Regulatory Issues Surrounding Therapeutic Use of Monoclonal Antibodies

Points to Consider in the Manufacture of Injectable Products Intended for Human Use

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1. Introduction

The therapeutic use of monoclonal antibodies offers virtually unlimited possibilities in situations in which currently available treatments are ineffective. Yet, with this promise come new special public health concerns. The introduction into humans of materials produced by an immortal, ofen xenogeneic, cell line produced in tissue culture or mouse ascites, which potentially reacts with components of human tissues, carries risks that at times may be hard to define. The Office of Biologics Research and Review has attempted to compile a list of problems, side effects, toxicities, and untoward reactions of which investigators and clinicians should be cognizant.

In addition, we have taken a multidisciplinary approach to devising strategies for minimizing or, hopefully, eliminating them. At this writing, there exist no formal published guidelines that, if adhered to, would ensure the product's approval or licensure. Nonetheless, a document called, "Points to Consider in the Manufacture of Injectable Monoclonal Antibody Products Intended for

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Human Use *In Vivo*'' has been disseminated to manufacturers, institutions, individual clinicians, and investigators involved in the development of monoclonal antibodies. These ''points'' are intended to serve as departure points for discussion and cooperation between regulators and manufacturers interested in the safety of these products.

Because the technology for hybridoma production is rapidly evolving, the information in that document and in this presentation is obviously subject to change. Every effort will be made by our office to have the content be up to date and consistent with the state of scientific knowledge in this area.

2. Development and Characterization of Hybridoma Cell Lines

Many satisfactory technical approaches to hybridoma establishment and propagation exist (1). At present, the vast majority involve immortalization of antibody-producing cells by chemically induced fusion with myeloma cells. Major emphasis has so far been placed on murine systems. It is hoped that principles discussed here may be applied to other species and to interspecies hybrids as well. With most license applications, certain elementary data (described and listed in Table 1) are helpful in orienting outside observers to the characteristics of the monoclonal. Obviously, the

Table 1
Development and Characterization of Cell Lines

Source, name, and characterization of parent myeloma immunoglobulin
Strain and tissue origin of the immune cell
Identification and characterization of the immunogen
Description of the immunization scheme
Description of the screening procedure used
Description of cell cloning procedures
Description of the seed lot system

source and type of parent myeloma is of importance. Its characterization with respect to any heavy or light chains that it synthesizes or secretes is useful. Similarly, the strain and tissue origin of the immune cell used for fusion, identification, and characterization of the immunogen, description of the scheme for immunization, and screening and cell cloning procedures need to be described.

Of particular importance is the description of the seed lot system used for establishing the primary and secondary lots. These seed cultures should be particularly well-characterized with respect to identity, stability, and known microbial contaminants. A description of how these seed cultures are maintained is of great interest and importance. It is thought that the presence of certain viral contamination, particularly with lymphocytic choriomeningitis virus, reovirus, polyoma, or murine leukemia virus, should disqualify the subsequent use of the seed lot for making monoclonal antibodies, but, in the absence of definitive data, controversy exists on this point.

A full description of the tissue culture facilities plus a description of the supplier, genotype, and husbandry of any animals used is helpful in evaluating production procedures. In addition, the procedures used to control contamination, the acceptance criteria for cells, tissue culture supernatants, or ascites, and the procedures used to purify the final product are important. A list of desired production procedure information is given in Table 2.

Table 2
Production Procedures

Tissue culture procedure
Supplier, genotype, husbandry of animals used
Steps taken to control viral, bacterial, mycoplasma contamination
Acceptance criteria for cells, tissue culture supernatants, or ascites
Procedures used to purify the final product
Procedure used to prepare and fill final containers

3. Quality Control Tests

Monoclonal antibodies intended for in vivo use should be as free as possible of nonimmunoglobulin contaminants. A definition of the measure of potency for each monoclonal antibody should be established. Steps should be taken to establish that the hybridoma secretion product remains unchanged; for example, biochemical and biophysical studies comparing the product to a retained sample of an initially well-characterized lot designated as an in-house reference standard is quite useful. An outline of quality controls that are applicable to hybridomas is given in Table 3. Principal among these is rigorous testing of immunologic specificity. Specificity should be tested after any chemical modification. Tests designed to test aggregation, denaturation, and fragmentation of

the immunoglobulin product should be performed. The degree of homogeneity should be established by appropriate tests. The immunoglobulin class and, when appropriate, the subclass should be determined, and the isoelectric focusing pattern of the antibody in each bulk lot should be measured relative to the in-house reference standard.

Table 3
Quality Control Tests

Immunologic specificity
Stability
Ig class and subclass
Isoelectric focusing
Sterility
Polynucleotide contamination
Viruses

3.1. Special Considerations Regarding Viral Contamination

Since monoclonal antibodies are the products of malignant hybridoma cell lines, they may contain viruses or viral nucleic acid that could adversely affect human recipients. Different modes of propagation, i.e., in tissue culture or in mouse peritoneal cavities, will introduce different problems of contamination. Special consideration should be given to attempts to exclude *potential* contamination in each case.

All quality assurance tests for possible viral contamination should be performed in qualified laboratories. Included among recommended tests are the following:

1. An appropriately controlled test for lymphocytic choriomeningitis (LCM) virus by either intracerabral inoculation into healthy weanling LCM-negative mice (2), or by isolation in sensitive cell cultures combined with immunofluorescence (3).
2. A mouse antibody production (MAP) test (2,4) that has the capacity to detect the following murine viruses: LCM; reovirus type 3; polyoma; pneumonia virus of mice; mouse adenovirus; minute virus of mice; mouse hepatitis; K; ectromelia; Sendai; and GD VII viruses, and other assay procedures that can detect the mouse salivary gland (murine CMV); EDIM; thymic; and LDH viruses. When such tests

are positive in the initial screening for any given murine virus, confirmation may be accomplished by additional approaches, e.g., complement fixation (5), immunofluorescence (6), hemagglutination inhibition (7), or enzyme-linked immunosorbent assay (8).

3. Appropriately controlled tests for murine leukemia viruses using the MCF assay (9), the XC test (10), the S+L−test (11), and reverse transcriptase (for retroviruses) assays utilizing both Mg^{2+} (12) and Mn^{2+} (13).
4. Process validation procedures may establish that viral and nucleic acid contaminants have been effectively excluded or inactivated from each bulk lot by the manufacturing processes employed. "Spiking" experiments with appropriately labeled viruses or nucleic acids may be useful in this regard (14). These procedures will be considered on a case-by-case basis as an alternative to routine product testing.

3.2. Special Considerations Regarding Nucleic Acid Contamination

A number of fluorescence enhancement assays exist that allow detection of nucleic acids. These are usually sensitive in the nanogram range (15–18). Considering that the major safety concerns of monoclonal antibody products are related to their derivation from a transformed cell line, hybridization analysis using nick-translated hybridoma cell DNA seems to be in order. This should allow sensitivity on the order of 10 pg of DNA/dose (19).

For the final filled product (Table 4), potency and stability need to be ascertained and documented. Purity should be confirmed via a method such as electrophoresis (compared with authentic standards). The protein concentration should be determined by a suitable assay. The product material should pass sterility tests, general safety tests, and the rabbit pyrogen or limulus amebocyte lysate assay

Table 4
Tests on Final Filling

Potency and stability
Electrophoretic migration
Protein concentration
Sterility
General Safety
Pyrogenicity (rabbit, limulus)
Identity

for the detection of pyrogens. Finally, an appropriate identity test should be designed and employed.

Tests designed to detect and quantify potential contaminants such as hypotensive substances or additives (antibiotics, chromatography reagents, leachable components, preservatives) should be performed. Penicillin should not be present, but minimal concentrations of other antibiotics may be acceptable.

Pristane, if used in the preparation of ascites fluid, should be shown to be absent.

For products being investigated under IND, stability need only meet the demands imposed by the clinical protocol. In the case of product license applications, studies to support the proposed dating period should be performed on the final product. The test should establish a valid dating period under realistic field conditions.

The extensive testing described above is intended for patient populations that do not have life-threatening disease. In cases of life-threatening disease or in other special circumstances, less extensive testing may be justifiable. Even in patient populations with life-threatening disease, LCM virus, reovirus type 3, and polyoma should be absent from the final bulk lot. Special caution should be exercised when dealing with immunocompromised patients, since no data are available concerning the effects of injection of murine viruses into humans. Immunosuppressive murine monoclonal antibodies administered to heavily immunosuppressed patients may predispose to fatal lymphoproliferative disorders and other clinical complications (20).

4. Preclinical Animal Testing

The toxicity of monoclonal antibodies should be evaluated by methods similar to those described for other therapeutic drugs (21). However, anaphylaxis and other allergic reactions may occur with xenogenic monoclonal antibodies, as with most heterologous proteins, but no available animal test system will adequately define these products as nonallergenic in humans. Hence, the risk of these reactions is best minimized by the use of well-characterized, nonaggregated products in carefully-designed and cautiously implemented clinical protocols. Toxicity studies should generally be performed on the hybridoma product using a number of animals in at least one species other than mice. The product should be ad-

ministered in a manner that gives proper attention to the conditions of the proposed clinical testing. When feasible, it should be given at the actual human dose (or multiple thereof per unit weight of the experimental animal), by the same route, and with the same frequency. Appropriate observations should be made that would be applicable to the particular product.

Additional pharmacologic studies would appear useful whenever they can be performed. Because of the peculiar nature of some monoclonal antibody products, certain pharmacological kinetic parameters may at times have to be determined by human clinical studies.

5. Preclinical Laboratory Testing

Monoclonal antibodies are homogeneous populations of immunoglobulin molecules having antibody combining sites that bind uniformly to discrete antigenic determinants. They may be prepared in very concentrated form and may exert highly potent effects. In some instances, the antigenic determinant may be expressed on human cells or tissues other than the intended target tissue, resulting in undesirable cross reactions. Accordingly, laboratory tests should be conducted to assess this possibility. When cross reactions are encountered, the resultant risk or hazard to potential recipients should be evaluated. One approach to testing for cross-reactivity is an immunohistological survey of human vital organs, blood components, and target cells or tissues that can be carried out using both frozen and chemically fixed adult or fetal tissue. Cross-reactivity may also be assessed in blood cells or representative cultured cell lines by clonogenic assays, microcytotoxicity testing, fluorescent antibody methods, radioautography, or other techniques. When cross reactions are encountered, they should be further studied to ascertain both their frequency and their intensity in tissue samples in a relevant human population.

If available, testing for in vivo cross reactivity should be done in an animal system that shares the same cross-reactive antigen or in an isolated human organ perfusion system. When such systems are not available, clinical studies may, nonetheless, be warranted. These should involve extensive histopathologic evaluations when possible and thorough biodistribution studies with reliably labeled samples of monoclonal antibody.

6. Special Considerations for Monoclonal Antibodies of Human Origin

For human–human hybridomas, human antibody producing lymphoblastoid cell lines, or mouse–human hybridomas, it is strongly recommended that steps that remove viruses and DNA be taken during purification of the product. In addition, tests should be performed at the seed lot stage to detect the presence of viruses (Table 5). The use of a cell line that is actively producing viruses

Table 5
Special Considerations for Mab of Human Origin

EBV
EDNA, VCA
Cocultivation with cord blood lymphocytes
DNA hybridization
CMV — tissue culture assay
HBV — HBsAg
Retroviruses
Reverse transcriptase
Electron microscopy
HTLV (when available)
Tissue culture safety test

is discouraged. For Epstein-Barr (EB) virus, a serological test for nuclear antigen (EBNA) should be done, unless the line is known to harbor EBV. If the line is positive for EBV, tests for antigens known to be associated with the virus should be performed. In addition, assays of supernatants or irradiated cells by cultivation with cord blood lymphocytes for 12 wk are in order. Optimally a DNA hybridization study at a sensitivity capable of detecting one EBV genome copy per cell should be performed on the supernatant. A 6-wk tissue culture assay with a blind subpassage at 3–4 wk in a human diploid fibroblast line should be employed to exclude the presence of cytomegaloviruses. A third generation licensed test for HBsAg should be done. Of particular concern at present is the possible presence of retroviruses. Currently, reverse transcriptase assays using both Mg^{2+} and MN^{2+} may be in order, along with competent electron microscopy on at least 2000 cells. A test for human retrovirus is probably imminent and, if a licensed test becomes available, it should be employed. Minimally, the donor of the immune cell

or cell line should be documented to be free of HTLV-III infection using a licensed test for antibody. Furthermore, a tissue culture safety test to detect other contaminating viruses may be performed with a series of established cell lines. These cell lines should include human diploid cells, human transformed cells such as Hela, simian cell lines such as Vero, and the monoclonal antibody producing cell line itself. A culture should be observed for the presence of changes attributable to growth of adventitious viral agents.

7. Summary and Conclusion

The Office of Biologics Research and Review advocates the use of monoclonal antibodies and other hybridoma-derived products under conditions in which optimal benefit/risk ratios are achievable. Addressing the points outlined here would seem to be a rational, attainable, and economically feasible endeavor for the vast majority of individuals intent on employing these products in humans. They are not intended, however, to obviate or supplant sound scientific and ethical principles. In our opinion, the use of highly purified products with optimal specificity as established by rigorous and copious experimental data represents the best possibility of ensuring their efficacious and therapeutic use.

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