AN INTRODUCTION TO MOLECULAR MEDICINE AND GENE THERAPY

Edited by

Thomas F. Kresina, PhD
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Dedicated with Love to my wife,
Laura Williams Cheever,
And my children,
Rachel Ann
Jenny Lynn
Rebecca Marie

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PREFACE

The research field of gene therapy and the clinical practice of medicine evolving from the research are fast moving, ever-changing disciplines. On an almost daily basis, there appears in the media a "breaking story" of a gene-based research finding. An implication of the story is that this research breakthrough will speedily transform, in the next few years, into a marvelous new therapy in molecular genetic medicine. *An Introduction to Gene Therapy and Molecular Medicine* provides a basis to interpret new clinical and basic research findings in the areas of cloning, gene transfer and targeting, the application of genetic medicine to clinical conditions, ethics, government regulation, genomics, and biotechnology and bioinformatics. The text provides the reader with fundamental and comprehensive basic as well as clinical research observations and findings relative to gene therapy and molecular medicine.

An Introduction to Gene Therapy and Molecular Medicine can be divided into three sections: basic science introductory Chapters 1 to 5; clinical application Chapters 6 to 12; and Chapters 13 to 15, and Appendix, addressing evolving issues related to gene therapy and molecular medicine. Each chapter, as well as the appendix, contains key concepts that the authors wish to leave the readers and a specific itemized listing of suggested readings in the field. The reading lists comprise state-of-the-art reviews, salient research articles, and articles useful for lay readership as well.

Chapter 1 is truly an overview of the field and the contents of the book. It presents in broad general terms the diseases targeted by gene therapy and the tools researchers use and the future needs of the field. It address what may be the "holy grail" of medicine, possible approaches for reversing the process of aging. A second approach to the aging issue is presented in Chapter 2. The techniques and usage of cloning are addressed. Chapter 3 provides a fundamental need for basic research in addressing human disease and the generation and use of animal models of disease. Specifically useful for gene therapy and molecular medicine are transgenic mouse models of human pathogenesis. Chapter 4 provides what appears to be endless detail related to vectors and their use in gene transfer. Chapter 5 rounds out the section on basic research by providing useful approaches to target genes to produce a specific desired expression of the gene.

Chapters 6 to 12 provide the clinical use of gene therapy approaches. Chapter 6 presents the use of gene therapy approaches to hematology with a special discussion of application of gene therapy using hemopoietic stem cells. Chapter 7 presents gene therapy in liver diseases describing approaches for inherited metabolic diseases as well as acquired infectious diseases such as hepatitis C. Chapter 8 presents impressive, realistic approaches to use gene therapy for the therapy of "broken hearts" and cardiovascular diseases. Chapter 9 presents equally challenging and con-

troversial molecular approaches for gene therapy of the brain and diseases of the central nervous system. Chapter 10 provides an overview of how gene therapy can be used in the treatment of cancer. Chapter 11 details gene therapy approaches for infectious diseases with a specific emphasis on utilization for the therapy of HIV infection. Chapter 12 provides a "disease contrast" in that it addresses an incredibly debilitating disease, rheumatoid arthritis, and how gene therapy can be used in amelioration of joint destruction.

The last section of the book provides individual presentations related to gene therapy and molecular medicine. Chapter 13 provides an update on the issue of federal regulation and oversight of gene therapy research. It is an issue that has been in the spotlight of late. Chapter 14 provides an ethical essay on gene therapy and the use of molecular medicine with an insight into health care rationing. Chapter 15 is a brief description of where the practice of molecular medicine is today. Finally, the appendix is an important presentation of some commercial aspects in molecular medicine and gene therapy. After all, if gene therapy is to reach the public in all walks of life a commercial venue is needed.

Thus, *An Introduction to Gene Therapy and Molecular Medicine* is a comprehensive manual that can be used as an aid through the rapidly moving field of gene therapy and its application in molecular medicine.

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CHAPTER 1

Molecular Medicine and Gene Therapy: An Introduction

THOMAS F. KRESINA, PH.D. and ANDREA D. BRANCH, PH.D.

INTRODUCTION

The use of genetics and genetic manipulation by humans for the therapy of human disease is a new and rapidly evolving field of both basic science and clinical medicine. The science of gene therapy is derived from significant research advances in the fields of genetics, molecular biology, clinical medicine, and human genomics. Thus, *gene therapy* can be defined as the use of genetic manipulation for treatment of disease. Experimental gene therapy research breakthroughs observed in model systems are modified for clinical or bedside use, forming the emerging practice of molecular medicine. Molecular medicine encompasses the elucidation of the genetic basis of disease, diagnosis of the disease, the design of an appropriate approach to disease management or therapy, the application of approved therapeutic protocols, and monitoring of clinical outcomes.

In the history of the practice of western medicine, initial concepts of disease were related to an imbalance in the persona or humus. Illness was treated on a whole-body or systemic level. As the practice of medicine advanced to and through the twentieth century, more information became available regarding the physiology of the body as well as its organ and tissue structure. Subsequently, advances were made into the cellular biology of health and disease. Most recently, research investigations opened insight into the genetic basis of inheritance and the biological processes at the molecular level. These were mainly in the genetics and molecular biology of selective breeding practices for plants and animals. The basic principles form a nidus for experimental treatments for human diseases.

The bases for this application to human disease are the successful development of the medical and surgical techniques in human organ transplantation, the western tradition of pharmacotherapy, and the continuing elucidation of the human genome and its regulatory elements. On what seems to be an almost daily basis, startling new molecular genetic discoveries are publicized. Some have profound moral

and ethical considerations, such as the cloning of sheep and primates. Others lead to a profound understanding of the pathogenesis of human disease, such as the identification of the mutation in the genes responsible for liver diseases, such as, hemochromatosis or, in pediatrics, Alagille syndrome. The cloning studies show us the new frontiers of genetic medicine and challenge us to use them wisely. The discoveries of mutant genes leading to disease pathology lend the promise of rapid diagnosis and potentially early clinical intervention allowing for better medical management. However, the discoveries of genes responsible for human pathology challenge us in the use of genetic population screening. The evolving field of genetic epidemiology can provide precise data on the incidence and prevalence of a specific inherited trait. The challenge here is to use this information ethically and in a medically beneficial manner (see Chapter 14).

GENETIC MANIFESTATIONS OF MOLECULAR MEDICINE

Gene therapy offers the potential of a one-time cure for devastating inherited disorders. It has application to many diseases for which current therapeutic approaches are ineffective or where the prospects for effective treatment are obscure. Current recombinant deoxyribonucleic acid (DNA) technologies allow for the rapid identification of genes and the facile manipulation of genetic material. This enables medical researchers to examine cellular physiology at a molecular level. Using these tools, scientists and clinicians can identify and determine a molecular basis of disease. There is a broad array of diseases in which specific protocols of gene therapy could provide novel therapeutic approaches. These are the "traditional genetic diseases" so called for their familiarity in clinical medicine (see Table 1.1). They consist of chromosomal disorders that are inherited as a single gene, Mendelian disorder (autosomal dominant, autosomal recessive, sex-linked recessive, or sex-linked dominant), and result from a mutation at a single locus. These compare to the multifactorially inherited disorders that involve multiple genes working in concert with known or enigmatic environmental factors.

Most diseases are complex and multifactorial. They result from a complex series of events involving changes in the level of expression of many genes and/or environmental factors and behavior. While many individual interventions may be partially effective at treating complex diseases, the greatest benefits are likely to be derived from combination therapies. Although complexity is the rule in human pathogenesis, many first-generation gene therapies are designed as a single intervention to correct a disease by adding a functional version of a single defective gene, as illustrated in Figure 1.1*a*. Such strategies, for example, have been used to introduce a specific gene into the liver cells of patients with familial hypercholesterolemia (see Chapters 6 and 7). But, it is estimated that only 2% of human diseases are thought to be caused by direct one-to-one Mendelian expression of a single gene. Even in these monogenetic diseases, clinical heterogeneity occurs, and it is often difficult to predict the progress of the clinical course of a patient. Patient-to-patient variation results from many factors, including differences in alleles, environment, and genetic background. While the precise cause of variable penetrance of a genetic lesion is usually not known, it likely reflects the genome's extensive series of "back-up" systems and feedback loops. For example, this premise has been

TABLE 1.1 Selected Inherited Disorders and Their Genetic Basis

Classification	Nomenclature	Characterization	Frequency
Autosomal aneuploidies newborns	Trisomy 13	Karyotype: 47,XX or XY +13 (extra copy)	1 per 12,000
	Trisomy 18	Karyotype: 47 XX or XY +18 (extra copy)	1 per 6000 newborns
	Trisomy 21 Down's syndrome	Karyotype: 47,XX or XY +21 (extra copy)	1 per 800 newborns ↑ incidence with age
Sex chromosome aneuploidies	Klinefelter's syndrome	Karyotype: 47, XXY plus variants	1 per 700 newborns males
	Triple X female	Karyotype: 47,XXX	1 per 1000 newborns
	Turner's syndrome	Karyotype: 45,X; 45X/46XX or 45X/46XY	1 per 1500 newborn females
	XYY male	Karyotype: 47,XXY	1 per 800 newborns
Autosomal dominant	Aniridia, type I	Chromosome 2 defect	1 per 80,000
	Aniridia, type II	Chromosome 11 defect	1 per 80,000
	Polycystic kidney disease	Chromosome 16 linkage	1 per 1250
	Charcot–Marie–Tooth	Two forms type I and II	1 per 2800
	Familial polyposis coli and Gardner's syndrome	Chromosome 5; adenomatous polyposis coli (APC) gene	1 per 8000
	Huntington's disease	Linked to chromosome 4p	1 per 3000
	Intrahepatic cholestasis	Vanishing bile ducts	
	Alagille syndrome	*Jagged 1* gene—20p12	1 per 70,000
	Byler's disease	18q21	familial
	Marfan's syndrome	Chromosome 15: FBN1 gene	1 per 20,000
	Myotonic dystrophy	19q13.2–q13.3	1 per 8000
	Neurofibromatosis		
	Type I	Chromosome 17: NF-1 gene 17q11.2	1 per 2000–5000
	Type II	Chromosome 22: NF-2 gene 22q12.2	
	Retinoblastoma	Deletion or rearrangement chromosome 13 RB-1 gene	1 per 20,000
	Pancreatitis hereditary	Chromosome 7 cationic trypsinogen gene PRSSI Two mutations: R117H & N21I	Familial

TABLE 1.1 (*Continued*)

Classification	Nomenclature	Characterization	Frequency
	Idiopathic	SPINKI-Chromosome 5 Missense mutation- N345	
Autosomal recessive	α_1-Antitrypsin deficiency	Chromosome 14 Multiple alleles based on phenotype M, S, Z, I	1 per 3500
	Cystic fibrosis	7q31–q32, CFTR gene Multiple alleles: Δ 508 ↑ Also R117H, R75Q, D1270N	1 per 2500 (Caucasians)
	Gaucher's disease Ashkenazic Jewish descent	N370S allele (nonneuropathic)	1 per 625
	Caucasian population	L444P allele neuropathic	
	Hemochromatosis	HFE gene C282Y and H63D mutations	1 per 300
	Thalassemia (α)	Globulin gene complex on chromosome 16 Two alles α-thal 1 α-thal 2	1 per 250–1000
	Thalassemia (β)	Chromosome 11 Two alleles β(+) IVS-I β(+) IVS-II	

shown in several lines of "knock-out" mice, which lack genes involved in key cellular processes (see Chapter 3). Such mice can be phenotypically normal. Thus, the genome has an impressive ability to compensate for a missing part. Because of this ability, the most effective treatments for single-gene diseases may not always be replacement of the single defective gene. Options may exist as illustrated in Figure 1.1*b*, where either a functional copy of a frankly defective gene could be added to correct a deficiency (yielding genotype 3) or expression of a compensatory gene could be enhanced (yielding genotype 4).

Monogenetic Disorders

Single-gene disorders are relatively infrequent in incidence but contribute significantly to the chronic disease burden. They include sickle cell anemia, the hemophilias, inherited immune deficiency disorders such as adenosine deaminase deficiency, hypercholesterolemia, severe combined immune deficiency syndrome, as well as the inherited disorders of cystic fibrosis, phenylkentouria, Duchenne's

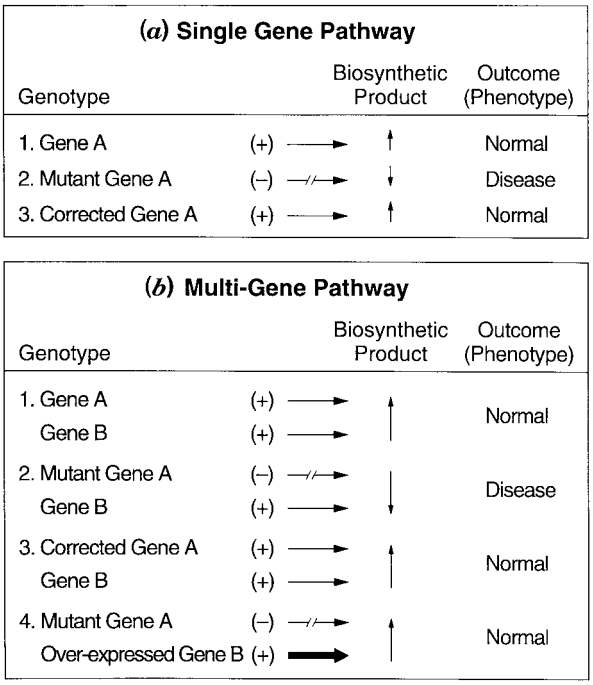

FIGURE 1.1 Pathology can result from a single gene defect, as illustrated in (*a*). More often, multiple genes are involved. In the latter case, a variety of gene therapy options may exist, as depicted in (*b*).

muscular dystrophy, emphysema, and fragile X syndrome. In deficiency disorders, pathology is a direct result of loss of function of the relevant protein. The straightforward application of gene therapy is replacement. Thus, the mutation needs to be identified and the normal gene isolated. In such situations, the transfer and (importantly) correct expression of the protein would benefit the patient, hopefully to the level of curative. In other dominantly inherited disorders where the presence of an abnormal protein interferes with the function and development of organ or tissue, only selective deletion of the mutant gene would be of benefit. Other diseases that are autosomal recessive (requiring two mutant alleles) manifest themselves in utero or at birth and thus require early diagnosis and intervention. Other difficulties in somatic gene therapy for monogenetic disorders are the necessity of direct therapy to a specific tissue or cell type, the number of cells or fraction of tissue needed to be transformed for therapy, and achievement of the therapeutic level of protein along with the long-term regulation of gene expression.

Mutifactorial Disorders

Multifactorial or polygenic disorders are well known because of their common occurrence in the population. In general, they involve several genes. An in-depth knowledge of the pathophysiology of the disease is required to discern the mechanism for therapy by gene-based therapeutic approaches. Examples of these disorders are coronary heart disease, diabetes mellitus, and essential hypertension.

Therefore, multifactorial disorders may not only have a complex genetic component but also be influenced by environmental factors. Elucidation of the pathophysiology of the disorder may suggest how the insertion of a specific gene may reverse or retard disease progression. For these diseases, it may be of most clinical importance to determine how a specific gene product influences tissue or cellular physiology. Currently, gene therapy for these disorders is in a relatively early stage of development.

When designing an appropriate approach to genetic disease management or gene therapy, it is important to ascertain the level of interactions between genes because the majority of diseases causing death in the United States result from processes influenced by many genes. These diseases are polygenic and/or epigenetic in origin. Epigenetic phenomena, such as imprinting, reflect the "state" of a gene and are influenced by environmental factors. Some measure of the magnitude of the gene expression changes that occur during a diseased state was provided by a recent comparison of gene expression profiles in normal and cancer cells (see Chapter 10). Using cellular DNAs (cDNA) as messenger ribonucleic acid (mRNA) surrogate markers of gene activation, it was found that almost 300 genes were expressed at significantly different levels in gastrointestinal tumors compared to normal tissue. The differential activation of such a large number of genes infers that all the genes will not be regulated through common mechanisms. Similar studies are now proceeding in the field of obesity research where the genetic basis of this disease is being elucidated. Thus, it is fundamental to the understanding of disease pathogenesis to identify all genes involved. Specific targeted interventions can then be aimed at the most accessible pathogenic targets. Since multiple experimental therapeutic approaches exist for treating even a "simple" monogenetic disorder, it will be most important to lay the groundwork for considering the potential numerous interventions for the multifactorial diseases that cause morbidity and mortality in the United States.

A specific example of the genetic manifestations of molecular medicine can be seen with the liver disease, α_1-antitrypsin deficiency (see Chapter 7). This liver disease results from a relatively common genetic lesion, in that, about 1 in 8000 infants born in the United States is homozygous for the most frequent mutant allele. Two entirely different organ-specific pathogenic processes can occur in these individuals. Liver injury can result from the accumulation of improperly folded α_1-antitrypsin protein in the endoplasmic reticulum of cells. Lung injury in the form of emphysema can result from the unrelenting proteolytic attack on lung elastin caused by the absence of α_1-antitrypsin. The severity of disease in individuals homozygous for the mutated gene is highly variable, indicating that the impact of the single-gene mutation depends on the "genetic background" of the individual. This example illustrates how the activity of compensatory genes can determine whether a genetic lesion becomes a genetic disease, suggesting that the up-regulation of compensatory genes might be an effective strategy for treating patients with certain genetic mutations.

For diseases that result in multiple organ-specific pathologies, one can question whether both organ pathologies can be cured by a gene therapy that merely adds a correct copy of the wild-type gene. In the case of the liver disease, α_1-antitrypsin deficiency, antisense strategies and ribozymes are being designed to destroy the mRNA of the mutant gene in an effort to eliminate the misfolded protein (see

Chapter 11). However, directed mutagenesis (induced by specialized oligonucleotides) is being explored as a way to repair the mutant gene and thereby "killing two birds with one stone" through the elimination of the aberrant protein as well as providing a source of functional polypeptide (gene product) at the same time.

GENE THERAPY AND PATTERNS OF GENE EXPRESSION

The clinical complexities of α_1-antitrypsin deficiency provide a window into the relationship between genotype and phenotype. The goal of somatic (nongermline) gene therapy is to achieve a healthy phenotype by manipulating gene expression. Gene therapy, thereby, corrects or compensates for genetic lesions or deficiencies whether inherited or acquired. Fully achieving this goal requires insight not only into the ways genes interact with each other, but also with the way genes interact with the environment. In biological systems, information flows in two directions—from the genome outward and from the extracellular milieu inward. Gene products perform important functions in this information transfer process. They serve as biosensors, forming a complex network that relays information about the intracellular and extracellular environment back to the genome. The genome can respond to the signals it receives in many ways, some of which are positive for the host and some of which could be detrimental to the host. For example, based on environmental stimuli the genome can up-regulate genes necessary for normal physiology, such as those encoding antiviral antibodies. Alternatively, the stimuli can up-regulate genes that accelerate a pathogenic process, such as those encoding autoantibodies. The goal of innovative medical interventions, such as gene therapy, is to accentuate the positive potential of gene expression and eliminate or circumvent the negative.

Because genes are linked to each other through an information network, it is often possible to alter the expression of one gene by manipulating the products of another. As presented in Figure 1.2, manipulation leads to the up-regulation of one

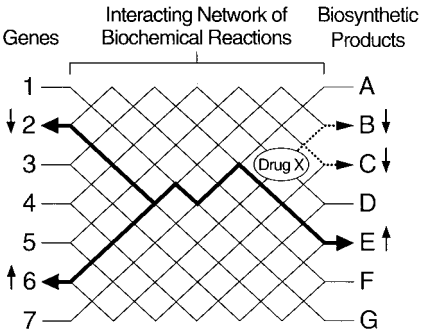

FIGURE 1.2 Schematic representation of a system in which genotype and phenotype are related by a complex network of interactions involving many proteins, RNAs, and reactants. Drug binding to a specific component leads to complex effects, lowering levels of some biosynthetic products, raising levels of others. Through a series of feedback loops, expression of some genes is up-regulated and of other genes down-regulated. (Adapted from Anderson and Anderson, Electrophoresis, 1996.)

gene and the down-regulation of another. Co-up-regulation and co-down-regulation can also take place. For example, the changes that occur in hypercholesterolemic patients (see Chapter 7) taking lovastatin provide an example of coordinately controlled gene expression. Mevacor (lovastatin) was developed to inhibit the enzyme, 3-hydroxy-3-methylglutaryl CoA reductase, and thereby lower plasma cholesterol levels. However, the biochemical reaction that has the greatest cholesterol-lowering effect occurs because lovastatin-induced enzyme inhibition produces a co-up-regulation of low-density lipoprotein receptor, which in turn removes low-density lipoprotein (LDL) cholesterol from plasma. Thus, a gene therapy protocol could follow this example and provide network effects or new interactions with environmental stimuli.

Infectious agents, such as human immunodeficiency virus (HIV) (see Chapter 11) and hepatitis C (HCV) (see Chapter 7), claim many lives in the United States. However, most death and disability in the United States is not caused by an infection but results from conditions causing chronic disabling diseases through an interplay of multiple genetic and environmental factors. These conditions include cardiovascular disease, malignant neoplasms, and cirrhosis. When the under (or over) expression of many different genes contributes to pathogenesis, it may be impossible to stop disease progression by replacing any single gene. However, it may be feasible to develop gene therapies to ameliorate these disease processes once they are fully understood at the molecular level.

Fortunately, knowledge of pathogenesis is taking a quantum leap forward because of several new techniques and technologies and the emergence of the field of "bioinformatics," which allow patterns of gene expression in diseased and healthy tissues to be determined (see the Appendix). As the molecular details of pathogenesis emerge and can be related to information about gene networks, the field of gene therapy may redefine its goals. Gene therapies may come to encompass all interventions specifically designed to promote health by altering patterns of gene transcription and translation.

Since patterns of gene expression vary from patient to patient, in part as a result of DNA polymorphisms, detailed information about the genotype of individual patients will be extremely important to consider when designing therapies. Advances in rapid DNA sequencing and gene expression analysis will soon reduce the cost of gathering data about a patient's genome and pattern of gene expression. This will pave the way for medical interventions tailor-made for an individual patient (see Chapter 15). Academic medical centers can contribute to the development of personalized medicine by providing high-quality specimen banks. They can establish interactive teams of scientists and physicians who are able to conduct the complex clinical trials needed to find the best matches between the expanding universe of therapeutic options and the genetic constitution of an individual patient.

GENE THERAPY AND MOLECULAR MEDICINE

A simple and concise definition of gene therapy (there are many) is the use of any of a collection of approaches for the treatment of human disease that rely on the transfer of DNA-based genetic material into an individual. Gene delivery can be

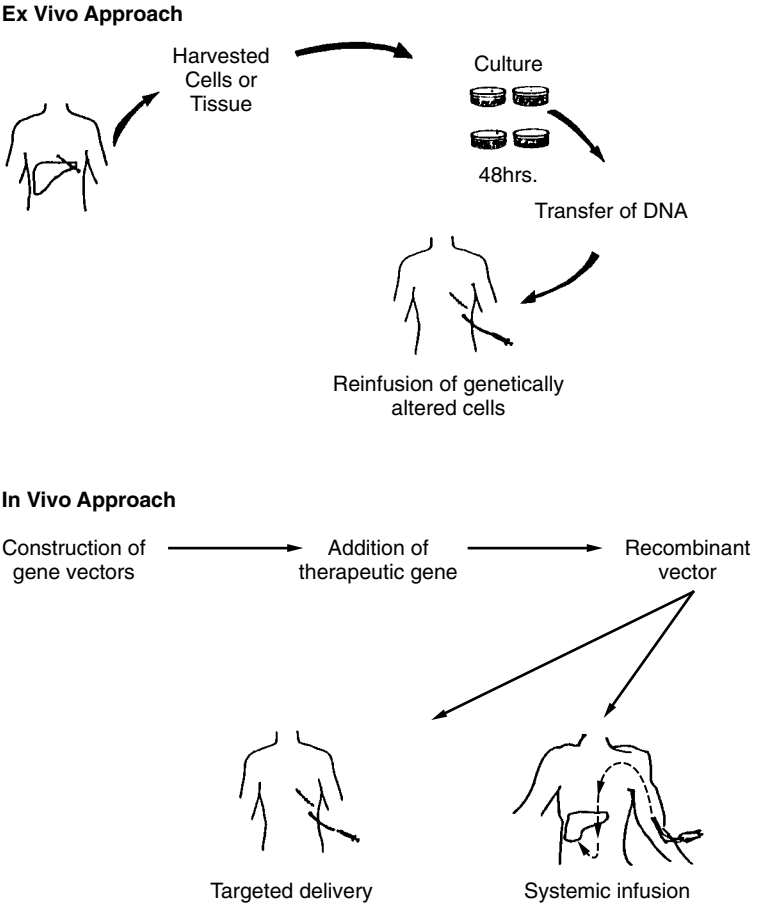

FIGURE 1.3 Two basic methods for delivery of genes. The upper panel shows the ex vivo approach. It requires removal of cells or tissue, culture of cells, and transfection. Successfully transformed cells are selected and returned to the patient where they home to the original location of removed cells or tissue. The lower panel shows the in vivo approach. A gene vector construct, suitable for the delivery of genes to the targeted cell or tissue, is generated. The therapeutic gene is incorporated onto the construct and the recombinant vector is delivered to the patient by any of a number of methods. The method of choice should be previously shown to provide the best level of transfection with minimal side effect.

performed in vivo through the direct administration of the packaged gene into the blood, tissue, or cell. Alternatively, the packaged DNA can be administered indirectly via ex vivo laboratory techniques (see Figure 1.3). Currently, somatic gene therapy, which targets nongermline cells (nonegg and nonsperm cells), is consistent with the extension of biomedical science and medical therapy in which treatment does not go beyond the individual. In altering the genetic material of somatic cells, gene therapy may correct the specific disease pathophysiology. Therapy to human germline cells, thereby modifying the genetic composition of an offspring, would

represent a departure from current medical practices in addition to presenting specific ethical issues (see Chapter 14).

Cancer

Cancer is a genetic disease that is expressed at the cellular level (see Chapter 10). The generation of neoplasia is a multistage process driven by inheritance and relatively frequent somatic mutation of cellular genes. These genes include oncogenes, tumor suppressor genes, and DNA repair genes. In a minority of individuals with cancer and in pediatric cases, germline mutations of tumor suppressor or DNA repair genes are the primary neoplastic events. Germline mutations result in all cells of an individual becoming at risk for cancer development and thus are not suitable for somatic cell gene therapy. But in both somatic and germline mutations, clonal selection of variant cells results in a population of cells with increasingly aggressive growth properties.

In individuals with only somatic gene mutations, the insertion of a gene (such as a tumor suppressor gene) would alter the phenotype of a malignant cell only if the mutation is not dominant. Additionally, the level of corrective cellular therapy (possibly as high as 100% correction of all tumor cells) would need to be determined as well as the issue of gene therapy in distal metastasis. Thus, substantial biological obstacles remain to be overcome in the application of gene therapy in certain forms of cancer. Based on these formidable problems, indirect therapies have been proposed. These include: gene transfer of cytokines or other immune mediators to augment host immune responses, the genetic modification of neoplastic cells to promote immunogenicity, the treatment of localized cancers with genes encoding viral or bacterial enzymes that convert prodrugs into toxic metabolites, or the transfer of genes that provide enhanced resistance to conventional chemotherapy (see Chapter 10).

Infectious Diseases

Chronic infectious diseases are suitable targets for gene therapy. These include viral, bacterial, and parasitic infections such as the hepatitis, herpesvirus infection, HIV and its analogs, human papillomavirus infection, mycoplasma infection, Lyme disease, malaria, rabies, and *Listeria* infection. Gene therapy strategies for diseases caused by rapidly proliferating infectious pathogens include intracellular immunization and polynucleotide vaccines. Gene-therapy-induced vaccination for these pathogens may represent an effective strategy by acting classically to "prime" innate immunity prior to exposure to the pathogen. Intracellular immunization seeks to transform cells into cells that are refactory to infection. Protocols may include ribozymes, antisense RNA, RNA decoys, intracellular antibodies, or genetic suppressor elements (see Chapter 11).

Genetic Vaccination

Polynucleotide or genetic vaccination seeks to attenuate the host's immune response, thus having both prophylatic and therapeutic potential. The physiologic basis for polynucleotide vaccines, either RNA or DNA, is the direct inoculation and

expression of specific pathogen gene(s) whose products are immunogenic and thus subsequently induce protective or neutralizing immunity. During the next decade, gene therapy may make its greatest contribution to medicine through the introduction of DNA vaccines. In part because DNA vaccines utilize simple vectors, they can be developed quicker than most other gene therapies. New and more effective vaccines are urgently needed in the United States and throughout the world to prevent infectious diseases. Furthermore, since they induce a broad range of immune responses, DNA vaccines may be useful in treating infectious diseases, such as chronic hepatitis B virus (HBV) infection, and it is hoped that they can be used to treat noncommunicable diseases, such as cancer and allergic reactions.

DNA vaccines have produced dramatic results in preclinical trials in many model systems, attesting to the simplicity and robustness of this technology. Immune responses have been generated against viral, bacterial, parasitic, allergy-inducing immunogens, and tumor-specific antigens. DNA vaccines are particularly useful for the induction of cytotoxic T cells. Furthermore, by varying the mode of delivery, it may be possible to select the type of immune response elicited by a DNA vaccine: intramuscular injection is associated with Th1-like helper cellular immune responses, while Th-2-like helper cellular immune responses are seen following progressive vaccinations in which DNA is literally "shot" into the epidermis with a gene gun.

Most DNA vaccines consist of a bacterial plasmid with a strong viral promoter, the gene of interest, and a polyadenylation/transcription termination sequence. The plasmid is grown in bacteria (*Escherichia coli*), purified and injected or blasted into target tissues of the recipient. The DNA is taken up, and its encoded protein is expressed. However, the plasmid does not replicate in mammalian cells, and it does not integrate into chromosomal DNA. This approach raises fewer concerns about mutagenesis and safety. The regulatory elements that have been used in DNA vaccines most frequently mediate high levels of gene expression in mammalian cell cultures or in transgenic mice. These include the human cytomegalovirus immediate/early promoter, the Rous sarcoma virus, and the SV40 virus early promoter, and the transcript termination/polyadenylation signal from either the SV40 virus or the bovine growth hormone 3′ untranslated region. Most vaccination vectors also contain an intron, which enhances expression of genes in mammalian cells. In some DNA vaccines, a cassette of CG dinucleotides is incorporated into the vector to boost immune responses, building on the discovery that DNA oligonucleotides containing centrally located CG dinucleotides stimulate B cells.

Rapid progress is being made toward the development of a DNA vaccine for HBV. It will be an interesting historical parallel if the first DNA vaccine for use in humans turns out to be for HBV. This is because the current HBV vaccine is the first vaccine produced from recombinant cells that is effective against a human virus. The yeast cells utilized for this vaccine were originally described in 1984 and contain an expression vector with an alcohol dehydrogenase I promoter with a segment encoding the HBV surface antigen of the adw subtype. Because the vaccine contains only a single viral protein, it is called a "subunit" vaccine, in contrast to vaccines comprised of attenuated live viruses or inactivated whole viruses, which contain many viral proteins. Unfortunately, the efficacy of the recombinant HBV vaccine has been difficult to duplicate in subunit vaccines for other infectious pathogens. Based on the ability to stimulate both T-cell and B-cell responses, it is

hoped that DNA vaccines will be effective against a broad spectrum of agents. Thus, it is hoped that they will be effective not only as preventive modalities but also as therapeutic vaccines. Therapeutic vaccines would be given to infected patients to stimulate immune clearance of established pathogens.

Organ Transplantation and Cellular Engineering

Organ Transplantation Organ and tissue transplantation are accepted treatments for end-stage organ damage. Current survival rates for major organ transplantation procedures range from 70 to 95% survival for 1 year to 30 to 75% for 5-year survival. These results indicate that the transplantation procedure itself is no longer a survival issue but that posttransplantation complications reduce long-term survival. Posttransplantation complications include acute and chronic allograft, rejection, infection, and the side effects of immunosuppresive treatments. Gene therapy approaches have been suggested as novel methods to control posttransplantation complications at the molecular level. Both ex vivo and in vivo approaches have been advanced.

For in vivo gene therapy, adenovirus vectors (see Chapter 4) have been used to obtain efficient gene transfer to the lung and heart in a posttransplantation setting. The efficacy of such procedures show the feasibility of genetic modification of the graft to reduce posttransplantation rejection, such as chronic graft vascular disease in cardiac allograft rejection, or other physiological processes. The graft rejection process could be modified by inserting specific genes of immunosuppressive molecules or by transfecting genes of antisense molecules to block expression of an important mediator of graft rejection. An example of a mediator to target would be an adhesion molecule. In addition to immune-mediated graft rejection, graft function is also important. Physiological processes could be modified for organ or tissue grafts that are malfunctioning. For instance, a liver allograft not producing therapeutic levels of factor VIII could be transfected with the gene for factor VIII.

The latter example has implications for ex vivo gene therapy approaches in organ transplantation. Organ, tissue, or cellular engineering could be performed on candidate grafts prior to transplantation during the cold storage time. This may be possible because recent studies have indicated that gene transfection may not be affected greatly by nonphysiological temperatures. Thus, organs or tissues may be transfected with genes of cytokines to reduce allorejection or other genes to suppress major histocompatibility (MHC) complex alloantigens or host MHC antigens. Studies, to date, have shown that transfection of immuno-modulating genes such as transforming growth factor beta (TGF-β) or interleukin 10 (IL-10) can induce local immunomodulation in transplanted vascularized organs or in cellular transplants such as pancreatic islet cells for diabetes.

Inherent in the ex vivo gene therapy technique is the opportunity to perform cellular engineering. Cells, tissues, or organs could be genetically modified or engineered to perform unique or specific functions. Host tolerance to a transplanted organ could be induced by the intrathymic administration of chimeric cells (part donor–part host phenotype; see Chapter 3). This would allow for a better "take" of the transplanted organ and less use of highly toxic immunosupressive regimens. Alternatively, the use of microencapsulated genetically engineered cells could be utilized. Microencapsulation is the procedure by which transduced cells secreting specific molecules are enclosed within microscopic, semipermeable containers. The