Numerous inherited human metabolic disorders are known (Table 1), most of which are recessive. Many have devastating effects that may include a combination of several clinical features, such as severe mental retardation, impairment of the peripheral nervous system, blindness, hearing deficiency, metabolic instability, organomegaly and gross disfiguration. Fortunately, most of these disorders are rare; however, the majority cannot be treated with drugs, and some are fatal. A few can be alleviated by adhering to a strict diet supplemented by specific nutrients. Although the enzyme deficiency that underlies these disorders may either affect many tissues or be restricted to a particular cell type, the deficient cell type is not always the one most affected: for example, deficiency of adenosine deaminase, a ubiquitous enzyme, results in the accumulation of toxic intermediates in purine metabolism and selectively kills T cells.

**Approaches to gene therapy for metabolic disorders: some considerations**

When designing approaches to gene therapy for metabolic disorders, several issues must be considered. Gene therapy for most metabolic disorders, unlike therapies for diseases such as the hemoglobinopathies, will probably not require strict gene regulation. For most enzymes deficiencies, clinical disease results only when enzyme activity is severely reduced: 5–25% of normal enzymatic activity will often protect from clinical disease. An example is hemophilia B, which results from a deficiency of the serine protease plasma clotting factor IX. Individuals who have <1% of normal factor IX clotting activity are at severe risk of spontaneous hemorrhage, those with 1–10% are at low to moderate risk, while those with 10–20% may have no apparent clinical disease. Exceptions to this general rule include some of the porphyrias, which are transmitted in an autosomal dominant manner and result in clinical disease with only a 50% reduction in enzymatic activity.

Treatment of certain metabolic disorders may be more complicated. One of the more common of the hepatic deficiencies is phenylketonuria (PKU). PKU most often results from a phenylalanine hydroxylase deficiency. "Glycogen storage deficiency type 1A". "Phenylketonuria". "Medium chain acyl CoA dehydrogenase".
deficiency, causing an inability to convert phenylalanine to tyrosine. PKU can result in mental retardation, but this can be prevented by a protein-restricted diet. All infants are therefore screened for PKU at birth so that those affected can be identified and started on dietary management before the onset of mental retardation. Unlike factor IX deficiency, which could in theory be treated by delivery of the normal gene into ectopic tissues, as Crigler-Najjar type 1, result in severe liver damage and, unless treated by liver transplantation, in death. The availability of the Gunn rat, a model for the human disease, has been important in trials for therapies of Crigler-Najjar type 1. An animal model is now also available for tyrosinemia type 1, in which absence of the hepatic enzyme fumarylacetoacetate hydrolase (FAH) leads to an accumulation of toxic metabolites that destroys hepatocytes.

Thus the underlying defect involved, the need for cofactors and the affected and target tissues are all important considerations in developing protocols for gene therapy for a particular disorder. Below, we discuss approaches to gene therapy for various metabolic disorders that have been undertaken in animal models and humans.

Vectors for gene therapy

One of the biggest obstacles to gene transfer has been the efficient transfer of genes to target tissues. A number of vectors, both viral and non-viral, have been developed for transferring therapeutic genes into primary cells.

Viral vectors

Retroviral vectors. To date, the vector that has received most attention is a recombinant retrovirus derived from the mouse Moloney leukemia virus (MMLV), which is replication-defective and allows cloning of promoter and cDNA sequences for expression. The cloned DNA is packaged into virions in cell lines that express the essential viral genes required, and virions recovered from the cell supernatant at concentrations of 10^5–10^7 p.f.u. ml^-1. Because these particles lack the genes necessary for replication and virion production, no additional virus can be produced by the cells they infect. When recombinant virus enters a cell, the RNA genome is reverse transcribed and the DNA product becomes integrated into the host chromosomal DNA. Efficient integration of the virus into the host cell requires cell replication, limiting the use of this vector to transferring genes into proliferating tissues. To date, MMLV-based vectors have been the most widely used in clinical gene therapy trials.

Adenoviral vectors. Adenoviral vectors have recently featured in gene therapy strategies. The linear double-stranded DNA adenovirus has a natural tropism for respiratory epithelium, but can also infect most other cell types, and preparations of the wild-type virus have been given orally as vaccines. The EIA region of the viral genome responsible for viral gene expression and replication can be deleted and replaced with therapeutic genes, and the replication-defective virus propagated in the human kidney cell line 293, which supplies the EIA products in trans. Virus is recovered after cell lysis, purified using cesium chloride gradients, can be concentrated to very high titer (10^{11–10^12} p.f.u. ml^-1), and is efficient at transferring genes into both non-dividing and dividing cells. However, the adenoviral genome does not integrate into host cell chromosomes and is slowly lost from infected cells. Because of their tropism for respiratory epithelium, these vectors are currently being tested for the treatment of cystic fibrosis in humans.

Other viral vectors. A number of other viral vectors are being developed for gene transfer. The adenovirus and associated virus is non-pathogenic and infects the respiratory epithelium. In certain cell types, the virus DNA appears to integrate at a specific location on human chromosome 19; however, it is less clear whether the recombinant vectors can integrate efficiently and whether their integration is site-specific. Two additional problems with these vectors are that the entire viral genome is only 5 kb, so the amount of transgenic DNA that can be delivered is limited, and that it has so far been difficult to produce recombinant vectors at high titer.

Non-viral vectors. Non-viral DNA-based vectors have been developed by a number of laboratories. Complexes of protein and DNA can be used to transfer genes into specific cell types by receptor-mediated endocytosis; this principle has been demonstrated in vivo and in vitro by using desialylated orosomucoid, which is normally taken up by the asialoglycoprotein receptor on hepatocytes. Transferin has also been used as a ligand for DNA transfer; however, unlike the asialoglycoprotein receptor, the transferin receptor is present on many cell types. The DNA–protein complexes are internalized but gene expression is low and transitory. The DNA is trapped in endosomes: Curiel et al.] demonstrated that treatment of transfected cells with an endosomal lysis agent such as an inactivated adenovirus increases gene expression from these vectors 2000-fold in vitro. Moreover, when the adenovirus is chemically linked to the DNA–protein complexes, gene expression is enhanced another tenfold. These strategies have been combined to produce high levels of transgene expression in cultured hepatocytes, but have not yet been shown to work efficiently in vivo.

Ex vivo versus in vivo gene therapy

Recombinant vectors have been used to deliver genes to various cells and tissues (Table 2). Here, we
Introduction of genes into hematopoietic cells

Ex vivo gene transfer by recombinant retroviral vectors has been used to reconstitute most types of hematopoietic stem cells in mice. However, much lower levels of gene transfer have been achieved in hematopoietic stem cells in dogs and non-human primates. Gene transfer into stem cells is important for therapy of genetic disorders, as a single successful transfer could result in permanent reconstitution. Transduction of stem cells in non-rodent models has been inefficient; however, a therapy has been developed for one form of severe combined immunodeficiency that results from adenosine deaminase deficiency. Although individuals with only 2–5% of normal enzyme activity have normal immune function, those more severely affected develop life-threatening opportunistic bacterial infections at an early age. Reconstitution of enzymatic activity in peripheral T lymphocytes by retrovirus-mediated gene transfer improves immune function in treated individuals. Because mature T lymphocytes are constantly being replaced, repeat treatments are needed. These findings, while encouraging, are not completely conclusive, since it is not yet certain that they are the result of gene therapy alone. Protocols are being designed for transducing hematopoietic stem cells in such a way that even at a low frequency of transduction, early progenitor lymphoid cells derived from transduced stem cells may have a selective advantage and repopulate the blood. However, since for most disorders, an endogenously based selection secondary to the disease process will not be available, methods for introducing genes into the stem cells must be improved. The recent development of strategies for isolating and characterizing bone marrow stem cells in mice and humans should be instrumental in this.

Several metabolic disorders are caused by the absence of specific lysosomal enzymes that degrade specific compounds, whose accumulation can cause organ dysfunction; some affect primarily visceral organs, others the central nervous system. Depending on the underlying defect, lysosomal deficiencies that cause dysfunction of the central nervous system may require gene transfer into cells of the viscera, such as hematopoietic cells or hepatocytes, or into the central nervous system.

The most common lysosomal storage disorder is Gaucher disease, a deficiency in β-glucocerebrosidase that causes accumulation of glucosylceramide in reticuloendothelial cells. Individuals with Gaucher disease develop hepatosplenomegaly and bone problems, and a rare subset who have a neuronopathic form develop neurological sequelae. Enzyme replacement therapy has had some success in treating the visceral manifestations of this disorder.

Gene therapy strategies also seem promising. Retroviral vectors that produce human β-glucocerebrosidase mRNA have been used to transduce mouse hematopoietic cells in vivo. Recently, a mouse model of the disease has been created by gene targeting. Mice that are completely deficient in the enzyme die soon after birth, and may parallel a rare neonatal or perinatal lethal form of the human disease. Although this particular model may not be useful in preclinical studies for the most common form of the disorder, mice carrying less severe mutations are being generated and may prove more appropriate. Clinical trials in which retroviral vectors are used to transfer β-glucocerebrosidase cDNA into bone marrow progenitors or stem cells are under way.

Sly syndrome, or mucopolysaccharidosis VII, is a lysosomal storage disorder in which β-glucuronidase deficiency results in the accumulation of sulphated glycosaminoglycans in the lysosomes of most cells, causing bone and joint abnormalities, hepatosplenomegaly and mental retardation. A mouse strain homozygous for the mutant allele *Gus"* has a similar disease, and has been used in preclinical gene therapy trials. Wolfe et al. transferred the β-glucuronidase cDNA by retrovirally mediated gene transfer into enzyme-deficient bone marrow progenitors, which were transplanted into affected mice. The low level of enzyme activity that resulted in the liver and spleen reduced the lysosomal storage lesions in these animals. A similar finding was obtained when autologous fibroblasts were transplanted using appropriate retroviral vectors and transplanted into these mice. The enzyme is secreted by the genetically modified fibroblasts and transported into tissues via the mannose-6-phosphate receptor. It is not yet clear whether similar approaches can also alleviate the effects of these disorders on other organ systems, including the central nervous system.

### Table 2. Targets for gene therapy

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Feasible approach</th>
<th>Approaches being tested in clinical trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematopoietic progenitor cells</td>
<td>+</td>
<td>Ex vivo</td>
</tr>
<tr>
<td>Peripheral lymphocytes</td>
<td>+</td>
<td>Ex vivo</td>
</tr>
<tr>
<td>Hepatocytes</td>
<td>+</td>
<td>Ex vivo</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>+</td>
<td>Ex vivo</td>
</tr>
<tr>
<td>Neuronal cells</td>
<td>+</td>
<td>Ex vivo</td>
</tr>
<tr>
<td>Keratinocytes</td>
<td>+</td>
<td>Ex vivo</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>+</td>
<td>Ex vivo</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>+</td>
<td>Ex vivo</td>
</tr>
<tr>
<td>Pulmonary epithelium</td>
<td>+</td>
<td>Ex vivo</td>
</tr>
<tr>
<td>Chondrocytes</td>
<td>+</td>
<td>Ex vivo</td>
</tr>
<tr>
<td>Cancer cells</td>
<td>+</td>
<td>Ex vivo</td>
</tr>
</tbody>
</table>

**REVIEWS**

*TIG* JULY 1994 VOL. 10 NO. 7 255
Introduction of genes into hepatocytes

Both ex vivo and in vivo methods have been developed for transferring genes into hepatocytes using vectors based on retroviruses, adenovirus and Herpes simplex virus, and DNA-protein complexes. To date, long-term constitutive expression of transgenes has only been demonstrated using recombinant retroviral vectors. Direct in vivo gene transfer into hepatocytes has been used to partially correct the coagulation defect in dogs deficient for factor IX (Ref. 38). Because retroviruses require cell division for gene transduction, a two-thirds partial hepatectomy was performed before gene transfer.

Transduction of hepatocytes with a retroviral vector that expresses canine factor IX was used to introduce the gene for human factor IX into dogs with hemophilia B, transiently ameliorating their clotting abnormalities and increasing their coagulation factor IX levels to protective levels (Fig. 1b). However, only transient expression of such transfected genes has been achieved so far. Recombinant adenoviral vectors have also been used to introduce the gene for factor IX into dogs with hemophilia B, transiently ameliorating their clotting abnormalities, and to transiently reverse the hypercholesterolemic effects of LDL receptor deficiency in mice.

Concluding remarks

Numerous monogenic metabolic disorders of humans are known. Although each individual disorder affects only a very small proportion of the population, many have devastating effects, resulting in death, multiple organ dysfunction or mental retardation. A number of therapies have been developed over the years in an attempt to treat patients with these disorders; most are designed to ameliorate the symptoms, rather than effect a cure. The possibility of therapy by gene transfer into somatic cells opens a new area of therapeutics and hope for individuals afflicted with these genetic disorders. Clearly, several technical hurdles must be overcome before successful and complete cures are possible for any of these diseases, and technologies must continually be improved upon if the many disorders are to be treated. Like all medical therapies, certain gene therapies will ameliorate some, but not all, symptoms of a particular disorder, and might improve the quality of life of affected individuals. Partial replacement of enzyme activity in a specific target tissue or a subset of affected tissues or cells may slow the accumulation of toxins in lysosomes, but not completely prevent it. Intervention may inhibit the progress of a chronic illness, but offer little in the way of reversing a pre-existing degeneration. For example, if a specific defect in the urea cycle has caused significant neurological impairment before gene therapy is begun, the achievement of metabolic homeostasis will not reverse the neurological sequelae. The same may be true for the treatment of muscular dystrophies: introduction of the appropriate normal gene may inhibit further muscle degeneration, but is less likely to reverse long-term muscle atrophy.

The ultimate goal of gene therapy for metabolic disease is to deliver a vector by non-invasive means such that the normal gene product will be produced in sufficient quantities in an appropriate cell type to alleviate all clinical manifestations of the disorder. It seems likely...
that no single vector system will be appropriate for treating all such disorders, or will cure all metabolic disease. The next several years will see the development of improved gene delivery systems that will satisfy stringent criteria. We can then look forward to improved treatments for patients affected by these hereditary metabolic disorders.

References

26. Weinthal, J. et al. (1991) *Bone Marrow Transplant.* 8, 403-412

Trends in Genetics gene therapy review series

This issue of *Trends in Genetics* contains the last in a series of reviews on gene therapy. These four reviews, which appeared in the issues April-July 1994, are:

- Gene therapy for infectious diseases: the AIDS model, by Eli Gilboa and Clay Smith
- Gene therapy for cancer, by Kenneth W. Culver and R. Michael Blaese
- Gene therapy for neurological disorders, by Theodore Friedmann
- Gene therapy for metabolic disorders, by Mark A. Kay and Suvio L.C. Woo