

PRODUCTION AND QUALITY CONTROL OF CYTOKINE PRODUCTS DERIVED BY BIOTECHNOLOGICAL PROCESSES

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PRODUCTION AND QUALITY CONTROL OF CYTOKINE PRODUCTS DERIVED BY BIOTECHNOLOGICAL PROCESSES

1. INTRODUCTION

Cytokines are a heterogeneous group of biologically active proteins or glycoproteins which regulate cell growth, differentiation and other functions. They are soluble (non-antibody) mediators which are active in very small quantities. Much research has centred on those cytokines which appear to modulate the growth and function of cells contained within the immune system.

The roles of cytokines in health and disease are likely to be complex. In general, measurable amounts of cytokines are rarely found in body fluids of healthy individuals. Much increased, detectable concentrations may, however, be found in body fluids during episodes of acute microbial infections and, in some cases, during the course of chronic invasive disease. It has been postulated therefore that cytokines are primarily involved in the induction and maintenance of host defence mechanisms against microbial infection and invasive disease. On the other hand, their continued presence in some chronic diseases may contribute to pathology.

Cytokines are highly pharmacologically active and thus may be effective agents for modifying biological responses (cytokines are often referred to as biological response modifiers). Since only human cytokines are likely to be used clinically, they should be of very low immunogenicity. So far, cytokines have mostly been used for the therapy of neoplastic disease (cancer). Clinical investigations, mainly carried out with interferon alpha or beta, have confirmed that cytokine treatment can be beneficial for a limited number of cancers. However, the full clinical potential of many cytokines, used individually or in combination with other agents, has yet to be explored.

2. POINTS TO CONSIDER IN MANUFACTURE

This note for guidance is intended to facilitate the collection and submission of data to support applications for marketing authorisation within the European Union for cytokine products derived from biotechnological processes. Cytokines may be produced in quantity by the large scale cultivation either of (i) transformed cell lines producing particular cytokines or (ii) prokaryotic or eukaryotic, including mammalian, cells in which the relevant cytokine genes have been inserted by rDNA techniques. The large scale production of cytokines by these procedures may affect the quality of particular cytokine products, and thus have implications for control testing. Although comprehensive characterisation of the final, purified cytokine product will be essential, particular emphasis must also be placed on "in-process" control and the consistency of the manufacturing process, a concept which has been very effective in the control of other biological products.

Requirements relating to establishments in which biological products are manufactured (e.g. Revised Requirements for Biological Substances No 1; WHO TRS 323) will apply to the manufacturers of cytokine products as will several of the general requirements for the

quality control of biological products. Thus, appropriate attention needs to be given to the quality of all reagents used in production, including components of fermentation or cultivation media; specifications for these are to be included in documentation and they must comply with any relevant European requirements. Tests for potency, abnormal toxicity, pyrogenicity and sterility, etc., which apply to products made by conventional methods, will also apply to products of biotechnological processes. It is undesirable to use in production agents which are known to provoke sensitivity reactions in certain individuals, such as, for example, penicillin or other beta-lactam antibiotics.

Certain factors may compromise the safety and efficacy of cytokine products; these should be given special attention and are outlined below:

- a) The use of a transformed cell line, particularly one of neoplastic origin, as the substrate for the manufacture of particular cytokines raises questions of safety. These questions, pertaining mainly to the potential oncogenicity of contaminating heterogeneous DNA derived from the substrate, have been debated for a number of years at many national and international scientific meetings. There is a growing international consensus that transformed cell lines can be used for the manufacture of biologicals, although their use as yet has only been approved in certain cases (e.g. the manufacture of interferon derived from the Namalwa human lymphoblastoid cell line) where the product may be subjected to rigorous purification.
- b) Cytokines encoded by naturally occurring genes expressed in foreign hosts may deviate structurally, biologically or immunologically from their natural counterparts. Such alterations can arise either at the genetic or post-translational level or during production or purification. Some cytokine products may be entirely novel in structure, since they may be produced by manipulation of naturally occurring genes or by chemical synthesis of new ones. These products may have enhanced biological properties and/or diminished undesirable characteristics compared with their naturally-occurring counterparts. It should be recognised, however, that they could have unexpected and undesirable biological properties.
- c) The choice of manufacturing procedure may influence the nature and range of potential contaminants. Examples of these are endotoxins in products expressed in bacterial cells and DNA or oncogenic potential in products expressed in transformed mammalian cells. Thus cytokine products may contain potentially hazardous contaminants which the purification processes must be shown to be capable of removing.
- d) Unintended variability in the culture during production may lead to changes which favour the expression of other genes in the host/vector system or which cause alterations in the polypeptide product. Such variation might result in decreased yield of the products and/or quantitative and qualitative differences in the impurities present in the product. Consequently, procedures to ensure the consistency of production conditions as well as the consistency of the final product are imperative.

Whilst the requirements set out below should be applied wherever they are appropriate for safety and efficacy, individual cytokine products may present particular quality control problems. Thus, the production and control of each cytokine product must be given careful individual consideration taking fully into account any special features.

3. MANUFACTURING REQUIREMENTS

3.1 Definitions

3.1.1 International name

A cytokine shall be named in accordance with international consensus. Proper names shall be the equivalent of the international name in the language of the country of origin. The use of the international name should be limited to suitably purified preparations.

3.1.2 Descriptive definition

A particular cytokine product shall be a preparation containing the intended cytokine prepared by a designated production process; the cytokine will be harvested in supernatants or extraction fluids derived from the cell substrate, purified and prepared in a form suitable for clinical application and should satisfy all the criteria specified in this note for guidance.

3.1.3 International standards and international unit

A number of international standards for particular cytokines have been or are being developed. These have assigned potencies in terms of biological activity which are quoted in International Units (IU).

3.2 Strategy for production

3.2.1 Cytokine production by transformed cell lines

A full description of the biological characteristics of the transformed cell line and any additions, e.g. the inducer and any enhancer or other substances (e.g. antibiotics) used in production, shall be given. The information includes:

- a) documentation concerning the origin of the cell line, and the nature of any known relevant condition of the donor, e.g. Burkitt's lymphoma, active infectious mononucleosis, infection with viruses or mycoplasma in general;
- b) data which can be used to establish the identity of the cell line;
- c) the growth characteristics of the cell line;
- d) data which document the stability of the cell line under the cultivation conditions used, especially if cells are to be sub cultured for an extended or indefinite period;
- e) if serum is included in the medium of production cell cultures, evidence that it is free from bacteria, fungi, mycoplasma and viruses.

3.2.2 Cytokine production by genetically engineered organisms – strategy for cloning and expression

- a) Expression vector and host cell: A description of the host cell and expression vector used in production should be given. This should include details of the origin and identification of the gene which is being cloned and the construction, genetics and structure of the expression vector. The method by which the vector is introduced into the host cell and the state of the vector within the host should be described. The association of the vector and host cell may be permanent, allowing continuous expression of the

product, or self-limiting, for example where the vector is an acceptable cytopathogenic virus.

- b) Sequence of the cloned gene: Full details of the nucleotide sequence of the gene insert and of flanking control regions of the expression vector should be provided. All relevant expressed sequences should be clearly identified. The DNA sequence of the cloned gene should normally be confirmed at the seed lot stage and at least once after a full scale fermentation. In certain systems, for example where multiple copies of the gene are inserted into the genome of a continuous cell line, it may be inappropriate to sequence the cloned gene at the production level. Under these circumstances, Southern blot analysis of total cellular DNA or sequence analysis of the mRNA may be helpful and particular attention should be paid to the characterisation of the final product.
- c) Expression: The strategy by which the expression of the relevant gene is promoted and controlled during production should be described in detail.

3.3 Validation and control of manufacturing process

3.3.1 Control of cell bank

It is essential that production is based on a well defined cell bank system involving a master cell bank and manufacturer's working cell bank(s) (MWCB). During the establishment of the cell bank, no other cell lines should be handled simultaneously in the same laboratory suite or by the same persons. The origin, storage, use and details of life expectancy at the anticipated rate of use must be described in full for all cell bank materials. Attention should be paid to the stability of the host-vector expression system in the cell bank under conditions of storage and recovery. Any known instability should be reported. New cell banks should be fully characterised.

A critical part of quality control is the full characterisation of cell bank material. Where higher eukaryotic cells are used for production, distinguishing markers of the cell, such as specific isoenzyme and immunological features or karyology will be useful in establishing the identity of the cell bank. Likewise, where microbial cultures are used, specific phenotypic features which form a basis for identification should be described.

Evidence that the cell bank is free from infective adventitious agents (viral, bacterial, fungal or mycoplasma) shall be provided. Special attention should be given to viruses which can commonly contaminate the species from which the cell line has been derived. For instance, cell lines of murine origin should be checked for contamination according to Annex I of the note for guidance *Production and Quality control of Monoclonal Antibodies*. Certain cell lines contain endogenous viruses, e.g. retroviruses, which may not readily be eliminated. The expression of these organisms, under a variety of conditions known to cause their induction, should be tested for and reported. Furthermore, the purification process should be shown to be capable of removing and/or inactivating any such virus which may be present in the cell bank as an endogenous agent or as part of the expression vector.

3.3.2 Production

Ideally not more than one cell line should be cultivated simultaneously in the same production area or the cultivation should be carried out in closed vessels with effective barriers which would prevent contamination with other adventitious agents or other cell

lines. If other cell lines are cultivated in parallel, records must be kept of the cell lines handled and evidence presented for the absence of cross-contamination between them.

- a) **Production at finite passage:** Details of the fermentation or culture used to manufacture the product should be provided. For each production run, the presence, extent and nature of any microbial contamination in the culture vessels immediately prior to all harvesting shall be thoroughly examined. Detailed information to confirm the adequate sensitivity of the methods used to detect contamination should be provided and acceptable limits of contamination set.

Maximum permitted passage levels for production should be defined and should be based on information concerning the stability of the host cell/vector system on serial sub-cultivation up to and beyond the level of production. Data on consistency of yield of the product should be presented. Criteria for the rejection of culture lots should be established.

Monitoring of the host cell/vector characteristics at the end of a number of production cycles should also be undertaken. For example, detailed information on plasmid copy number and degree of retention of the expression vector within the host cell, as well as restriction mapping of the vector containing the gene insert, may be of value.

- b) **Continuous culture production:** This approach should only be undertaken when special consideration has been given to the control of production based on continuous culture. Where it is undertaken, monitoring of the production system is necessary throughout the life of the culture. The required frequency and type of monitoring will depend upon several factors including the nature of the expression system and product. Information should be obtained on the molecular integrity of the gene being expressed and of the phenotypic and genotypic characteristics of the host cell after long term cultivation. Evidence should be provided that the yield does not vary beyond defined limits and that the nature and quality of the product does not change with respect to specific parameters. The acceptance of harvests for further processing should be clearly linked to the schedule of monitoring applied. The period of continuous cultivation should be specified and this should be based on information concerning the stability of the system and consistency of the product up to and beyond this limit. In cases on long term continuous cultivation, the cell line and product should be completely re-evaluated at intervals based on information concerning the stability of the system and the characteristics of the product.

A clear definition of a “batch” of product for further processing should be provided. Regular tests for microbial contamination should be performed in relation to the strategy for harvesting. Criteria for rejection of harvests and premature termination of the culture should be defined.

3.4 Purification of the product

3.4.1 Methods

Methods used to purify the product should be described in detail. Procedures which make use of affinity chromatography, for example employing monoclonal antibodies, should be accompanied by appropriate measures to ensure that these substances, or any additional potential contaminants arising from their use, do not compromise the quality and safety of the final product. Attention is drawn to the note for guidance *Production and Quality Control of Monoclonal Antibodies*.

3.4.2 Validation of the purification procedure

The capacity of the purification procedure to remove unwanted host cell derived proteins, nucleic acids, viruses and other impurities should be investigated thoroughly, as should the reproducibility of the purification process as regards its ability to remove specific contaminants and the consistent composition of the purified product with respect to any impurities which may be present. Laboratory scale pilot studies which mimic the production process using, for example, a carefully selected group of viruses which exhibit a range of physico-chemical features relevant to their behaviour during the process of purification, or radioactively labelled DNA intentionally mixed with the crude preparation (spiking) should be undertaken. A reduction factor for such contaminants at each stage of purification should be established by using, if necessary, concentrations of DNA and viruses in excess of that expected during normal production.

3.5 Final processed product

3.5.1 Characterisation of the purified cytokine

Rigorous characterisation of the cytokine by chemical and biological methods will be essential. Routine detailed characterisation of the final product may be required if the nature of the expression system makes it impossible to characterise the gene at the production level.

Particular attention should be given to using a wide range of analytical techniques exploiting different physico-chemical properties of the molecule; for instance, size, charge, isoelectric point, amino acid composition and hydrophobicity. It may be desirable to include suitable tests to establish that the product has the desired conformational structure and state of aggregation. Examples of techniques suitable for such purposes are: polyacrylamide gel electrophoresis, isoelectric interaction or affinity chromatography; peptide mapping; amino acid analysis; light scattering; UV spectroscopy; circular dichroism and other spectroscopic techniques. Additional characterisation of the product using, for example, immunochemical techniques may provide valuable information. Biological and immunological characterisation should include as wide a range of techniques as possible appropriate to the anticipated biological activity, use, system of administration of the product and duration of treatment. The determination of the specific activity of highly purified material is of particular value (units of activity/weight of product).

Sufficient sequence information to characterise the gene product adequately should be obtained. The degree of sequence verification required will depend on the extent of other characterisation tests. For some purposes, partial sequence determination and peptide mapping may suffice, for others full sequence determination may be necessary. Attention should be paid to the possible presence of N-terminal methionine, signal or leader sequences and other possible N- and C- terminal modifications (for instance acetylation, amidation or partial degradation by exopeptidases). Other post-translational modifications, such as glycosylation should be indicated. Special consideration should be given to the possibility that such modifications are likely to differ from those found in a natural counterpart and may influence the biological and pharmacological properties of the product.

3.5.2 Purity

Data should be provided on contaminants which might be present in the final processed product. The level of contamination considered as acceptable and criteria for acceptance or rejection of a production batch should be given. It is important that the techniques used to demonstrate purity be assessed using as wide a range of methods as possible, including physico-chemical and immunological techniques. Particular emphasis should be placed on tests for viral and nucleic acid contamination and for other unwanted materials of host origin, as well as on materials which may have been added during the production or purification processes.

3.6 Routine batch control of bulk product

A comprehensive analysis of the initial batches of a product should be undertaken to establish consistency with regard to identity, purity and potency. Thereafter, a more limited series of tests may be appropriate as outlined below.

3.6.1 Consistency

An acceptable number of successive batches of the purified cytokine bulk solution should be characterised as fully as possible to determine consistency of composition. The studies should include biological, chemical and immunological methods to characterise and assay the cytokine and methods to detect and identify impurities. Any differences which occur between batches should be noted. The data obtained from these consistency studies should be used as the basis for product specification.

3.6.2 Identity

A selection of the tests used to characterise the purified cytokine (see section 3.4) should be used to confirm the product identity for each batch. The methods employed should include tests for the anticipated biological activity as well as physico-chemical and immunological methods. Depending on the extent of other identification tests, sequence verification of a number of amino acids at the N- and C- terminus or other methods such as peptide mapping should be performed.

3.6.3 Purity

The purity of each batch should be established and be within specified limits. The analysis should include sensitive and reliable assays for DNA of host cell origin applied to each batch of product prepared from continuous lines of transformed mammalian cells. Strict upper limits should be set for DNA in the product. It is recommended that DNA analyses are also performed on each batch of product obtained from other eukaryotic cells, and limits set for DNA content, until further information on safety is obtained. DNA of prokaryotic expression systems (e.g. of vector or plasmid origin) should be tested for when considering the quality and safety of the product. For products to be administered chronically, or in high doses, the residual cellular proteins should also be determined by an assay with appropriate sensitivity (e.g. ppm) and limits be established for these.

3.6.4 Potency

The potency of each batch of the cytokine product should be established (e.g. units of biological activity per ml) using, wherever possible, an appropriate national or international reference preparation calibrated in units of biological activity (see section 3.7).

In addition, information on specific activity (units of biological activity per unit weight of product) is of considerable value and should be reported. A highly purified reference preparation is required to standardise measurements of specific activity (see section 3.7).

It is recommended that correlations between potency measurements, involving biological tests, and the results of physico-chemical methods of assay are made and the information reported. If possible, batches should be calibrated using accurate physico-chemical tests, and the biological assays used to confirm – within stated limits – that the product is biologically potent.

3.7 Specification and reference materials

The studies described in section 3.5 will contribute to a definitive specification for the product when considered together with the information obtained from the examination of successive batches, as indicated under section 3.6.

A suitable batch of the cytokine product, preferably one which has been clinically evaluated, should be fully characterised in terms of its chemical composition, purity, potency and biological activity, including where possible full amino acid sequencing, and retained for use as a chemical and biological reference material.

When appropriate, the biological activity of the cytokine product and its physical characteristics, including the amino acid sequence, should be compared with that of a highly purified preparation of the naturally occurring molecule.

4. FINISHED PRODUCT

Where appropriate, the product in final containers should be shown to comply with the requirements of the relevant European directives. In circumstances where this is not possible, the omission of tests should be justified by the manufacturer.

The manufacturer shall take samples from each final lot for the following tests.

4.1 Sterility test

Sterility shall be tested according to Ph. Eur. requirements.

4.2 Identity test

Cytokine products shall be identified as the intended cytokine by appropriate methods as approved by the competent authority. Methods such as immunoassays or neutralisation assays are useful in this context.

4.3 Potency

The test for potency should utilise a bioassay unless otherwise justified. An appropriate reference preparation should be tested in parallel. These tests shall be performed on samples representative of the final filling lots. Statistical analysis of the data should show that the mean potency value obtained has the confidence limits accepted by the competent authority.

4.4 Safety tests

Each final lot shall be tested for safety in mice and guinea pigs as required by the European Pharmacopoeia.

4.5 Test for pyrogenic substances

Each final lot shall be tested for pyrogenic substances. It should be noted that some cytokines may themselves induce a pyrogenic response in vitro and, where applicable, this should be taken into account when evaluating the product.

4.6 Test for preservatives/additives

Each final lot shall be tested to determine the levels of preservatives/additives. The tests used and the permitted concentration shall be approved by the competent authority.

4.7 Moisture content

For lyophilised products, the acceptable level of moisture content per container shall be approved by the competent authority.

4.8 Colour, clarity and pH

The colour, clarity, and pH of the cytokine solution in the final containers or in the re-constituted final containers should be approved by the competent authority.

GLOSSARY

1. Master Cell Bank

A quantity of cells able to produce the intended cytokine stored at -70°C or below in aliquots of uniform composition, one or more of which is used for the production of a Manufacturer's Working Cell Bank.

2. Manufacturer's Working Cell Bank (MWCB)

A single uniform suspension of cells which have been dispensed in a single working session into a number of containers which are stored at -70°C or below. Cells revived from one or more of these containers are used as a source of Production Cell Cultures.

3. Production Cell Cultures

The cell cultures derived from one or more containers of MWCB which in the production process are able to form or are induced to form the intended cytokine.

4. Inducer

A substance added to a cell culture which leads to or stimulates the production of the intended cytokine.

5. Enhancers

Any substances added to an induced cell culture which improve the final yield of the intended cytokine.

6. Single Harvest

The biological material prepared from a single production run.

7. Purified Cytokine Solution

The resultant cytokine solution from a single harvest, which has been taken through a designated purification process.

8. Purified Cytokine Bulk Solution

Either the purified cytokine solution or the resultant of blending two or more batches of purified cytokine solution.

9. Formulated Cytokine Bulk Solution

The purified cytokine bulk solution to which excipients, e.g. stabilisers, have been added.

10. Final Bulk

The formulated cytokine bulk solution (or the finished biological material) present in the container from which the final containers are filled.

11. Final Lot

A collection of sealed final containers that are homogeneous with respect to the risk of contamination during filling or preparation of the finished product. A final lot must therefore consist of finished material obtained during one working session from a single final bulk.

12. Manufacturers Reference Material

Numerous samples from one or more final lots of material which has been shown to be active in clinical use, or are directly related to such material e.g. purified cytokine bulk solution, and which has been fully characterised in ways to be specified by the competent authority, appropriately stored by the manufacturer to serve as a reference material for several years. For certain critical tests, such reference material shall be included in parallel with each lot of production material, which must match the specification of the reference batch within limits to be agreed by the competent authority.