DRAFT
APRIL 10, 1985

Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology

Office of Biologics Research and Review

Center for Drugs and Biologics

Table of Contents

I. Introduction

This document provides suggestions for evaluating safety, purity, and potency of new drugs and biologics produced by recombinant DNA technology. The suggestions expressed herein are expected to change with time as new knowledge is acquired, and should not be regarded as being either definitive or all-inclusive. Accordingly, the points discussed below should be interpreted as those that manufacturers of such products are generally expected to consider during development of new drugs and biologics, in filing notices of claimed investigational exemptions for new drugs (IND), in new drug applications (NDA), and in license applications.

II. General Considerations

General regulations for biologics (e.g. 21 CFR, Chapter I, Subchapter F) and drugs (e.g. 21 CFR, Chapter I, Subchapters C and D) also pertain to products produced by recombinant DNA technology where applicable. Specific concerns relevant to particular products should be discussed with the appropriate Office on a case-by-case basis. New license applications or new drug applications are required before marketing products made with recombinant DNA technology, even if the active ingredient in the product is thought to be identical in molecular structure to a naturally occurring substance or a previously approved product produced in an established manner.

The production of new drugs and biologics by recombinant DNA technology should generally follow the NIH Guidelines for Research Involving Recombinant DNA Molecules. In addition, manufacturers wishing to export their products from the United States should consult the appropriate guidelines published by agencies such as the World Health Organization, and the National Institute for Biological Standards and Control (United Kingdom).

III. Expression Systems

Recombinant DNA technology involves the systematic arrangement and manipulation of specific segments of nucleic acid for construction of composite molecules which, when placed into an appropriate host environment, will yield a desired product. There are three general methods for obtaining a specific coding segment: (a) reverse transcription of mRNA to complementary DNA; (b) isolation of genomic DNA or RNA; or (c) chemical synthesis.

The manufacturer should provide a description of the method used to prepare the segment coding for the desired product, including both the cell type and origin of the source material. A detailed nucleotide sequence analysis, and a restriction enzyme digestion map of the cloned segment should also be included. If a cloned polynucleotide contains more information than coding sequences, i.e. introns or flanking sequences, then these additional sequences should be adequately characterized.

The construction of the vector used for expression of the cloned nucleotide segment into its respective product should also be described. This description should include a detailed explanation of the source and function of the component parts of the vector, e.g. origins of replication, antibiotic resistance genes, promoters, enhancers, whether or not the product is being synthesized as a fusion protein. A restriction enzyme digestion map indicating at least those sites used in construction of the vector should be provided.

The host cell system which will generate the product is coordinated to fit the expression vector. It is, therefore, important that a description of the source, relevant phenotype and genotype of the host be provided, including

literature references. If the host cell is of mammalian origin then it should be thoroughly characterized.[1] Various methods can be utilized to transfer an expression vector into its host, such as transfection, transduction, infection, microinjection, etc. The mechanism of transfer, copy number, and the physical state of the vector inside, the host cell, integrated or extrachromosomal, should be provided.

IV. Master Cell Bank

The Master Cell Bank is a designated seed lot, from which all subsequent seed lots are made. A seed lot consists of aliquots of a single culture, stored in a manner which gives a reasonable assurance of genetic stability. In most cases, a single host cell containing the expression vector should be cloned to give rise to the Master Cell Bank. The cloning history and methodology should be described. If new Master Cell Banks are to be generated periodically by expression vector transfer and clonal selection, acceptance criteria for both the new clones and the product produced by these clones should be described. The stability of both the host cell and expression vector should be investigated. In particular, the fidelity of the nucleotide sequence encoding the expression product in the Master Cell Bank should be verified. Whenever clonal selection is used to construct a new seed lot, DNA sequence analysis of the coding region should be performed.

In cases in which there is multiple integration into the host cell genome of the DNA sequences expressing the protein product, thus making these sequences difficult to characterize, the mRNA encoding the specific product should be cloned and the anticipated coding sequence of the product should be verified, for each Master Cell Bank.

The identity and purity of the cells in each seed lot should be assured by isoenzyme analysis, auxotrophy, antibiotic resistance, and karyology, as appropriate.

Each seed lot should be characterized for adventitious agents including mycoplasma, bacteria, fungi, viruses, and virus-like particles.[1]

V. Production

The cells used in each production run should be characterized by analysis of relevant phenotypic or genotypic markers, and tested for adventitious agents in samples taken just prior to termination of culture. Additionally, a detailed restriction enzyme digestion map of the expression vector and the nucleotide sequence of the insert encoding the expression product should be determined after full scale culture at least once for each Master Cell Bank.

The procedures and materials used for cell growth and induction of product expression should be described in detail.

Data on the consistency of yield of the product from full-scale culture should be maintained, and criteria for the rejection of culture lots should be established.

Penicillin and other beta-lactam containing antibiotics may derivatize proteins and generally should not be used in production runs because of the risk of hypersensitization in product recipients. Similarly, caution should be exercised in the use of such materials as phenylmethylsulphonylfluoride (PMSF, a protease inhibitor), β-propiolactone, formaldehyde and other protein derivatizing chemicals, since multiple exposure to derivatized proteins may lead to undesirable immune responses in recipients of the final product.

VI. Purification

The methodology of harvesting, extraction, and purification should be

described in detail, and the removal of any undesirable chemicals introduced by these procedures should be demonstrated.

The extent of purification of recombinant DNA products should be consistent with the intended use of the product. Drugs and biologics which are to be administered repeatedly or at high concentrations should be adequately pure to prevent the development of undesired immune or toxic reactions to contaminants. Although recombinant DNA products may be demonstrated to be 99% pure by physicochemical characterization, special attention should be directed toward the removal of certain contaminants which may be present in small amounts. The purification process should be designed to specifically eliminate detectable viruses, microbial and nucleic acid contamination and undesirable antigenic materials.

The use of antibodies for affinity purification of recombinant DNA products deserves special comment. The antibodies should be shown to be free from unwanted biologically active substances such as DNA and viruses as described in Section VII, C.[2] Methods used for the coupling of the antibody to the column matrix and the removal of contaminants from the affinity column should be described. Several production lots of the final product should be examined for the absence of detectable immunoglobulin protein.

VII. Characterization of the Product

Evidence for identity, purity, and stability of the product in comparison with reference preparations may be derived from the results of a wide variety of tests. The specific tests that will adequately characterize any particular product on a lot to lot basis will depend on the nature of the product. Some examples of tests which may be useful during product development or lot to lot testing are described below.

A. Physicochemical Characterization of Proteins

1. Amino Acid Composition Analysis

The complete amino acid composition of the peptide or protein should include accurate values for methionine 1/2-cystine and tryptophan, which may require sample preparation procedures other than hydrolysis in 6N HCl or chemical modification of proteins and analysis of derivitized amino acids. The amino acid composition presented should be the average of at least three (3) separate hydrolysates of each lot number.

For small proteins or peptides with molecular weight less than 10,000, the demonstration of nearly integral ratios of amino acids would support arguments of peptide purity.

For proteins with molecular weight in excess of 10,000 the amino acid composition analysis may not provide as useful information in support of the purity of the product as for the small proteins or peptides. However, integral values for those amino acid residues generally found in low quantities» such as tryptophan and/or methionine, could be obtained and used to support arguments of purity.

2. Partial Sequence Analysis

Where possible, partial amino terminal (15 residues) and carboxy terminal sequence analyses can serve as important criteria for the identity of recombinant DNA produced proteins or peptides. The sequence data presented in tabular form should include the total yield for every amino acid at each cycle, as well as the repetitive yield for the major sequence(s). In several cases. unexpected heterogeneity in the amino termini and carboxy termini of proteins produced by recombinant DNA technology has been observed by using protein sequence analysis.

3. Peptide Mapping

Peptide mapping can provide a very discriminating comparison between a recombinant DNA product and an authentic sample of the natural product or a reference preparation. In conjunction with amino acid composition and sequence analysis of each peptide, peptide mapping can provide precise evidence for the identity of a protein. For proteins containing disulfide bonds, peptide mapping often can be used to verify the correct arrangement of disulfide bonds in the final product.

4. Polyacrylamide Gel Electrophoresis (PAGE) and Isoelectric Focusing

PAGE and isoelectric focusing are valuable techniques for verifying identity, purity and apparent molecular weight of proteins and peptides. The PAGE analysis should include the use of denaturing conditions with and without exposure to reducing agents, and with appropriate molecular weight standards or reference preparations.

It is preferable to analyze samples on slab gels stained by an appropriately sensitive method: for example, silver stain is generally more sensitive than Coomassie blue for the detection of very small quantities of proteins and is useful in identifying nonprotein materials such as nucleic acid, carbohydrate and lipid which may be present.

For peptides of molecular weight less than ca. 8,000, most PAGE methods may not be sufficiently accurate for molecular weight estimates.

5. High Performance Liquid Chromatography (HPLC)

HPLC is a useful method to determine the purity of a protein or peptide, to evaluate its molecular configuration and, under some circumstances, to confirm its identity. HPLC may be especially useful in

characterizing and quantitating specific impurities in the final product, and in peptide mapping.

6. Circular Dichroism and Optical Rotatory Dispersion (CD and ORD)

A comparison of the CD or the ORD spectrum of the material prepared by recombinant DNA technology with the corresponding spectrum of the native material or a reference preparation may support conformational similarity.

7. Other Characterization

Additional physicochemical characterizations may be appropriate for recombinant DNA products containing carbohydrates, DNA, lipids, and other nonprotein components.

B. Biological Tests for Identity and Potency

A comparison of the recombinant DNA product to the natural product or reference preparation in a suitable bioassay will provide additional evidence relating to the identity and potency of the recombinant DNA product. Various types of bioassay may be used. In vitro assays are usually faster, less expensive, and more precise than animal studies, yet adequate testing of a biological product may involve animal studies.

Most vaccines produced by recombinant DNA techniques should be compared to the natural substance or reference preparations with respect to their ability to promote immune responses in animals. Non-vaccine recombinant DNA products may be compared with the natural product in pharmacokinetic studies including tissue distribution and clearance mechanism. The extent, frequency and methods of animal testing should be determined on a case-by-case basis.

C. Tests for Contaminants

Reliable and sensitive tests will be needed to assay for trace contamination and product related impurities in the final product on a lot to lot basis. Although physiochemical characterization can ensure a high degree of purity of a recombinant product, tests for trace contaminants will rely heavily on biological indicator systems.

1. Pyrogen Contamination

Pyrogenicity testing should be conducted by injection of rabbits with the final product or by the limulus amebocyte lysate (LAL) assay.[3] Criteria comparable to those adopted for acceptance of the natural product should be used for the recombinant DNA product.

Certain biological pharmaceuticals are pyrogenic in humans despite having passed the LAL test and the rabbit pyrogen test. This phenomenon may be due to materials which appear to be pyrogenic only in humans. To attempt to predict whether human subjects will experience a pyrogenic response, human blood mononuclear cells can be cultured in vitro with the final product and the cell culture fluid injected into rabbits. A fever in the rabbits indicates that the product contains substances which may be pyrogenic in humans.[4]

2. Viral Contamination

Tests for viral contamination should be appropriate to the cell substrate and culture conditions employed.[1] Absence of detectable adventitious viruses contaminating the final product should be demonstrated.

3. Nucleic Acid Contamination

Removal of nucleic acid at each step in the purification process may be demonstrated in pilot experiments by examining the extent of

elimination of added host cell DNA. Such an analysis would provide the theoretical extent of the removal of nucleic acid during purification.

Direct analyses of nucleic acid in several production lots of the final product should be performed by hybridization analysis of immobilized contaminating nucleic acid utilizing appropriate probes, such as both nick-translated host cell and vector DNA. This method ought to provide sensitivity on the order of 10 picograms per dose. Theoretical concerns regarding transforming DNA derived from the cell substrate will be minimized by the general reduction of contaminating nucleic acid.

4. Antigen Contamination

Products which are administered repeatedly or in large doses should be assayed for trace antigenic constituents and product related impurities (e.g. aggregates or degradation products) likely to contaminate the final product. Tests such as Western blots, radioimmunoassays and enzyme-linked immunosorbant assays using high affinity antibodies raised against the product, host cell lysates, appropriate subcellular fractions, and culture medium constituents, should be used to detect contaminating antigens. Because the detection of antigens will be limited by the specificity and sensitivity of the antisera used, these immunoassays will complement but not replace silver stain analysis of SDS-PAGE gels. Patients given large or repeated doses of a product should be monitored for the production of antibodies to contaminating antigens.

5. Microbial Contamination

Appropriate tests should be conducted for microbial contamination that demonstrate the absence of detectable bacteria (aerobes and anaerobes), fungi, yeast and mycoplasma in the final product.

D. Preclinical Toxicity Evaluation

The specific preclinical testing needs are best addressed on a case-by-case basis with the appropriate Office. Appropriate animal tests. which might include those for carcinogenicity, teratogenicity and effects on fertility may be necessary for a product in which the active ingredient is radically altered from the natural substance.

VIII. Modified Protein Products

Using recombinant DNA procedures it may be possible to modify the structure of proteins to enhance their desired biological properties and/or diminish undesirable ones. Any substance that is not a natural constituent of the human body may be antigenic and also may cause unknown and possibly adverse biological effects. The use of such a product in humans depends on a careful assessment of its new benefits compared to the risks identifiable during its preclinical and clinical evaluation.

IX. Clinical Trials

Clinical trials will be necessary for products derived from recombinant DNA technology to evaluate their safety and efficacy.

[1]See "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals." Office of Biologics Research and Review, Center for Drugs and Biologics. FDA. (Federal Register, Vol. 49, N0. 110. June 6, 1984).

[2]See "Points to Consider in the Manufacture of Injectable Monoclonal Antibody Products Intended for Human Use In Vivo," Office of Biologics Research and Review, Center for Drug and Biologics, FDA. (Federal Register, Vol. 49, p. 1138, January 9, 1984).

[3]Hochstein. H.D., Elin, R.J., Cooper, J.F., Seligmann, Jr., E.R., and Wolff, S.M. (1973). Bull. Parenteral Drug Assoc., 27, 139-148.

[4]Dinarello. C.A., (1974) "Endogenous pyrogen" in Methods for Studying Mononuclear Phagocytes, Adams. D., Edelsan, P., and Koren, H., Eds., pp. 629-639, Academic Press.