Review

Gaucher disease as a paradigm of current issues regarding single gene mutations of humans

Ernest Beutler

Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037

ABSTRACT Gaucher disease is a glycolytic storage disease caused by a deficiency in activity of the catabolic enzyme glucocerebrosidase. Over 35 different mutations have been documented, including missense and nonsense point mutations, splicing mutations, deletions and insertions, a fusion gene, and examples of gene conversion. Gaucher disease is most common in the Ashkenazi Jewish population, in which just five of the mutations in this population account for 98% of the disease-producing alleles. Each of these mutations is found in the context of a single haplotype, a finding consistent with a single origin of each mutation. Although it is clear that these mutations provide a selective advantage in the Jewish population and thus constitute a balanced polymorphism, the nature of the advantage is unknown. Gaucher disease can be treated symptomatically, by administration of the missing enzyme, by allogeneic bone marrow transplantation, and potentially by gene transfer into hematopoietic stem cells. Increasing understanding of this disease has, as in other genetic disorders, created a host of social and ethical dilemmas regarding matters such as the cost of treatment for rare diseases and the advantages and disadvantages of population-targeted genetic screening.

In 1902 Sir Archibald Garrod suggested that the excretion of homogentisic acid in alkaptonuria was the result of the genetic absence of a single enzyme (1). Although single gene defects are, no doubt, abundant throughout the plant and animal kingdoms, it is in humans that the great diversity of such mutations has become the most widely appreciated. A single ill individual not uncommonly becomes the object of intense and extensive investigation and as a result hundreds of human diseases due to deficiency of a single enzyme have been catalogued (2). Until only a decade ago studies of such disorders were limited largely to investigation of the residual protein and of the more indirect results of the deficiency. In only a few disorders, most notably the hemoglobinopathies, was there sufficient residual protein available to make the determination of the underlying defect of the amino acid sequence possible. Al-

though by 30 years ago it was recognized that some enzyme deficiencies were caused by different mutations affecting the same gene, attempts to distinguish mutant enzymes from each other in different families were often flawed by the fact that the mutant enzymes were unstable and therefore that reproducible results were difficult to obtain (3).

The development of a variety of facile techniques for the detection of mutations at the DNA level has greatly enhanced our understanding of single gene defects. In several human disorders, including classical hemophilia, cystic fibrosis, factor IX deficiency, glucose-6-phosphate dehydrogenase deficiency, Tay-Sachs disease, and Gaucher disease, the extent of the heterogeneity of disease-causing mutations has become apparent. As a result, more accurate diagnosis and better assessment of prognosis has become possible. At the same time, the techniques of molecular biology have made new treatment options available.

Invariably, as we answer questions that were until recently unanswerable, new questions arise to tax our ingenuity. The study of Gaucher disease, which has occupied our laboratory and clinic for the past 25 years, illustrates what we have been able to learn about such single gene defects and poses questions that need to be answered.

Definition and Clinical Manifestations

Gaucher disease is one of a group of lysosomal storage disorders. The glycosphingolipid glucosylceramide (glucocerebroside) accumulates in macrophages through the body because of an inherited deficiency in the activity of a lysosomal acid β -glucosidase (glucocerebrosidase). Thus, major organs that are affected include the liver and spleen, which may become greatly enlarged, and the bone, which may be subject to fractures. Marrow involvement often results in anemia and a lowered platelet count. Only in rare cases, designated as type II (acute neuronopathic; infantile) and type III (chronic neuronopathic; juvenile), is there involvement of the central nervous system. Most patients have type I dis-

ease, in which the nervous system is spared.

History

Described by Phillipe Gaucher in 1882, the disease that bears his name was initially believed to be a neoplasm of the spleen. However, the familial occurrence of Gaucher disease was recognized as early as 1901 (4) and the fact that it was a metabolic abnormality was discovered in 1907 (5). The pathologic hallmark of the disease was the presence of macrophages engorged with lipid. Although first thought to be kerafin or galactocerebroside (6, 7), the lipid was ultimately correctly identified as glucosylceramide (glucocerebroside) (8). Storage diseases result from deficiency of a catabolic enzyme, the storage material representing the substrate of the missing enzyme. Thus, it was found that the defective enzyme in Gaucher disease is the acid β -glucosidase that normally functions to remove the β -glucose from glucocerebroside (9, 10).

Identification of the underlying enzymatic defect opened the door to greater understanding and better diagnosis of the disease. Diagnosis could now be made by measuring the enzymatic activity of the peripheral blood leukocytes (11, 12). Purification of the enzyme proved difficult, largely because it was bound to the lysosomal membrane and because it required a cofactor for activity (13, 14). Once purified, the enzyme was used for trials of enzyme replacement therapy (15, 16).

Purification of the enzyme ultimately made possible cloning of the gene (17, 18), elucidation of its structure (19), and identification of most of the mutations that cause the disease (20). The possibility now exists that the gene can be used to treat the disease (21–24). It is these newer developments and their implications that are the major focus of this brief review.

The Glucocerebrosidase Gene

The gene for glucocerebrosidase is located on the long arm of chromosome 1. Localization of the gene was first estab-

lished by studying cell hybrids (25, 26) and was subsequently confirmed by using DNA probes (27, 28). The gene has been assigned to the same band (q21) occupied by the pyruvate kinase gene (29). We have recently found that there is a linkage disequilibrium between the polymorphic sites in the pyruvate kinase and glucocerebrosidase genes. This implies that the genetic distance between these genes is very short, indeed.

A glucocerebrosidase pseudogene exists 16 kb downstream from the functional gene. There is a high degree of homology between the pseudogene and the functional gene, and the promoter of the pseudogene is still active (30, 31). However, the pseudogene has many point mutations and deletions that preclude it from directing synthesis of active protein. The glucocerebrosidase cDNA has two start codons, both of which are used in synthesizing protein (32). No matter which start codon is used, the enzyme contains a leader sequence that is apparently cleaved posttranslationally (27). Eleven widely distributed polymorphic sites have been documented to exist in the glucocerebrosidase gene (33). Surprisingly, all of these are in complete linkage disequilibrium. It is difficult to understand how this state of affairs came about, since sequential occurrence of n mutations would be expected to create $n + 1$ haplotypes even if no crossovers had occurred. An additional polymorphism, apparently limited to Africans, and one limited to White populations have been superimposed on the matrix of 11 polymorphisms.

Gaucher Disease Mutations

A variety of mutations causing Gaucher disease have been found (Table 1). Included are point mutations, insertional mutations, deletions, and one splicing mutation.

Population Genetics

The spectrum of mutations encountered in the Ashkenazi Jewish population, in which Gaucher disease is relatively common (see below), and in the non-Jewish population, in which it occurs much less frequently, is quite different (Table 2). In the Jewish population the G¹²²⁶ mutation is by far the most common and the GG⁸⁴ mutation is second most common, representing 77.2% and 13.2%, respectively, of Gaucher disease mutations in 100 Jewish patients we have studied (36). In another series of 88 patients, these two represented 72.7% and 10.2% of the mutations, respectively (52). In contrast, the C¹⁴⁴⁸ mutation is the most common in the non-Jewish population (38.1%), with the G¹²²⁶ mutation being the second most common in the European non-Jewish community (33%), but being unknown in

patients of Asian or African ethnic origin (53). Table 3 shows the haplotype of patients with the three mutations that are most common in the Jewish population. The G¹²²⁶ allele is always found in the context of the P_v1.1⁻ haplotype, the GG⁸⁴ mutation is found in the P_v1.1⁺ haplotype, and the IVS2(+1) mutation is always found in the P_v1.1⁻ haplotype. These findings are consistent with, and indeed argue for, a single origin for each of these mutations. Since they are now common in the Ashkenazi Jewish popu-

lations, they must have provided a selective advantage to those who carried it, an advantage large enough to allow the frequency of the gene in the population to increase, even though those who had two abnormal genes, and hence Gaucher disease, clearly had decreased fitness. In contrast to the situation in the Jewish population, in which the gene has become quite common, it has remained rare in the rest of the European population, suggesting that the selective advantage that it conferred was unique to Jews.

Table 1. Gaucher disease mutations

cDNA*	Amino acid*	Genomic*	Nucleotide substitution	Amino acid substitution	Ref.
Point mutations and insertions					
72 del	NA	1023	C → del	NA	34
84	NA	1035	G → GG	NA	35
IVS2(+1)	NA	1067	G → A [†]	NA	36
476	120	3060	G → A	Arg → Gln	37
481	122	3065	C → T	Pro → Ser	34
535 } [‡]	140	3119	G → C	Asp → His	38
1093 }	326	5309	G → A	Glu → Lys	
586	157	3170	A → C	Lys → Gln	38, 39
751	212	3545	T → C	Tyr → His	34
754	213	3548	T → A [†]	Phe → Ile	40
764	216	4113	T → A	Phe → Tyr	41
983	289	4332	C → T	Pro → Leu	42
1043	309	5259	C → T	Ala → Val	39
1053	312	5269	G → T	Trp → Cys	39
1085	323	5301	C → T	Thr → Ile	42
1090	325	5306	G → A [†]	Gly → Arg	43
1141	342	5357	T → G	Cys → Gly	43
1192	359	5408	C → T	Arg → Stop	§
1193	359	5409	G → A	Arg → Gln	44
1208	364	5424	G → C	Ser → Thr	39
1226	370	5841	A → G	Asn → Ser	45
1297	394	5912	G → T	Val → Leu	46
1342	409	5957	G → C [†]	Asp → His	43, 46
1343	409	5958	A → T	Asp → Val	46
1361	415	5976	C → G	Pro → Arg	47
1390	425	6375	A → G	Lys → Glu	44
1448	444	6433	T → C [†]	Leu → Pro	48
1504	463	6489	C → T	Arg → Cys	49
1549	478	6628	G → A	Gly → Ser	34
1603	496	6682	C → T	Arg → Cys	44
1604	496	6683	G → A	Arg → His	34
Recombination events that cause Gaucher disease: Location of crossover event(s) [¶]					
>455 <475 } [‡]		>3039 <3059 } [‡]			39
>754		>3548			
>1225 <1263		>5588 <5878			43
1263–1317 del		5879–5933 [†]			34
>1317 <1343		>5932 <5957			43, 50
>1343 <1388		>5957 <6272**			51
>1343 <1388		>5957 <6272			34, 43, 49, 50

NA, not applicable.

*Nucleotide or amino acid position.

[†]Pseudogene sequence.

[‡]Both found in one gene.

[§]E.B. and T. Gelbart, unpublished data.

[¶]Only approximate ranges can be given, since the pseudogene and functional gene contain long identical sequences.

^{||}The first range represents crossover from gene to pseudogene; the second represents crossover from pseudogene to gene. This region contains 7 mutations, only 6 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.

**Physical fusion with loss of intergenic segment.

Table 2. Occurrence of Gaucher disease mutations in Jewish and non-Jewish patients

Mutation	Occurrence in Jewish patients	Occurrence in non-Jewish patients
G ¹²²⁶	187	37
C ¹⁴⁴⁸	8	47
GG ⁸⁴	30	1
IVS2(+1)	6	1
T ¹²⁹⁷	5	0
XOVR	1	5
A ¹⁶⁰⁴	3	1
T ¹⁵⁰⁴	0	6
C ⁷⁵¹	1	0
A ¹⁵⁴⁹	0	1
A ⁴⁷⁶	0	1
T ¹⁰⁸⁵	0	1
T ⁴⁸¹	0	2
Del ⁷²	0	1
A ⁷⁶⁴	0	2
C ¹³⁴²	0	1
Del	0	1
?	3	20

XOVR designates a fusion gene resulting from recombination between the glucocerebrosidase gene and pseudogene. ? indicates that none of the 29 mutations tested was present.

The puzzle of what advantage that partial glucocerebrosidase deficiency might provide or have provided to Ashkenazi Jews is made even more intriguing by the fact that deficiencies of at least two other enzymes of lipid catabolism are extraordinarily common in the Ashkenazi Jewish population. These are the hexosaminidase A and sphingomyelinase deficiencies that cause, respectively, Tay-Sachs disease and Niemann-Pick disease. Infectious diseases have been a major cause of death and thus any gene that produced resistance against an important infection would thrive in the population. In Africa, where malaria has been a great killer, a number of single gene traits have emerged at high frequency because they protect the erythrocyte against the malaria parasite. Included are glucose-6-phosphate dehydrogenase deficiency and sickle cell trait (54, 55). Because tuberculosis was probably a major killer in the densely populated urban ghetto, it has been suggested that resistance to this infection might have been the selective factor that made these lipid storage diseases common in Jews (56). Since tuberculosis is now a relatively uncommon disease, this hypothe-

FIG. 1. Relationship between age and severity of symptoms of 120 unrelated patients with several common Gaucher disease mutations. The clinical severity score was calculated as described (58). The 12 patients with the C¹⁴⁴⁸/C¹⁴⁴⁸ genotype were the youngest and had the most severe symptoms. The 69 patients with the G¹²²⁶/G¹²²⁶ genotype tended to be older and had the mildest symptoms, but there was considerable overlap between the severity of disease observed in different genotypes.

sis is difficult to test, but efforts to do so have, thus far, yielded negative results.

Genotype-Phenotype Correlations

A relationship exists between the type of mutation and the clinical manifestation of the disease (57) (Fig. 1). Presence of the G¹²²⁶ mutation appears to preclude the development of neurological disease, a single case of putative neurologic involvement notwithstanding (59). Homozygotes for the G¹²²⁶ mutation generally have relatively late onset disease often with only minor disease manifestations, although occasional exceptions are found. In contrast, the C¹⁴⁴⁸ mutation is more drastic and is frequently associated with neuronopathic disease. More severe, still, is the GG⁸⁴ frameshift mutation, which not surprisingly is associated with complete lack of enzyme (35) and has not been found in the homozygous form. It is of interest from this point of view that mice homozygous for targeted disruption of the gene die before birth (60).

It is not surprising that a correlation exists between the severity of Gaucher disease mutations and severity of the disease phenotype. What is more difficult to explain is the degree of variability that exists among patients that have the same disease genotype. To some extent this has been obvious merely from clinical observations: there is often considerable discordance between the disease severity observed in sibs, who must have inherited the same two disease-producing alleles.

But the frequency with which the same alleles produce different disease manifestations in Gaucher disease and other diseases caused by single gene defects has become more obvious as genotyping has become commonplace. For example, we have studied a 64-year-old patient with the G¹²²⁶/GG⁸⁴ genotype with much milder disease manifestations than a 30-year-old woman with the G¹²²⁶/G¹²²⁶ genotype. Yet, the patient with the G¹²²⁶/GG⁸⁴ genotype is essentially hemizygous for the G¹²²⁶ allele for which the other patient has two copies, since the GG⁸⁴ mutation is a frameshift mutation at the 5' end of the gene. Moreover, the sister of the homozygous patient who has the same G¹²²⁶/G¹²²⁶ genotype has scarcely any disease manifestations at all.

Such variability in phenotypic expression has been studied most extensively, perhaps, in patients with sickle cell disease, a disorder in which the molecular change was identified decades ago (61). Here the defective protein is a heteropolymer, composed of α and β chains, with the mutation localized to the β chain. It is reasonable to suppose that in this situation genetic variability of the α chain might be a factor that affects the phenotype. And so it is. But the effect is a very minor one. Each of the many factors that have been studied, including the β -locus haplotype and the amount of fetal hemoglobin, has only a slight impact on the clinical manifestations of this disease (62, 63). The same pattern is emerging in all single gene defects and, as discussed below, becomes an important confounding factor in making treatment decisions and in offering counseling to families who carry these mutations.

Table 3. Distribution of the *Pv1.1* haplotype among patients with three common Gaucher disease genotypes

Gaucher disease mutation	<i>Pv1.1</i> ⁻ / <i>Pv1.1</i> ⁻	<i>Pv1.1</i> ⁻ / <i>Pv1.1</i> ⁺	<i>Pv1.1</i> ⁺ / <i>Pv1.1</i> ⁺
G ¹²²⁶ /G ¹²²⁶	63	0	0
G ¹²²⁶ /GG ⁸⁴	0	24	0
G ¹²²⁶ /IVS2(+1)	6	0	0

Treatment

Disorders due to inherited enzyme deficiencies can be treated at several different levels, including mere symptomatic

treatment, substrate deprivation, supplying a missing product, exogenous administration of the defective enzyme, allogeneic transplantation with genetically normal tissue, and, potentially, replacement of the defective gene.

Many of these treatment approaches have been used with some success in the treatment of Gaucher disease, and a considerable amount of work has been done to make possible gene transfer as a treatment modality for the disease.

Symptomatic Treatment. Symptomatic management of a genetic disease is generally a low-technology approach that does not alter the fundamental disease process. Yet, it can be extraordinarily helpful in improving the quality of life of the patient and thus is not to be disdained. In Gaucher disease, surgical removal of the spleen almost always corrects the thrombocytopenia that is present in many patients. Patients who are totally disabled by osteonecrosis can be rehabilitated by joint replacement. While such a symptomatic therapeutic approach is helpful, it is clearly only an interim measure while treatments that can cause actual regression of the primary cause of disease manifestations are developed.

Exogenous Administration of the Defective Enzyme. History. Since the suggestion of De Duve (64) that exogenous replacement of missing enzyme may be a successful approach to the treatment of lysosomal storage diseases, type 1 Gaucher disease has appeared as an unusually promising candidate for such therapy. This is the case because there is no primary central nervous system involvement and the target cells are macrophages, cells that are usually in direct contact with the flowing blood.

Early attempts at replacement therapy were disappointing due to an insufficient supply of enzyme that could be targeted efficiently to macrophages. Brady and co-workers (15, 65–67) administered unmodified placental acid β -glucosidase intravenously to two patients with Gaucher disease. Liver biopsies before and after enzyme administration appeared to show a decrease in the amount of liver glycolipid, but, in view of the heterogeneous disposition of glucocerebroside in liver (16), the results are difficult to interpret, and in any case no clinical response was reported. Our administration of smaller amounts of enzyme targeted to the reticuloendothelial system by encapsulation in erythrocyte ghosts that were coated with immunoglobulin (16) appeared to result in the reduction of liver size as judged by technetium scanning. However, results of the study of six additional patients using this technique (68–72) were disappointing. Trials of enzyme replacement were also undertaken using liposome-entrapped enzyme (73). Alarm-

ing symptoms occurred after some liposome infusions and the therapeutic results were equivocal (74).

Mannose terminated glucocerebrosidase. With the realization that the uptake of glycoproteins occurred by way of carbohydrate-specific receptors (75) and in particular that macrophages contained mannose-specific receptors (76), attempts were made to target acid β -glucosidase to macrophages by modifying the oligosaccharides of the enzyme (77–79). Industrial-scale production of human placental enzyme modified so as to expose more terminal mannose residues (Ceredase; alglucerase) made available sufficient material for prolonged clinical administration of large amounts of acid β -glucosidase. Clear-cut clinical responses could be demonstrated (80, 81) and readily confirmed (82–85).

In anemic patients, the hemoglobin concentration of the blood begins to increase within the first few months of enzyme therapy. Regression of organomegaly is generally evident within the first 6 months of treatment, with an average decrease of excess liver volume by 20–25%.

Pharmacology. The plasma half-life of alglucerase is ≈ 11 min (82) and, based on studies of macrophages loaded with alglucerase *in vitro* (86), the intracellular half-life is 4–8 h. Although a portion of the modified enzyme binds to the classic mannose receptor on macrophages, *in vitro* binding studies show that the enzyme binds principally to another, quite distinct mannose-dependent receptor. Not only are there ≈ 20 times as many copies of this receptor as of the classic receptor on macrophages, it is also much more widely distributed. It is present on peripheral blood leukocytes, endothelial cells, and COS cells. It is apparent that most of the alglucerase that is infused will be bound by this type of receptor rather than by the classic mannose receptor. Not surprisingly, then, very little of the administered enzyme is found in bone marrow cells, even immediately after marrow infusion (20). Thus, the reason for the very satisfactory clinical response to alglucerase is not clear. It may be that the small amount that is taken up by macrophages is sufficient to decrease the glucosyl ceramide burden or that the enzyme removes the glycolipid from sites other than macrophages.

Dosage regimens. The dose of alglucerase used in the early studies (81) and recommended by the commercial producer and the Food and Drug Administration (87) is 60 units per kg of body weight every 2 weeks. Treatment of a 70-kg patient according to this schedule at the current market price of alglucerase is \$380,000 per year for enzyme alone. Thus, the preparation is extremely costly and well out of the price range of many

patients, particularly in developing countries (88). For this reason, it is desirable to administer the preparation in as efficient a manner as possible. The pharmacologic properties of the preparation, particularly the short half-life and the presence of low-affinity receptors that compete with the high-affinity classic mannose receptors for alglucerase suggest that frequent administration of the enzyme would be more effective than infrequent dosing. Thus, a treatment protocol under which only about one-fifth to one-fourth as much enzyme is administered has been introduced (82–84, 89) and found to give results that are comparable to those obtained with infrequent administration of large doses of alglucerase.

Allogeneic Transplantation. Macrophages are progeny of the hematopoietic stem cell, and it has been shown that marrow transplantation replaces not only the circulating blood cells but also the macrophages of the liver (90), lung (91), and, very likely, brain (92). The ultimate test of whether the phenotype of a genetic disease is expressed largely or entirely in the macrophage is to perform allogeneic bone marrow transplantation after ablating the patient's own marrow. Since the macrophages that then populate the body carry the correct genetic information, signs of the disease should disappear. Allogeneic marrow transplantation has been performed a number of times in patients with type 1 Gaucher disease with marked amelioration of the disorder and with disappearance of Gaucher cells from the marrow (93, 94). The response of patients with neuronopathic disease has been less definitive (95, 96).

However, the risks of allogeneic transplantation are considerable, both because marrow ablation produces a period of pancytopenia and immunocompromise and because of the risk of graft-versus-host disease. The mortality risk of the procedure is of the order of 10%, even in patients who are in good general medical condition at the time transplantation is carried out. It is higher in the severely compromised patients who would be candidates for transplantation. Autologous transplantation also carries a considerable risk because marrow ablation is required, but since graft-versus-host disease cannot occur when patients are transplanted with their own marrow, it is considerably safer than allogeneic transplantation. Of course, autologous stem cells will only correct the disease if a functioning normal copy of the gene or cDNA has been transferred to them.

Gene Transfer. The enzyme deficiency of cultured skin fibroblasts and lymphoblasts from patients with Gaucher disease is readily corrected by retrovirus-mediated transfer of the glucocerebrosidase cDNA (21, 97, 98). Efficient transfer of the cDNA into hematopoietic progen-

itor stem cells is a more difficult task, but it has been achieved in murine (22–24, 99, 100) and to some extent in human (101) systems. In some cases, prolonged expression in macrophages of mice transplanted with transduced marrow cells has been documented (23, 24).

Although such studies engender some optimism that gene transfer therapy of Gaucher disease might be implemented some time in the future, there are a number of reasons to doubt that such treatment will become practical in the near future. Gene transfer in mice is easier to accomplish than gene transfer in primates (102, 103). Once it is established that expression can be achieved in primates, the availability of enzyme replacement therapy for Gaucher disease makes it more difficult to justify the risks inherent in marrow ablation in the treatment of this disorder than in disorders for which there is no other specific treatment. An example of such a genetic disorder is chronic granulomatous disease (104) (see below).

Ethical Issues

Advancing knowledge of Gaucher disease and other genetic disorders has created ethical dilemmas.

Population-Based Screening. It is well established that screening a population that is at risk for an autosomal recessive disorder can be very useful. The disease that has been studied most extensively in this regard is Tay–Sachs disease. Here screening of the Jewish population has resulted in a marked decrease in the birth incidence of the disorder (105). However, the dismal prognosis of Tay–Sachs disease is highly predictable. The vast majority of newborns who lack hexosaminidase A in their serum are condemned to a short, neurologically impaired life, although there are rare genetic variants of hexosaminidase A in which this is not true (106). The Gaucher disease phenotype is much more variable than the Tay–Sachs phenotype. There is a broad range of possible clinical outcomes even within a given Gaucher disease genotype (Fig. 1), and we do not know which patient will be at one extreme and which will be at the other. This creates a major problem in genetic counseling. We can identify a fetus with the Gaucher disease genotype, but we cannot give the parents a confident prediction of the clinical course. If we screen and counsel, then the information that we can provide is often not useful and only produces anxiety. If, on the other hand, we do not screen and a baby with Gaucher disease is born, the parents may justifiably resent the fact that they were not provided with information about the possible outcome, even though the technology to do so exists. Their resentment

may be greater if the child goes through repeated, prolonged, and painful medical examinations before the diagnosis is established.

Treatment. The cost of high-tech treatment of rare diseases is one that our society has not faced. The orphan drug laws encourage development of treatments for rare diseases. In the United States most insurance plans cover any treatment that is licensed by the Food and Drug Administration. The fixed costs of developing drugs increases progressively and is essentially independent of the size of the patient population. In comparison with other serious diseases caused by single gene mutations, Gaucher disease is very common. However, from an economic point of view, the target population is very small. Thus, the fixed development costs are very high in proportion to the number of patients to be treated. This, at least in part, accounts for the very high cost of the drug (107).

The high cost of alglucerase has created difficult problems for patients in developed countries and impossible ones in underdeveloped countries. Patients have been dropped from their insurance plans. Others will soon exhaust the maximum amount of their lifetime insurance coverage, commonly \$1,000,000. Insurance plans sometimes seek any excuse to refuse to authorize treatment. As a consequence, there is relatively little relationship between the severity of disease and whether or not a patient receives treatment.

Even if the problem posed by alglucerase therapy were manageable, and it is not, more difficult problems could arise with other diseases due to single gene mutations. The number of patients with each form of mucopolysaccharidosis, Fabry disease, or triosephosphate isomerase deficiency, to name only three of hundreds of such disorders, is 1 or 2 orders of magnitude smaller than the number of patients with Gaucher disease. Yet, the cost that would attend developing and licensing a treatment would be just as high. Thus, the cost of the treatment might be millions of dollars per year instead of several hundred thousand, as is the case with alglucerase today.

The Future

The challenges that we face in better understanding and treating Gaucher disease are similar to those with many other single gene defects.

The understanding of phenotypic variability is particularly important. If we understood why one patient with the G¹²²⁶/G¹²²⁶ genotype never develops clinically significant disease while another becomes disabled by severe bone lesions and organomegaly, we could counsel and treat more effectively. As

noted above, the same problem exists in the case of sickle cell disease, where it is difficult or impossible to arrive at a rational decision regarding the advisability of relatively risky marrow transplantation (108).

The study of Gaucher disease has proven the feasibility and utility of DNA-based diagnosis, particularly in a population in which the mutant genes are polymorphic. But searching one by one for all known mutations is impractical and will, of course, always fail to detect the increasingly rare mutations that have not yet been encountered. What are needed in the study of Gaucher disease and other mutations are more powerful techniques for detection of any deviation from normal in the sequence of a gene. The method of single-stranded conformation polymorphism analysis (109) is a good start but is neither simple nor powerful enough to find all mutations.

Another area in which progress needs to be made is in treatment. Ultimately, gene transfer into hematopoietic stem cells will probably provide the solution. Barriers that need to be overcome relate largely to the likelihood that a clinical cure will depend on replacement of most or all of the macrophages with transduced cells. This requires not only very efficient transduction but also, if gene transfer is performed *in vitro*, the destruction of the patient's own stem cells. The requirements for effective treatment of some other single gene disorders—e.g., adenosine deaminase deficiency, chronic granulomatous disease, and mucopolysaccharidoses—are less stringent. Here correction of the defect in just a few cells may provide great clinical benefit. In chronic granulomatous disease, for example, a clinical cure may be achieved, since only a few normal granulocytes are needed to prevent the infections that plague these children.

In the meanwhile, further knowledge is needed to design enzymes that are directed efficiently to lysosomal targets. The rational approach of infusing a mannosidase-rich enzyme has proved disappointing. It binds to the wrong receptor and is not efficiently delivered. Intracellular trafficking is proving to be increasingly complex, and a more complete understanding of the receptors and pathways that might be used is needed.

This is manuscript number 7879-MEM from The Scripps Research Institute. This work was supported by National Institutes of Health Grants DK36639 and RR00833 and the Sam Stein and Rose Stein Charitable Trust Fund.

1. Garrod, A. E. (1902) *Lancet* ii, 1616–1620.
2. McKusick, V. A. (1983) *Mendelian Inheritance in Man: Catalogs of Autosomal Dominant, Autosomal Recessive,*

- and X-Linked Phenotypes (The Johns Hopkins Univ. Press, Baltimore).
3. Fairbanks, V. F., Nepo, A. G., Beutler, E., Dickson, E. R. & Honig, G. (1980) *Blood* **55**, 216–220.
  4. Brill, N. E. (1901) *Am. J. Med. Sci.* **121**, 377–392.
  5. Marchand, F. (1907) *Münch. Med. Wochenschr.* **54**, 1102–1124.
  6. Epstein, E. (1924) *Biochem. Z.* **145**, 398–424.
  7. Lieb, H. (1924) *Hoppe-Seyler's Z. Physiol. Chem.* **140**, 305–324.
  8. Aghion, H. (1934) Ph.D. thesis (Paris).
  9. Brady, R. O., Kanfer, J. N. & Shapiro, D. (1965) *Biochem. Biophys. Res. Commun.* **18**, 221–225.
  10. Patrick, A. D. (1965) *Biochem. J.* **97**, 17C–18C.
  11. Kampine, J. P., Brady, R. O. & Kanfer, J. N. (1967) *Science* **155**, 86–88.
  12. Beutler, E. & Kuhl, W. (1970) *Lancet* **i**, 612–613.
  13. Pentchev, P. G., Brady, R. O., Hibbert, S. R., Gal, A. E. & Shapiro, D. (1973) *J. Biol. Chem.* **248**, 5256–5261.
  14. Dale, G. L. & Beutler, E. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 4672–4674.
  15. Brady, R. O., Pentchev, P. G., Gal, A. E., Hibbert, S. R. & Dekaban, A. S. (1974) *N. Engl. J. Med.* **291**, 989–993.
  16. Beutler, E., Dale, G. L., Guinto, E. & Kuhl, W. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4620–4623.
  17. Sorge, J., West, C., Westwood, B. & Beutler, E. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7289–7293.
  18. Tsuji, S., Choudary, P. V., Martin, B. M., Winfield, S., Barranger, J. A. & Ginns, E. I. (1986) *J. Biol. Chem.* **261**, 50–53.
  19. Horowitz, M., Wilder, S., Horowitz, Z., Reiner, O., Gelbart, T. & Beutler, E. (1989) *Genomics* **4**, 87–96.
  20. Beutler, E. (1992) *Science* **256**, 794–799.
  21. Sorge, J., Kuhl, W., West, C. & Beutler, E. (1986) *Cold Spring Harbor Symp. Quant. Biol.* **60**, 1041–1046.
  22. Kohn, D. B., Nolte, J. A., Weinthal, J., Bahner, I., Yu, X. J., Lilley, J. & Crooks, G. M. (1991) *Hum. Gene Ther.* **2**, 101–105.
  23. Correll, P. H., Colilla, S., Dave, H. P. G. & Karlsson, S. (1992) *Blood* **80**, 331–336.
  24. Ohashi, T., Boggs, S., Robbins, P., Bahnson, A., Patrene, K., Wei, F.-S., Wei, J.-F., Li, J., Lucht, L., Fei, Y., Clark, S., Kimak, M., He, H., Mowery-Rushton, P. & Barranger, J. A. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 11332–11336.
  25. Shafit-Zagardo, B., Devine, E. A., Smith, M., Arredondo, G. A. F. & Desnick, R. J. (1981) *Am. J. Hum. Genet.* **33**, 564–575.
  26. Barneveld, R. A., Keijzer, W., Tegelaers, F. P. W., Ginns, E. I., Geurts van Kessel, A., Brady, R. O., Barranger, J. A., Tager, J. M., Galjaard, H., Westerveld, A. & Reuser, A. J. J. (1983) *Hum. Genet.* **64**, 227–231.
  27. Ginns, E. I., Choudary, P. V., Tsuji, S., Martin, B., Stubblefield, B., Sawyer, J., Hozier, J. & Barranger, J. A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7101–7105.
  28. Sorge, J., Gelbart, T., West, C., Westwood, B. & Beutler, E. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 5442–5445.
  29. Collins, A., Keats, B. J., Dracopoli, N., Shields, D. C. & Morton, N. E. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 4598–4602.
  30. Reiner, O., Wigderson, M. & Horowitz, M. (1988) *DNA* **7**, 107–116.
  31. Sorge, J., Gross, E., West, C. & Beutler, E. (1990) *J. Clin. Invest.* **86**, 1137–1141.
  32. Sorge, J. A., West, C., Kuhl, W., Treger, L. & Beutler, E. (1987) *Am. J. Hum. Genet.* **41**, 1016–1024.
  33. Beutler, E., West, C. & Gelbart, T. (1992) *Genomics* **12**, 795–800.
  34. Beutler, E., Gelbart, T. & West, C. (1993) *Genomics* **15**, 203–205.
  35. Beutler, E., Gelbart, T., Kuhl, W., Sorge, J. & West, C. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 10544–10547.
  36. Beutler, E., Gelbart, T., Kuhl, W., Zimran, A. & West, C. (1992) *Blood* **79**, 1662–1666.
  37. Graves, P. N., Grabowski, G. A., Eisner, R., Palese, P. & Smith, F. I. (1988) *DNA* **7**, 521–528.
  38. Eyal, N., Firon, N., Wilder, S., Kolodny, E. H. & Horowitz, M. (1991) *Hum. Genet.* **87**, 328–332.
  39. Latham, T. E., Theophilus, B. D. M., Grabowski, G. A. & Smith, F. I. (1991) *DNA Cell Biol.* **10**, 15–21.
  40. Kawame, H. & Eto, Y. (1991) *Am. J. Hum. Genet.* **49**, 1378–1380.
  41. Beutler, E. & Gelbart, T. (1990) *Ann. Hum. Genet.* **54**, 149–153.
  42. He, G.-S., Grace, M. E. & Grabowski, G. A. (1992) *Hum. Mutat.* **1**, 423–427.
  43. Eyal, N., Wilder, S. & Horowitz, M. (1990) *Gene* **96**, 277–283.
  44. Kawame, H., Hasegawa, Y., Eto, Y. & Maekawa, K. (1992) *Hum. Genet.* **90**, 294–296.
  45. Tsuji, S., Martin, B. M., Barranger, J. A., Stubblefield, B. K., LaMarca, M. E. & Ginns, E. I. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2349–2352, and correction (1988) **85**, 5708.
  46. Theophilus, B. D. M., Latham, T., Grabowski, G. A. & Smith, F. I. (1989) *Nucleic Acids Res.* **17**, 7707–7722.
  47. Wigderson, M., Firon, N., Horowitz, Z., Wilder, S., Frishberg, Y., Reiner, O. & Horowitz, M. (1989) *Am. J. Hum. Genet.* **44**, 365–377.
  48. Tsuji, S., Choudary, P. V., Martin, B. M., Stubblefield, B. K., Mayor, J. A., Barranger, J. A. & Ginns, E. I. (1987) *N. Engl. J. Med.* **316**, 570–575.
  49. Hong, C. M., Ohashi, T., Yu, X. J., Weiler, S. & Barranger, J. A. (1990) *DNA Cell Biol.* **9**, 233–241.
  50. Latham, T., Grabowski, G. A., Theophilus, B. D. M. & Smith, F. I. (1990) *Am. J. Hum. Genet.* **47**, 79–86.
  51. Zimran, A., Sorge, J., Gross, E., Kubitz, M., West, C. & Beutler, E. (1990) *J. Clin. Invest.* **85**, 219–222.
  52. He, G.-S. & Grabowski, G. A. (1992) *Am. J. Hum. Genet.* **51**, 810–820.
  53. Beutler, E. & Gelbart, T. (1993) *Br. J. Haematol.*, in press.
  54. Luzzatto, L., O'Brien, S., Usanga, E. & Wanachiwanawin, W. (1986) in *Glucose-6-Phosphate Dehydrogenase*, eds. Yoshida, A. & Beutler, E. (Academic, Orlando, FL), pp. 181–193.
  55. Nagel, R. L. (1990) *Blood Cells* **16**, 321–339.
  56. Rotter, J. I. & Diamond, J. M. (1987) *Nature (London)* **329**, 289–290.
  57. Zimran, A., Sorge, J., Gross, E., Kubitz, M., West, C. & Beutler, E. (1989) *Lancet* **ii**, 349–352.
  58. Zimran, A., Kay, A. C., Gelbart, T., Garver, P., Saven, A. & Beutler, E. (1992) *Medicine (Baltimore)* **71**, 337–353.
  59. Sidransky, E., Tsuji, S., Martin, B. M., Stubblefield, B. & Ginns, E. I. (1992) *Am. J. Med. Genet.* **42**, 331–336.
  60. Tybulewicz, V. L. J., Tremblay, M. L., LaMarca, M. E., Willemsen, R., Stubblefield, B. K., Winfield, S., Zablocka, B., Sidransky, E., Martin, B. M., Huang, S. P., Mintzer, K. A., Westphal, H., Mulligan, R. C. & Ginns, E. I. (1992) *Nature (London)* **357**, 407–410.
  61. Conley, C. L. (1980) in *Blood, Pure and Eloquent*, ed. Wintrobe, M. M. (McGraw-Hill, New York), pp. 319–371.
  62. Nagel, R. L. (1991) *Semin. Hematol.* **28**, 180–201.
  63. Powars, D. R. (1991) *Semin. Hematol.* **28**, 202–208.
  64. De Duve, C. (1964) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **23**, 1045–1049.
  65. Brady, R. O., Gal, A. E. & Pentchev, P. G. (1974) *Life Sci.* **7**, 1235–1248.
  66. Brady, R. O., Pentchev, P. G. & Gal, A. E. (1975) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 1310–1315.
  67. Pentchev, P. G., Brady, R. O., Gal, A. E. & Hibbert, S. R. (1975) *J. Mol. Med.* **1**, 73–78.
  68. Beutler, E. (1981) *Trends Biochem. Sci. Rev.* **6**, 95–97.
  69. Beutler, E., Dale, G. L. & Kuhl, W. (1980) in *Enzyme Therapy in Genetic Diseases*, ed. Desnick, R. J. (Liss, New York), Vol. 2, pp. 369–381.
  70. Beutler, E. & Dale, G. L. (1979) in *Covalent and Non-covalent Modulation of Protein Function*, eds. Atkinson, D. & Fox, C. F. (Academic, New York), pp. 449–461.
  71. Dale, G. L. & Beutler, E. (1982) in *Advances in the Treatment of Inborn Errors of Metabolism*, eds. Crawford, M. A., Gibbs, D. A. & Watts, R. W. E. (Wiley, New York), pp. 77–91.
  72. Beutler, E., Dale, G. L. & Kuhl, W. (1977) *N. Engl. J. Med.* **296**, 942–943.
  73. Belchetz, P. E., Crawley, J. C. W., Braidman, I. P. & Gregoriadis, G. (1977) *Lancet* **ii**, 116–117.
  74. Gregoriadis, G., Weeraratne, H., Blair, H. & Bull, G. M. (1982) in *Gaucher Disease: A Century of Delineation and Research*, eds. Desnick, R. J., Gatt, S. & Grabowski, G. A. (Liss, New York), pp. 681–701.
  75. Ashwell, G. & Morell, A. G. (1974) *Adv. Enzymol.* **41**, 99–128.
  76. Achord, D., Brot, F., Gonzalez-Noriega, A., Sly, W. & Stahl, P. (1977) *Pediatr. Res.* **11**, 816–822.
  77. Furbish, F. S., Steer, C. J., Barranger, J. A., Jones, E. A. & Brady, R. O.

- (1978) *Biochem. Biophys. Res. Commun.* **81**, 1047-1053.
78. Furbish, F. S., Steer, C. J., Krett, N. L. & Barranger, J. A. (1981) *Biochim. Biophys. Acta* **673**, 425-434.
  79. Doeber, T. W., Wu, M. S., Bugianesi, R. L., Ponpipom, M. M., Furbish, F. S., Barranger, J. A., Brady, R. O. & Shen, T. Y. (1982) *J. Biol. Chem.* **257**, 2193-2199.
  80. Barton, N. W., Furbish, F. S., Murray, G. J., Garfield, M. & Brady, R. O. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1913-1916.
  81. Barton, N. W., Brady, R. O., Dambrosia, J. M., Di Bisceglie, A. M., Doppelt, S. H., Hill, S. C., Mankin, H. J., Murray, G. J., Parker, R. I., Argoff, C. E., Grewal, R. P. & Yu, K.-T. (1991) *N. Engl. J. Med.* **324**, 1464-1470.
  82. Beutler, E., Kay, A., Saven, A., Garver, P., Thurston, D., Dawson, A. & Rosenbloom, B. (1991) *Blood* **78**, 1183-1189.
  83. Beutler, E. (1991) *N. Engl. J. Med.* **325**, 1354-1360.
  84. Kay, A. C., Saven, A., Garver, P., Thurston, D. W., Rosenbloom, B. F. & Beutler, E. (1991) *Trans. Assoc. Am. Phys.* **104**, 258-264.
  85. Fallet, S., Sibille, A., Mendelson, R., Shapiro, D., Hermann, G. & Grabowski, G. A. (1992) *Pediatr. Res.* **31**, 496-502.
  86. Sato, Y. & Beutler, E. (1993) *J. Clin. Invest.* **91**, 1909-1917.
  87. Food and Drug Administration (1991) *FDA Med. Bull.* **21**, 6-7.
  88. Zimran, A., Hadas-Halpern, I. & Abrahamov, A. (1991) *N. Engl. J. Med.* **325**, 1810-1811.
  89. Figueroa, M. L., Rosenbloom, B. E., Kay, A. C., Garver, P., Thurston, D. W., Koziol, J. A., Gelbart, T. & Beutler, E. (1992) *N. Engl. J. Med.* **327**, 1632-1636.
  90. Gale, R. P., Sparkes, R. S. & Golde, D. W. (1978) *Science* **201**, 937-938.
  91. Thomas, E. D., Ramberg, R. E., Sale, G. E., Sparkes, R. S. & Golde, D. W. (1976) *Science* **192**, 1016-1018.
  92. Hickey, W. F. & Kimura, H. (1988) *Science* **239**, 290-292.
  93. Rapoport, J. M. & Ginns, E. I. (1984) *N. Engl. J. Med.* **311**, 84-88.
  94. Hobbs, J. R., Shaw, P. J., Jones, K. H., Lindsay, I. & Hancock, M. (1987) *Lancet* **i**, 1111-1115.
  95. Tsai, P., Lipton, J. M., Sahdev, I., Najfeld, V., Rankin, L. R., Slyper, A. H., Ludman, M. & Grabowski, G. A. (1992) *Pediatr. Res.* **31**, 503-507.
  96. Svennerholm, L., Erikson, A., Groth, C. G., Ringdén, O. & Månsson, J.-E. (1991) *Dev. Neurosci.* **13**, 345-351.
  97. Sorge, J., Kuhl, W., West, C. & Beutler, E. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 906-909.
  98. Choudary, P. V., Tsuji, S., Martin, B. M., Guild, B. C., Mulligan, R. C., Murray, G. J., Barranger, J. A. & Ginns, E. I. (1986) *Cold Spring Harbor Symp. Quant. Biol.* **51**, 1047-1052.
  99. Nolta, J. A., Sender, L. S., Barranger, J. A. & Kohn, D. B. (1990) *Blood* **75**, 787-797.
  100. Correll, P. H., Fink, J. K., Brady, R. O., Perry, L. K. & Karlsson, S. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 8912-8916.
  101. Nolta, J. A., Yu, X. J., Bahner, I. & Kohn, D. B. (1992) *J. Clin. Invest.* **90**, 342-348.
  102. van Beusechem, V. W., Kukler, A., Heidt, P. J. & Valerio, D. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 7640-7644.
  103. Bodine, D. M., McDonagh, K. T., Brandt, S. J., Ney, P. A., Agricola, B., Byrne, E. & Nienhuis, A. W. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 3738-3742.
  104. Smith, R. M. & Curnutte, J. T. (1991) *Blood* **77**, 673-686.
  105. Blitzer, M. G. & McDowell, G. A. (1992) *Clin. Lab. Med.* **12**, 463-480.
  106. Beutler, E. (1979) *Am. J. Hum. Genet.* **31**, 95-105.
  107. Goldman, D. P., Clarke, A. E. & Garber, A. M. (1992) *Int. J. Technol. Assess. Health Care* **8**, 583-587.
  108. Beutler, E. (1991) *Semin. Hematol.* **28**, 263-267.
  109. Orita, M., Iwahana, H., Kanazawa, H., Hayashi, K. & Sekiya, T. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2766-2770.