Appendix D
Biotechnology

Introduction

Recent advances in recombinant DNA (rDNA) technology, cell fusion technology (monoclinal antibodies), and bioprocessing technology (biological production) promise to make earlier diagnosis possible and improve disease prevention and treatment. The ability to develop more definitive diagnostic technologies and predictive tools, as well as more cost-effective therapeutics, gives biotechnology the potential to reduce the severity and burden of chronic disease among the elderly.

New methods of biotechnology may improve pharmaceutical development and production in a variety of ways, perhaps most importantly by increasing the supply, variety, and quality of products now being marketed by making them more effective, convenient, safe, or economical.

Monoclinal antibody and rDNA technologies can complement each other. For example, monoclinal antibodies (MAbs) can be used to identify and purify new compounds, and rDNA technology can be used to biologically produce them. Yet without advances in bioprocess technology, the production process by which genetically manipulated micro-organisms are adapted for large-scale industrial use, rDNA-based pharmaceutical development is not possible.

Cell fusion—monoclinal antibodies

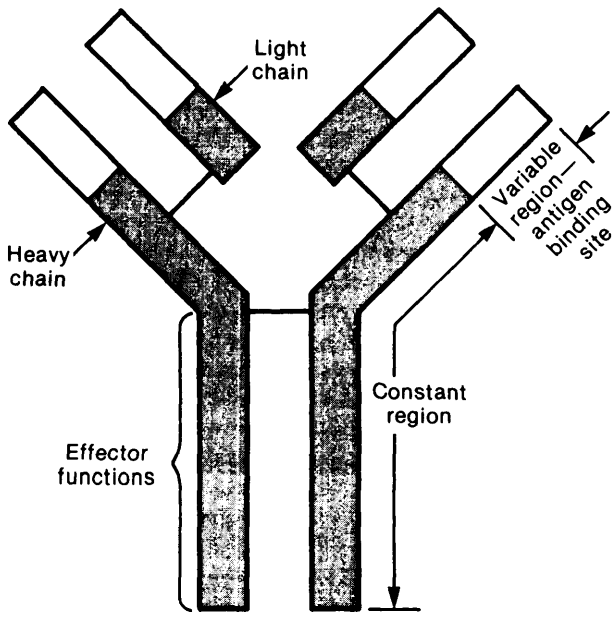
Cell fusion, the artificial joining of cells, combines the desirable characteristics of different types of ceils into one cell. Through cell fusion, the traits for immortality and rapid proliferation (derived from certain cancer cells), and the traits for production of specific antibodies (derived from specialized cells of the immune system) are combined in the new cell line (hybridoma) that results from the fusion. These hybridomas produce large amounts of monoclinal antibodies—antibodies that are specific to only one kind of antigen. Because of their specificity and their ability to "home in" on specific kinds of cells, MAbs can be used for a variety of pharmaceutical applications, ranging from diagnostic assays, passive vaccines, and drug delivery (i.e., chemotherapy) to body imaging and purification.

HOW ANTIBODIES WORK

Production of antibodies is one aspect of a complex of biological reactions known as the immune response. Antibodies are produced by specialized cells (B lymphocyte cells) found in the spleen, lymph nodes, and blood. Bzells recognize substances foreign to the body (antigens) and respond by producing antibodies that specifically recognize and bind to the antigens. Antigens can be almost any substance recognized by the body as foreign—bacteria, viruses, or even chemicals. The binding of the antibody to the antigen results in breakdown of the antigen, thereby removing the antigenic response in the body.

Structurally, all antibodies have the same basic Y-shape—two "heavy" chains and two "light" chains (see fig. D-l). Each heavy and light chain has a "variable" and a "constant" region. The variable region contains the site that recognizes and binds to a specific antigen in much the same manner as a lock to a key. This site varies greatly from antibody to antibody, allowing for a wide range of antigens to be recognized by the large number of different antibodies that are naturally produced by the body. **The constant region of the antibody is associated with effecter functions, such as the secretion of antibodies from the B-cells, and "signaling" to the immune system after the antibody binds with the target antigen. Thus, if the antigen is a complex macromolecule (e.g., a protein) with many an-**

Figure D-l.—Structure of an Antibody Molecule



SOURCE: Office of Technology Assessment

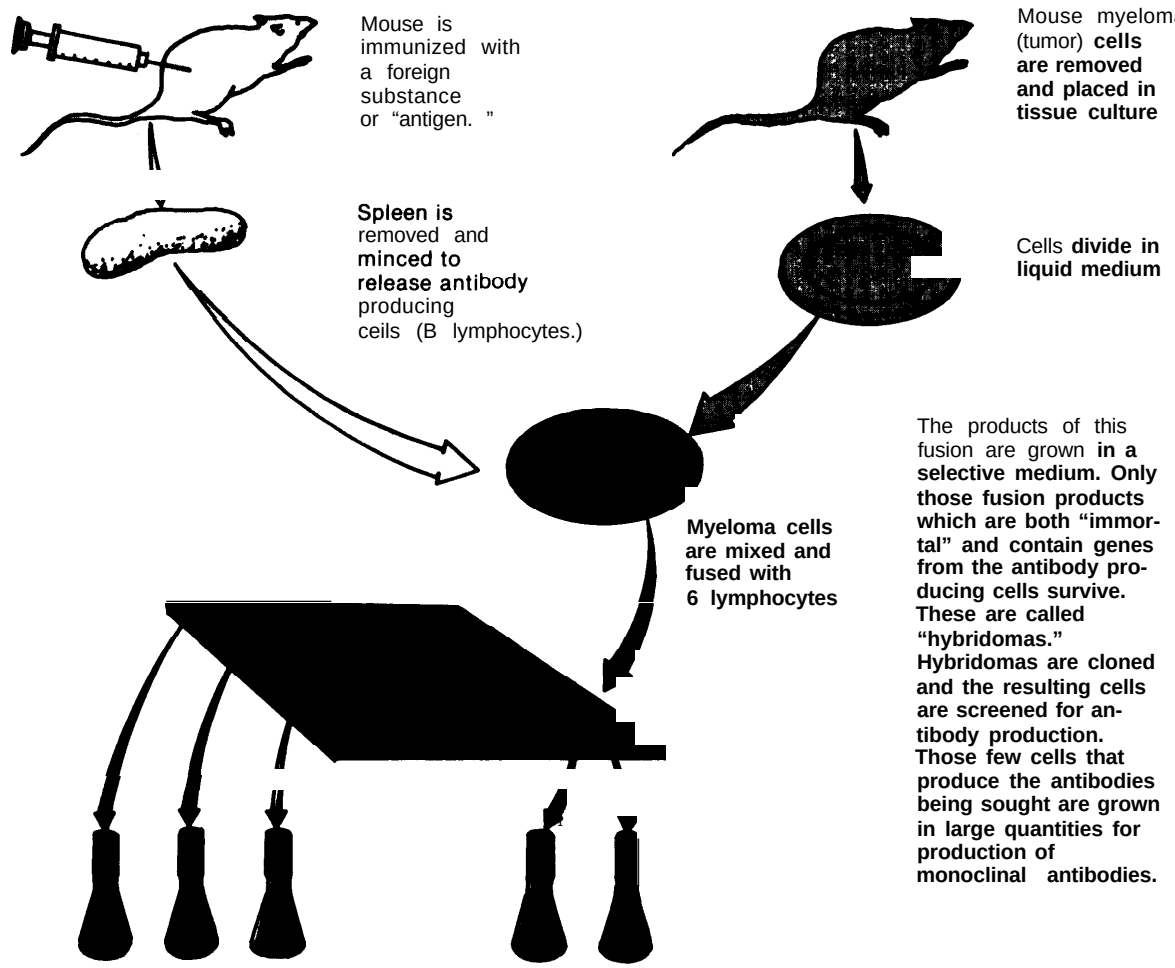
tigenic **determinants, a large number of different B-cells, and a variety of antibodies specific to those determinants will be produced. By virtue of a very complex genetic scheme, millions of potential antibodies are possible, but each Bwell is committed to only one antibody. When the appropriate antibody-producing B-cell contacts and "recognizes" its antigen, it clones a set of identical cells, all of which produce the genetically programmed antibody for the life of the cells.**

THE MAKING OF ANTIBODIES

Antibodies have long been important tools for clinicians and researchers who use an antibody's specificity to identify particular molecules or cells and to separate them from mixtures. The conventional production method by which antibodies are made for diagnostic, therapeutic, and investigational purposes is to inject an antigen into a laboratory animal and, after an immune response is triggered, to collect antiserum (blood serum containing antibodies) from the animal. This method of antibody production results in production of many antibodies of several classes and various specificities. Moreover, the limited life span of the animal prohibits large-scale production of reproducible antibodies.

Cell fusion technology and the hybridoma-producing MAbs that result allow for the production of an homogeneous and therefore reproducible reagent in almost unlimited quantities. Figure D-2 illustrates the method used to prepare MAbs. As in vitro diagnostic tools, murine MAbs (MAbs derived from mice) are very valuable, but as clinical tools for in vivo diagnosis and treatment of human disease, their use is limited by the fact that mouse protein is antigenic in humans and eventually would be rejected by **the** body as a foreign

Figure D-2.—Preparation of Monoclinal Antibodies



SOURCE: Office of Technology Assessment, adapted from Y. Baakin, "In Search of the Magic Bullet," *Technology Review,* October 1982, pp. 19-23.

substance. Thus, for in vivo application, MAbs must be produced from a human myeloma/human spleen cell fusion. Several investigators and many new biotechnology firms have reported development of human myelomas that are suitable for hybridoma preparation, and successful fusions apparently result from using these cell lines (17).

DIAGNOSIS AND DETECTION USING MAbs

The ability of MAbs to '(zero in" or target very specific antigens could theoretically give rise **to as** many detection tests as there **are** antigens. Clinical trials for MAb-based diagnostics **are** under way in medical centers all around the country and the Food and Drug Administration (FDA) has already approved some 30 MAb-based diagnostic kits. Although the greatest potential diagnostic application of MAbs may be for cancer, MAbs are being used most successfully in diagnostic kits to test for viral and bacterial infection and other factors. MAbs are also expected to find expanded application in detecting, purifying, and/or measuring such things as enzymes, hormones, plasma proteins, drugs, and micro-organisms, leading to better prevention and treatment of chronic disease.

Viral and Bacteria] Infections.-A major problem in clinical microbiology is the inability to rapidly identify the particular agent responsible for clinical disease. In many instances, infectious organisms require from 3 days to 3 weeks for culturing. As a result, physicians often prescribe a broad-spectrum drug rather than wait for laboratory data. MAb technology and probe technology (discussed later in this section) offer the potential for rapid and highly specific tests to replace current culture techniques for direct detection of infectious organisms.

Infectious diseases are a major cause of hospitalization in the United States, accounting for one-quarter of all hospital admissions (11). While hospitalized, patients with weakened or deteriorated immune function (e.g., elderly patients or patients undergoing chemotherapy) are often plagued by "opportunistic" infections that may jeopardize recuperation. Several days may pass before the infection can be diagnosed and treatment initiated. Many U.S. companies are developing MAb-based diagnostic products for both viral and bacterial infections—including two major pathogens, Websiella and ***Pseudomonas.*** These very accurate diagnostics can be read rapidly (often within hours).

Use of MAbs in virology has made it possible to identify new substrains of many viruses and to make distinctions between isolates from different parts of the world (11). The ability to identify and differentiate among the substrains is expected to help in the preparation of more effective influenza vaccines, Vaccines against such viruses will be especially useful among the elderly population, in whom influenza can be fatal.

Cancer. -Cancer cells have characteristic proteins called tumor-associated antigens as cell surface markers or as internal cellular markers that may be shed into the blood or other body fluids. Although no antigen that occurs exclusively in cancer cells has been identified, tumor-associated antigens that are relatively restricted to cancer cells, or to certain kinds of cancers (as opposed to normal cells), have been identified. Additionally, certain types of malignancies, such as B-celI tumors, possess quite distinctive markers which allow useful characterization. At this time MAbs have been generated to recognize antigens associated with almost all human malignancies and have been made and used in clinical trials for a number of different cancers. MAbs are also being made against carcinoembryonic antigen, a proposed marker for colon cancer, as well as antigens such as alpha-fetoprotein, a protein that can be used to diagnose early stages of liver cancer (14). TWO MAb-based diagnostic kits have already been approved by the FDA for carcinoembryonic antigen, as have other kits for prostatic acid prosphatase and human chorionic gonadotropin, hormones used as indicators for prostatic or testicular cancer. In other cases, MAb reagents are being used to identify tumor cells by staining tissue specimens.

The currently available range of human MAbs is likely to be greatly extended in the next few years as more and more labs make use of hybridoma techniques. New MAbs are likely to prove useful in diagnosis and subtyping of carcinomas, sarcomas, and other related neoplasms (16). Moreover, a greater number of MAbs will be available to monitor the level of antigens in the body, thereby determining the effectiveness of treatment and extent of diseases.

Radioimmunoimaging.–The process of locating tumors in the body, large or small, early or late, can be facilitated by injecting radiolabeled MAbs specific to tumor markers into the body. The MAbs can then be read with ordinary imaging equipment and the location of the tumor identified. Radiolabeled MAbs can also be used to track metastasis of cancers. One of the attractions of radioimmunoimaging is that it has the potential not only to provide information about the size and location(s) of tumors, but to determine whether the cancer has metastasized. For example, MAbs can be developed that distinguish malignant from normal cells in the peripheral blood and bone marrow of patients with acute lymphocytic leukemia (15).

Imaging can also help physicians to determine which type of therapy may be appropriate. For example, knowing where the MAb has distributed throughout

the body will tell the clinician whether it would be appropriate to attach a therapeutic agent to that same antibody. If there is sufficient localization, and if the antibody is not localizing at some other crucial tissue that would be irreparably damaged by the therapy, immunotherapy might be the treatment of choice.

In addition to their application in locating tumors, radiolabeled MAbs specific to fibrinogen are also being developed to locate and characterize blood clots.

Heart Attack. -Myocardial infarction, a blood clot-generated heart attack, causes death of heart tissue cells. When heart tissue dies, cardiac myosin (contractile muscle fiber) is released into the bloodstream. MAb diagnostics, based on MAb affinity for myosin, are being developed to signal the beginning of myocardial infarction (13) and to assess the extent of permanent myocardial damage shortly after a heart attack occurs (5). A radiolabeled MAb could be used to identify the damaged part of the heart muscle and to quantify the percentage of muscle damaged. Such a test would enable doctors to decide whether a patient is still at risk from infarction and to prescribe appropriate treatment much earlier.

Brain Disorders.-The main biochemical abnormalities that occur in aging and in dementing disorders, especially senile dementia, appear to involve defects in neurotransmitter synthesis. MAbs have recently been used to isolate specific proteins that are present only on a small subset of neurons in the brain. MAbs can thus be used to detect and quantify minute amounts of neurotransmitters present in different regions of the brain. They are expected to gain increased use in isolating many of the small peptides that appear to function as neurotransmitters. The ability to quantify the level of these neurotransmitters would be useful in studying normal functioning under different circumstances and in examining the transmitter imbalances associated with a variety of neurological disorders like dementia of the Alzheimer type. Detection of distinctive abnormal cellular components, such as the "paired helical filaments" of Alzheimer disease, may also provide earlier and more certain diagnosis.[1]

Development of MAbs promises to make the identilcation and tracing of cell components more precise. Using MAbs, scientists can locate and study cells and cell types in the nervous system that share a common function by identifying particular molecules in the midst of complex nerve tissue. Transmitter receptor makeup is being investigated by this technique with potential benefits for diagnosing or treating diseases. It is expected that MAbs will soon help answer fundamental questions about neurobiology, such as how nerve terminals recognize and interact with correct target cells, perhaps resulting in a wider understanding of brain disorders.

[1]See ch. 3 for a discussion of the aging brain and dementia.

PURIFICATION

Because of their unique properties of homogeneity, specificity, and affinity, MAbs can be used effectively to purify molecules, especially proteins. Various important proteins, including leukocyte interferon, are already being purified using MAbs (17). Many others, including a number of protein "growth factors," have been isolated and are being characterized, with the possibility that they may soon be candidates for production by rDNA technology. Furthermore, large-scale production of very pure active vaccines, using MAbs to bind and isolate the antigens, is now possible.

PREVENTION AND TREATMENT USING MAbs

Applications of MAbs to prevent or treat diseases are being pursued on two fronts: 1) administration of MAbs as passive vaccines to protect against specific diseases; and 2) coupling cytotoxic agents (e.g., diptheria toxin, ricin—a plant-derived toxin-or cobra venom) to MAbs that direct the agents to diseased cells (i.e., for drug delivery) (3).

Vaccines.-The technology being used to develop MAbs for diagnosing bacterial and viral infections is also being used to develop MAbs for passive vaccines and treatment of these infections. Very few viruses can be treated with drugs. Because viruses are parasites that live and reproduce inside human cells, it is nearly impossible to develop a drug that can search out and selectively kill the virus without harming the patient.

All viral infections and some bacterial infections are resistant to antibiotics. Worldwide medical research is focused on finding ways to manufacture MAbs for hundreds of known viruses. Human MAbs are currently being developed for the treatment of problematic bacterial infections that are often resistant to conventional antibiotics and commonly occur in hospitalized patients with long-term illnesses (e.g.,E. coli, Klebsiella, and staphylococcus).

Immunotoxins.—With the advent of MAbs, the promise of a "magic bullet" approach to cancer therapy has been revived, In this case, the magic bullet is a tumor-specific antibody to which a toxic substance (immunotoxin) has been chemically linked (2). MAbs produced against unique antigens on the surface of, for example, leukemia cells can be linked to plant or bacterial toxins. Such immunotoxins may then be used for the selective and specific elimination of cells bearing the target antigen on their surface (9). Unlike conventional forms of cancer treatment, which kill both

healthy and cancerous cells and often create serious side effects, it maybe possible to design MAbs tagged with a chemotherapeutic agent that will bind only to proteins on the surface of cancer cells. Several research groups have reported success in using antibodydirected cytotoxic agents (14), marking the first time that tumor cells have been targeted for treatment while leaving normal cells unaffected.[2] The success of these experiments gives credence to the belief that antibody drug, antibody-hormone, antibody-enzyme, or antibody-toxin conjugates may prove therapeutically useful in a variety of disease conditions.

Plasmapheresis. -The specific binding properties of MAbs give rise to potential applications in treating some of the autoimmune diseases-conditions in which the body mistakenly identifies some of its own substances as foreign and manufactures antibodies against them. In myasthenia gravis, for example, antibodies are generated against the acetylcholine receptors in the neuromuscular junction, resulting in progressive weakening of the skeletal muscles. In a process akin to kidney dialysis, MAbs could potentially be used to purify the blood and remove these antibodies, thus improving the condition of the patient. Similarly, MAbs may prove useful in extracting factors implicated in arthritis.

Recombinant DNA

DNA (deoxyribonucleic acid), a universal genetic code, carries all of the information necessary to direct each and every function of every living organism; it contains the complete plan for life itself. Recombi. nant DNA, which includes gene cloning (reproduction), is a technique used to join DNA from different organisms for a specified purpose (e.g., production of a protein). It allows direct manipulation and alteration of the information coded in the genes so that the productive capabilities of the cell can be directed. Genes, composed of different arrangements of DNA, contain the information necessary for the creation and production of specific cellular proteins-compounds that perform most of the necessary functions of the cell. Gene expression is the mechanism whereby the genetic information of a cell is decoded and processed in order to manufacture a product, usually a protein.[3]

Through rDNA, genes from a human cell that are responsible for the production of a desired protein can be inserted in a micro-organism where the protein is expressed (i.e., produced) in large quantities as the micro-organism reproduces (see table D-l). The ability to develop micro-organisms that produce either new pharmaceuticals (e.g., vaccines against cancer) or large quantities of otherwise scarce pharmaceutical compounds (e.g., hormones that regulate immune response or calcium deposition) could revolutionize existing health care. Finally, the availability of pure substances (e.g., proteins) may enable researchers to answer more questions concerning cell biology and medicine.

DIAGNOSIS AND PREVENTION USING rDNA

DNA Probes. -Probes are powerful tools that can be used to recognize and bind to the inherent property of any cell—its DNA. The genetic information encoded by the DNA of each species is unique and, as such, can be used to identify that species. DNA is a double-stranded, helical molecule composed in part of four nucleotide bases—adenine (A), cytosine (C), guanine (G), and thymine (T) (see fig. D-3). When these bases pair up to form the rung-like structures of the DNA molecule, they do it exactly the same way every time—A always pairs with T and C always pairs with G. The pairing is accurate, but not very strong. Thus, when the DNA "unzips," leaving a series of unpaired bases on each strand, a strand with the corresponding sequence of nucleotide bases must be found before another double helix can be formed.

DNA probe technology is based on the fact that DNA is composed of two parallel, complementary strands which are uniquely matched and held together by chemical bonds. If separated, the two complementary strands can find each other and rejoin (known as hybridization), even in the presence of a large number of noncomplementary molecules.

To make a probe, a specific segment of DNA is removed from a biological sample, or a segment of DNA is synthesized to match a segment of DNA thought to exist in the patient. This piece of DNA is then labeled with a substance that allows researchers or clinicians to follow it as it searches for a complementary strand of DNA that has been obtained from the patient and treated so that the double-stranded DNA is separated. If the probe "pairs up" with the complementary strand

[2] 'An investigator at Scripps Clinic recently succeeded in linking cobra-venom factor (CVF) which is itself nontoxic, but is found in cobra venom, to MAbs directed against a specific surface antigen found on a human melanoma cell line. These CVF-conjugated MAbs were subsequently able to specifically kill the melanoma cells. In clinical trials at Johns Hopkins University and Hospital, radioactive iodine has been tagged to MAbs specific to liver tumor cells. Many of the patients have experienced remissions and shrunken tumors; one has been in remission for 3% years (8).

[3] Different proteins perform different functions Many are expected to be useful in the prevention and treatment of chronic conditions among the elderly. Enzymes, for example, are proteins that catalyze biological reactions. Examples include thrombolytic and fibrinolydc enzymes that dissolve blood clots. Hormones, some of which are also proteins, control regulatory functions (e.g., insulin is a protein hormone that regulates sugar metabolism and other functions; and interferon, an immune regulator', regulates the response of cells to viral infections), Other proteins have other spdalized functions (growth factors, for example, regulate the growth of a variety of different body cells such as nerves and bones).

Table D=I.—Some Proteins With Possible Pharmaceutical Applications Being Developed With Recombinant DNA Technology

Class/substance	Size (number of amino acids)	Function	R&D status	Project sponsors	Applications
Human growth regulators:					
Growth hormone (GH)	191-198	Promotes growth	Cloned, expressed, 1979	Genentech (U.S.)/ Kabigen AB (Sweden) UCSF/Eli Lilly (U. S.)	Growth promotion; healing burns, fractures; cachexia
Somatostatin	14	Inhibits GH secretion	Cloned, expressed, 1977	UCSF/Genentech	Adjunct to insulin
Somotomedins	44-59	Mediates action of GH	Cloned, expressed, 1982	Chiron (U. S.)	Growth promotion, regulation
Growth hormone releasing factor (GRF)	44	Increases pituitary GH release	Isolated, sequenced, synthesized, 1982	Salk Institute (U. S.)	Growth promotion
Cacium regulators:					
Calmoduiin	148	Mediated calcium's effects	Determined to be unprofitable	None	Numerous applications in basic research; hypertension
Caicitonin	32	Inhibits bone resorption	rDNA production	Genentech, Amgen (U. S.)	Bone disease therapy
Parathyroid hormone (PTH)	84	Mobilizes calcium; prevents calcitonin excretion	Cloned, but no production	Massachusetts General Hospital	Osteoporosis therapy; calcium metabolism
Reproductive hormones:					
Luteinizing hormone (LH)	Beta chain; 115b	Females: induces ovulation Males: stimulates androgen secretion	Cloning in progress (glycoprotein)	Integrated Genetics (U. S.)/Serono Labs (Itaiy)	Antifertility
Folicle-stimulating hormone (FSH).	Beta chain; 115	induces ovarian growth	Cloning in progress (glycoprotein)	integrated Genetics/ Serono Labs	Reproductive services
Human chorionic gonadotrophin (HCG)	Beta chain; 147	Like LH; more potent	Cloning in progress (glycoprotein)	integrated Genetics/ Serono Labs	Pregnancy testing
Relaxin .,	52	Dilation of birth canal; relaxation of uterus	Cloning in progress (non-glycoprotein)	Genentech	Soften bone connective tissue of reproductive tract; antiarthritic (?)
Neuroactive *peptidex*					
-Endorphin	31	Analgesia	Cloned, expressed	Amgen, others	Analgesia
Enkephalins	5	Analgesia	Cloning in progress	Agen, others	Analgesia
Pancreatic endorphin. . ., .	N.A.[c]	Undetermined	Cloning in progress	Endorphin, Inc.	Analgesia, particularly in childbirth
L y m t					
Interleukin-2	133	Promotes T-cell growth, activity	Cloned, expressed	Ajinomoto Co. (Japan) Japanese Cancer Institute Immunex (U. S.) Cetus (U. S.) Chiron Genex (U. S.) Biogen (U. S.) Genetics Institute (U. S.) Interferon Sciences (U. S.) Quidel (U. S.)	Maintain T-cell cultures; immunotherapy
Thymosin (fraction 5)	10-150	Promotes maturation of bone marrow cells, T-ceil differentiation	Purified, sequenced	George Washington University	Immunodeficiency diseases
Thymosin (alpha 1)	28	Promotes T-helper and T-amplifier functions	Purified, sequenced cloned, 1979	Hoffmann-La Roche (Switz,) Genentech	Systemic lupus erythmatosis; other immune disorders
Thymic hormone factor (THF)	9	Promotes T-helper and T-amplifier functions	N.A.	N.A.	Antiviral protection in immunosuppressed patients
Thymic factor (TFX) ,.. ,.. .	40	Restores delayed-type hypersensitivity	N.A.	N.A.	Cancer treatment
Thymopoietins	49	Inhibits B-ceil differentiation	N.A.	Ortho Pharms. (U. S.)	Reversing immunodeficiencies

Table D-l. -Some Proteins With Possible Pharmaceutical Applications Being Developed With Recombinant DNA Technology—continued

Class/substance	Size (number of amino acids)	Function	R&D status	Project sponsors	Applications
Microphage inhibitory factor (MIF)	N.A.	Inhibits microphage migration	Cell fusion	Denki Kagaku (Japan)	Immunotherapy
Respiratory system:					
Alpha-l -antitrypsin	45,000 molecular weight	Prevents destruction of alveolar walls by elastase	rDNA in yeast	Zymos Corp. (U.S.)/ Cooper Laboratories (Us.)	Emphysema treatment

[a] Armor Pharmaceutical Co. the source of salmon calcitonin in the United States, does not believe that rDNA technology offers significant advantages over chemical synthesis for the production of salmon calcitonin at the present time A New Drug Application is pending for human calcitonin, but this product is 20 times less than salmon calcitonin for the same effects Hence, the economics of human calcitonin production are less advantageous than those of salmon calcitonin production

[b] Most reproductive hormones thus studied are glycoproteins consisting of two polypeptide chains All share a common (89 amino acids long) alpha chain. Biological activity is manifested in the beta chain, and most cloning efforts focus on producing the biologically active component

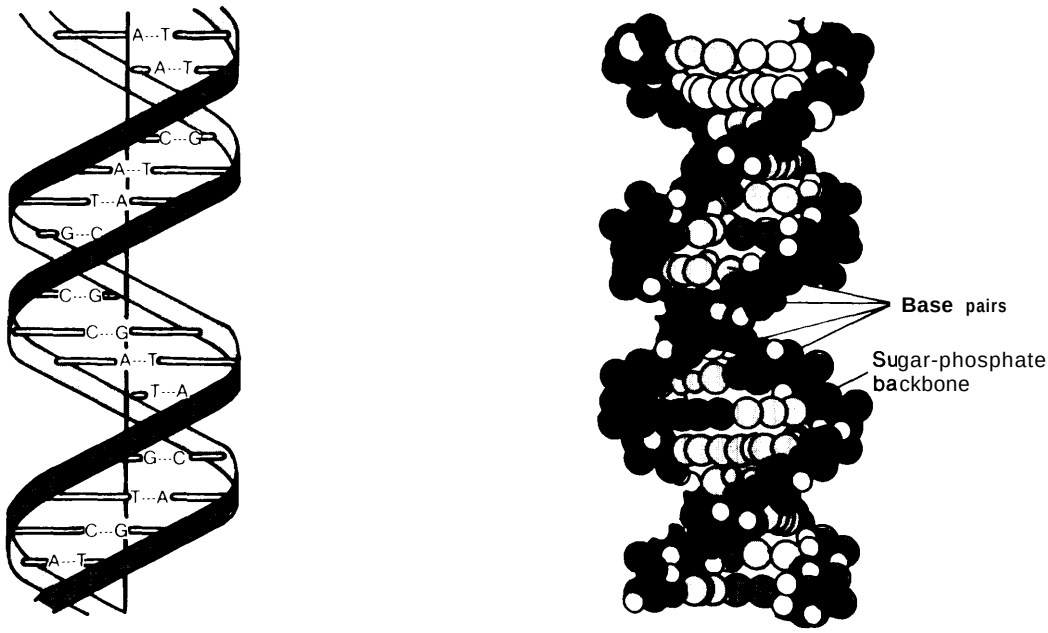
C„A = Information not available

SOURCE Office of Technology Assessment

of DNA from the patient (i.e., if it finds a sequence in the DNA that matches its own), the existence of the organism or gene is confirmed (see fig. D-4). Thus, based on the hybridization property, probes can provide new methods for not only diagnosing infectious diseases but also for predicting genetic disorders and isolating minute quantities of chemicals.

- Viral and bacterial infections. The greatest commercial use of probes appears to be in detecting, within hours or even minutes, viral and bacterial infections. For instance, a probe obtained from a pathogenic organism (e.g., cytomegalovirus (CMV)) can be used to identify the presence of that virus within human ceils, thus allowing specific

Figure D-3.- The Structure of DNA

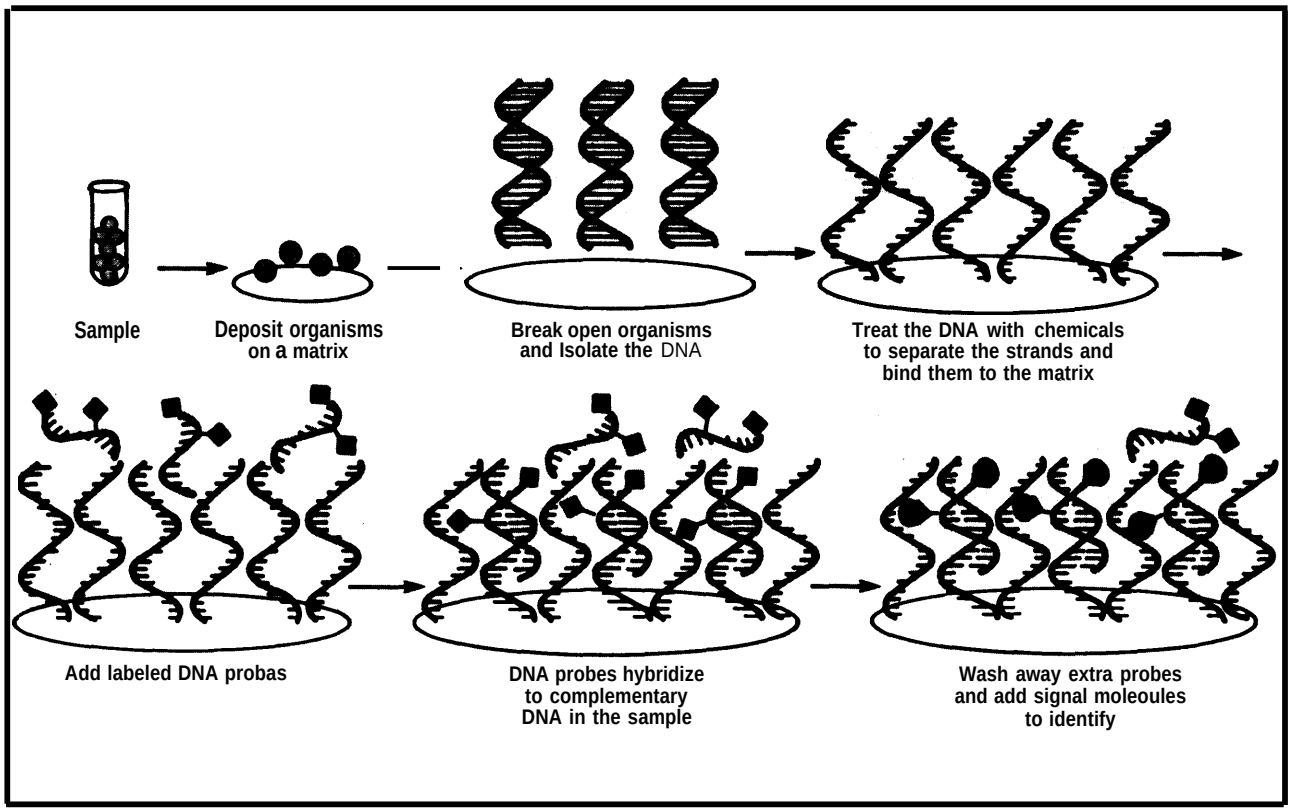


A schematic diagram of the DNA double helix. A three-dimensional representation of the DNA double helix.

The DNA molecule is a double helix composed of two chains. The sugar-phosphate backbones twist around the outside, with the paired bases on the inside serving to hold the chains together.

SOURCE" Office of Technology Assessment.

Figure D-4.—DNA Probe Filter Assay



SOURCE: A. Klausner and T. Wilson, "Gene Detection Technology Opens Doors for Many Industries," Biotechnology, August 1983; Ron Carboni, N. Y., NY., artist.

diagnosis based on whether or not the probe "pairs up" with the DNA in the cell. Currently, probes are being designed to detect a variety of viruses, including CMV, hepatitis B, rotavirus, and herpes.

- Genetic disorders. More than 3,000 human disorders result from defects in the basic genetic makeup of individuals (18). DNA probes are being designed to detect the genes that can cause some of the diseases. By screening individuals for genetic disorders (e.g., Huntington's disease), probes can serve as an early warning system for some diseases. If, in the future, more genes that are responsible for predisposing people to disease (e.g., cancer, mental disorders, heart attack) can be identified, doctors may use probes to identify those at risk. Harvard scientists have recently uncovered a rare inherited genetic defect that predisposes cholesterol buildup in tissues, which leads to heart attacks (7). The defective gene responsible for the disease was identified with a probe. In the future, scientists expect to identify genes that predispose individuals to common pathologies like cardiovascular disease, cancer, and mental disorders. Doctors could then use probes to identify persons who are at risk (4).
- Characterization and isolation of proteins. Aside from their use in screening for disposition to disease, probes are also being used to characterize and isolate many small peptides that act as neurotransmitters or regulators of the release of hormones from the pituitary gland. For example, probes are being used to unravel the mechanics of the brain's control of blood circulation and pressure.

The brain controls blood pressure in two ways: by controlling the release of peptides and hormones that adjust blood circulation in response to emotion and behavior, and by controlling the nerve cells that innervate the blood vessels and the heart. By using DNA probes, researchers are attempting to trace the networks and the dynamics of the interacting transmitters and peptide hormones involved in the brain's control of circulation with the hope of one day being able to control blood pressure. Probes might also ultimately be

used to indicate changes in cellular DNA that accompany aging.

- Cancer. Recent discoveries indicating that some human tumor cells have identifiable oncogenes have spurred research and development efforts in this area (4). Earlier this year, the National Cancer Institute announced a 3-year, $1.8 million grants program to develop genetic techniques for cancer diagnosis, including DNA probes for oncogenes.

Vaccines. -At present, most vaccines are made from the organisms that cause the particular disease they are intended to prevent. These organisms (pathogens) are killed or otherwise treated ("attenuated") to make them nonvirulent. They are then injected as a vaccine. The body responds to the injection by producing antibodies against antigens on the surface of the attenuated organism. These antibodies then circulate in the system, protecting the body from an invasion by a live organism of the same kind. Thus, the immunity is conferred.

Limitations associated with current vaccines include: contracting the disease from incompletely attenuated viruses, incomplete immunization due to changes in the strains of the pathogen, and inefficient immune response to nonliving organisms.

Recombinant DNA is being applied to the production of vaccines that do not use the genetic material responsible for the pathogen's virulence. Instead, the gene for a surface protein of the pathogen is isolated and cloned, and the surface protein is used as the vaccine. Instead of the whole organism, only the surface protein responsible for eliciting the antibody response is used. This permits greater quantities of purer antigens to be used.

Among the elderly, influenza can often lead to death due to respiratory complications. Viral vaccines are being developed through rDNA for influenza types A and B. More effective vaccines such as these could prevent or minimize the effects of viral infections among the elderly,

Several research institutes have begun animal trials to test the efficacy of new vaccines targeted against melanoma and other cancers of the lung, breast, colon, and rectum. The vaccine, a short segment of DNA specific to the hormone human chorionic gonadotropin (hCG), is designed to simulate patients' immune systems to attack the hormone, which plays a role in tumor development. Many tumors, but not all, produce hCG. For instance, about 86 percent of all lung cancers produce hCG, and 50 to 60 percent of all breast cancers produce hCG, which not only helps ensure the survival of the tumor by fending off the body's immune system, but also works as a growth hormone to spur its development. Thus, if a vaccine could be delivered so that the immune system would fight hCG, further development of the tumor might be halted. More practically, the vaccine could be delivered after the tumor is removed to ensure complete eradication of the tumor cells.

TREATMENT USING rDNA

Therapeutic proteins normally present in human blood (e.g., clotting factors, antibodies, enzymes, certain hormones) are available only in limited supply; the only possible sources are animal or human blood, tissues, or urine. Recombinant DNA promises to provide an alternative route to the production of proteins for therapeutic purposes.

Thrombosis. -Thrombosis, the blockage of blood vessels by blood clots, is a major cause of disabling diseases and death (12). Blood clots, which are made of fibrin, platelets, a mixture of blood cells, red cells, and leukocytes, can cause heart attacks when lodged in the heart. When lodged in the brain, clots can cause strokes; in the lungs they can cause pulmonary embolisms.

Use of rDNA to make new and safer anticoagulants could reduce the incidence and severity of diseases induced by thrombosis by restoring blood flow to the affected heart muscle, thereby preventing or limiting permanent damage. Tissue plasminogen activator (TPA) and kidney plasminogen activator, for example, are two naturally occurring enzymes currently being produced via rDNA, They are being used to treat a wide variety of severe cardiovascular disorders, including heart attacks and arterial blockages. Both enzymes are specific for fibrin, the protein of which clots are made, and both are being developed for injection to dissolve potentially fatal blood clots. Two patients at Washington University in St. Louis, MO, and five at the University of Leuven in Belgium have received TPA treatment. When the TPA reached the heart, it caused dissolution of the clots that were causing the myocardial infarction. If TPA proves effective in further studies, it could be used in emergency rooms and ambulances to treat heart attack victims.

TPA has certain advantages over the two enzymes (urokinase and streptokinase) in current use. First, neither urokinase nor streptokinase are specific for blood clots (i.e., fibrin) (12). Both destroy other blood proteins. In order to avoid hemorrhaging, cardiac catheterization and coronary angiography are necessary to administer the enzymes directly to the site of the clot. Furthermore, streptokinase, because it does not occur naturally in humans, often elicits an allergic reaction that makes repeated treatments impractical.

Heart Attacks.—Renal renin, an enzyme produced by the kidneys, governs the release of angiotensin, which constricts blood vessels and raises blood pressure. Because the kidneys secrete only small amounts of renin that vanish quickly, the amount produced is insufficient to allow amino acid sequencing. With rDNA it should be possible to express human renin in host organisms and to accumulate enough for structural studies (6). Once renin's threedimensional structure is determined, an analog can be developed that would bind to the renin receptor site, thereby inhibiting renin's effect. Renin analogs are being developed through rDNA with the hope that high blood pressure may soon be better controlled.

Emphysema. —Emphysema is caused in part by the gradual attack of lung tissue by natural enzymes (10). This lung deterioration can be accelerated by environmental factors such as air pollutants, bacteria, or cigarette smoke, or it can be caused by a congenital deficiency of alpha-l antitrypsin (AAT)—a protein which counteracts degradative enzymes produced by the body to destroy foreign particles in the lungs. Without AAT, surplus enzymes attack lung tissue itself, causing emphysema. The availability of AAT could potentially be used to correct the condition in patients with the deficiency, as well as to prevent further deterioration in those with emphysema.

Necrologic Disorders. -Recent attention has focused on the variety of peptides found in the brain and other parts of the body that are assumed to function as neurotransmitters or neuromodulators. Included in this group are chemicals important in modifying pain, emotional response, and muscle tone; peptides implicated in seizure and postseizure events; and others active in controlling brain mechanisms of cardiovascular regulation and food intake. With the use of MAbs and DNA probes to isolate and characterize these peptides, coupled with **rDNA to produce sufficient quantities for research (instead of purifying extracts made from large quantities of brain tissue), doctors may one day be in charge of a revolutionary arsenal of new compounds able to treat conditions (e.g., dementia) arising from necrologic disorders.**

Cholecystokinin, for example, a peptide found in the gastrointestinal tract, was also recently found in the brain (I). Studies of genetically obese mice, which are obese because of excessive food intake, show the brains of these mice to contain three to four times less cholecystokinin than the brains of normal mice. Adding the peptide to the systems of the obese mice reduced their voracious eating. Information derived from such experiments may in the future serve to treat the problem of human obesity.

Immune Regulation **and Modification** (lymphokines and thymic hormones) .—Lympokines, such as interleukin-2 and interferon, are immune mediators and are produced by white blood cells (lymphocytes) when these cells are exposed to foreign bodies or alien cells. They regulate the response of the cells to microbial infections and cancer proliferation. Their presence (or absence) is thus crucial to the body's immune system.

The importance of lymphokines in preventing disease and understanding cellular function is fostering widespread research on these compounds. It is hoped that research efforts may lead to the use of lymphokines to stimulate the patient's own immune system to combat disease. For example, patients who are undergoing chemotherapy or radiation therapy may die from an unrelated infection such as pneumonia because their immune systems have been severely damaged. Interleukin-2, a lymphokine now being produced through rDNA, has been shown to restore immune balance in mice undergoing chemotherapy. The same results are now being sought for human applications.

Interferon, a class of immune regulators being produced through rDNA, is being considered for various pharmaceutical applications, including treatment for viral infections and cancer. In some clinical trials, interferon has inhibited tumor cell growth, although its effects on inhibiting tumor metastasis are better established than its ability to cause regression of primary tumors **(17).**

Thyrnic *Hormones:* The thymus appears to be an endocrine organ with the capacity to synthesize many different hormone-like products. These individual hormones probably act to regulate selective aspects of T-cell differentiation (into killer, helper, and suppressor T-cells). The rationale for employing thymic hormones therapeutically in adults is based on the observation that circulating thymic hormone levels decrease dramatically with age as the thymus involutes, decreasing the efficiency of immune function. It has been suggested that the increased incidence of autoimmune diseases and cancers in the elderly population might reflect the loss of homeostatic control governed by the thymus ***(16).***

Clinical trials using thymic peptides suggest that thymic hormones exert immunorestorative effects when administered to patients with T-cell immunity. Randomized trials in cancer patients have indicated

*T-cells are lymphocytes produced by the thymus Some T-cells recognize antigens and send messages to B-cells, which start making antibodies. Some T- cells assist B-cells in making antibodies; others suppress the same process; still others are themselves capable of seeking out antibody-bearing cells and killing them

that thymosin fraction 5 (which contains a number of active peptides) may be efficacious when administered as an adjunct to conventional chemotherapy or radiation therapy by reducing the immunosuppressive side effects of radiation and chemotherapy and helping the patient to mount a response to the disease. Other thymic factors (i.e., thymic humoral factor and thymic factor X) have shown promise in treating a variety of infectious diseases caused by adenoviruses. Potential therapeutic applications are also being investigated for autoimmune diseases. It is thought that some thymic hormones may help normalize the aberrant immuno - regulatory cell activity that is characteristic of these diseases.

Conclusion

Many future pharmaceuticals, which cannot yet be identified, are likely to emerge during this decade from the basic work in biotechnology now under way. Their development would provide new therapies for many of the conditions that impair the functional ability of many of today's elderly. The advances arising from current basic research are expected to generate both new products and greater understanding of the aging process. This understanding should lead to significant reductions in the severity of chronic illness among the elderly and in the cost of health care for this growing segment of the U.S. population.

Appendix D references

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