Application of monoclonal antibodies in medicine

Medowelie Mathew Thokchom Shawankumar Singh

Post Graduate Trainee : Dept. of the Biochemistry State Medical officer

Regional Institute of Medical Sciences, Imphal, Manipur Govt. of Manipur

 mathewmedowelie@gmail.com thshawan2015@gmail.com

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 B-cell is specifically stimulated by one particular epitope and allowed to proliferate and produce antibodies having the same antigenic specificity, they are called monoclonal antibodies (mAbs). The production of monoclonal antibodies is done by Hybridoma technology discovered in 1975 by Georges Kohler of West Germany and Cesar Milstein of Argentina. Modern day research on mAbs have its widespread uses in clinical medicine for both diagnostic and therapeutic purposes. Its uses in ELISA, immunohistochemistry and western immunoblotting has redefined in the diagnosis of various diseases. Use of mAbs in cancer treatment, immune diseases and many bacterial and viral diseases is increasing. The future prospect of this review is to understand its application in the trend of diseases and its role in research in clinical diagnostic and therapeutic fields.

Keywords – Monoclonal antibody; hybridoma technology; ELISA; western immunoblotting

**I. INTRODUCTION**

Antibody or immunoglobulin is a glycoprotein, produced from activated B cells (plasma cells) in response to an antigen. Monoclonal antibodies(mAb) are antibodies with **i**dentical antigenic specificity & derived 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 for which they were awarded Noble Prize in 1984. In this technique, an antigen primed B-cells and immortal cells are fused together to form a hybrid cells(hybridoma). A hybridoma has the ability to multiply indefinitely and produce a large population or clone of identical cells, and can produce antibodies of 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 produce its own antibodies. The antigen-primed B cells are obtained from spleen of mice. The antigen-primed B cells and the mutated myeloma cells are then fused in polyethylene glycol. The hybrid cells are selected by growing these cells in HAT medium(Hypoxanthine, Aminopterine, 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.

In 1986, the first licensed murine monoclonal antibody, Orthoclone OKT3 (muromonab-CD3) was approved for use in preventing kidney transplant rejection. Adalimumab (Humira), used for the treatment of rheumatoid arthritis, was the first fully human antibodyapproved by the U.S. Food and Drug Administration (approved in 2002).

Since the discovery of hybridoma technique, various modifications have been developed to produce monoclonal antibodies by recombining human and mouse proteins to produce human monoclonal antibodies which are less immunogenic than the murine monoclonal antibodies. They are categorized as;

1. Chimeric mAb: the variable region of the antibody is of mouse origin and the constant region is of human origin(65%). It has less antigenicity without altered specificity, therefor less immunogenic.
2. Humanized mAb: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 mAb: The whole antibody is of human origin.

**II. APPLICATION OF MONOCLONAL ANTIBODIES**

1. **DIAGNOSTICS APPLICATIONS:** Monoclonal antibodies are used in different techniques such as ELISA, flow cytometry, immunohistochemistry, western blotting, radioimmunology assay.

**a). Monoclonal antibodies in diagnostic histopathology:** With the help of monoclonal antibodies, tissues and organs can be classified based on their expression of certain defined markers. For example, Prostate-specific antigen, placental alkaline phosphatase, human chorionic gonadotrophin, α-fetoprotein, and others are organ-associated antigens and the production of monoclonal antibodies against these antigens helps in determining the nature of a primary tumor.

**b). Enzyme linked immunosorbent assay:** Many immunological assays have been developed for the diagnosis of infectious diseases through the detection of either antigen or anti-antibodies in sera of infected animals. In this regard, the specific epitopes are targeted by the monoclonal antibodies 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.

**c). Western immunoblotting:** It is a method in molecular biology or biochemistry or immunogenetics to detect protein in a given sample of tissue homogenate or extract, which is normally used with a high antibody directed against the desired antigen. Western blotting was developed based on monoclonal antibody for the detection of viruses such as Cytomegaloviruses and HIV detection.

**B. THERAPEUTIC APPLICATIONS**

**a). Cancer therapy:** The concept of using monoclonal antibodies to selectively target tumors was proposed by Paul Ehrlich.

**Mechanism of monoclonal antibodies for the treatment of cancer:**

**i. Altering signal transduction in the downstream intracellular pathways**. Various cell surface receptors, expressed by cancer cells such as, EGFR or ErbB1, ErbB2 or HER-2/Neu, HER-3, and HER-4 are over-expressed in epithelial malignancies originating from the colon, breast, lung, and head and neck resulting in rapidly proliferating disease and increased metastatic potential. Downstream activation of the receptor and increasing receptor internalization inhibited by Anti-EGFR antibodies bind to the receptor domain of the EGFR receptor. Thus, cancer cell cycle is inhibited by antibodies and cause apoptosis.

**ii. Antibody-dependent cell cytotoxicity (ADCC).** The cancer cells that are coated by antibodies resulted in immune-mediated destruction. The effector cells in the antibody-dependent cytotoxicity include macrophages, NK cells, and neutrophils. ADCC occurs when the Fab and Fc portions of the mAb engage both tumor cell antigen and an activating FcγR, respectively, thus creating a bridge from the tumor cell to the effector cell. Target cell recognition is then coupled to a lytic attack on the target cell mounted by effector cells.

**iii. Complement-mediated cytotoxicity (CDC).** CDC results from a cytolytic cascade mediated by a series of complement proteins, resulting in lysis of the antibody-bound cell.

**iv. Soluble ligand neutralization**. Antibodies can bind to circulating proteins and interfere with their ability to find their targets to help facilitate the growth of the tumors. Bevacizumab, a fully human mAb binds and inactivates the biological activity of VEGF-A, inhibiting angiogenesis, and thus, tumor growth and proliferation.

**v. Cytotoxic drug delivery**. Tumor-targeted monoclonal antibodies are linked to Cytotoxic agents to deliver them specifically to the tumor cells which is preferable for its limited systemic side effect. Trastuzumab emtansine (Kadcyla) for HER-2 positive metastatic breast cancer, Inotuzumab ozogamicin (Bespona) for CD-22 + B cell precursor for ALL are such examples.

**Table 1: Monoclonal antibodies used in clinical oncology**

|  |  |  |  |
| --- | --- | --- | --- |
| Antibody name | Target | Antibody format  | 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 |

**b). Infectious diseases:** Palivizumab, the sole mAb currently on the marketplace for the treatment of infectious diseases, was developed as a prophylactic treatment against the viral disease Respiratory Syncytial Virus (RSV). The clearance of a virus infection is typically related to T cell-mediated adaptive immunity. CD8+ T cells act by killing virus-infected cells, thus preventing viral replication and reducing the viral load. Additionally, antibodies can promote the killing of infected cells expressing viral proteins on their surface through the activation of natural killer (NK) cells that mediate ADCC, additionally to their viral neutralization properties. Inmazeb (atoltivimab, maftivimab, and odesivimab), a mixture of three mAbs is the first FDA-approved treatment for Zaire-ebolavirus (Ebola virus) infection in adult and paediatric patients. The REGEN-CoV-2 which is a cocktail of two mAbs of 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.

**c). 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 mother's immune system which produce 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 small number of Rh+ve foetal cells into mother's blood during the late pregnancy and delivery activate the Rh specific B cells producing plasma cells and memory cells. The plasma cell 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 remain. These cells are a threat to any subsequent pregnancy. Activation of these memory cells in the subsequent pregnancy by the Rh +ve foetal cells leads to the production of the anti-Rh IgG antibodies. These Abs cross the placenta and haemolyse the foetal RBCs leading to anaemia and jaundice. This can at times be fatal to the foetus depending upon the severity. 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.

**d). Autoimmune diseases:** MAbs may potentially be used to suppress the immune system after transplant or to induce tolerance to transplanted organs or tissues. OKT3, a murine IgG2a antibody to human CD3, and antibodies to CD25 (Il-2 receptor) have been used to reduce allograft rejection. Daclizumab, a humanized antibody that targets the anti-IL-2 receptor, may reduce the risk of acute rejection of a renal transplant and also lower cytomegalovirus infection rates among transplant recipients. The inflammatory bowel disorder known as Crohn disease has also been treated with MAb therapy. A chimeric IgG1k antibody, infliximab (Remicade), acts by binding to soluble and transmembrane tumor necrosis factor-alpha(TNF-alpha), preventing it from binding to its receptors on activated macrophages. The anti-TNF-alpha antibody may provide relief to patients with moderately to severely active Crohn’s disease. In addition, the FDA recently approved infliximab for the treatment of rheumatoid arthritis in combination with methotrexate.

**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, Crohn’s disease. Ulcerative colitis |
| Basiliximab | IL-2 | Chimeric | Acute rejection of kidney transplants |
| Daclizumab | IL-2 | Humanized | Acute rejection of kidney transplant |
| Omalizumab | IgE | Humanized | Asthma  |

**III. CONCLUSION**

 The development of monoclonal antibodies with the specificity of immune responses is one of the most successful application to medicine till date. Fully humanised monoclonal antibodies have reduces the risk of allergy and increased the effectiveness of mAb treatment in many disease conditions. Monoclonal antibodies are being utilized for diagnostic purposes like ELISA, immunohistochemistry and western immunoblotting etc. On the other hand, diseases that are global issues such as cancer, AIDS, immune diseases and other bacterial and viral diseases are being treated using monoclonal antibodies. Furthermore, the technology for the production of monoclonal antibody should be adopted in the developing countries and readily available for use.

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