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Biotech Pharmaceuticals and Biotherapy: An Overview

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ABSTRACT Broadly, the history of pharmaceutical biotechnology includes Alexander Fleming's discovery of penicillin in a common mold, in 1928, and the subsequent development—prompted by World War II injuries—of large-scale manufacturing methods to grow the organism in tanks of broth. Pharmaceutical biotechnology has since changed enormously.

Two breakthroughs of the late 1970s became the basis of the modern biotech industry: the interspecies transplantation of genetic material, and the fusion of tumor cells and certain leukocytes. The cells resulting from such fusion—hybridomas—replicate endlessly and can be geared to produce specific antibodies in bulk.

Modern pharmaceutical biotechnology encompasses gene cloning and recombinant DNA technology. Gene cloning comprises isolating a DNA-molecule segment that corresponds to a single gene and synthesizing ("copying") the segment. Recombinant DNA technology, or gene splicing, comprises altering genetic material outside an organism—for example, by inserting into a DNA molecule a segment from a very different DNA molecule—and making the altered material (recombinant DNA) function in living things.

Recombinant DNA technology enables modifying microorganisms, animals, and plants so that they yield medically useful substances, particularly scarce human proteins (by giving animals human genes, for example). This review, however, focuses not on pharmaceutical biotechnology's methods but on its products, notably recombinant pharmaceuticals. It describes various types of biotech pharmaceuticals, their safety and effectiveness relative to the safety and effectiveness of conventionally produced pharmaceuticals, and the regulation of biotech pharmaceuticals.

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INTRODUCTION

In the context of this review, "biotechnology" refers to the use of living things or parts of living things to create or modify drugs and other substances; to modify food crops and other macroscopic organisms; or to adapt microorganisms to agricultural, medical, or other purposes.

Biotechnology encompasses such disparate processes as industrial fermentation, gene therapy, and cloning. The medical repercussions of advances in biotech have been impressive, but the implications of those advances for human health are no less than staggering.

Biotechnology produces biotherapeutic agents on industrial scales. These agents include both novel agents and agents formerly available only in small quantities. Crude vaccines were used in antiquity in China, India, and Persia. For example, vaccination with scabs that contained the smallpox virus was a practice in the East for centuries. In 1798 English country doctor Edward Jenner demonstrated that inoculation with pus from sores due to infection by a related virus could prevent smallpox far less dangerously. Humankind has benefited incalculably from the implementation of vaccination programs.

Insulin replacement therapy has been in use for decades. Before Canadian physiologists Frederick

Banting and Charles Best discovered and isolated insulin in 1921, nearly all persons diagnosed with diabetes died within a few years after the diagnosis. In the mid-1960s several groups reported synthesizing the hormone.

Virtually all biotherapeutic agents in clinical use are biotech pharmaceuticals. A biotech pharmaceutical is simply any medically useful drug whose manufacture involves microorganisms or substances that living organisms produce (e.g., enzymes). Most biotech pharmaceuticals are recombinant—that is, produced by genetic engineering. Insulin was among the earliest recombinant drugs.

Genetic engineering—also known as bioengineering, gene splicing, and recombinant DNA technology—comprises altering DNA molecules outside an organism and making the resultant molecules function in living things. Multicellular organisms that have been genetically engineered to produce substances medically useful to humans include cows, goats, sheep, and rats, and corn, potato, and tobacco plants. Genetic engineering is revolutionizing medicine: enabling mass production of safe, pure, more effective versions of biochemicals the human body produces naturally.

Genetic engineering is central to modern biotherapy's backbone: pharmaceutical biotechnology. Pharmaceutical biotechnology involves using microorganisms, macroscopic organisms, or hybrids of tumor cells and leukocytes:

- to create new pharmaceuticals;
- to create safer and/or more effective versions of conventionally produced pharmaceuticals; and
- to produce substances identical to conventionally made pharmaceuticals more cost-effectively than the latter pharmaceuticals are produced.

For example, before the development of recombinant human insulin—which became the first manufactured, or commercial, recombinant pharmaceutical in 1982—animals (notably pigs and cattle) were the only nonhuman sources of insulin. Animal insulin, however, differs slightly but significantly from human insulin and can elicit troublesome immune responses. Recombinant human insulin is at least as effective as insulin of animal origin, is safer than animal-source insulin, and can satisfy medical needs more readily and more affordably.

Pharmaceutical biotechnology's greatest potential lies in gene therapy. Gene therapy is the insertion of genetic material into cells to prevent, control, or cure disease. It encompasses repairing or replacing defective genes and making tumors more susceptible to other kinds of treatment.

The FDA approved more biotech drugs in 1997 than in the previous several years combined. The laundry list of human health conditions for which the FDA has approved recombinant drugs includes AIDS, anemia, certain cancers (Kaposi's sarcoma, leukemia, and colorectal, kidney, and ovarian cancers), certain circulatory problems, certain hereditary disorders (cystic fibrosis, familial hypercholesterolemia, Gaucher's disease, hemophilia A, severe combined immunodeficiency disease, and Turner's syndrome), diabetic foot ulcers, diphtheria, genital warts, hepatitis B, hepatitis C, human growth hormone deficiency, and multiple sclerosis.

Table 1 lists biotech pharmaceuticals that the U.S. Food and Drug Administration (FDA) has approved.

I. Types of Biotech Pharmaceuticals

Many biotech pharmaceuticals are similar or identical to proteins that healthy human bodies produce routinely for normal functions. In addition to gene-therapy drugs, there are seven major types:

1. Cytokines

Cytokines are hormonelike molecules that can control reactions between cells. They activate immune-system cells such as lymphocytes and macrophages.

Table 1: Some Approved Biotech Drugs.

Product	Year of First U.S. Approval	Approved for:
recombinant human insulin	1982	diabetes mellitus
recombinant somatrem (human growth hormone) for injection	1985	human growth hormone (hGH) deficiency in children
recombinant interferon alfa-2b	1986	hairy cell leukemia
	1988	genital warts
	1988	Kaposi's sarcoma
	1991	hepatitis C
	1992	hepatitis B
recombinant interferon alfa-2a	1986	hairy cell leukemia
	1988	Kaposi's sarcoma
Muromonab-CD3	1986	reversal of kidney transplant rejection
	1993	reversal of heart and liver transplant rejection
recombinant hepatitis B vaccine	1986	hepatitis B prevention
recombinant somatropin for injection	1987	human growth hormone (hGH) deficiency in children
Alteplase	1987	acute myocardial infarction
	1990	acute massive pulmonary embolism
Epoetin alfa (rEPO, Epogen)	1989	anemia of chronic renal failure
recombinant hepatitis B vaccine	1989	hepatitis B
interferon alfa-n3	1989	genital warts
adenosine deaminase	1990	severe immunodeficiency in infants
interferon gamma-1b	1990	chronic granulomatous disease
filgrastim (rG-CSF)	1991	neutropenia caused by chemotherapy
	1994	bone marrow transplantation
	1994	chronic, severe neutropenia
sargramostim (yeast-derived GM-CSF)	1991	bone marrow transplantation
Aldesleukin (interleukin-2)	1992	renal cell carcinoma
Staumonab pendetide (OncoScint)	1992	colorectal and ovarian cancers
recombinant antihemophiliac factor (rAHF)	1992	hemophilia A
recombinant interferon beta-1b	1993	relapsing, remitting multiple sclerosis
dornase alpha (Pulmozyme)	1993	cystic fibrosis
Pegaspargase	1994	lymphoblastic leukemia
imiglucerase for injection (Cerezyme, recombinant lucocerebrosidase)	1994	Gaucher's disease
abciximab (ReoPro)	1994	prevention of blood clotting
Humulin 70/30 (biosynthesized human insulin)	1996	diabetes mellitus
Humatrope	1996	adult- or childhood-onset growth hormone deficiency
Serostim	1996	AIDS wasting associated with catabolism, weight loss, or cachexia
Saizen	1996	human growth hormone deficiency in children
Nutropin	1996	Turner's syndrome
Infanrix (vaccine)	1997	diphtheria and tetanus toxoids absorbed
coagulation factor IX (recombinant)	1997	factor IX deficiencies (Christmas disease)
Novolin 70/30 (biosynthesized human insulin)	1997	diabetes mellitus
Velosulin human (semisynthesized purified human insulin)	1997	diabetes mellitus
Genotropin	1997	human growth hormone deficiency in adults
Oprelvekin (Neumega)	1997	prevention of thrombocytopenia
Rituximab (Rituxan)	1997	follicular B-cell non-Hodgkin's lymphoma
Becaplermin (Regranex Gel)	1997	diabetic foot ulcers
daclizumab (Zenapax)	1997	acute renal allograft rejection
Nutropin AQ	1997	human growth hormone deficiency in adults

Sources include:

(1) Biotechnology Industry Organization (BIO). Biotechnology Drug Products: Washington, DC: BIO (undated, received in Jan 1995).

(2) Pharmaceutical Research and Manufacturers of America (PhRMA). 1995 survey: biotechnology drug research has come of age. In: Biotechnology Medicines in Development; Washington, DC: PhRMA; 1995: 20–21.

(3) U.S. Food and Drug Administration (FDA). Center for Drug Evaluation and Research webpage.

(4) U.S. Food and Drug Administration (FDA). Center for Biologics Evaluation and Research webpage.

Cytokines that have recombinant variants or versions include those described below.

- Interferons are potent cytokines that act against viruses and uncontrolled cell proliferation, which is the primary hallmark of cancer. Virtually all conventional chemotherapeutic agents act directly on cancer cells. When interferons act on cancer cells, however, they do so indirectly—by affecting the functioning of the immune system. The FDA has approved certain recombinant interferons for the treatment of several diseases, including AIDS-related Kaposi's sarcoma, hairy cell leukemia, hepatitis B, and genital warts.

- Interleukins function as messengers between leukocytes. Interleukin-2 (IL-2) stimulates T lymphocytes. The FDA has approved a recombinant variant of IL-2, aldesleukin (Proleukin), for treating renal cell carcinoma. The antitumor effect of IL-2 and its recombinant variant is directly proportional to how much of the agent is administered. Endogenous IL-2 is scarce; aldesleukin can be mass-produced but has adverse side effects at relatively low levels of administration. (1)

- Granulocyte-colony stimulating factor (G-CSF) stimulates the bone marrow to produce neutrophils (antibacterial leukocytes). The FDA has approved a recombinant variant of G-CSF, filgrastim, for controlling infections in patients on anticancer drugs that suppress immune responses, in patients undergoing bone-marrow transplantation, and in patients with neutropenia.

- Granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulates the bone marrow to produce neutrophils and macrophages. The FDA has approved its recombinant equivalent, sargramostim (Leukine), for administration to cancer patients who, because intensive chemo- and/or radiotherapy destroyed their bone marrow, have undergone a transplant. Sargramostim is administered until the transplanted marrow can produce leukocytes adequately without such stimulation. By keeping leukocyte levels high enough to control infections, sargramostim can hasten recovery.

2. Enzymes

Below are descriptions of recombinant enzymes and diseases against which they are effective.

- *Alteplase.* The process of dissolving blood clots in the circulatory system involves conversion of the protein plasminogen to the proteolytic enzyme plasmin. A recombinant version of one of the enzymes that accelerate this conversion can contribute to the treatment of heart attacks, strokes, and pulmonary emboli. This recombinant enzyme is recombinant tissue-type plasminogen activator (alteplase). The effects of alteplase are more localized than those of other enzymes used to dissolve blood clots (streptokinase and urokinase); thus, in theory, alteplase would cause less bleeding throughout the body. (2)

- *Dornase alfa.* Cystic fibrosis (CF) is a genetic disorder marked by excessive mucous secretions and frequent lung infections. About half of those with CF live fewer than 29 years. In 1995 approximately 20,000 to 25,000 persons in the U.S. had the disease. (3) A DNA-splitting enzyme produced by the body, deoxyribonuclease I (DNase I), can break down DNA that is outside cells, but not DNA that is within intact cells. In contrast, dornase alfa (Pulmozyme), a recombinant variant of DNase I in aerosol form, *can* break down intracellular DNA. Decomposition of the intracellular DNA in the excessive mucous secretions that dispose persons with CF to lung infections can make the secretions less adhesive to airways. Dornase alfa can thus decrease the incidence and duration of both lung infections and hospital stays in CF patients. It is the first new drug the FDA has approved in 30 years for the management of CF.

- *Imiglucerase.* Gaucher's disease, characterized by bone destruction and enlargement of the liver and spleen, is due to an hereditary deficiency of glucocerebrosidase. A variant of this enzyme is

obtainable from human placentas. But 20,000 placentas would provide only a year's supply for a single patient, at a cost of $160,000 annually (4), and everyone with the disease has a lifelong need for such an enzyme. The FDA has approved a recombinant variant of glucocerebrosidase, imiglucerase, that should end the supply problem.

3. Hormones

Recombinant human insulin became the first manufactured, or commercial, recombinant pharmaceutical in 1982, when the FDA approved human insulin for the treatment of cases of diabetes that require the hormone. Before the development of recombinant human insulin, animals (notably pigs and cattle) were the only nonhuman sources of insulin. Animal insulin, however, differs slightly but significantly from human insulin and can elicit troublesome immune responses. The therapeutic effects of recombinant human insulin in humans are identical to those of porcine insulin, and it acts as quickly as porcine insulin, but its immune-system side effects are relatively infrequent. Further, it can satisfy medical needs more readily and more affordably.

Other recombinant hormones include those described below.

- *Lispro.* Regular insulin ordinarily must be injected 30 to 45 minutes before meals to control blood glucose levels. Lispro (Humalog)—a recombinant insulinlike substance—is faster-acting than regular insulin. Because injection of lispro is appropriate within 15 minutes before meals, using it instead of regular insulin may be more convenient for some patients. (5)

- *Epoetin alfa.* Erythropoietin (EPO), a hormone produced by the kidneys, stimulates the bone marrow to produce red blood cells. The FDA has approved recombinant EPO—epoetin alfa—for the treatment of anemia due to chronic renal failure.

- *Recombinant human growth hormone.* Human growth hormone (hGH) is used to counter growth failure in children that is due to a lack of hGH production by the body. Before the introduction of recombinant hGH the hormone was derived from human cadavers. Cadaver-derived hGH was susceptible to contamination with slow viruses that attack nerve tissue. Such infective agents caused fatal illnesses in some patients. Recombinant hGH has greatly improved the long-term treatment of children whose bodies do not produce enough hGH.

4. Clotting Factors

Inadequate bodily synthesis of any of the many clotting factors can prevent effective clotting. The FDA has approved two clotting-related recombinant drugs: abciximab for the prevention of blood clotting as an adjunct to angioplasty, and recombinant antihemophiliac factor (rAHF) for the treatment of hemophilia A. Hemophilia A is a lifelong hereditary disorder characterized by slow clotting and consequent difficulty in controlling blood loss, even from minor injuries. About 20,000 persons in the United States alone have this condition, which is due to a deficiency of antihemophiliac factor (AHF, or factor VIII). Before the introduction of rAHF, treatment of hemophilia A required protein concentrates from human plasma. Such concentrates could contain contaminants (e.g., HIV), and the lifetime treatment of a single patient required thousands of blood contributions.

Persons with hemophilia B lack factor IX. They require either factor IX concentrates from pooled human blood or factor IX from cell cultures (some of which are genetically engineered). In July 1997 Scotland's Roslin Institute announced the birth of the first genetically engineered sheep clone. The clone carries a human gene for factor IX, and it gives milk that contains the factor. (Other multicellular organisms that have been genetically engineered to produce substances that are or may be medically useful to humans include cows, goats, and rats, and corn, potato, and tobacco plants.) (6) (7)

5. Vaccines

In every modern vaccine the main or sole active

ingredient consists of killed microorganisms, nonvirulent microorganisms, microbial products (e.g., toxins), or microbial components that have been purified. All these active ingredients are antigens: substances that can stimulate the immune system to produce specific antibodies. Such stimulation leaves the immune system prepared to destroy bacteria and viruses whose antigens correspond to the antibodies it has learned to produce. Although conventionally produced vaccines are generally harmless, some of them may, rarely, contain infectious contaminants. Vaccines whose active ingredients are recombinant antigens do not carry this slight risk.

More than 350 million persons worldwide are infected with the virus that causes hepatitis B, a major cause of chronic inflammation of the liver, cirrhosis of the liver, and liver cancer. (8) Hepatitis B kills a million people each year worldwide. About 1.25 million Americans harbor the hepatitis B virus (HBV); 30 percent of them will eventually develop a serious liver disease. About 300,000 children and adults in the U.S. become infected with HBV each year, and 5,000 Americans die annually from liver disease caused by the virus. The first hepatitis B vaccine available in the U.S. was made with derivatives of plasma from persons with chronic HBV infections. A recombinant vaccine—whose sole active ingredient is a recombinant (and thus uncontaminated) antigen—has replaced it. Use of this vaccine is very cost-effective—especially in North America, since interferon treatment of hepatitis B is very expensive.

The Ebola virus, first identified in 1976, causes Ebola hemorrhagic fever, one of the deadliest viral diseases known. About 50–90 percent of patients infected with the Ebola virus consequently die. In 1997 American researchers announced that an experimental recombinant vaccine against the virus had proved effective in mice and guinea pigs.

Because of immune-system inadequacy, some groups—infants and young children, for example—tend to respond poorly to vaccination against certain bacterial infections (e.g., streptococcal pneumonia). Preliminary research suggests that antibacterial vaccines that contain specific antibodies are more effective against such diseases than are comparable conventional vaccines, which do not contain antibodies. (9)

Although vaccines traditionally have been designed to prevent only infectious diseases, the development of individualized vaccines—vaccines made from the cancer cells of each patient—to restrain, prevent the recurrence of, or cure some forms of cancer is promising. Researchers at the U.S. National Cancer Institute have demonstrated that a special vaccine plus interleukin-2 can shrink tumors in patients with metastatic melanoma. (10) The vaccine used in this study contained a melanoma-antigen variant more effective than the original antigen at attracting to cancer sites T lymphocytes that are destructive to tumors.

Another prospect is effective inoculation by ingestion. In February 1998 U.S. researchers announced that they had genetically engineered potatoes to produce a “vaccine” against cholera. (11) Every year five million people contract cholera, and 200,000 die from it. The “vaccine” is a nontoxic, relatively heat-stable protein that can elicit an immune response even when it is ingested as a potato constituent.

6. Monoclonal Antibodies

All the antibodies the immune system normally produces in response to a specific antigen are capable of marking (binding to) that antigen, but these antibodies—termed “polyclonal”—are varied, not identical. Monoclonal antibodies (MoAbs) that share a specific antigenic target are identical and are more sensitive to that target than are polyclonal antibodies for the same antigen. MoAbs are the products of hybridomas—cells that result from the biotech fusion of bone-marrow tumor cells and B lymphocytes. Hybridomas can be geared to produce specific MoAbs continuously.

Theoretically, a MoAb designed for a particular antigen on cancer cells can initiate an immune response that would destroy cancer cells without

harming normal cells. At least 26 MoAbs are undergoing clinical testing as anticancer agents (12), but the medical potential of MoAbs extends to many other diseases.

For example, the FDA has approved the MoAb drug muromonab-CD3 for the treatment of immune-system rejection of transplanted hearts, kidneys, and livers. Muromonab-CD3 restrains immune response and thus increases the likelihood that the transplant will function. More recently, the FDA approved the immunosuppressant daclizumab (Zenapax) for the prevention of kidney-transplant rejection. Daclizumab's active ingredient is a "humanized" MoAb; 90 percent of the MoAb's amino-acid structure is human. Thus, the likelihood of an allergic reaction to it is low.

Another MoAb, infliximab (cA2), appears effective against Crohn's disease, an immune-system disorder marked by intestinal inflammation. (13) Infliximab is specific for a factor in the development of the disease.

The medical utility of MoAbs is not limited to therapeutics. Because of their ability to bind to specific antigens, MoAbs have been used for many years to identify antigen-carrying disease agents and to locate them in the human body. Recently, British researchers designed MoAbs that may be useful in determining whether cancer has spread from breast tissue to axillary lymph nodes. The spread of cancer to other parts of the body is likelier if the cancer has spread to lymph nodes than if it has not. Traditionally, determining whether the lymph nodes have been affected involves surgery. But using radiolabeled MoAbs specific to antigens on malignant cells enables locating such cells with an instrument comparable to a Geiger counter and may decrease the need for surgery.

The ability of MoAbs to bind to, and thus tag, specific proteins also makes them potentially useful in the diagnostic imaging of internal organs and tumors.

OTHER BIOTECH DRUGS

Listed below are a few of the hundreds of other biotech drugs that are either in clinical use or undergoing scientific investigation.

- Biotech vaccines undergoing investigation include vaccines for acellular pertussis (whooping cough), AIDS, herpes simplex, Lyme disease, and melanoma.

- Two new recombinant interferons are undergoing investigation: consensus interferon, for treating hepatitis C; and recombinant beta interferon 1a, for multiple sclerosis.

- Recombinant PTK (protein tyrosine kinase) inhibitors may have therapeutic utility against diseases marked by cell proliferation, such as cancer, atherosclerosis, and psoriasis. Protein tyrosine kinases contribute to cell division and are the targets of these biotech drugs.

- Recombinant human interleukin-3 is undergoing clinical investigation as an adjunct to traditional cancer chemotherapy.

- Two recombinant growth factors (cytokines that regulate cell division) are undergoing major clinical trials: recombinant human insulin-like growth factor (rhIGF-1) and recombinant human platelet-derived growth factor-BB (PDGF). PDGF can contribute to wound healing.

- In December 1997 the FDA approved clinical testing of a recombinant version of the cytokine myeloid progenitor inhibitory factor-1 (MPIF-1). MPIF-1 can keep certain normal cells, including many immunologically important cells, from dividing and can thus protect them from anticancer drugs that target rapidly multiplying cells. When such anticancer drugs affect normal cells that divide rapidly, hair loss, nausea, and immunosuppression can result.

- Injecting the recombinant protein fibroblast growth factor (FGF-1) into the human myocardium increases the blood supply to the heart by inducing blood-vessel formation. (14) Such treatment, called a "biologic bypass" or "biobypass," does not require surgery. FGF-1 is injectable nonsurgically into the myocardium by cardiac catheterization. A biobypass may benefit persons with coronary artery disease whose arteries are not reparable surgically. (A gene-therapy form of biobypass, VEGF gene therapy, is described below.)

- In January 1998 advisors to the FDA recommended that the agency approve Apligraf, a recombinant skin replacer, for the treatment of leg ulcers due to poor circulation; and DermaGraft, another such product, for the treatment of diabetic ulcers. About 800,000 diabetic foot ulcers occur in the U.S. annually, and they lead to most of the lower-leg amputations that approximately 60,000 diabetics undergo each year. Traditionally, patients with chronic skin ulcers or severe burns have had only two treatment options: skin grafts, which depended on how much healthy skin they had, and temporary protective coverings made of dead cells. The FDA approved Apligraf in May 1998, and trials of the product against bedsores may begin this year.

Table 2 describes several other biotech pharmaceuticals undergoing clinical investigation.

Table 2: Miscellaneous Biotech Pharmaceuticals Undergoing Clinical Investigation.

Drug	Description
recombinant factor VIIa	clotting factor for treatment of hemophilia A and B
Pixykine	colony stimulating factor designed to contribute to the prevention of deficiencies of neutrophils and platelets. (Such deficiencies can result from anticancer chemo- and radiotherapy.)
Auriculin anaritide	for acute renal failure
Hirudin	for acute heart problems
ILl-2 fusion toxin (DAB_{389}IL-2)	for cutaneous T-cell lymphoma
platelet aggregation inhibitor	for prevention of complications after angioplasty
recombinant human leutinizing hormone	for fertility enhancement (follicular stimulation)
recombinant osteogenic protein-1	for bone fractures in which the ends fail to unite
recombinant human thyroid stimulating hormone	useful in the detection and treatment of recurrent thyroid cancer

Source: Pharmaceutical Research and Manufacturers of America (PhRMA). 1995 survey: biotechnology drug research has come of age. In: Biotechnology Medicines in Development. Washington, DC: PhRMA; 1995: 2–18.

GENE THERAPY

Pharmaceutical biotechnology's greatest potential lies in gene therapy. Gene therapy is the insertion of genetic material into cells to prevent, control, or cure disease, especially genetic disorders. It encompasses repairing or replacing defective genes and making tumors more susceptible to other kinds of treatment. Thus, gene therapy's potential for preventing and curing disease is vast. It has proved somewhat useful in the treatment of certain rare genetic diseases, such as cystic fibrosis and familial hypercholesterolemia.(15)

Carriers of therapeutic genes include:

- harmless viruses that have undergone genetic alteration and can carry selected genetic material into human cells; and
- liposomes—injectable microscopic fatty globules that can enclose and protect DNA segments (e.g., a "suicide gene" for insertion into cancer cells.) (16)

Existing modes of gene therapy can restrain the replication of pathogenic microorganisms, can eliminate defective cells, and can increase the resistance of normal cells to drugs harmful to them

(e.g., certain anticancer agents). (17) For example, the Multiple Drug Resistance (MDR) gene enables production of a protein that removes various foreign chemicals from cells. Introduction of the MDR gene into the bone-marrow cells of patients with advanced cancer seems safe and may protect their bone marrow from the toxic side effects of chemotherapy. It may thus make high-dose chemotherapy safer and improve recovery.

Another anticancer strategy undergoing investigation, antiangiogenesis gene therapy, involves introducing genetic material to a limited area to decrease the formation of blood vessels there. (18) Decreasing angiogenesis at the site of a tumor decreases the tumor's ability to grow and spread.

A form of gene therapy with the opposite effect on blood-vessel formation has also been developed. Preliminary research suggests that "therapeutic angiogenesis," or VEGF gene therapy, may be effective against sensory neuropathy (19) (specifically, a loss of feeling in the feet) and critical limb ischemia (an arterial disease marked by a decrease in the supply of oxygen-rich blood to the legs). Such a decrease can result in gangrene and the need for amputation. "VEGF" stands for vascular endothelial growth factor, a protein that can induce angiogenesis. Scientists have modified a relatively harmless respiratory virus so that it bears the gene for VEGF. Injection of the material that carries the VEGF gene directly into defective parts of the heart might eventually supersede surgical procedures used to treat coronary artery disease. (20) As many as 600,000 cardiac patients a year might benefit from VEGF gene therapy.

(Viruses can elicit an immune response, and in any case using viruses to convey genes is not a very accurate means of sending genetic material to target cells. In chimeraplasty, an experimental mode of gene therapy, chimeraplasts—"repairman" molecules that are hybrids of RNA and recombinant DNA—convey the gene. [21] Chimeraplasty may enable gene transmission that is more accurate than viral or microbial gene transmission.)

In January 1998 researchers reported that introduction of the active gene for human telomerase reverse transcriptase (hTRT)—a vital component of the enzyme telomerase—into normal human cells had resulted in a marked increase in the cells' life span without making the cells otherwise abnormal (e.g., cancerous) (22) Most human cells do not produce hTRT but contain all the other components of telomerase. (23) Normal cells that lack telomerase can replicate only about 50 times. Each time one divides, it loses DNA from its telomeres (the natural, protective ends of its chromosomes). Without telomerase, which is key to the synthesis of telomeres, shortening of the telomeres ultimately brings cell division to a halt, whereupon the cell dies. Because the hTRT gene of sperm cells, egg cells, and cancer cells is active, they can divide perpetually. It is theoretically possible to destroy cancer cells safely by neutralizing telomerase or by modifying the hTRT gene. Controlling various age-related disorders, such as heart disease, with the hTRT gene may also be feasible. (24) Specific cells from a patient could be rejuvenated and then cultured to replace, for example, the patient's hardened arterial tissue or burned or wrinkled skin.

II. Safety and Effectiveness

Many biotech agents are identical to, or differ only slightly from, proteins the human body produces naturally; thus, biotech pharmaceuticals tend to have a lower potential for adverse reactions than do conventionally produced pharmaceuticals.

DRUG DELIVERY

Many biopharmaceutical substances lack stability and/or are not absorbable in a medically useful form through the gastrointestinal tract, the lungs, or the skin. In the gastrointestinal tract, for example, digestive chemicals normally break down protein products. Even injection may not ensure effective delivery to the target cells. To be effective, many injected drugs need to survive transport through the liver and encounters with enzymes. Therefore, how biopharmaceuticals are delivered is very critical.

Drug-delivery innovations relevant to biopharmaceuticals include those described below.

- *Liposomes.* A liposome is a microscopic fatty droplet designed to carry a therapeutic substance, especially to specific bodily tissues. The liposomal outer membrane and the outer membrane of the target cell can fuse, whereupon the liposome empties into the cell. Liposomal encapsulation of a therapeutic substance enables increasing the accumulation of the active ingredient in target tissues and controlling the spread of the active ingredient to nontarget tissues, where it might do harm.

- *Immunotoxins.* An immunotoxin is a combination of a monoclonal antibody and a toxic (e.g., anticancer) substance. Because it responds only to specific antigens, the MoAb component limits the toxic effects of the immunotoxin to target (e.g., tumor) cells.

- *Prodrugs.* A prodrug is any medical compound designed to work only after the body or a specific type of tissue in the body has activated it. Prodrugs are useful when the "active" drug is too toxic for nonspecific or general distribution to bodily tissues, when absorption of the "active" drug is poor, or when the body breaks down the "active" drug prematurely. For example, a prodrug that can be activated by only one type of enzyme will work only in tissues that produce that enzyme. Such a prodrug can thus spare nontarget tissues toxic effects. The introduction into tumor cells of genes for enzymes that can activate anticancer prodrugs—a prodrug-activating gene therapy—has been well studied. (25)

- *Polyethylene glycol.* Frequent injections of a therapeutic protein can result in harmful immune responses. Adding polyethylene glycol (PEG) to therapeutic proteins increases their stability in the body and lengthens the time they stay in the bloodstream, thus decreasing the number of injections needed. PEG can contribute to the treatment of severe combined immunodeficiency disease (SCID). SCID, an hereditary disorder, renders even ordinarily trivial infections so deadly to children that institutionalization or isolation is necessary for their survival. Neither bone marrow transplants nor daily infusions of leukocytes—the conventional treatments—are always effective against SCID. Deficiency of the enzyme adenosine deaminase (ADA) causes about one third of all cases. Adding PEG to recombinant ADA enables effective *weekly* infusions, as PEG slows the breakdown of ADA in the body.

  PEG likewise slows the breakdown of another enzyme, L-asparaginase, which the body produces naturally. Pegaspargase, a combination of PEG and recombinant L-asparaginase, can improve the condition of children with lymphoblastic leukemia.

BIOTECH PHARMACEUTICAL PURITY

Nearly all biotech agents are proteins and have to be isolated from proteinaceous substances. Thus, the most common impurities in recombinant drugs are proteinaceous. Protein impurities can cause allergic reactions or make the therapeutic effects of the drug different from the intended therapeutic effects.

A slight difference between a recombinant protein and its endogenous counterpart can elicit an adverse immune response. Recombinant protein preparations derived from bacterial cultures may also contain small amounts of nitrogen-containing bacterial contaminants that can elicit an adverse response. (26)

Contamination occurs about as often in the manufacture of products from traditional cell cultures as in the manufacture of products from recombinant cultures. Adherence to modern standards of manufacture can keep such contamination infrequent. (27) In any case, even low-level microbial contamination of recombinant cultures is easily detectable. (28)

BIOTECH PHARMACEUTICAL STABILITY

Protein molecules are larger and less stable than the molecules of conventionally produced pharmaceutical agents.

Stability is particularly important with larger protein molecules, because their *in vivo* effects often depend on their three-dimensional structure. (29) Even without a change in the order and kind of the amino acid components, a change in the three-dimensional structure of a biotech product can render it medically useless. For example, at low concentrations, interferons, interleukins, and certain other biotech molecules have a tendency to adhere to glass and plastic. Such adsorption may denature the molecule, and a loss of potency can result. This is often preventable by coating the insides of containers used in drug administration with human serum albumin before placing the drug in the containers. (30)

The shell of water around a protein molecule critically affects its structure. (31) Removal of all water from a protein usually changes its structure irreversibly. Thus, freeze-drying of biotech proteins is complicated and care must be given to prevent denaturation. A common practice is the use of humectants to increase the stability of biotech protein powders.

Expiration-dating of pharmaceuticals is based on tests of the drug's pre-administration stability. Generally, estimates of a pharmaceutical's shelf life are based on "accelerated" testing, in which the temperature and humidity are considerably higher than the temperature and humidity recommended for commercial storage. But because heat can affect protein structure, the utility of accelerated testing for expiration-dating biotech pharmaceuticals is very limited. To establish expiration dates for protein-based pharmaceuticals, manufacturers necessarily conduct real-time stability studies on such preparations under recommended storage conditions.

III. Regulation of Biotech Pharmaceuticals

Regulatory agencies such as the U.S. Food and Drug Administration (FDA) oversee sales of "human therapeutics" and other lawful products categorized as drugs and presented for application to humans. Regulatory approval of any such product must precede its sale. To obtain FDA approval, manufacturers must submit to the agency voluminous information about the product, including reports of scientific findings concerning medical effectiveness, purity, stability, and side effects (e.g., due to impurities or high dosing). By the time approval has been obtained, a company may have spent five to ten years and more than $200 million seeking it.

The consensus of many national and international groups is that biotech risk is primarily a function of product characteristics, and that it is not a function of rDNA technology. (32) In other words, these organizations have decided that biotech pharmaceuticals should be judged according to the components (e.g., active ingredients and contaminants) and the effects (e.g., side effects) of each pharmaceutical, and not according to how they were made. Consistent with this consensus, the FDA's approach to recombinant drugs and other biotech pharmaceuticals is the same as its approach to conventional biologicals.

In the United States, the Environmental Protection Agency (EPA) and the National Institutes of Health (NIH) also influence pharmaceutical biotech research. The EPA regulates releases of recombinant microorganisms into the environment, and the NIH repeatedly updates biotech research guidelines that recipients of federal funds must follow. (33) Many biotech researchers who do not receive such funds also follow these guidelines.

CONCLUSION

Recombinant DNA technology is revolutionizing medicine, i.e., enabling mass production of safe, pure, more effective versions of biochemicals the human body produces naturally. Through gene therapy, the potential of biotech pharmaceuticals for curing chronic and "incurable" diseases and improving the human condition is limitless. With sensible regulatory requirements and expeditious

product review by regulatory agencies, biotech pharmaceuticals can within decades become unprecedented preventers and relievers of human suffering.

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