58 Gene Therapy

John S. Lazo and Jennifer Rubin Grandis

Most drugs used today are designed to treat symptoms rather than cure the underlying disease. Notable exceptions include cytotoxic chemotherapeutic agents, as described in Chapter 56, and agents that restore or modulate hormone function, as outlined in Chapter 57. However, increased understanding of the molecular and genetic etiology of diseases may permit permanent modification of organ function by drug-oriented methods. The first disease-associated gene, β-globin, was cloned over 25 years ago. It is now theoretically possible to isolate, sequence, and analyze genes causally associated with many heritable and acquired human diseases, including cystic fibrosis, Duchenne's muscular dystrophy, and Gaucher's disease. Moreover, with the complete sequencing of the human genome, many of the estimated 100,000 human genes may become candidates for genetic manipulations. Thus, it is now possible to propose molecular pharmacological and genetic approaches to therapy . Many of these approaches fall under the general rubric of *gene therapy.*

Germ cell gene therapy will require considerable discussion about ethical issues and extensive information before it can be applied to humans, but somatic cell gene therapy in humans is now being extensively explored. During the past 5 years in the United States alone, more than 500 human gene therapy clinical trials aimed at treating conditions ranging from inherited disorders such as cystic fibrosis to cancer and AIDS, have been approved by the Office of Biotechnology Activities (OBA, formerly the Recombinant DNA Advisory Committee) of the National Institutes of Health. Nearly 3500 patients have been enrolled in these studies (Fig. 58.1).

With few exceptions, gene therapy was considered safe if not particularly effective until the death of an 18-year-old man in 1999, the first fatal outcome for a patient in a phase I gene therapy protocol. This death has stimulated a substantial review of the oversight mechanisms in human gene transfer research. One of the first successes of gene therapy was reported in 2000, when three infants with a fatal form of severe combined immunodeficiency syndrome (SCID) received ex vivo gene therapy with a recombinant mouse leukemia viral vector encoding the γC receptor gene. After 10 months, γC transgene expression in T- and NK cells was detected and T-, B-, and NK-cell counts and function were comparable to those of age-matched controls.

Although numerous obstacles must be overcome before gene therapy will be routinely employed, a rigorous approach to investigating the safety and efficacy of gene transfer will ensure that clinical strategies employing genetic manipulation are rationally incorporated into the therapeutic armamentarium.

GENE THERAPY: DEFINITION AND GOALS

The broadest definition of human gene therapy includes the in vivo (direct administration of the gene therapy formulation) and ex vivo (transfection of cells in tissue culture by gene therapy followed by administration of the transfected material into the patient) transfer of defined genetic material to cells of patients. Principles of gene therapy include transfer of one or more transgenes to prevent a disease, prevent an adverse consequence of a disease, or facilitate recovery from the consequence. Although most of the controversy and excitement have centered on the transfer of functional genes, the therapeutic potential of genes that abrogate aberrant function (e.g. antisense and ribonucleic acid–based strate-

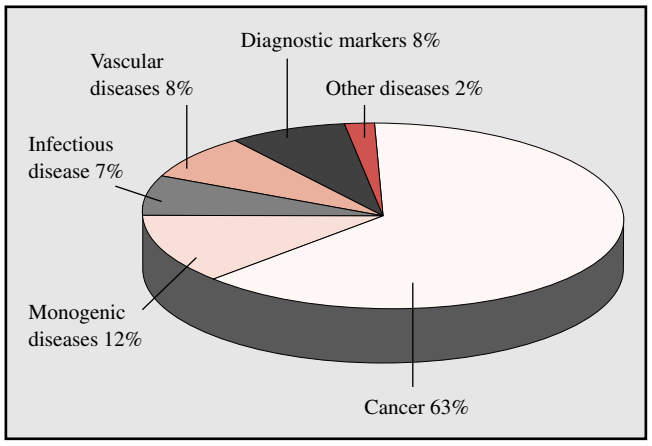

FIGURE 58.1
Proposed uses or targets of human gene therapy trials. During the past 5 years in the United States more than 500 human gene therapy clinical trials have been approved by the Office of Biotechnology Activities of the National Institutes of Health.

gies) should also be considered. Two fundamental approaches underlie the basis of gene therapy. In the first, genetic material is introduced into cells to alter the cellular phenotype but not the genotype. This is typified by the transfer of unintegrated DNA, antisense oligomers, and ribozymes. In this regard, gene therapy has many of the attributes and problems of conventional endocrine or antimicrobial therapy with respect to efficiency of targeting and the duration of effect. A second approach seeks permanent alteration of the genotype of the cell, leading to a modified phenotype that prevents or alters a disease state. In this setting, gene therapy will permanently modify organ function.

Theoretically, mutated or nonfunctional genes could be excised and replaced, and new genes with desired functions could be permanently inserted into the genome. Stable integration of an antisense DNA might also be desirable in some circumstances. Because of the technical difficulties associated with the delivery of nucleic acid–based products selectively to specific target cells in vivo, more experimental information is available for ex vivo human gene therapy.

ANTISENSE

The antisense approach is use of nucleic acids to reduce the expression of a specific target gene. As shown in Figure 58.2, a small piece of DNA, an oligodeoxynucleotide that is in the reverse orientation (antisense) to a portion of a target messenger RNA (mRNA) species, is introduced into a cell and a DNA–RNA duplex is formed by complementary Watson-Crick base pairing. Cessation of protein synthesis then may result from the rapid

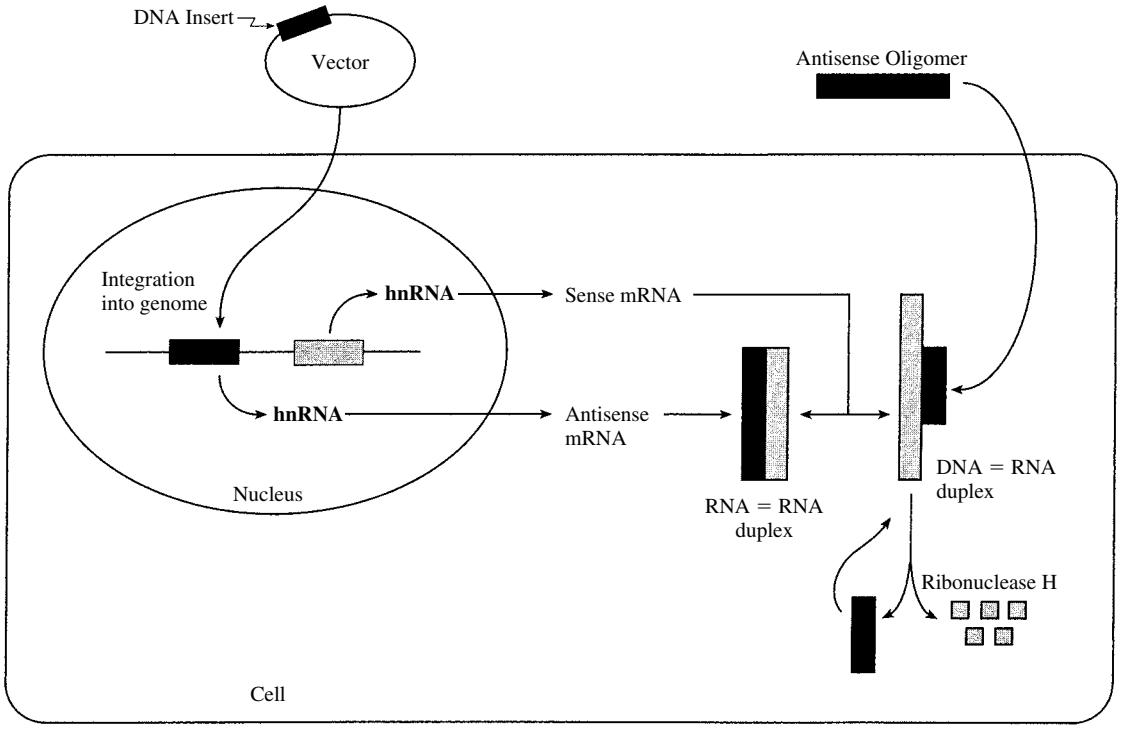

FIGURE 58.2
Translation arrest or nuclease digestion by exogenously applied antisense oligonucleotides or by antisense mRNA produced from DNA delivered by a plasmid. Heterogeneous nuclear RNA is hnRNA.

degradation of the mRNA species due to activation of ribonuclease H or disruption of translation. Cells and organisms protect themselves against foreign DNA and RNA by producing nucleases that degrade phosphodiester bonds in oligodeoxynucleotides. Chemical modification of the phosphodiester moiety can produce nuclease-resistant oligomers. In the two most common chemical analogues, the backbone phosphate is replaced either with a methyl group to form a methyl phosphonate or with a sulfur group to form a phosphorothioate (Fig. 58.3). These modifications grant extra stability to the oligonucleotides, allowing for a longer half-life in vivo.

The antisense RNA can also be generated within cells after delivery via a plasmid or attenuated virus containing a suitable promoter that controls expression of the antisense strand using methods of gene insertion described later (Fig. 58.2). In addition to the strict antisense strategies, several related approaches have been considered. Catalytic RNA, catalytic DNA, or ribozymes capable of degrading complementary mRNA may decrease translation of targeted sequences. Oligomers designed to interact with genes directly via Hoogsteen hydrogen binding in a triplex formation have been suggested as a means of disrupting transcription (Fig. 58.4). Transcription factor decoys that are duplexes designed to bind to a particular transcription factor and prevent its normal function are another approach examined in the context of NFκB blockade. These strategies, like antisense itself, do not require integration into the genome, and thus they share the pharmacological problems of absorption, distribution, metabolism, and elimination of any traditional drug not based on nucleic acid.

GENE EXCISION AND REPLACEMENT

Diseases at a genetic level can result from several causes, including (1) mutation in a gene, (2) loss of expression of a gene, (3) elevated expression of a gene, or (4) expression of a pathogenic viral or foreign gene. In each case, gene replacement or excision therapy might be desirable. Theoretically, the disease gene could be replaced through a homologous recombination event. Depending on the design of the replacement gene, it also would be possible to engineer stop codons or nonsense sequences into the internal domains of a gene to ensure loss of protein production. Excision of an entire

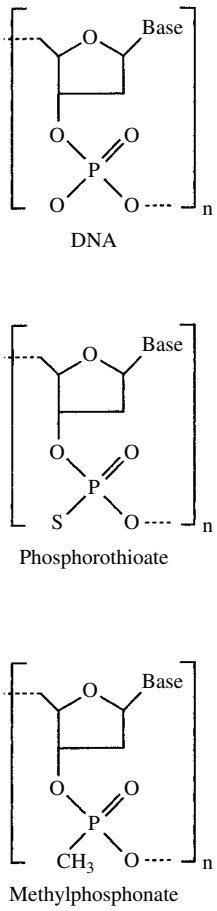

FIGURE 58.3
Chemical structures of oligodeoxynucleotides and the analogues used in gene therapy.

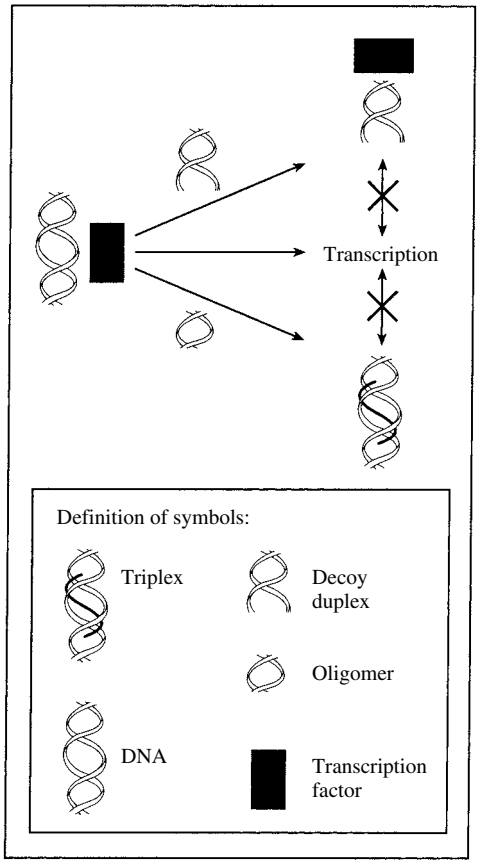

FIGURE 58.4
Theoretical mechanism of transcription disruption by oligomers.

gene also is feasible. This strategy, however, requires extremely efficient and specific homologous recombination events in the target cell population. Such strategies have allowed for the development of knockout animals, but to date have not been practical for human somatic cell gene therapy. Ongoing investigations are exploring the feasibility of inducible vectors, use of the cre-lox system, or cell type specific promoters to optimize gene expression in target cells.

GENE ADDITION

A more practical approach has been to permit the introduced genes to integrate into the genome in a site-nonspecific manner. The newly added gene could then function to provide a missing or mutated gene product (Fig. 58.5*A*). *This is the approach of most current gene therapy protocols and is exemplified by the development of clinical trials for adenosine deaminase (ADA) defi-*

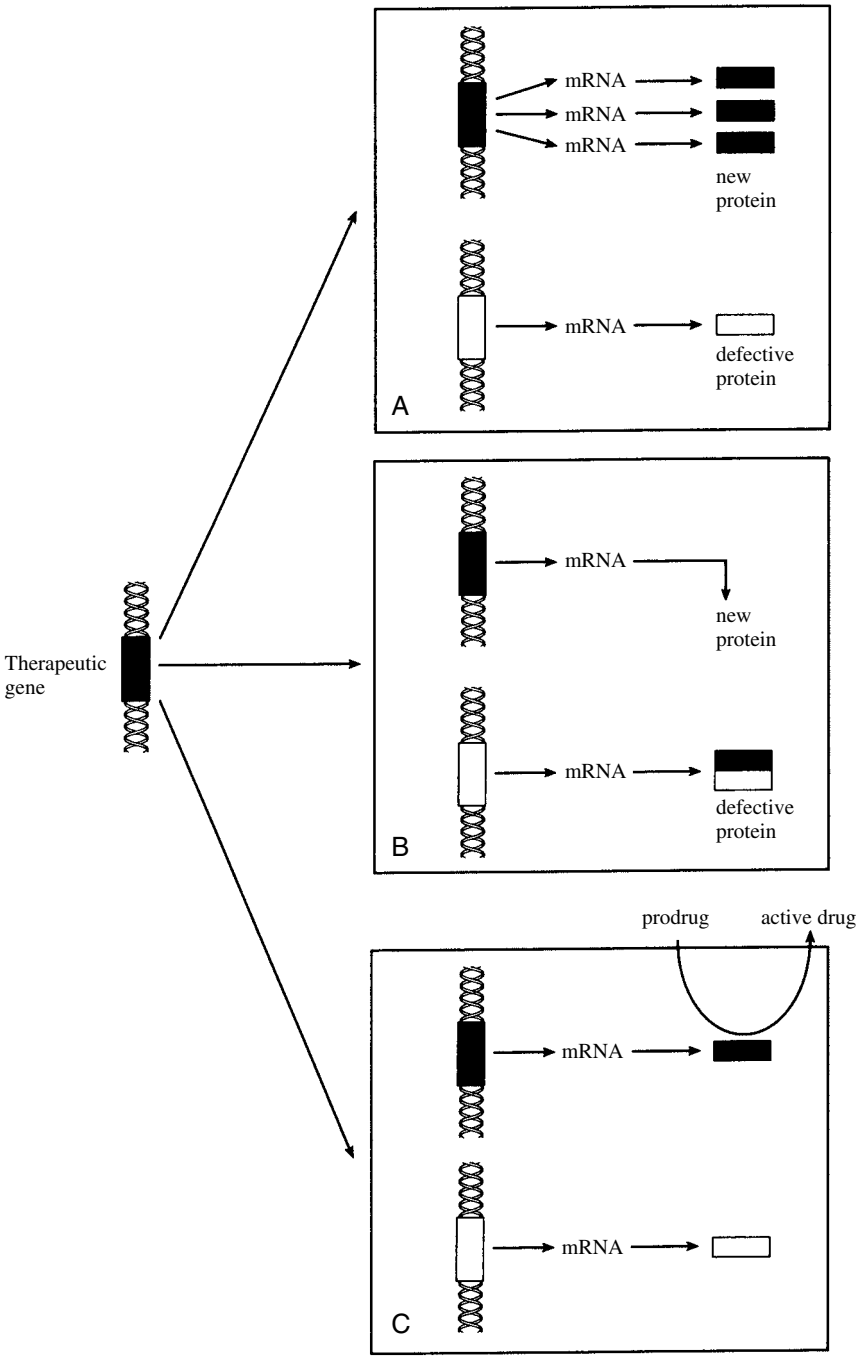

FIGURE 58.5
Possible mechanisms by which inserted therapeutic genes may alter cellular function. **A.** Gene addition with return to a normal phenotype. **B.** Dominant-negative or phenotype deletion. **C.** Gene addition to a unique phenotype, such as an enzyme that activates a prodrug.

ciency, which is an example of inherited SCID. ADA is a reasonable target for these reasons: (1) It is an autosomal recessive disorder in which a defect in a single gene produces absence of or diminished ADA activity with fatal combined immunodeficiency. (2) ADA expression is characteristic of a normal maintenance gene with considerable variation in the normal ADA levels, suggesting that stringent regulation of expression is unnecessary. (3) A significant level of expression is not required to correct the phenotype. (4) Ex vivo gene transfer studies can be conducted. (5) Replacement of ADA may reduce the production of toxic DNA metabolites and thus provide a growth advantage for transfected cells.

For ethical reasons, children enrolled in these clinical trials have also received standard therapy of enzyme infusions, so the results of these studies have been difficult to interpret and are controversial. Nevertheless, there is some evidence that the ex vivo gene transfer approach may evoke a biological response relevant to the treatment of ADA deficiency. Such interpretations have stimulated efforts to use the ex vivo strategy for other monogenic disorders, such as familial hypercholesterolemia, hemophilia B, and Gaucher's disease.

Alternatively, the introduced gene could generate a protein that acts to block or suppress the function of another undesirable protein in a dominant-negative manner (Fig. 58.5*B*). Last, the introduced gene could result in the production of an entirely new and unique protein that provides the recipient cell with a desirable phenotype (Fig. 58.5*C*). In theory, an enzyme required for the metabolic activation of a prodrug could be expressed, leading to the desired pharmacological activity near the genetically altered cell. This approach is used in cancer gene therapy in which tumor cells are transfected with a gene encoding for an enzyme such as thymidine kinase in the presence of systemic administration of a nontoxic prodrug. The transfected enzyme in the tumor cells converts the prodrug, such as ganciclovir, to an active cytotoxic compound. Theoretically, such an approach selectively kills tumor cells and is nontoxic to untransfected cells. Clinical trials to assess the safety and efficacy of enzyme–prodrug cancer therapy are under way.

DELIVERY SYSTEMS

In many cell types it is feasible to deliver nucleic acids and genes by a variety of methods when the cells are grown in tissue culture (Table 58.1). Nonetheless, some cells, such as pneumocytes and neurons, are not readily isolated from humans and do not grow well in vitro. Furthermore, for many diseases it is essential to alter the phenotype of a significant proportion of the total cell population, making ex vivo gene therapy of limited use.

There is general agreement that no ideal delivery system is available for in vivo gene therapy. Direct or intratumoral injection of plasmid DNA or antisense oligomers without a viral vector has been attempted. Expression of genes using traditional nonviral vectors has been low compared to viral strategies. Nonetheless, recent breakthroughs in nonviral delivery systems, including the gene gun, electroporation and naked DNA, suggest that nonviral gene therapy can achieve local expression of therapeutic genes at levels equivalent to those of viral vectors.

Although the mechanism remains undetermined, the injection of naked DNA into skeletal muscle has demonstrated relatively high transfection efficiency. In this setting, DNA is precipitated onto the surface of microscopic metal beads (e.g., gold) and the microprojectiles are accelerated and penetrate intact tissue to several cell layers.

TABLE 58.1 Vectors Approved for Human Use by the U. S. Office of Biotechnology Activities

Vector	Advantages	Disadvantages
Nonviral		
Liposomes	No replication risk, nonimmunogenic, useful for plasmids or viruses	Limited efficiency
Naked or particle-mediated DNA	No replication risk	Moderate efficiency, nonspecific cell targeting
Viral		
Retrovirus	Efficient transfer, manufacturing easy, most commonly used	Small DNA capacity (9 kb), random DNA insertion, targets only dividing cells, replication risk
Adenovirus	Infects nonproliferating cells, nonintegrating	Immunogenic, small DNA capacity (7.5 kb), replication risk, repeated injections required for long-term expression
Adeno-associated virus	Low immunogenicity, targets nonproliferating cells, may have discrete genome insertion sites	Difficult to manufacture, low titer
Herpesvirus	Targets central nervous system, low immunogenicity	Difficult to manufacture, host toxicity

In preclinical trials, efficiency remains low, but expression has been noted to last for several weeks, and there has been no significant inflammatory response.

Some investigators have used electrical current (*electroporation*) to improve DNA (or drug) entry into tumor cells with some preliminary success. Liposomes are attractive vehicles for gene delivery, since they can carry plasmid, antisense, or viral DNA. Compared with viral approaches, however, liposomes remain relatively inefficient at facilitating gene transfer, although their safety profile remains more desirable. Some of the attributes and limitations of the nonviral methods are listed in Table 58.1.

Because viruses can efficiently integrate into the genome, many clinical trials are exploring the use of *replication-defective recombinant viral vectors* and delivery systems. Retroviruses contain their genetic information as a double-strand DNA genome that is transcribed, and the single-strand proviral DNA product is stably integrated into the host genome. Recombinant DNA technology has been used to remove deleterious viral genes involved in replication, and the resulting vector is replication defective, nonpathogenic, and unable to produce infectious particles. Ideally, with a retroviral vector, only a single administration should be required because the gene should be permanently retained and expressed. No clinical evidence of mutagenesis has emerged from the clinical trials performed to date, but the number of patients treated and the time of exposure has been limited.

Adenoviral vectors have also been used in human trials. These vectors enter cells by either an adenovirus fiber–specific receptor or a surface integrin receptor. They efficiently transfer genes in nonreplicating and replicating cells. Nonetheless, immunological responses to viruses have been noted with adenoviral vectors. *Replication-selective adenovirus vectors* have been introduced to optimize infection of target cells and minimize infection of normal cells. Over 200 cancer patients have been treated to date in more than 10 clinical trials with little evidence of toxicity reported. Replication, however, has generally been transient (<10 days), with limited efficacy observed when the gene therapy was administered as a single agent. More encouraging antitumor effects have been observed when the gene therapy was combined with cytotoxic chemotherapy. Further modifications are likely to be required before there can be general application of adenoviral vectors for cancer therapy.

DISEASE APPLICATION AND FUTURE DIRECTIONS

Antisense clinical trials, most with phosphorothioates, have been directed toward blocking viral production in patients with AIDS or genital warts, disrupting the functionality of protooncogenes in cancer, blocking immune cell activity after kidney transplantation, treating rheumatoid arthritis, or influencing autoimmune diseases. Studies to date have not reported marked clinical efficacy, which might be due to protein binding and poor entry into cells. Additional chemical modifications and possibly the use of carriers, such as liposomes, may improve drug delivery and utility.

A proportion of the human gene therapy trials approved by the OBA seek to correct a single-gene defect, such as adenosine deaminase deficiency, glucocerebrosidase deficiency in Gaucher's disease, or the mutated chloride transport gene in cystic fibrosis. The major difficulties limiting success have been immunogenicity associated with the vector delivery system, low transfection efficiency, and transient transgene expression.

Most human gene therapy trials are designed to express a new gene product that facilitates the correction of a disease process, such as cancer. Almost half of the current gene therapy–based protocols in the United States are aimed at boosting the immune response to tumor antigens. Thus, there are attempts to express the lymphokine interleukin-2 in tumor cells to stimulate a natural immune response against the producing tumor cell and its malignant neighbors. In other types of studies, malignant cells infected with a vector that encodes a tumor suppressor gene, p53, lead to growth arrest, apoptosis or enhanced sensitivity to cytotoxic agents. Others have used vectors encoding the herpesvirus protein thymidine kinase that target cells for killing when exposed to the antiviral prodrug ganciclovir; this is known as *suicide gene therapy.* Similarly, attempts are being made to produce HIV-infected cells that express thymidine kinase or other enzymes that activate the nontoxic prodrugs to cytotoxic compounds. Disruption of viral functions with decoy molecules that compete with, sequester, or cleave products produced by HIV also is being examined.

Most of these trials have been early phase I or II studies that are designed to evaluate safety rather than efficacy of the gene therapy formulation. Results of ongoing and pending phase III studies will more precisely place the role of gene therapy in a clinical context. Although the feasibility of human gene transfer has been demonstrated in the completed clinical trials, there has been a paucity of evidence to support the efficacy and reliability of gene transfer approaches. Future gene therapy studies will capitalize on preclinical efforts to improve cellular targeting, gene transfer efficiency, and sustained expression. Regulation of the expression of the introduced transgene would be desirable, and use of cell type–specific promoters, such as the actin or surfactant promoter, or drug-controlled promoters, such as the tetracycline promoter, are being examined in preclinical models.

Study Questions

1. Severe combined immunodeficiency (SCID) syndromes are excellent models for gene therapy because of the genetic basis of these disorders and significant advances in the technology to transfer therapeutic genes into hematopoietic precursor cells. For all these reasons, which of the following syndromes represents an ideal candidate for gene therapy?
   (A) B-cell deficiency
   (B) DiGeorge's syndrome
   (C) γC Deficiency
   (D) Adenine deaminase deficiency
   (E) T-cell deficiency
2. All of the following are desirable characteristics in the design of a gene therapy vector EXCEPT
   (A) Ability to produce at high titer on a commercial scale
   (B) Ability to transfect both dividing and nondividing cells
   (C) Ability to produce site-specific integration into the chromosome of the target cell
   (D) Ability to limit size of genetic material it can deliver
   (E) Ability to deliver only certain cell types
3. A patient with ornithine transcarbamylase (OTC) deficiency is being treated in a gene therapy clinical trial. The gene therapy approach for this disease is primarily designed to
   (A) Replace the enzyme ornithine transcarbamylase
   (B) Decrease the accumulation of ammonia
   (C) Eliminate the need for a modified diet
   (D) Target a protooncogene
   (E) Enhance the immune system
4. A 25-year-old hemophiliac is interested in receiving gene therapy. He should contemplate all of the following approaches EXCEPT
   (A) Intravenous infusion of a retroviral vector expressing the B-domain-deleted factor VIII
   (B) Ex vivo transfection of autologous fibroblasts transfected with a plasmid encoding B-domain-deleted factor VIII
   (C) Intravenous adenoviral-mediated delivery of factor VIII
   (D) Adeno-associated virus (AAV) vector delivered to skeletal muscle
   (E) Retroviral vector expressing B-domain deleted factor VIII transfected into dermal fibroblasts that are then reimplanted
5. A patient with advanced inoperable squamous cell carcinoma of the head and neck receives a replication-selective adenovirus on a gene therapy clinical trial. The rationale for the use of this treatment:
   (A) Deletion of viral genes will reduce toxicity of the viral vector to normal cells.
   (B) Deletion of a p53 inhibitory protein will be selective for tumors that have lost p53 function.
   (C) Deletion of a key regulatory sequence will allow for induction of the therapeutic gene in tumor cells.
   (D) Results of preclinical studies suggest that only tumor cells are affected by this treatment.
   (E) Clinical results support that only patients with p53 mutations in their tumors respond to the treatment.

ANSWERS

1. **C.** SCID-X1 (γC deficiency) is an optimal model for gene therapy because there is little γC gene transcription regulation; γC expression is ubiquitous and constitutive among different hematopoietic lineages; and γC exerts no autonomous function.
2. **D.** The vector should have no size limit to the genetic material it can deliver. The coding sequence of a therapeutic gene can vary from several hundred base pairs to more than 10,000 base pairs. In addition, the requirement for appropriate regulatory sequences may be required for efficient transduction and expression of the therapeutic DNA. The ability to produce a high titer on a commercial scale is essential to carry out large-scale tests. It is necessary to be able to transfer genes in nonreplicating and replicating cells. It is also important to optimize delivery to target cells and minimize delivery to normal cells.
3. **A.** OTC is a metabolic enzyme required to break down ammonia. Total lack of this enzyme leads to death shortly after birth owing to a buildup of ammonia. The partial presence of OTC also leads to accumulation of ammonia, which can be controlled by drugs and dietary intake. The genetic cause of this disease, its morbidity, and the need for rapid production of OTC by adenoviral vectors may extend the life span of OTC-deficient newborns to allow for drug treatment and dietary manipulation. Jesse Gelsinger, the 18-year-old patient who was the first patient to die on a phase I gene therapy trial, had OTC deficiency.
4. **C.** Systemic administration of adenoviral vectors has not been used in the treatment of hemophilia because of the transient gene expression and immunogenic consequences of adenoviral delivery. All of the other approaches are under investigation or have been published in the literature on treatment of hemophilia.

5. B. dl1520 (Onyx-015) was the first adenovirus developed with deletion of a gene encoding a p53-inhibitory protein, E1B-55kD, theoretically making it selective for tumor cells that have lost p53 function. Controversial data demonstrate that the mechanism of selectivity is more complex than originally thought. In addition, clinical results have demonstrated responses in patients whose tumors did not have mutant p53.

SUPPLEMENTAL READING

Huber BE and Lazo JS. (eds.). Gene therapy for neoplastic diseases. Ann N Y Acad Sci 1994;716;1–351.

Fischer A et al. Gene therapy for human severe combined immunodeficiencies. Immunity 2001;15:1–4.

Somia N and Verma IM. Gene therapy: Trials and tribulations. Nature Rev Genet 2000;1:91–99.

Friedman T, Noguchi P, and Mickelson C. The evolution of public review and oversight mechanisms in human gene transfer research: Joint roles of the FDA and NIH. Curr Opin Biotechnol 2001;12:304–307.

Mannucci PM and Tuddenham EGD. The hemophilias: From royal genes to gene therapy. N Engl J Med 2001;344:1773–1784.

Kirn D. Clinical research results with dl1520 (Onyz-015), a replication-selective adenovirus for the treatment of cancer: What have we learned? Gene Therapy 2001;8:89–98.

McCormick F. Cancer gene therapy: Fringe or cutting edge? Nature Rev Cancer 2001;1:130–141.

CASE Study Cystic Fibrosis and Gene Therapy

Kris Allen was diagnosed with cystic fibrosis (CF) shortly after birth. Genetic analysis revealed that he had the most common form of dysfunction of the CF transmembrane conductance regulator gene (CFTR) leading to faulty processing and protein trafficking. His therapy to date has consisted of palliative treatments, such as daily physiotherapy to improve chest and lung function, pancreatic enzyme replacement, and a high calorie diet. Conventional treatment of his recurrent pulmonary disease is less and less effective, and he is interested in gene therapy. What would be a logical strategy for this patient?

ANSWER: Aerosol delivery of the CFTR gene. Both viruses and liposome–DNA complexes are capable of successful CFTR gene transfer to the nasal and airway epithelia of patients with CF. In fact, gene transfer to the airways is one of the few areas where liposome–DNA complexes match the expression obtained using viral vectors without the viruses' inflammatory side effects. Current trials are aimed at optimizing gene delivery with reduced toxicity to produce sustained correction of the epithelial transport defect.