

A review on Monoclonal antibody and its application in biotechnology

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Abstract

The production of monoclonal antibodies was invented by Cesar Milstein and Georges J. F. Kohler in 1975 and they got a Nobel Prize for this work in 1984. Hybridoma technology is the method useful for the production of large quantity of identical antibodies these antibodies are known as monoclonal antibodies. The production of monoclonal antibody is done by the administration of antigen in mouse which produces an immune response. The B-cells producing antibodies are then harvested from the injected mouse. The harvested B-cells are then fused with B cancer cells which remain immortal. This produces hybrid cell line called hybridoma which possesses the antibody-producing ability of the B-cell. The hybridomas can be grown in culture with one viable cell which produces cultures having genetically identical hybridomas. It produces monoclonal antibodies. It retains the ability to grow in tissue culture and do not possess antibody producing capability. Monoclonal antibodies are used to track cancer antigens and, alone or linked to anticancer agents, to attack cancer metastases. The monoclonal antibody known as OKT3 is saving organ transplants threatened with rejection, and preventing bone marrow transplants. The advantage of this process it combine the qualities of the two different types of cells; the ability to grow continually, and to produce large amounts of pure antibody. Monoclonal antibody is also useful for the treatment of non-infectious diseases such as immune disease, arthritis, cancer etc.

Key Words- Monoclonal antibody production, hybridoma technology, application

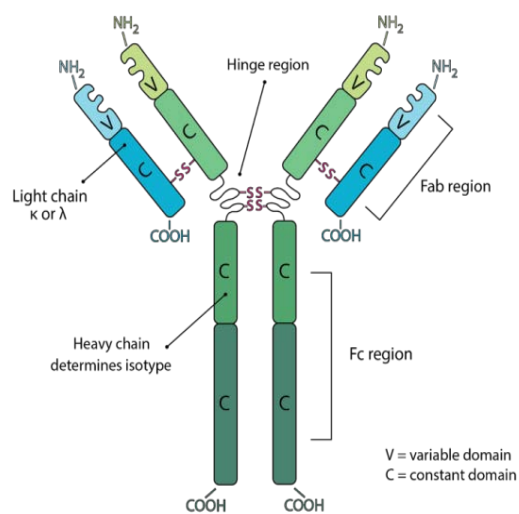
INTRODUCTION

Hybridomas are cells that have been engineered to produce a desired antibody in large amounts, to produce monoclonal antibodies. (1,2) Monoclonal antibodies can be produced in specialized cells through a technique now popularly known as **hybridoma technology**. (1) Hybridoma technology was discovered in 1975 by two scientists, Georges Kohler of West Germany and Cesar Milstein of Argentina and they got the noble prize for this work in 1984.

The production of one MAb, using the hybridoma technology, costs between \$8,000 and \$12,000. Monoclonal antibodies is valuable for the analysis of parasites antigen Monoclonal antibodies are being used to track cancer antigens and, alone or linked to anticancer agents, to attack cancer metastases. The monoclonal antibody known as OKT3 is saving organ transplants threatened with rejection, and preventing bone marrow transplants from setting off graft-versus-host disease. The mAbs have various applications in the fields of cell biology, immunology, biotechnology and medicines. They are also being used in vivo imaging techniques of different kinds of diseases (3,4). mAbs are important diagnostic reagents used in biomedical research, microbiological research in the diagnosis of Hepatitis, AIDS, influenza, herpes simplex, infections and in the treatment of such diseases and cancer (5).

Antibodies are glycoprotein molecule present in the serum. They are produced in response to antigen which are either protein or polysaccharide molecules which may be foreign to the body. Antibodies are secreted by a class of

blood cells known as B-lymphocytes. Each antibody produced is specific to that particular antigen which has stimulated its production. Antibodies may be classified five major classes such as – **IgG (monomer)**, **IgA (Dimer)**, **IgM (Pentamer)**, **IgD (monomer)**, **IgE (monomer)**. Antibodies are also called as **Immunoglobulin (Ig)** and the structure of antibody contains two chain such as – Heavy chain, Light chain and constant region ,variable region and they also contain di sulphide bond etc (6).

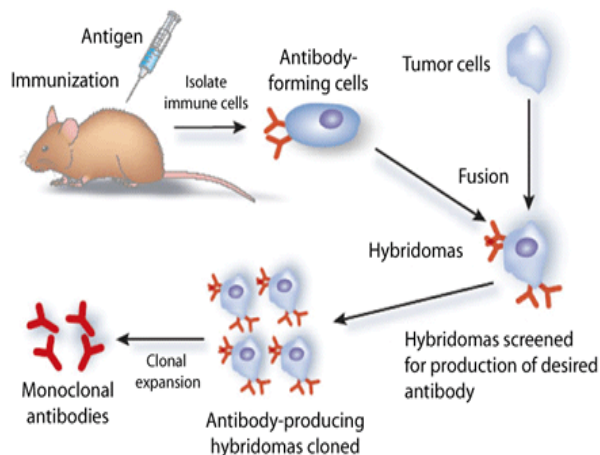


Diag. Structure of antibody

Procedure for the Production of monoclonal antibody

Basic steps involved in the production of a monoclonal antibody:

1. Immunization (Immunize the animal)
2. Cell fusion process
3. Selection of hybridomas
4. Screening the products
5. Cloning and propagation
6. Characterization and storage



Diag. Production of monoclonal antibody

1. Immunize the animal

The first step in hybridoma technology is to immunize an animal (mouse), with appropriate antigen. The antigen, along with an adjuvant like Freund's complete or incomplete adjuvant is injected subcutaneously. The injections at multiple sites are repeated several times. This enables increased stimulation of B-lymphocytes which are responding to the antigen. Three days prior to killing of the animal, a final dose of antigen is intravenously administered. The immune-stimulated cells for synthesis of antibodies have grown maximally by this approach. The concentration of the desired antibodies is assayed in the serum of the animal at frequent intervals during the course of immunization.

When the serum concentration of the antibodies is optimal, the animal is sacrificed. The spleen is aseptically removed and disrupted by mechanical or enzymatic methods to release the cells. The lymphocytes of the spleen are separated from the rest of the cells by density gradient centrifugation.

2. Cell Fusion process:

The thoroughly washed lymphocytes (spleen cell) are mixed with HGPRT negative myeloma cells. The mixture of cells is exposed to polyethylene glycol (PEG) for a short period (a few minutes), since it is toxic. PEG is removed by washing and the cells are kept in a fresh medium. These cells are composed of a mixture of hybridomas (fused cells), free myeloma cells and free lymphocytes.

Condition for Fusion Procedure:

Before carrying out the fusion the following condition should be met for the mouse system:

- Myeloma cells in the logarithmic growth phase
- Ratio of 2-5 lymphocyte per myeloma cell

- 40% PEG (1000dal); PEG should be protected for cell toxicity
- Fusion at 37°C, pH 7.5 -8.0, 3 minute

3. Selection of Hybridomas:

When the cells are cultured in HAT medium only the hybridoma cells grow, while the rest will slowly disappear. This happens in 7-10 days of culture. Selection of a single antibody producing hybrid cells is very important. This is possible if the hybridomas are isolated and grown individually. The suspension of hybridoma cells is so diluted that the individual aliquots contain on an average one cell each. These cells, when grown in a regular culture medium, produce the desired antibody.

4. Screening the Products:

The hybridomas must be screened for the secretion of the antibody of desired specificity. The culture medium from each hybridoma culture is periodically tested for the desired antibody specificity. The two techniques namely ELISA and RIA are commonly used for this purpose.

In both the assays, the antibody binds to the specific antigen (usually coated to plastic plates) and the unbound antibody and other components of the medium can be washed off. Thus, the hybridoma cells producing the desired antibody can be identified by screening. The antibody secreted by the hybrid cells is referred to as monoclonal antibody.

5. Cloning and Propagation:

The single hybrid cells producing the desired antibody are isolated and cloned. Two techniques are commonly employed for cloning hybrid cells:

- limiting dilution method
- soft agar method.

Limiting dilution method: In this procedure, the suspension of hybridoma cells is serially diluted and the aliquots of each dilution are put into micro culture wells. The dilutions are so made that each aliquot in a well contains only a single hybrid cell. This ensures that the antibody produced is monoclonal.

Soft agar method: In this technique, the hybridoma cells are cultured in soft agar. It is possible to simultaneously grow many cells in semisolid medium to form colonies. These colonies will be monoclonal in nature. In actual practice, both the above techniques are combined and used for maximal production of MABs.

6. Characterization and Storage:

The monoclonal antibody has to be subjected to biochemical and biophysical characterization for the desired specificity. It is also important to elucidate the MAB for the immunoglobulin class or sub-class, the epitope for which it is specific and the number of binding sites it possesses.

The stability of the cell lines and the MABs are important. The cells (and MABs) must be characterized for their ability to withstand freezing, and thawing. The desired cell lines are frozen in liquid nitrogen at several stages of cloning and culture.

Applications (1,7)

➤ Diagnostic Applications:

Monoclonal antibodies have revolutionized the laboratory diagnosis of various diseases. For this purpose, MAbs may be employed as diagnostic reagents for biochemical analysis or as tools for diagnostic imaging of diseases.

MAbs in Biochemical Analysis: Diagnostic tests based on the use of MAbs as reagents are routinely used in radioimmunoassay (RIA) and enzyme-linked immunosorbent assays (ELISA) in the laboratory. These assays measure the circulating concentrations of hormones (insulin, human chorionic gonadotropin, growth hormone, progesterone, thyroxine, triiodothyronine, thyroid stimulating hormone, gastrin, renin), and several other tissue and cell products (blood group antigens, blood clotting factors, interferon's, tumor markers).

In recent years, a number of diagnostic kits using MAbs have become commercially available. Now it is useful for diagnosis the various disease:

- **Pregnancy:** Pregnancy by detecting the urinary levels of human chorionic gonadotropin.
- **Cancers:** Cancers estimation of plasma carcinoembryonic antigen in colorectal cancer, and prostate specific antigen for prostate cancer. Besides diagnosis, estimation of tumor markers is also useful for the prognosis of cancers. That is a gradual fall in a specific tumor marker is observed with a reduction in tumor size, following treatment.
- **Hormonal disorders:** Hormonal disorders analysis of thyroxine, triiodothyronine and thyroid stimulating hormone for thyroid disorders.

➤ Therapeutic Applications:

Monoclonal antibodies have a wide range of therapeutic applications. MAbs are used in the treatment of cancer, transplantation of bone marrow and organs, autoimmune diseases, cardiovascular diseases and infectious diseases.

The therapeutic applications of MAbs are broadly grouped into 2 types:

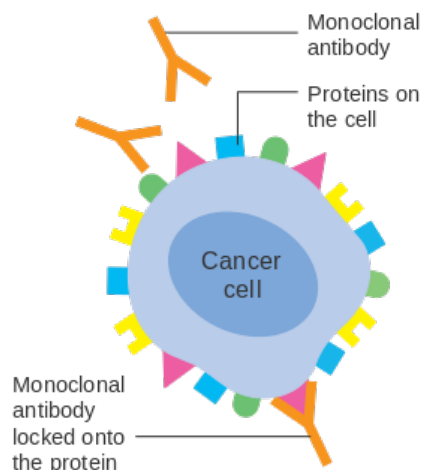
- Direct use of MAbs as therapeutic agents
- MAbs as targeting agents

Direct use of MAbs as therapeutic agents

Monoclonal antibodies can be directly used for enhancing the immune function of the host. Direct use of MAbs causes minimal toxicity to the target tissues or the host.

In destroying disease-causing organisms: MAbs promote efficient opsonization of pathogenic organisms (by coating with antibody) and enhance phagocytosis. In fact, MAbs were found to protect chimpanzees against certain viral (hepatitis B-virus) and bacterial (*E. coli* Haemophilus influenza, *Streptococcus* sp and *Pseudomonas* sp) infections.

In the treatment of cancer: MAbs, against the antigens on the surface of cancer cells, are useful for the treatment of cancer. The antibodies bind to the cancer cells and destroy them. This is brought out by antibody—dependent cell-mediated cytotoxicity, complement-mediated cytotoxicity and phagocytosis of cancer cells (coated with MAbs) by reticuloendothelial system.



Diag. Monoclonal antibody bound to cancer cell protein

The patients suffering from leukemia, colorectal cancer, lymphoma and melanoma have been treated with MAbs. However, there was a wide variation in the success rate. A monoclonal antibody specific to the cells of leukemia is used to destroy the residual leukemia cells without affecting other cells. MAbs are used in vitro to remove the residual tumor cells prior to autologous bone marrow transplantation (transplantation of the patient's own bone marrow cells, due to non-availability of a suitable donor).

Limitations for direct use of MAbs in cancer:

1. The MAbs produced in mice and directly used for therapeutic purposes may lead to the development of anti-mouse antibodies and hypersensitivity reactions.
2. All the cancer cells may not carry the same antigen for which MAb has been produced. Thus, MAbs may not be attached to some cancer cells at all.
3. The free antigens (of target cells) present in the circulation may bind to MAbs and prevent them from their action on the target cells

In the treatment of AIDS: Immunosuppression is the hall mark of AIDS. This is caused by reduction in CD₄ (cluster determinant antigen 4) cells of T-lymphocytes. The human immunodeficiency virus (HIV) binds to specific receptors on CD₄ cells by using surface membrane glycoprotein (gp120).

Genetic engineers have been successful to attach Fc portion of mouse monoclonal antibody to human CD₄ molecule. This complex has high affinity to bind to membrane glycoprotein gp120 of virus infected cells. The Fc fragment induces cell-mediated destruction of HIV infected cells (Fig. 17.7).

In the treatment of autoimmune diseases: Autoimmune diseases like rheumatoid arthritis and multiple sclerosis are of great concern. Some success has been reported in the clinical trials of rheumatoid arthritis patients by using MAbs directed against T-lymphocytes and B-lymphocytes.

MAbs as Targeting Agents in Therapy

Toxins, drugs, radioisotopes etc., can be attached or conjugated to the tissue-specific monoclonal antibodies

and carried to target tissues for efficient action. This allows higher concentration of drugs to reach the desired site with minimal toxicity. In this way, MABs are used for the appropriate delivery of drugs or isotopes.

MABs in drug delivery: In general, the drugs are less effective in vivo (in the living body) when compared to in vitro (in laboratory when tested with cultured cells). This

is mainly due to the fact that sufficient quantity of the drug does not reach the target tissue. This problem can be solved by using tissue-specific MABs. The drugs can be coupled with MAB (directed against a cell surface antigen of the cells, say a tumor) and specifically targeted to reach the site of action (Fig. 17.9A).

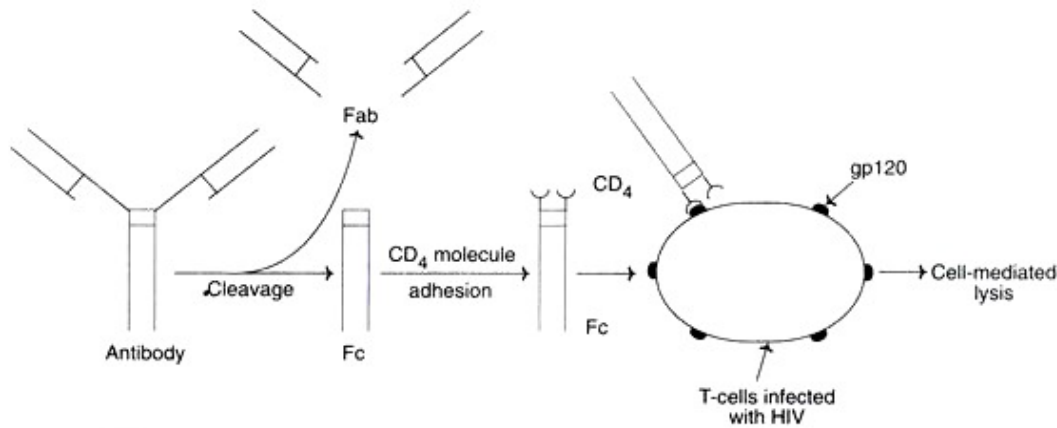


Fig. 17.7 : Modified monoclonal antibody in the treatment of AIDS.

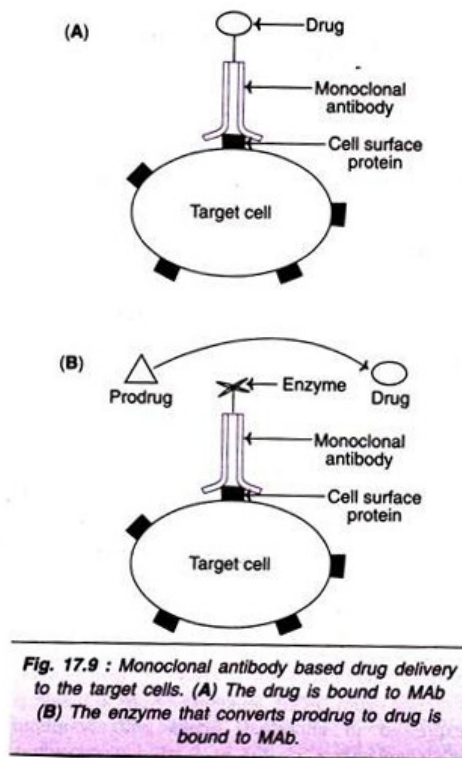


Fig. 17.9 : Monoclonal antibody based drug delivery to the target cells. (A) The drug is bound to MAB (B) The enzyme that converts prodrug to drug is bound to MAB.

In the treatment of certain diseases, a pro-drug (an inactive form of the drug) can be used. This can be enzymatically converted to active drug in the target tissues. For this purpose, the enzyme (that converts pro-drug to drug) is coupled with MAB that is directed against a specific cell surface antigen (Fig. 17.9A). This approach, referred to as antibody-directed enzyme pro-drug therapy (ADEPT), allows an effective delivery of the drug to the cells where it is required.

The some examples of enzymes that have been used in ADEPT:

- i. Alkaline phosphatase for the conversion of phosphate pro-drugs.
- ii. Carboxy peptidase for converting inactive carboxyl pro-drugs to active drugs.
- iii. Lactamase for hydrolyzing β -lactam ring containing antibiotics.

MABs in radio immunotherapy (RAIT): The radioisotopes can be coupled to MABs that are directed against tumor cells. This allows the concentration of radioactivity at the desired sites and a very efficient killing of target cells (tumor cells). The advantage with radio immunotherapy is that conjugated complex need not penetrate the cells, as is required in immunotoxin therapy. The limitation is that the neighbouring normal cells may also get damaged or killed. This can be minimized by using radioisotopes with short half-lives. Yttrium-90 with a half-life of 64 hours is a suitable isotope to be employed in RAIT. Due to shortage in the supply of yttrium-90, indium-111 is more commonly used.

Protein Purification: Monoclonal antibodies can be produced for any protein. And the so produced MAB can be conveniently used for the purification of the protein against which it was raised. MABs columns can be prepared by coupling them to cyanogen bromide activated Sepharose (chromatographic matrix). The immobilized MABs in this manner are very useful for the purification of proteins by immunoaffinity method.

Different **advantages** of using **MABs for protein purification**. These include the specificity of the MAB to bind to the desired protein, very efficient elution from the chromatographic column and high degree of purification. Immunoaffinity chromatography is routinely used for the purification of recombinant interferon's. The efficiency of this technique will be obvious from the fact that by a single step, it is possible to achieve more than 5,000 fold purification of interferon- α_2 .

CONCLUSION

Hybridomas are cells that have been engineered to produce a desired antibody in large amounts. Hybridoma technology is a patented technology its capacity to production of major industrial needs for a faster, lower cost and better quality process and Due to the presence of desired immunity, monoclonal antibodies are used in the diagnosis of diseases such as AIDS, it is also useful in pregnancy test kits. Monoclonal antibodies are specific antibodies arc now it is essential tool of biomedical research and great commercial and medical value. For example it is useful for the detection of (ABO) blood groups.

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