Secreted enzyme could then be taken up by cells with mannose receptors. Such a strategy would not provide permanent correction of the disorder, as the transplanted transformed cells would probably have a finite life span, but it might constitute an intermediate step toward more permanent correction of the disease.

Conclusions

The application of modern technology has made possible more effective diagnosis and treatment of Gaucher disease, the most common glycolipid storage disease. This has created a number of ethical and economic dilemmas for which there are no simple solutions. Similar difficulties are likely to arise as we learn more about some of the less common storage diseases. Further technologic advances and better understanding of phenotype-genotype relations in this group of disorders may provide solutions to some of the problems that we now face.

REFERENCES AND NOTES

1. R. J. Desnick, in *Gaucher Disease: A Century of Delineation and Research*, R. J. Desnick, S. Gatt, G. A. Grabowski, Eds. (Liss, New York, 1982), p. 1.
2. In only two reported cases a deficiency of glucocerebrosidase cofactor saposin C was found to cause the disease [D. Schnabel, M. Schröder, K. Sandhoff, *FEBS Lett.* **284**, 57 (1991)].
3. P. G. Pentchev, R. O. Brady, H. E. Blair, D. E. Britton, S. H. Sorrell, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3970 (1978); P. G. Pentchev, B. Neumeyer, L. Svennerholm, C. G. Groth, R. O Brady, *Am. J. Hum. Genet.* **35**, 621 (1983), E. Beutler, W. Kuhl, J. Sorge, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 6506 (1984); J. M F. G. Aerts *et al.*, *Biochem. J* **269**, 93 (1990); M. E. Grace, P. N. Graves, F. I. Smith, G. A. Grabowski, *J Biol. Chem.* **265**, 6827 (1990).
4. E. Beutler and W. Kuhl, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7472 (1986); M. E. Grace *et al*, *Am. J. Hum. Genet.* **49**, 646 (1991).
5. G. A. Grabowski *et al.*, *Am. J. Hum. Genet.* **37**, 499 (1985); T. Ohashi, *et al.*, *J. Biol. Chem.* **266**, 3661 (1991).
6. P. A. Ockerman and P. Kohlin, *Scand. J. Clin. Lab. Invest.* **22**, 62 (1968), E. Beutler and W. Kuhl, *Lancet* i, 612 (1970), G. L. Dale, E. Beutler, P. Fournier, P. Blanc, J. Liautaud, in *Enzyme Therapy in Genetic Diseases. 2*, R. J. Desnick, Ed. (Liss, New York, 1980), p. 33; B. A. Butcher *et al.*, *Clin Chim. Acta* **184**, 235 (1989); E. Beutler and A. Saven, *Blood* **76**, 646 (1990)
7. E. Beutler and W. Kuhl, *J. Lab. Clin. Med.* **76**, 747 (1970), G. A. Grabowski, T. Dinur, S. Gatt, R. J. Desnick, in *Gaucher Disease: A Century of Delineation and Research*, R. J. Desnick, S. Gatt, G. A. Grabowski, Eds. (Liss, New York, 1982), p. 573.
8. J. Sorge, C West, B. Westwood, E. Beutler, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7289 (1985); J. Sorge, T. Gelbart, C. West, B. Westwood, E. Beutler, *ibid.*, p 5442; S. Tsuji *et al.*, *J. Biol. Chem.* **261**, 50 (1986).
9. J. A. Sorge, C. West, W. Kuhl, L. Treger, E. Beutler, *Am. J. Hum. Genet.* **41**, 1016 (1987), E. Beutler *et al.*, in *Lipid Storage Disorders. Biological and Medical Aspects*, R. Salvayre, L. Douste-Blazy, S. Gatt, Eds. (Plenum Press, New York, 1988), p. 19.
10. T. Dinur *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 1660 (1986).
11. Expression has been achieved in transformed murine fibroblasts [J. Sorge, W. Kuhl, C. West, E. Beutler, *ibid.* **84**, 906 (1987); *Cold Spring Harbor Symp. Quant. Biol.* **60**, 1041 (1986)], murine hematopoietic cells [P. H Correll, J. K. Fink, R. O. Brady, L. K. Perry, S. Karlsson, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 8912 (1989)], COS cells [G. A. Grabowski, W. R. White, M. E. Grace, *Enzyme* **41**, 131 (1989)], *Spodoptera frugiperda* cells infected with baculovirus [B. M. Martin *et al.*, *DNA* **7**, 99 (1988); G. A. Grabowski, W. R. White, M. E. Grace, *Enzyme* **41**, 131 (1989)], and human hematopoietic cells [J K. Fink, P. H Correll, L. K. Perry, R O. Brady, S. Karlsson, *Proc. Natl Acad Sci U.S.A.* **87**, 2334 (1990); D. B. Kohn *et al.*, *Hum. Gene Ther.* **2**, 101 (1991)].
12. B. Shafit-Zagardo, E. A. Devine, M Smith, G. A. F. Arredondo, R. J. Desnick, *Am. J. Hum. Genet.* **33**, 564 (1981); R. A Barneveld *et al.*, *Hum. Genet.* **64**, 227 (1983); E. I. Ginns *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7101 (1985).
13. M. Horowitz *et al.*, *Genomics* **4**, 87 (1989).
14. A Zimran *et al.*, *J. Clin. Invest* **85**, 219 (1990)
15. E. Beutler, C. West, T. Gelbart, *Genomics* **12**, 795 (1992).
16. O. Reiner and M. Horowitz, *Gene* **73**, 469 (1988).
17. J Sorge, E. Gross, C. West, E. Beutler, *J. Clin. Invest.* **86**, 1137 (1990).
18. F Harada *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 8091 (1987); E. Mornet *et al.*, *Am. J. Hum. Genet.* **48**, 79 (1991)
19. E. Beutler, T. Gelbart, W. Kuhl, A. Zimran, C. West, *Blood* **79**, 1662 (1992).
20. A. Zimran *et al.*, *Lancet* ii, 349 (1989).
21. An earlier portion of this experience has been published [A. Zimran, T. Gelbart, B. Westwood, G A. Grabowski, E. Beutler, *Am. J. Hum. Genet.* **49**, 855 (1991); (*19*)].
22. A. Zimran, T. Gelbart, E. Beutler, *Am. J. Hum. Genet.* **46**, 902 (1990)
23. J. I. Rotter and J. M Diamond, *Nature* **329**, 289 (1987), J. Diamond, *Discover* **12**, 60 (1991).
24. A. Zimran, *personal communication.*
25. C. De Duve, *Fed. Proc.* **23**, 1045 (1964).
26. R. O. Brady, P. G. Pentchev, A. E. Gal, S. R. Hibbert, A. S. Dekaban, *N. Engl. J. Med.* **291**, 989 (1974).
27. E. Beutler, G. L. Dale, E. Guinto, W. Kuhl, *Proc. Natl Acad. Sci. U.S A.* **74**, 4620 (1977), E Beutler, *Trends Biol. Sci.* **6**, 95 (1981).
28. P E. Belchetz, J. C. W. Crawley, I. P Braidman, G. Gregoriadis, *Lancet* ii, 116 (1977).
29. D. T. Achord, F. E. Brot, C. E. Bell, W. S. Sly, *Cell* **15**, 269 (1978)
30. F. S Furbish, C. J. Steer, J. A. Barranger, E. A. Jones, R. O. Brady, *Biochem. Biophys. Res. Commun.* **81**, 1047 (1978), C. J. Steer, F. S. Furbish, J. A Barranger, R. O. Brady, E. A. Jones, *FEBS Lett.* **91**, 202 (1978), F. S Furbish, C. J. Steer, N. L. Krett, J A. Barranger, *Biochim. Biophys Acta* **673**, 425 (1981); T. W. Doebber *et al.*, *J. Biol. Chem.* **257**, 2193 (1982).
31. N. W. Barton *et al*, *N. Engl. J. Med.* **324**, 1464 (1991)
32. E Beutler *et al.*, *Blood* **78**, 1183 (1991).
33. *Wall Street Journal*, 23 December 1991, p. B1, *New York Times*, 22 January 1992, p. C19 (N); Genzyme Corporation (Boston) has suggested [*Wall Street Journal*, 24 December 1991, p. A2] that the dose and therefore the cost of aglucerase could rapidly be reduced. Although one may hope that this will prove to be the case, there is no

evidence at present bearing on this assertion.
34. M. R. Lennartz, T. E. Wileman, P. D. Stahl, *Biochem. J.* **245**, 705 (1987); R. W. Jansen *et al.*, *J. Biol. Chem.* **266**, 3343 (1991); M. L. Figueroa, Y. Sato, A. Kay, E. Beutler, *Clin. Res.*, in press. The fact that there is a linear relation between enzyme infusion rate and plasma concentrations has been interpreted as indicating that only a single class of receptors is present [N. W. Barton *et al.*, *N. Engl. J. Med.* **325**, 1811 (1991)]. However, the number of nonspecific binding sites is so large that most of the enzyme is cleared by binding to such sites. In fact, we have found that cultured macrophages and the marrow macrophages of patients being treated with macrophage-targeted glucocerebrosidase incorporate scarcely any of the exogenous enzyme, calling into question the actual distribution of the administered enzyme.
35. E. Beutler *et al.*, *Blood* **78**, 1183 (1991); E. Beutler, A. C. Kay, A. Saven, P. Garver, D. W. Thurston, *N. Engl. J. Med.* **325**, 1809 (1991), A. C. Kay *et al.*, *Trans. Assoc. Am. Phys.* **104**, 258 (1991), A. Zimran, I. Hadas-Halpern, A. Abrahamov, *N. Engl. J. Med.* **325**, 1810 (1991).
36. R. W. Jansen *et al.*, *J. Biol. Chem.* **266**, 3343 (1991); B. Smedsrod, J Melkko, L. Risteli, J. Risteli, *Biochem. J* **271**, 345 (1990)
37. J. M. Rappeport and E. I. Ginns, *N. Engl. J. Med.* **311**, 84 (1984); A. Erikson, *Acta Paediatr. Scand.* **326**, 7 (1986); J. R. Hobbs, *Enzyme* **38**, 194 (1987).
38. J. S. O'Brien, S. Okada, A. Chen, D. L. Fillerup, *N. Engl. J. Med.* **283**, 15 (1970); A. Cao *et al.*, *Am J. Hum. Genet.* **33**, 592 (1981).
39. R. Navon, B. Padeh, A. Adam, *Am. J. Hum. Genet.* **25**, 287 (1973); J. Vidgoff, N. R. M. Buist, J. S. O'Brien, *ibid.*, p. 372; R. Navon, B. Geiger, Y. Ben-Yoseph, M. C. Rattazzi, *ibid.* **28**, 339 (1976), E. Conzelmann, H. J. Kytzia, R. Navon, K. Sandhoff, *ibid.* **35**, 900 (1983); R. Navon, Z. Argov, A. Frisch, *ibid.* **24**, 179 (1986).
40. D. P. Goldman, A E. Clarke, A. M. Garber, *Internatl. J. Tech. Assess. Health Care*, in press.
41. E. Beutler and J. Sorge, *Exp. Hematol.* **18**, 857 (1990); S. Karlsson, *Blood* **78**, 2481 (1991); D. B. Kohn *et al.*, *Hum Gene Ther.* **2**, 101 (1991).
42. P. N. Graves, G. A. Crabowski, R. Eisner, P. Palese, F. I. Smith, *DNA* **7**, 521 (1988).
43. E. Beutler, T. Gelbart, W. Kuhl, C. West, unpublished data.
44. N. Eyal, N. Firon, S. Wilder, E. H. Kolodny, M. Horowitz, *Hum. Genet.* **87**, 328 (1991).
45. T. E. Latham, B. D. M. Theophilus, G. A. Grabowski, F. I. Smith, *DNA Cell Biol.* **10**, 15 (1991).
46. H. Kawame and Y. Eto, *Am. J. Hum. Genet* **49**, 1378 (1991).
47. E. Beutler and T. Gelbart, *Ann. Hum. Genet.* **54**, 149 (1990).
48 N. Eyal, S. Wilder, M. Horowitz, *Gene* **96**, 277 (1990).
49. S. Tsuji *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2349 (1988).
50. B. D. M. Theophilus, T Latham, G. A. Grabowski, F. I Smith, *Nucleic Acids Res.* **17**, 7707 (1989).
51. M. Wigderson *et al.*, *Am. J. Hum. Genet.* **44**, 365 (1989).
52. S. Tsuji *et al.*, *N. Engl. J. Med.* **316**, 570 (1987), N. Dahl, M. Lagerstrom, A. Erikson, U. Pettersson, *Am. J. Hum. Genet.* **47**, 275 (1990).
53. C. M. Hong, T. Ohashi, X. J. Yu, S Weiler, J. A. Barranger, *DNA Cell Biol.* **9**, 233 (1990)
54. E. Beutler, T. Gelbart, W. Kuhl, J. Sorge, C. West, *Proc. Natl. Acad. Sci U.S.A.* **88**, 10544 (1991).
55. T. Latham, G. A. Grabowski, B. D. M. Theophilus, F I. Smith, *Am. J. Hum. Genet.* **47**, 79 (1990)
56. Supported by NIH grants DK36639 and RR00833 and the Sam Stein and Rose Stein Charitable Trust Fund. This is manuscript 7252-MEM from The Scripps Research Institute; The author is grateful to T. Gelbart, W. Kuhl, and C. West for their assistance in preparing this manuscript and to B. Beutler for helpful suggestions.

Molecular Genetics of Epidermolysis Bullosa

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Blisters following minor trauma characterize epidermolysis bullosa, a group of hereditary diseases of the skin. In the simplex type, epidermal basal cells are fragile, and mutations of genes encoding keratin intermediate filament proteins underlie that fragility. In the dystrophic types, the causative mutation appears to be in the gene encoding type VII collagen, which is the major component of anchoring fibrils. These recent findings afford solid evidence that at least one function of the cytoskeletal intermediate filament network is the provision of mechanical stability and that anchoring fibrils indeed do anchor the epidermis to the underlying dermis.

Job 7:5—*My skin closeth up and breaketh out afresh.*

Skin diseases present a particular fascination and, by their visibility, cause particular embarrassment for the afflicted. They are so common that all of us have had some personal experience with them on our own skin or at least on the skin of others we see about us. Hence, it is not surprising that skin disease was so prominent among Job's trials. Because the eye can make such fine discriminations, dermatologists can appreciate an enormous number of skin diseases, and many of these are inherited. Among the latter, a group of diseases marked by blistering, epidermolysis bullosa, is quite prominent, less for their frequency and more for their distinctive appearance. Quite recently, our understanding of the defects underlying this group has advanced markedly through the application of the techniques of molecular genetics. As is typical for such studies, new understanding of normal functioning of skin molecules is inherent in the identification of the defects. Less typical, however, is that some of the tissue-specific technology that might be necessary for gene therapy of epidermal disease already exists: removal of skin, in vitro culture of keratinocytes, and reattachment of epidermal sheets at their normal site are techniques already used routinely for treatment of patients with burns. Hence, skin disease would seem an especially promising substrate for DNA-based therapies. In this review, I will focus on epidermolysis bullosa (EB), on the defective cytoskeletal keratins underlying EB simplex and the defective dermal collagen underlying dystrophic EB, and will mention some of the array of hereditary skin diseases, the underlying defects of which are likely to be discovered soon by further applications of molecular genetics.

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Hereditary Blisters of the Skin

The term "epidermolysis bullosa" was attached a century ago to a clinically heterogeneous group of disorders characterized by blistering of the skin following minimal to inapparent trauma. Onset is early in life, often neonatally or during infancy, and, except for the rather uncommon EB aquisita, the disease is heritable. The inheritance may be dominant or recessive, and the disease may be severe or mild and may be generalized or localized to a portion of the skin surface. Most importantly, blisters may heal with mutilating scarring or with no scarring at all (*1*).

Although some workers had noted histologic heterogeneity previously, subclassification of EB was rationalized three decades ago when electron microscopy permitted accurate assessment of the blister site in the skin (Fig. 1) (*2*). Pearson initially and subsequently Anton-Lambrecht and others established that the split in all variants of EB simplex (termed "simplex" because healing generally occurs without scarring) is within the basal cells of the epidermis, such that the base of the blister contains remnants of the basal plasma membrane and the roof contains the nucleus and supranuclear portion of the basal cells. This site is quite different from that of ordinary friction blisters, in which the split is considerably higher, in the upper spinous cells of the epidermis (*3*). In junctional EB (one variety of which had been termed EB letalis because of its dismal prognosis) the split is within the basement membrane zone in the lamina lucida, and blisters heal with varying degree of scarring. In dystrophic EB, so called because chronic cycles of blistering invariably lead to scarring and, in more severe cases, to disability, inanition, and death, the split is in the upper dermis.