www.pharmaerudition.org

ISSN: 2249-3875

International Journal of Pharmaceutical Erudition

Research for Present and Next Generation

NOV. 2020

Vol: 10 Issue:03

(30-40)

ISSN 2249-3875

International Journal of Pharmaceutical Erudition

Review Article

MONOCLONAL ANTIBODY: AN OVERVIEW

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Antibodies are important tools used by many investigators in their research and have led to many medical advances. Monoclonal antibodies (MAb) are important reagents used in biomedical research, in diagnosis of diseases, and in treatment of such diseases as infections and cancer.Like many other great discoveriesin science and medicine, the productionof monoclonal antibodies waspartly accidental. A specific, antibody-producing, mortal plasma cell was hybridized with an immortal, non-specific, antibody-producing, myeloma cell, and the hybrid continued to produce the specific antibody. And thus the era of hybridoma technologywas launched. These antibodies are produced by cell lines or clones obtained from animals that have been immunized with the substance that is the subject of study.Ortho-clone(OKT3) was the first MAb to be licensed for human use.Choosing the method of producing monoclonal antibodies are consistent with recommendations in the Guide for the Care and Use of Laboratory Animals.The development of hybridoma monoclonalantibody (MAb) technology has had a majorimpact on clinical and laboratory medicine. Ithas allowed the production of essentiallyunlimited quantities of pure, homogeneousantibodies against a large variety of antigens.A major advantage of using MAb rather than polyclonal antiserum is the potential availability of almost infinite quantities of a specific monoclonal antibody directed toward a single epitope. Mabs are used in renal, hepatic, cardiac transplantation, infectious disease, autoimmune disease and in reaction against digoxin and tetanus toxins.

Key Words: MAbs, Hybridoma, Polyclonal Antiserum, Autoimmune disease.

INTRODUCTION

Monoclonal antibodies are monospecific antibodies that are the same because they are made by identical immune cells that are all clones of a unique parent cell, in contrast to polyclonal antibodies which are made from several different immune cells. Monoclonal antibodies have monovalent affinity, in that they bind to the same epitope. Monoclonal antibodies have found extensive use in the diagnosis and treatment of various diseases such as infections and cancer. These antibodies are produced using cell lines or clones which are obtained from animals, which then are immunized with the substance, that is the subject of the study.The cell lines are produced by fusing B cells from the immunized animal with myeloma cells (Köhler and Milstein 1975). To produce the desired MAb, the cells must be grown in either of two ways: by injection into the peritoneal cavity of a suitably prepared mouse (the in vivo, or mouse ascites, method) or by in vitro tissue culture. Further processing of the mouse ascitic fluid and of the tissue-culture supernatant might be required to obtain MAb with the required purity and concentration. The mouse ascites method is generally familiar, well understood, and widely available in many laboratories; but the mice require careful watching to minimize

ISSN 2249-3875

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the pain or distress induced by excessive accumulation of fluid in the abdomen or by invasion of the viscera. The in vitro tissue-culture method would be widely adopted if it were as familiar and well understood as the mouse ascites method and if it produced the required amount of antibody with every celline; but in vitro methods have been expensive and time-consuming relative to the costs and time required by the mouse ascites method and often failed to produce the required amount of antibody even with skilled manipulation. Modern in vitro methods have increased the success rate to over 90% and have reduced costs.

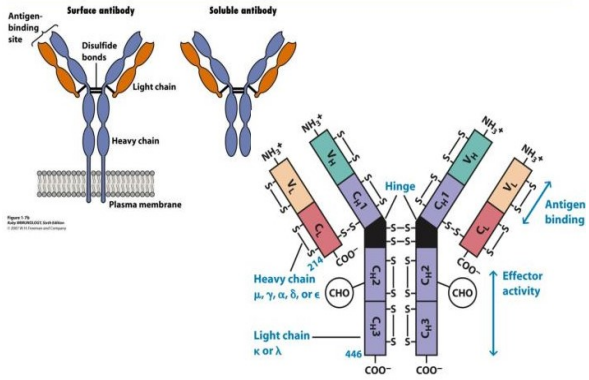
We believe that choosing the method of producing monoclonal antibodies should be consistent with other recommendations in the Guide for the Care and Use of Laboratory Animals.

Recommendation 1: There is a need for the scientific community to avoid or minimize pain and suffering by animals. Therefore, over the next several years, as tissue-culture systems are further developed, tissue-culture methods for the production of monoclonal antibodies should be adopted as the routine method unless there is a clear reason why they cannot be used or why their use would represent an unreasonable barrier to obtaining the product at a cost consistent with the realities of funding of biomedical research programs in government, academe, and industry. This could be accomplished by establishing tissue-culture production facilities in institutions.

Recommendation 2: The mouse ascites method of producing monoclonal antibodies should not be banned, because there is and will continue to be scientific necessity for this method.

Recommendation 3: When the mouse ascites method for producing MAb is used, every reasonable effort should be made to minimize pain or distress, including frequent observation, limiting the numbers of taps, and prompt euthanasia if signs of distress appear.

Recommendation 4: MAb now being commercially produced by the mouse ascites method should continue to be so produced, but industry should continue to move toward the use of tissue-culture methods.



PRODUCTION OF MONOCLONAL ANTIBODIES:

Production of monoclonal antibodies involves *in vivo* or *in vitro* procedures or combinations thereof. Before production of antibodies by either method, hybrid cells that will produce the antibodies are generated. The generation of MAb-producing cells requires the use of animals, usually mice. The procedure yields a cell line capable of producing one type of

antibody protein for a long period. A tumor from this "immortal" cell line is called a hybridoma. Development of the hybridoma technology has reduced the number of animals (mice, rabbits, and so on) required to produce a given antibody but with a decrease in animal welfare when the ascites method is used.

Step 1: Immunization of Mice and Selection of Mouse Donors for Generation of Hybridoma Cells:

Mice are immunized with an antigen that is prepared for injection either by emulsifying the antigen with Freund's adjuvant or other adjuvant or by homogenizing a gel slice that contains the antigen. Intact cells, whole membranes, and microorganisms are sometimes used as immuno-gens. In almost all laboratories, mice are used to produce the desired antibodies. In general, mice are immunized every 2-3 weeks but the immunization protocols vary among investigators. When a sufficient antibody titer is reached in serum, immunized mice are euthanized and the spleen removed to use as a source of cells for fusion with myeloma cells.

Step 2: Screening of Mice for Antibody Production:

After several weeks of immunization, blood samples are obtained from mice for measurement of serum antibodies. Several humane techniques have been developed for collection of small volumes of blood from mice (Loeb and Quimby 1999). Serum antibody titer is determined with various techniques, such as enzyme-linked immunosorbent assay (ELISA) and flow cytometry. If the antibody titer is high, cell fusion can be performed. If the titer is too low, mice can be boosted until an adequate response is achieved, as determined by repeated blood sampling. When the antibody titer is high enough, mice are commonly boosted by injecting antigen without adjuvant intra-peritoneally or intravenously (via the tail veins) 3 days before fusion but 2 weeks after the previous immunization. Then the mice are euthanized and their spleens removed for in vitro hybridoma cell production.

Step 3: Preparation of Myeloma Cells:

Fusing antibody-producing spleen cells, which have a limited life span, with cells derived from an immortal tumor of lymphocytes (myeloma) results in a hybridoma that is capable of unlimited growth. Myeloma cells are immortalized cells that are cultured with 8-azaguanine to ensure their sensitivity to the hypoxanthine-aminopterin-thymidine (HAT) selection medium used after cell fusion. A week before cell fusion, myeloma cells are grown in 8-azaguanine. Cells must have high viability and rapid growth. The HAT medium allows only the fused cells to survive in culture.

Step 4: Fusion of Myeloma Cells with Immune Spleen Cells:

Single spleen cells from the immunized mouse are fused with the previously prepared myeloma cells. Fusion is accomplished by co-centrifuging freshly harvested spleen cells and myeloma cells in polyethylene glycol, a substance that

ISSN 2249-3875

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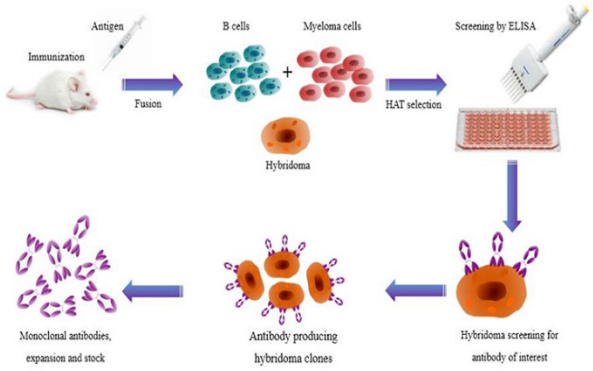
causes cell membranes to fuse. As noted in step 3, only fused cells will grow in the special selection medium. The cells are then distributed to 96 well plates containing feeder cells derived from saline peritoneal washes of mice. Feeder cells are believed to supply growth factors that promote growth of the hybridoma cells (Quinlan and Kennedy 1994).

Step 5: Cloning of Hybridoma Cell Lines by "Limiting Dilution" or Expansion and Stabilization of Clones by Ascites Production:

At this step new, small clusters of hybridoma cells from the 96 well plates can be grown in tissue culture followed by selection for antigen binding or grown by the mouse ascites method with cloning at a later time. Cloning by "limiting dilution" at this time ensures that a majority of wells each contain at most a single clone. Considerable judgment is necessary at this stage to select hybridomas capable of expansion versus total loss of the cell fusion product due to under population or inadequate in vitro growth at high dilution. In some instances, the secreted antibodies are toxic to fragile cells maintained in vitro. Optimizing the mouse ascites expansion method at this stage can save the cells. Also, it is the experience of many that a brief period of growth by the mouse ascites method produces cell lines that at later in vitro and in vivo stages show enhanced hardiness and optimal antibody production (Ishaque and Al-Rubeai 1998). Guidelines have been published to assist investigators in using the mouse ascites methods in these ways (Jackson and Fox 1995).



production of monoclonal antibody by immunizing mice

IN VITRO PRODUCTION OF MONOCLONAL ANTIBODIES

METHODS:

1. Batch Tissue-Culture Methods:

The simplest approach for producing MAb in vitro is to grow the hybridoma cultures in batches and purify the MAb from the culture medium. Fetal bovine serum is used in most tissue-culture media and contains bovine immunoglobulin at about 50 mg/ml.

In most cases, hybridomas growing in 10% fetal calf serum (FCS) can be adapted within four passages (8-12 days) to grow in less than 1% FCS or in FCS-free media. However, this adaptation can take much longer and in 3-5% of the cases the hybridoma will never adapt to the low FCS media. After this adaptation, cell cultures are allowed to incubate in commonly used tissue-culture flasks under standard growth conditions for about 10 days; MAb is then harvested from the medium.

The above approach yields MAb at

concentrations that are typically below 20 mg/ml.

batch tissue-culture methods are technically relatively easy to perform, have relatively low start-up costs, have a start-to-finish time (about 3 weeks) that is similar to that of the ascites method, and make it possible to produce quantities of MAb comparable with those produced by the mouse ascites method.

The disadvantages of these methods are that large volumes of tissue-culture media must be processed, the MAb concentration achieved will be low (around a few micrograms per millilitre), and some MAb are denatured during concentration or purification (Lullau and others 1996).

2. Semipermeable – Permeable - Based Systems:

Growth of hybridoma cells to higher densities in culture results in larger amounts of MAb that can be harvested from the media. The use of a barrier, either a hollow fiber or a membrane, with a low-molecular-weight cut-off (10,000-30,000 kD), has been implemented in several devices to permit cells to grow at high densities (Evans and Miller 1988; Falkenberg and others 1995; Jackson and others 1996). These devices are called semipermeable-membrane-based systems.

The objective of these systems is to isolate the cells and MAb produced in a small chamber separated by a barrier from a larger compartment that contains the culture media. Culture can be supplemented with numerous factors that help optimize growth of the hybridoma (Jaspert and others 1995). Nutrient and cell waste products readily diffuse across the barrier and are at equilibrium with a large volume, but cells and MAb are retained in a smaller volume (1-15 ml in a typical membrane system or small hollow-fiber cartridge). Expended medium in the larger reservoir can be replaced without losing cells or MAb; similarly, cells and MAb can be harvested independently of the growth medium. This compartmentalization makes it possible to achieve MAb concentrations comparable with those in mouse ascites.

The advantage of membrane-based systems is that high concentrations of MAb can be produced in relatively low volumes and fetal calf serum can be present in the media reservoir with only insignificant crossover of bovine immunoglobulins into the cell chamber. A disadvantage is that the MAb may be contaminated with dead cell products. Technical difficulty is slightly more than that of the batch tissue culture methods but should not present a problem for laboratories that are already doing tissue culture. The total MAb yield from a membrane system ranges from 10-160 mg according to Unisyn literature.

Advantages of In-Vitro Methods:

• In-vitro methods reduce the use of mice at the antibody-production stage (but can use mice as a source of feeder cells when antibody

ISSN 2249-3875

International Journal of Pharmaceutical Erudition

generation is under way).

• In-vitro methods are usually the methods of choice for large-scale production by the pharmaceutical industry because of the ease of culture for production, compared with use of animals, and because of economic considerations.

• In-vitro methods avoid the need to submit animal protocols to IACUCs.

• In-vitro methods avoid or decrease the need for laboratory personnel experienced in animal handling.

• In-vitro methods using semi permeable-membrane-based systems produce MAb in concentrations often as high as those found in ascitic fluid and are free of mouse ascitic fluid contaminants.

Disadvantages of In-Vitro Methods:

It should be noted that each of the items below pertains to only a fraction (3- 5%) of hybridomas, but they indicate some of the difficulties associated with in vitro methods.

• Some hybridomas do not grow well in culture or are lost in culture.

• In-vitro methods generally require the use of FCS, which limits some antibody uses. The use of in vitro methods for MAb production generally requires the use of FCS, which is a concern from the animal-welfare perspective.

• The loss of proper glycosylation of the antibody (in contrast with in vivo production) might make the antibody product unsuitable for in vivo experiments because of increased immunogenicity, reduced binding affinity, changes in biologic functions, or accelerated clearance in vivo.

• In general, batch-culture supernatants contain less MAb (typically 0.002-0.01) per milliliter of medium than the mouse ascites method

• In batch tissue-culture methods, MAb concentration tends to be low in the supernatant; this necessitates concentrating steps that can change antibody affinity, denature the antibody, and add time and expense.

• Most batches of MAb produced by membrane-based in vitro methods are contaminated with dead hybridoma cells and dead hybridoma-cell products, thus requiring early and expensive purification before study.

• MAb produced in vitro might yield poorer binding affinity than those obtained by the ascites method.

• In-vitro culture methods are generally more expensive than the ascites method for small-scale or medium-scale production of MAb (Hendriksen and de Leeuw1998; Jackson and others 1996; Peterson Peavey 1998; Marx 1998; Lipman 1997).

• The number of MAb produced by in vitro methods is limited by the amount of equipment that it is practical to have available.

TYPES OF MONOCLONAL ANTIBODIES

Throughout the progression of monoclonal drug development there have been four major antibody types developed: murine, chimeric, humanized and human.

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1. Murine monoclonal antibodies (suffix – *omab*):

Initially, murine antibodies were obtained by hybridoma technology, for which Kohler and Milstein received a Nobel prize. However, the dissimilarity between murine and human immune systems led to the clinical failure of these antibodies, except in some specific circumstances. Major problems associated with murine antibodies included reduced stimulation of cytotoxicity and the formation complexes after repeated administration, which resulted in mild allergic reactions and sometimes anaphylactic shock.

2. Chimeric and humanized monoclonal antibodies (suffixes *-ximab*, *-zumab* respectively):

Chimeric antibodies are composed of murine variable regions fused onto human constant regions. Human gene sequences, taken from the kappa light chain and the IgG1 heavy chain, results in antibodies that are approximately 65% human. This reduces immunogenicity, and thus increases serum half-life.

Humanized antibodies are produced by grafting murine hypervariable regions on amino acid domains into human antibodies. This results in a molecule of approximately 95% human origin.

However, it has been shown in several studies that humanized antibodies bind antigen much more weakly than the parent murine monoclonal antibody, with reported decreases in affinity of up to several hundredfold. Increases in antibody-antigen binding strength have been achieved by introducing mutations into the complementarity determining regions (CDR), using techniques such as chain-shuffling, randomization of complementarity determining regions and generation of antibody libraries with mutations within the variable regions by error-prone PCR, E. coli mutator strains, and site-specific mutagenesis.

3. Human monoclonal antibodies (suffix -*umab*):

The general scheme of Human monoclonal antibodies is produced using transgenic mice or phage display libraries. Human monoclonal antibodies are produced by transferring human immunoglobulin genes into the murine genome, after which the transgenic mouse is vaccinated against the desired antigen, leading to the production of monoclonal antibodies, allowing the transformation of murine antibodies in vitro into fully human antibodies. The heavy and light chains of human IgG proteins are expressed in structural polymorphic (allotypic) forms. Human IgG allotype has been considered as one of the many factors that can contribute to immunogenicity.

APPLICATIONS OF MONOCLONAL ANTIBODIES:

1. Diagnostic applications of monoclonal antibodies:

1.1 Diagnostic reagents:

Monoclonal antibody based diagnostic reagents include products for detecting pregnancy, diagnosing infections protozoan, bacterial and

viral pathogens; monitoring therapeutic drug levels, detecting heart damage, matching histocompatibility antigens; detecting diabetes and detecting tumor cells.

Many of these test kits utilize strips of paper impregnated with an appropriate monoclonal antibody. Diagnostic products are relatively inexpensive to produce and are projected to have growing markets.

1.2 Diagnostic imaging:

It employs the use of planar gamma camera to detect the two dimensional distribution in the body of the gamma emitting radioisotopes conjugated to MAbs. Recently camera utilizing single photon emission computed tomography (SPECT) has been used to give more sensitive three dimensional evaluations.

Various imaging applications of MAbs are as follows:

a. Cardiovascular disease:

- Myocardial infarction
- Deep-vein thrombosis
- Atherosclerosis

b. Sites of bacterial infections:

c. Cancer

- Solid tumor (antitumor associated antigen)
- Locating and sizing
- Detection of occult tumors
- Determining suitability of MAbs
- Monitoring response in therapy

d. Cell surface markers

e. Hormones

f. Immuno-metric assay procedures

g. Rapid detection of drugs in urine

2 Therapeutic applications:

2.1 General:

- Radioisotope immune-conjugates: These MAbs conjugates deliver cytotoxic doses of radioactivity to target cells.
- Toxin and drug immune-conjugates: MAbs are being evaluated for the delivery of potent toxin like ricin, abrin, pokeweed antiviral protein, gelonin, diphtheria toxin and pseudomonas endotoxins and drugs to target cells while reducing toxicity to non-target tissues.

2.2 Transplantations:

- Organ transplantation: Like renal, hepatic, cardiac and combined kidney pancreas transplants. Ortho-clone(OKT3) was first MAb to be licensed for human use.

2.3 Infectious disease:

- Against microorganisms
- Against parasites

2.4 Autoimmune disease:

MAbs directed against B and T lymphocytes used for therapy of autoimmune disease such as rheumatoid arthritis and multiple sclerosis.

2.5 Murine Antidotes:

MAbs have been produced which react against digoxin and tetanus toxins.

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