Human Insulin from Recombinant DNA Technology

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Summary. Human insulin produced by recombinant DNA technology is the first commercial health care product derived from this technology. Work on this product was initiated before there were federal guidelines for large-scale recombinant DNA work or commercial development of recombinant DNA products. The steps taken to facilitate acceptance of large-scale work and proof of the identity and safety of such a product are described. While basic studies in recombinant DNA technology will continue to have a profound impact on research in the life sciences, commercial applications may well be controlled by economic conditions and the availability of investment capital.

During 1982, human insulin of recombinant DNA origin was approved by the appropriate drug regulatory agencies in the United Kingdom, the Netherlands, West Germany, and the United States. This new source guarantees a reliable, expandable, and constant supply of human insulin for diabetics around the world.

The research, development, and production of human insulin by recombinant DNA technology ushers in a new era in pharmaceuticals, agricultural products, and industrial chemicals by establishing the feasibility of commercial production of a gene product initiated at a laboratory level of expression. I shall review how human insulin became the first human health product of this technology. I will also discuss some of the special problems, in terms of regulatory environment and public opinion, that had to be overcome in order to bring it to the current stage of development.

Sources of Insulin

Eli Lilly and Company has been involved in the development and manufacture of insulin and other products for diabetics since 1922. In that year our scientists began working with Frederick G. Banting and his associates at the University of Toronto to develop a standardized and clinically acceptable insulin product. Banting had just begun to extract relatively crude insulin from animals and inject it into his diabetic patients.

In the early 1970's we began to be concerned about a possible shortage of insulin. Until now, the world's insulin needs have been derived almost exclusively from pork and beef pancreas glands, which were collected as by-products from the meat industry. This supply changes with the demand for meat and is not responsive to the needs of the world's diabetics. Indeed, from 1970 to 1975, the supply of pancreas glands in the United States declined sharply (*1*) and remained on a plateau at that lower level in succeeding years. There is no accurate way to predict availability of future supplies of glands, although we predicted that the demand for insulin would continue to increase. Our concern was whether or not there would be a time when the supply of bovine and porcine pancreas glands might not be sufficient to meet the needs of insulin-dependent diabetics. Although it is difficult to obtain substantiated figures for a nonreportable disease, we estimate that there are 60 million diabetics in the world—more than half of them in less developed countries. In the developed countries, some 4 million diabetics, 2 million in the United States, are treated with insulin.

Today, the diabetic population is growing more rapidly than the total population. While the U.S. population is increasing at a rate of about 1 percent per year and the world population at slightly more than 2 percent, the annual rate of increase of insulin-using diabetics in this country has been 5 to 6 percent in recent years, and a similar pattern may hold true worldwide (*2*).

Several factors contribute to this accelerated growth of the insulin-using diabetic population. One factor, of course, is the availability of insulin, which enables diabetics, who often did not survive beyond their teens, to live long, productive—and reproductive—lives. Because of the genetic etiologic component of diabetes, the offspring of diabetics are likely to suffer from the disease as well. Other factors that contribute to growth of the diabetic insulin-using population include improved methods of detection, greater public awareness of the disease and its symptoms, less reliance on oral forms of therapy, and changes in dietary habits.

Because of the uncertainty of the insulin supply and the forecasts of rising insulin requirements, it seemed not only prudent but a responsibility as well for the scientific community and insulin manufacturers to develop alternatives to animal sources for supplying insulin to the world's diabetics. Lilly established several internal committees of scientists to examine various solutions to the problem. They considered augmentation of insulin production from pancreas glands, transplantation of islet of Langerhans cells, chemical synthesis, beta cell culture, directed-cell synthesis, and cell-free biosynthesis, as well as insulin replacements. These discussions touched on the technology called genetic engineering.

The function of DNA in a cell is to serve as a stable repository of coded information that can be replicated at the time of cell division to transmit the genetic information to the progeny cells and to encode the information necessary to synthesize proteins and other cell components. There are several ways of performing genetic engineering, some of which have been practiced for many years by geneticists. The first is mutation. Mutations in DNA can be either spontaneous, due to environmental factors and errors in DNA replication, or they can be induced in the laboratory by physical and chemical agents. Mutations can lead to a change in the structure of the product coded for by the gene in question; sometimes this change in structure is so great that the product is

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vastly different. Other mutations may affect the regulatory elements that control the expression of the structural gene, leading, in some instances, to increased or decreased production of gene products. A key point is that mutagenesis is an essentially random technique.

A second type of genetic engineering, recombination, has also been used for a long time. Recombination refers to exchange of a section of DNA between two DNA molecules. Recombination of DNA fragments from different organisms can occur by the mating of two organisms—a process called conjugation—where DNA is physically transferred from one organism to another. This is a process occurring in nature, which can be duplicated in the laboratory. Two other natural processes whereby cells exchange DNA in nature are transformation and transduction. Recombination may also occur following the use of a technique known as protoplast fusion, where one literally strips off the outer cell wall of cells of fungi and bacteria. This phenomenon only occurs in the laboratory and allows the remaining protoplasts, which now have just a cell membrane enclosing the components of the cell, to fuse together. The fused protoplasts contain the DNA molecules of both parents, and exchange of sections of DNA can now occur as these cells regenerate and divide. All these recombination processes involve random exchange of DNA sequences, and this exchange is generally, but not always, limited to members of a single species of organism.

In 1972, Jackson, Symons, and Berg (*3*) described the biochemical methods for cutting DNA molecules from two different organisms, using restriction enzymes, and recombining the fragments to produce biologically functional hybrid DNA molecules. In 1973, Cohen, Chang, and Boyer (*4*) reported that they could make a hybrid molecule that would express the foreign DNA within it as though it were a part of the original molecule's natural heritage (*5*). That profoundly significant accomplishment also generated major concern over potential biohazards.

Regulating DNA Research

The Berg Committee was formed, and it responded to concerns about conjectural risk associated with recombinant DNA research in 1974 by calling for a moratorium and deferral of certain types of recombinant DNA research until the scientific community could evaluate the risks and benefits associated with it (*6*). Work was halted—including work in my own organization—until after the Asilomar Conference in 1975 (*7*).

The majority of scientists invited to the Asilomar Conference were molecular biologists from government and academia; those with expertise in infectious disease or those from industry with extensive knowledge of large-scale fermentation processes and other techniques that require careful methods of containing potentially harmful materials were underrepresented. As a result, many of us believe that the guidelines defined at Asilomar were unnecessarily restrictive. An example was the establishment of the 10-liter limit. To those of us in industry, this restriction was never considered reasonable. We were accustomed to handling containment problems at much larger volumes. But few of the scientists at Asilomar conceived of performing large-scale fermentation with recombinant organisms; as noted in the British journal *Nature*, "[In 1975] even optimists would have predicted that it would be a decade before genetic engineering would be commercially exploited" (*8*). This volume limit was established because it was regarded as probably the largest volume that could be conveniently handled in an experimental laboratory by conventional laboratory centrifuges. It was clear that the 10-liter limit excluded industrial-level activity. It was not suggested that increased volume of a culture of a safe organism would result in any increased risk. The conjectural nature of the early concerns soon became clear, and broader participation in research decisions by those expert in infectious diseases, containment, and risk-assessment, as well as more practical experience, led inevitably to revisions and continued relaxation of restrictions around the world.

In June 1976, the National Institutes of Health (NIH) announced guidelines for recombinant DNA work, marking the end of the 2-year moratorium on this type of research. Only research financed by the federal government was subject to the guidelines, for which Lilly, with other companies, NIH, the Pharmaceutical Manufacturers Association (PMA), the Food and Drug Administration (FDA), and the Department of Health, Education and Welfare (HEW), was actively involved in developing compliance procedures.

At Lilly, work on DNA recombination, which had been under way before Jackson, Symons, and Berg (*3*) published their work, resumed vigorously. We contracted with a new California company, Genentech, Inc., for specific work on human insulin. Genentech subcontracted the synthesis of the human insulin gene to the City of Hope Medical Center, which succeeded in its mission. Scientists at Genentech inserted the genes for both chains of insulin into a K-12 strain of *Escherichia coli* and after isolation and purification, the A and B chains were joined by disulfide bonds to produce human insulin. In the Lilly Research Laboratories, we have also used recombinant DNA technology to produce human proinsulin, the insulin precursor.

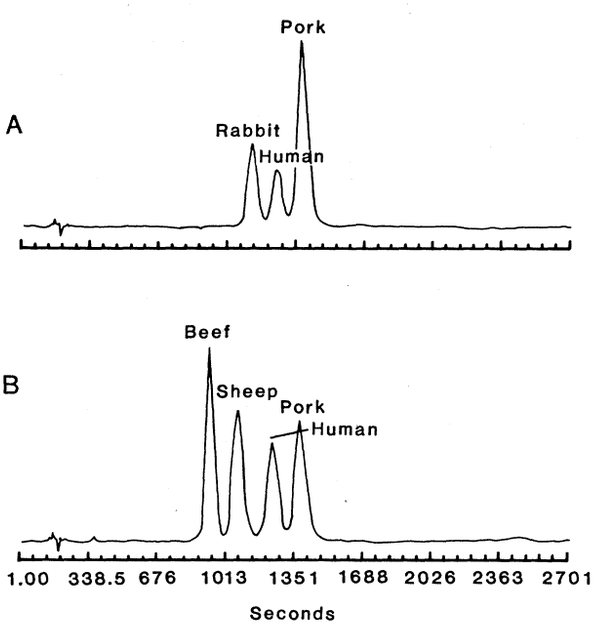

Fig. 1. HPLC chromatograms of insulins which differ by one or more amino acids.

Human Insulin

The successful expression of human insulin (recombinant DNA) in *E. coli* was announced on 6 September 1978. This was a first step. Although we had been successful in obtaining expression of the hormone under laboratory conditions and scale, we still faced the equally difficult challenge of achieving satisfactory production of the purified product on a commercial scale. The process we used in accomplishing large-scale production has been described (*9–11*), but it may be useful to touch on some of the methods that we employed to prove that the product produced was indeed human insulin.

High-performance liquid chromatography (HPLC) techniques developed at Lilly can detect proteins that differ by a single amino acid (*10*), and HPLC tests showed that human insulin (recombinant DNA) is identical to pancreatic human insulin and that it is close to, but not the same as, pork insulin, which differs from the human by one amino acid; beef, which differs by three amino acids; and sheep, which differs at four residue positions (Fig. 1). A chromatogram of human insulin (recombinant DNA), pancreatic human insulin, and a mixture of the two, showed that they were superimposable and identical (Fig. 2). HPLC has become an important analytical tool to determine structure and purity and is now considered to be a more precise measurement of potency than the rabbit assay, although most government regulatory agencies around the world still emphasize the rabbit potency assay.

A measure of the correct tertiary structure and appropriate folding is the circular dichroic spectrum. The spectrum for porcine insulin and for human insulin (recombinant DNA) were found to be identical. X-ray crystallographic studies further revealed the structural integrity of the recombinant molecule (*12*). We also found the amino acid composition of human insulin (recombinant DNA) and pancreatic human insulin to be identical (Table 1). In addition, we compared polyacrylamide gel electrophoresis for human insulin (recombinant DNA), pancreatic human insulin, and pork insulin, as well as isoelectric focusing gels for these three insulins.

Another technique that we found useful for ensuring that we had the appropriate disulfide bonds and lacked other types of protein or peptide contaminants was HPLC of a specifically degraded sample. There is a staphylococcal protease that cleaves insulin in a specific way at five sites—always next to glutamic acid, except for one site between serine and leucine. After treating the insulin with the protease, we looked for and identified the various peptide fragments by HPLC (Fig. 3) and found none that were not derived from insulin.

In the end, we employed 12 different tests to establish that what we had produced was human insulin. We believe the correlation among three of the tests was particularly important—the radioreceptor assay, the radioimmunoassay, and HPLC. Moreover, the pharmacologic activity of human insulin (recombinant DNA), as demonstrated by a rabbit hypoglycemia test, showed a response essentially identical to pancreatic human insulin.

Another serious question remained to be answered—namely that of the potential contamination of the product with trace amounts of antigenic *E. coli* peptides. Relevant to this question is the difference in starting materials between human insulin of recombinant DNA origin and pancreatic animal insulins. The glandular tissue is collected in slaughterhouses, with no control over bacterial contamination. The desired gene product is isolated from a few cells of the islets of Langerhans, which make up less than 1 percent of the glands; thus more than 99 percent of the tissue represents tissue contaminants and undesirable materials. The common protein contaminants of the animal insulins are other pancreatic hormones or proteins, many of which are highly immunogenic.

In contrast, with recombinant DNA production of human insulin, almost 100 percent of the cells (*E. coli*) produce the desired gene product. Because of the method of manufacture, none of the pancreatic contaminants of the animal insulins are found in the human insulin of recombinant origin. The issue of proteinaceous contamination derived from the bacterial host cell was addressed through some experiments that were made possible by running large-scale fermentations of the production strain of *E. coli*, which contains the production plasmid with the code for the insulin chain sequence deleted. The small quantities of peptides isolated after applying the chain purification and disulfide linking process to the "blank" preparation were shown not to be antigenic except in complete Freund's adjuvant (*13*); in addition, no changes in amount of antibody to *E. coli* peptides were detected in serum from patients who had been treated with human insulin for more than a year (*14*).

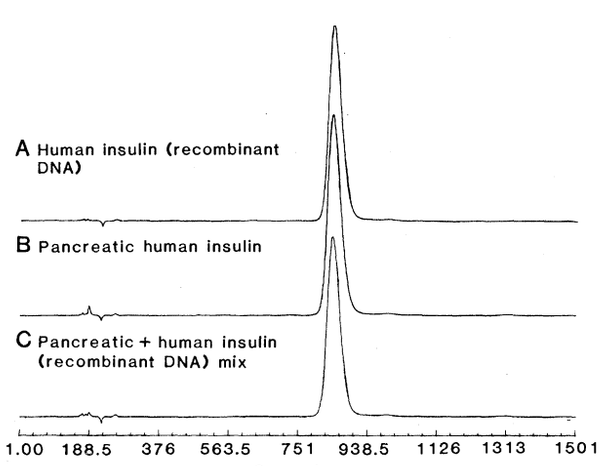

Fig. 2. HPLC chromatograms of human insulin (recombinant DNA), pancreatic human insulin, and mixtures of the two showing identity.

Commercial Production

As we were scaling up this new technology for commercial production, we recognized that there would be external problems and forces with which to contend. Because this would be the first human health care product resulting from recombinant DNA techniques, we expected that many people would perceive that there were risks associated with this new scientific tool. We also recognized that the existing regulatory systems had not been designed to cope with the new technology. The public's concern reached such levels that some communities, most notably Cambridge, Massachusetts, passed ordinances regulating recombinant DNA research (*15*). In Congress, several bills were introduced to regulate the research. Some of these would have subjected all recombinant DNA research, public or private, to federal regulation (*16*). It was probably fortunate that none of the bills was enacted into law, as former Representative Paul Rogers (D–Fla.) noted: "I think Congress was right [in not regulating rDNA research]. Congress did a good service in airing the issue, but there wasn't a necessity to pass a law" (*17*).

The regulatory system, too, adapted well to this unexpected challenge to its flexibility. On 22 December 1978, the FDA had published in the *Federal Register* a "Notice of Intent to Propose Regulations" governing recombinant DNA work. But, by the time that the FDA's Division of Metabolism and Endocrine Drug Products convened a conference on the development of insulin and growth hormone by recombinant DNA techniques (*11*) in mid-1980, attitudes had changed, and the regulations were never promulgated.

Concerns about the containment of potentially harmful organisms fell under the purview of the Recombinant DNA Advisory Committee (RAC) and the National Institute of Occupational Safety and Health (NIOSH). RAC, established in 1974 by the secretary of HEW, had 11 members, all of whom were scientists. In December 1978, 14 more members were added to RAC; all of the new members were nonscientists. It was apparent that the nonscientists would have to rely heavily on the scientists to develop their understanding of the new technology. Lilly scientists participated actively in all aspects of public discussion, through testimony in both houses of Congress, participation in the open forum of the National Academy of Sciences (*18*), and in meetings of RAC, and by submitting comments and amendments to NIH for its guidelines.

In June 1979, Lilly made the first application to RAC for an exception to the rule limiting recombinant DNA work to 10-liter volumes. At its meeting in September 1979, RAC recommended that our request to scale up production of bacteria-derived insulin be approved, and a month later the director of the NIH granted us permission to use 150-liter containers. In 1980, permission to expand to 2000-liter containers was granted. This was a major step toward a production type of operation; the submission to RAC contained detailed engineering specifications for equipment and monitoring systems as well as descriptions of the proposed operating procedures. Because of the unprecedented volume increase in the handling of cultures of recombinant organisms, the scale-up request was preceded by a visit to our plant by a group consisting of RAC representatives and NIH officials; they came to see for themselves how we could handle containment problems. With the experience gained at these intermediate levels, we are now routinely using 10,000-gallon fermentors.

Throughout 1980, there were several other positive developments. NIH published in the *Federal Register* draft guidelines on physical containment recommendations for large-scale uses of organisms containing recombinant DNA molecules. This draft was not formally a part of the guidelines, but it did serve as a model for persons preparing submissions to RAC for large-scale fermentations with recombinant organisms. About the same time, the National Institute of Allergy and Infectious Diseases sponsored a workshop on risk assessment. Among the issues discussed were risks associated with pharmacological action of hormones from recombinant organisms populating the human intestinal tract, medical surveillance of workers involved in large-scale fermentation of recombinant organisms, pathogenesis of approved recombinant hosts, and containment practices in commercial-scale fermentation facilities. Most participants indicated that there was little or no risk involved in these practices. A few months later, the industrial practices subcommittee of the Federal Interagency Advisory Committee (FIAC), a working group of representatives from all the cabinet-level departments as well as all federal agencies that are in any way affected by recombinant DNA issues invited Lilly to make a formal statement. Bernard Davis of the Harvard Medical School and I submitted a document on the safety of *E. coli* K-12, the reliability of commercial-scale equipment, operator training, and other topics; this was favorably received. NIOSH also published a favorable report of its on-site inspection of Lilly Research Laboratories' recombinant DNA research facilities and procedures for large-scale fermentations of recombinant organisms.

In July 1980, we began clinical trials of our human insulin in the United Kingdom. Within weeks, similar tests were under way in West Germany and Greece and, finally, in the United States. Plants, specifically designed for the large-scale commercial production of human insulin (recombinant DNA), were built at Indianapolis (Fig. 4) and at Liverpool in the United Kingdom. On 14 May 1982, we filed our new drug application for human insulin with the FDA.

Clinical studies with human insulin (recombinant DNA) indicate its efficacy in hyperglycemic control. It appears to have a slightly quicker onset of action than animal insulins. In double-blind transfer studies with animal insulins, patients previously treated with mixed

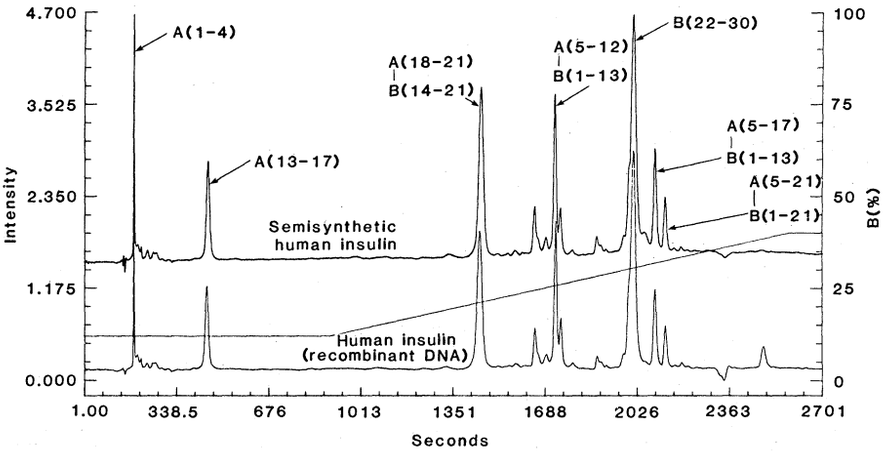

Fig. 3. HPLC chromatograms of peptide fragments from the A and B chains of semisynthetic human insulin and human insulin (recombinant DNA) after treatment with a specific staphylococcal protease. These chromatograms indicate the correctness of the disulfide bridges and the lack of any other major peptide components.

Table 1. Amino acid compositions of human insulins. Molar amino acid ratios with aspartic acid as unity [actual aspartic acid yields were 160 nanomoles per milligram for human insulin (recombinant DNA) and 156 nanomoles per milligram for pancreatic human insulin (*10*)].

Amino acid	Recombinant DNA	Pancreatic
Aspartic acid	3.00	3.00
Threonine	2.77	2.77
Serine	2.56	2.63
Glutamic acid	7.11	7.10
Proline	1.03	0.99
Glycine	3.98	3.98
Alanine	0.97	0.99
Half-cystine	5.31	5.43
Valine	3.76	3.71
Isoleucine	1.66	1.61
Leucine	6.16	6.14
Tyrosine	3.91	3.90
Phenylalanine	2.99	2.91
Histidine	1.97	1.99
Lysine	0.97	0.97
Ammonia	6.89	6.95
Arginine	1.00	1.00

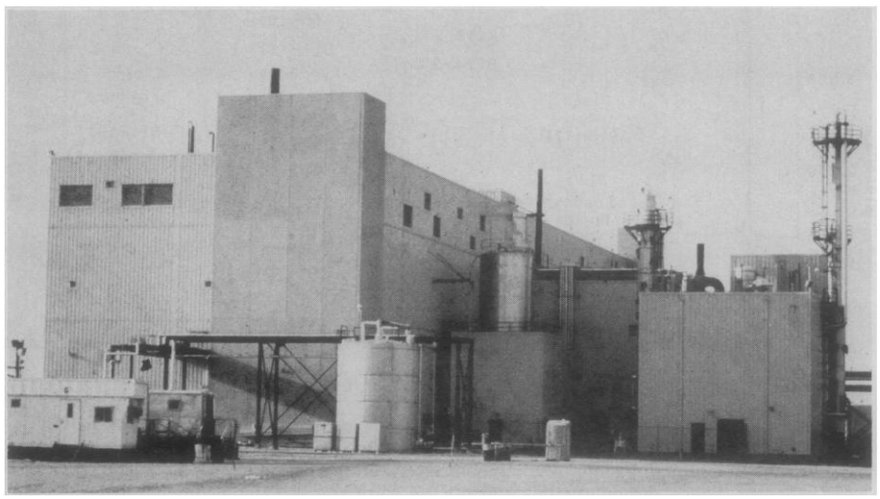

Fig. 4. The new production plant in Indianapolis for human insulin produced by recombinant DNA technology.

beef-pork insulin had a 70 percent decrease of bound insulin in comparison with a base line. Species-specific binding of human, pork, and beef insulin at 6 months decreased by 61, 58, and 57 percent, respectively. In patients previously treated with pork insulin, the bound insulin decreased by 30 percent in control subjects treated with pork insulin, and by 51 percent in patients transferred to human insulin. Species-specific binding of beef and human insulins decreased equally whether patients were maintained on purified pork insulin or switched to human insulin. Species-specific binding for pork insulin, however, remained constant in both groups (*19*). The clinical importance of these findings remains to be clarified in long-term studies. Occasional patients hypersensitive to animal insulins and semisynthetic human insulin derived from pork insulin tolerated human insulin (recombinant DNA) well. Recombinant technology now permits us to study human proinsulin and mixtures of human proinsulin and insulin much as they are secreted by the beta cell. These studies may provide an improved modality of therapy in diabetes.

The power of recombinant DNA technology resides in its high degree of specificity, as well as the ability it provides to splice together genes from diverse organisms—organisms that will not normally exchange DNA in nature. With this technology, it is now possible to cause cells to produce molecules they would not normally synthesize, as well as to more efficiently produce molecules that they do normally synthesize. The logistic advantages of synthesizing human insulin, growth hormone, or interferon in rapidly dividing bacteria, as opposed to extracting these from the tissues in which they are normally produced, are obvious.

We have shown the practicality of using recombinant technology to produce proteins of pharmacological interest as fermentation products. This was accomplished without adverse environmental impact or increased risk to workers. At this point it seems reasonable to speculate about the future of this new technology.

Impact of the Technology on Industry

A whole growth industry largely dependent on investor interest has developed. Through newsletters, conferences to develop research strategies, market estimates, and so forth, these investors supposedly predict which projects will be brought to fruition through this new biotechnology. It is difficult to estimate the extent to which these prognostications will reflect economic and scientific reality, but there are some items of fact that appear to be supported by fairly simple logic.

In the biomedical area there will certainly be other proteins and peptides of pharmacological interest produced. Some of these are likely to result from new discoveries as additional genes are cloned. As an example, perhaps the most interesting aspect of the cloning of the interferon genes is that they represent a family of genes that code for a large number of interferons, leading to the possibility of producing hybrid molecules that have not been seen in nature. It seems unlikely that interferons should be unique in this respect among cytokines or other biologically interesting messengers.

The technology will probably permit the mapping of the entire human genome during the next decade. Medical geneticists have laboriously mapped human genes by studying electrophoretic variants or phenotypic expression of disease tracked through family trees. It is now possible to isolate individual human chromosomes on a preparative scale, followed by establishment of gene banks or libraries for each chromosome. The work should advance rapidly with an enormous potential impact on new medical research and the understanding of human biology. In addition, it seems likely that eventually we will understand the mechanism of gene control and regulation which, combined with information now being unraveled concerning potent tumor-specific oncogenic DNA sequences, clearly suggests major applications in our understanding of oncology and differentiation. Consider, for example, the recent finding that the point mutation in a normal human gene that leads to the acquisition of transforming properties is due to a single nucleotide change from guanylate to thymidylate. This codon change results in a single amino acid substitution of valine for glycine in the 12th amino acid residue of the T24 oncogene encoded p25 protein; it appears to be sufficient to confer transforming properties on the T24 human bladder oncogene (*20*).

Assumptions can be made about applications to agriculture as well. It seems incontrovertible that in some areas, for example, the amount of productive land is decreasing because of the fall of water tables and sometimes increasing salinity of ground water. Moreover, the number of people producing crops is decreasing while the population dependent upon them continues to increase. Recombinant technology, in combination with conventional plant breeding, plant cell culture, and regeneration, may well result in the production of new plants. Such plants could increase the productivity of existing farmland as well as permit farming on land currently considered to be nonproductive. Equally important applications are technically feasible in the animal husbandry area, and many other types of applications—in the fermentation industry, industrial chemicals, environmental clean-up—have been suggested.

We can certainly debate how rapidly these further developments will occur and whether or not they will be economically feasible. However, we must all be

impressed with the speed with which the technology has progressed since 1974 and can be confident that if we invest wisely, this rate will be maintained or even increased.

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21. Grateful acknowledgment is made to the scores of Lilly associates who have made significant contributions to this project. As in any major technological development of this type, the laboratory, engineering, production, and logistic requirements that had to be met involved the close cooperation of all levels of our administrative and technical staff.

Monoclonal Antibodies for Diagnosis of Infectious Diseases in Humans

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Within its unique ecological niche the human organism serves as a biological reservoir for a vast array of microorganisms, ranging from viruses and bacteria to fungi and multicellular parasites (*1*). Most of the host's interactions with these microorganisms are without consequence to health, because physical barriers in the body (such as the gut) or the immune system maintain the microorganisms at a tolerable level. However, in instances of immune nonresponsiveness, or in circumstances involving infection with highly pathogenic organisms, this normal equilibrium is upset and the health of the host is threatened. When this occurs, there is a pressing need to rapidly and specifically identify the overgrowing organisms with a view to designing therapies capable of either restoring the appropriate biological balance or entirely eliminating the pathogens from the body. This need for specific, rapid diagnosis and prompt, targeted therapy has become of paramount importance with (i) the dramatic increase of antibiotic-resistant bacteria in our society, (ii) the growing importance of progressive infections in the immunocompromised host, and (iii) the advent of newer forms of antiviral therapy.

Conventional Diagnosis of Infectious Diseases

Infectious diseases are generally diagnosed by four methods (*2*): (i) microscopic examination of tissue specimens and exudates, with the visual identification of either virus-infected cells (that may show inclusion bodies), bacteria, fungi, or parasites; (ii) culture methods, with the use of selective growth media that allow the amplification of small numbers of organisms that can be tested for susceptibility to potential therapeutic agents; (iii) immunological identification in tissues or body fluids of antigens associated with specific pathogens; or (iv) measurement of specific antibodies produced in the patient as a result of infection with an organism.

Given the complexity of infectious diseases, it is not surprising that no one diagnostic method has proved optimal for all situations. Instead, depending on the particular infection, laboratories commonly use a combination of two or more of the four different diagnostic methods. Thus, while microscopic identification may yield an unequivocal diagnosis of a multicellular parasite, this method is of limited value in identifying viruses. Culture methods, in contrast, provide an unambiguous determination of infectivity and are extremely sensitive; however, these methods also tend to be labor intensive and to involve lengthy incubation periods, and many common pathogens are difficult to grow in culture. Direct identification of pathogens by antibodies, while providing a rapid and specific method for diagnosis, is dependent on the specificity and strength of the antiserums used, which are known to vary considerably. In addition, since many microorganisms are antigenically related to each other, antibodies may demonstrate cross-reactions between pathogenic and nonpathogenic forms.

Monoclonal Antibody Technology

Recently, monoclonal antibody techniques have provided an opportunity to reevaluate the role of immunological methods for the diagnosis of infectious diseases. As a result of the pioneering studies of Kohler and Milstein (*3*), it is now possible to create immortal cloned

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