

THE APPLICATION OF MONOCLONAL ANTIBODIES IN MEDICINE

Abstract

An antigen usually has many epitopes and may stimulate specific B-cells and bind to the specific epitope. Each stimulated B-cell undergoes proliferation and produces a clone of B-cells which then differentiates into plasma cells. Plasma cells in turn produce a mixture of antibodies specific to various epitopes. However, if only one particular epitope stimulates one B cell and allowed to proliferate and produce antibodies having the same antigenic specificity, they are referred to as monoclonal antibodies (mAbs). The Hybridoma technology developed in 1975 by Georges J. F. Kohler and C. Milstein produces monoclonal antibodies. Recent research on mAbs have its widespread uses in clinical medicine for both diagnostic and therapeutic purposes. Its uses in ELISA, immunohistochemistry and western immunoblotting have redefined in the diagnosis of various diseases. Uses of mAbs in cancer treatment, immune diseases and many bacterial and viral diseases are increasing. The future prospect of this review is to understand its application in the trend of diseases and its utility in clinical diagnostic and therapeutic fields.

Keywords: Monoclonal antibody, hybridoma technology, ELISA, western immunoblotting

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I. INTRODUCTION

Immunoglobulin or antibody is a glycoprotein synthesized from activated B-cells or plasma cells in response to an antigen. Monoclonal antibodies(mAbs) are antibodies with identical antigenic specificity obtained from a single B-cell clone.

In 1975, G. Kohler and C. Milstein discovered a technique called hybridoma technology (fusion of normal cells and malignant cells) for the production of monoclonal antibodies, and were awarded the Nobel Prize in 1984. In this technique, an antigen-primed B-cell and an immortal cell are combined together to form a hybrid cell(hybridoma). A hybridoma can multiply indefinitely and generate a huge population or clones of identical cells, and can produce antibodies of the same antigenic specificity. The immortal cell is a double mutated myeloma cell i.e. Hypoxanthine Guanine Phosphoribosyl Transferase (HGPRT) gene and immunoglobulin gene loci have been rendered inactive. Such a myeloma cell cannot use the purine salvage pathway nor can generate its own antibodies. The antigen-primed B-cells are obtained from the spleen of mice. The antigen-primed B-cells are then fused with the mutated myeloma cells using polyethylene glycol. The hybrid cells are selected by culturing in HAT medium(Hypoxanthine, Aminopterin, Thymidine). The unique feature of this HAT medium is that only the hybrid cells will survive indefinitely thereby producing specific monoclonal antibodies of murine origin.

A Murine monoclonal antibody, Muromonab-CD3 (trade name Orthoclone OKT3), was the first to be approved for use in acute rejection of kidney transplant. Adalimumab, used for the treatment of patients diagnosed with rheumatoid arthritis, was the first FDA approved fully human monoclonal antibody developed in 2002.

Newer techniques have been developed for the monoclonal antibody production by recombining mouse and human proteins to produce human monoclonal antibodies which are less immunogenic than the murine monoclonal antibodies. They are categorized as;

1. Chimeric monoclonal antibodies: The variable region of the antibody belongs to mouse and the constant region belongs to human (65%). It is less antigenic without altered specificity, therefore less immunogenic.
2. Humanized monoclonal antibodies: Here, only the sequences of the CDRs are of mouse origin, while the rest of the antibody are of human origin(>90%).
3. Fully human monoclonal antibodies: The whole antibody is of human origin.

II. THE APPLICATION OF MONOCLONAL ANTIBODIES

1. **Diagnostics applications:** Monoclonal antibodies are used in different techniques such as ELISA, flow cytometry, immunohistochemistry, western blotting and radioimmunity assay.
 - **Diagnostic histopathology:** Monoclonal antibodies are used to classify tissues and organs based on the expression of certain biomarkers. For example, prostate-specific antigen, placental-ALP, HCG, alpha-fetoprotein etc. which are organ-specific antigens, the monoclonal antibodies against these antigens are used to identify tumors of such organs.

- **Enzyme linked immunosorbent assay:** Many immunological tools developed for the diagnosis of infectious diseases are through the detection of antigens or antibodies in the sera of infected individuals. In this regard, monoclonal antibodies target the specific epitopes and are detected by the presence of change in colour or fluorescence. Example includes detection of HIV, Hepatitis B, Tuberculosis, Typhoid and others such detection of pregnancy, blood group identification etc.
- **Western immunoblotting:** In molecular biology or biochemistry or immunogenetics, monoclonal antibodies are used to detect proteins in a given sample of tissue extract. Western blotting which uses monoclonal antibody was developed for the detection of viruses such as Cytomegaloviruses and HIV.

2. Therapeutic Applications

Cancer therapy: Monoclonal antibodies as anti-cancer agents act by the following mechanisms

- **Interfering with intracellular signalling pathways.** Many receptors expressed on cancer cell surface such as ErbB1, ErbB2 or HER-2/Neu, HER-3, and HER-4 are up-regulated in tumors arising from epithelial cells of the colon, breast, lung, and head and neck leading to a rapidly proliferating disease with increased metastatic potential. Monoclonal antibodies bind and downregulate these receptors thereby inhibiting the cancer cell proliferation leading to apoptosis.
- **Antibody-dependent cellular cytotoxicity (ADCC):** The Fab portion of a monoclonal antibody interacts with the tumor cell antigen while its Fc portion is recognised by effector cells such as NK cells, macrophages and neutrophils. This interaction forms a connection between the effector cells and the target tumor cells leading to the destruction of the target tumor cells by the effector cells.
- **Complement-dependent cytotoxicity (CDC):** Monoclonal antibody-coated target tumor cells activate the complement cascade which causes target tumor cell lysis by the formation of membrane attack complex.
- **Neutralization of soluble ligand:** Certain soluble proteins can bind to their target receptors on the tumor cells and facilitate the proliferation of tumors. Monoclonal antibodies can bind such soluble proteins and neutralize its action.
- **Delivery of Cytotoxic drug:** Monoclonal antibodies targeted for a tumor are conjugated with cytotoxic drugs specifically against the tumor cells with limited systemic side effect.

Table 1: Monoclonal antibodies used in clinical oncology

mAbs names	Target	Antibody type	Application
Cetuximab	EGFR	Chimeric	Colorectal, breast. Lung cancer
Panitumumab	EGFR	Human	Colorectal cancer
Nimotuzumab	EGFR	Humanized	Head and neck cancer
Rituximab	CD-20	Chimeric	Non-Hodgkin lymphoma
Ofatumumab	CD-20	Human	Chronic Lymphocytic Leukemia
Trastuzumab	HER2	Humanized	Breast cancer
Ipilimumab	CTLA-4	Human	Metastatic melanoma

Infectious diseases: The monoclonal antibody Palivizumab is used as a prophylactic treatment for Respiratory Syncytial Virus (RSV) infection. T-cell mediated adaptive immunity is primarily responsible for the clearance of the virus. Additionally, monoclonal antibodies can neutralize the virus infected cell expressing specific proteins by the activation of natural killer (NK) cells. Inmazeb (atoltivimab, maftivimab, and odesivimab), a three monoclonal antibody mixture, is used for the treatment of Zaire-ebolavirus (Ebola virus) infection in paediatric and adult patients. The REGEN-CoV-2 which is a cocktail of two monoclonal antibodies (Casirivimab+Imdevimab), has been approved for treatment and prevention of SARS-CoV-2 infection. Monoclonal antibodies have been used to provide passive immunity against acute attack of diseases like Rabies and Tetanus.

Rh-incompatibility: Pregnancy by Rh+ve husband leading to Rh+ve foetus in a Rh-ve mother leads to Rh incompatibility. The foetal Rh+ve cells enter the mother's circulation during delivery and sensitize the maternal immune system which produces anti-Rh antibodies and memory cells. The antibodies can destroy the foetal cells if they come in contact with them. For example, the leakage of a small number of Rh+ve foetal cells into the maternal blood during the late pregnancy and delivery activate the Rh specific B cells producing plasma cells and memory cells. The plasma cells secrete IgM Abs and then die due to short life span. The secreted IgM clear the foetal Rh+ cells from the mother circulation but memory cells persist. These cells are a threat to any following pregnancy. Activation of these memory cells in the following pregnancy by the Rh +ve foetal cells leads to the production of anti-Rh IgG antibodies. These Abs cross the placenta and hemolyze the foetal RBCs leading to anaemia and jaundice. This can at times be fatal to the foetus depending upon the severity. The Administration of anti-Rh antibodies at around 28 weeks of pregnancy or within 48-72 hours of first delivery will prevent this Rh incompatibility in all subsequent pregnancies by rapidly clearing the foetal cells from the mother's circulation without giving a chance to activate the mother's immune system and producing memory cells.

Autoimmune diseases: Monoclonal antibodies have been used to suppress the immune response in autoimmune diseases and for prevention transplant rejection.

Table 2: Monoclonal antibodies used in autoimmune diseases

Antibody name	Target	Antibody format	Application
Infliximab	TNF-alpha	Chimeric	Rheumatoid arthritis, Crohn's disease, ankylosing spondylitis
Adalimumab	TNF-alpha	Human	Rheumatoid arthritis, Ulcerative colitis, Crohn's disease.
Basiliximab	IL-2	Chimeric	Acute rejection of kidney transplant
Daclizumab	IL-2	Humanized	Acute rejection of kidney transplant
Omalizumab	IgE	Humanized	Asthma

Some of the monoclonal antibodies used in the autoimmune diseases and transplant rejection are mentioned in the Table 2.

III. CONCLUSION

The development of monoclonal antibodies is one of the most successful application in the field of medicine till date. Fully humanised monoclonal antibodies have been developed with reduced immunogenicity and increased effectiveness. Monoclonal antibodies are being utilized for diagnostic purposes like ELISA, immunohistochemistry and western immunoblotting etc. On the other hand, diseases that are an issue globally such as cancer, AIDS and autoimmune diseases are being treated using monoclonal antibodies. Furthermore, the newer techniques used for the production of the monoclonal antibody should be adopted in the developing countries and readily available for use.

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