The application of monoclonal antibodies in medicine

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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 with the same antigenic specificity, they are referred to as monoclonal antibodies (mAbs). The Hybridoma technology developed in 1975 by Georges Kohler of West Germany and Cesar Milstein of Argentina 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 role in clinical diagnostic and therapeutic fields.

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

**I. INTRODUCTION**

Immunoglobulin or antibody, a glycoprotein is produced from activated B cells or plasma cells in response to an antigen. Monoclonal antibodies(mAbs) are antibodies with identical antigenic specificity is 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 for which they were awarded Nobel Prize in 1984. In this technique, an antigen primed B-cell and immortal cell are fused together to form a hybrid cell(hybridoma). A hybridoma has the ability to multiply indefinitely and produce a large population or clone 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 produce its own antibodies. The antigen-primed B cells are obtained from the spleen of mice. The antigen-primed B-cells and the mutated myeloma cells are then fused using 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.

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

Since the discovery of hybridoma technique, certain modifications have been developed for monoclonal antibodies 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, therefor 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, radioimmunology assay.

**a). Diagnostic histopathology:** Tissues and organs can be classified based on their expression of certain defined markers using mAbs. For example, The prostate-specific antigen, placental ALP, HCG, α-fetoprotein, and others which are organ specific antigens and the monoclonal antibodies against these antigens aid in finding the nature of a primary tumor.

**b). 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, the specific epitopes are targeted by monoclonal antibodies and 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:** In molecular biology or biochemistry or immunogenetics, mAbs are used to detect protein in a given sample of tissue extract, which is used normally with an antibody directed against the desired antigen. Western blotting which is based on the monoclonal antibody was developed for the detection of viruses such as Cytomegaloviruses and HIV.

**B. THERAPEUTIC APPLICATIONS**

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

**The mechanism of mAbs as anti-cancer:**

**i. Altering signal transduction in intracellular pathways**. Many cell surface receptors, expressed by cancer cells such as, ErbB1, ErbB2 or HER-2/Neu, HER-3, and HER-4 are usually over-expressed in epithelial malignancies arising from the colon, breast, lung, and head and neck leading to rapidly proliferating disease with increase in metastatic potential. Downstream regulation of the receptor and receptor internalization inhibited by Anti-ErbB1 antibodies bind to the receptor domain of the ErbB1 receptor. Thus, the antibodies inhibit the cancer cell cycle and cause apoptosis.

**ii. Antibody dependent cell cytotoxicity (ADCC):** The cancer cells that are coated with antibodies lead to immune-mediated destruction. The effector cells in the antibody dependent cell cytotoxicity include macrophages, neutrophils and NK cells. ADCC happens when the Fab and Fc portions of the mAbs interact with both the tumor cell antigen and an activating FcγR, thus forming a bridge from the effector cell to the tumor cell. Target cell recognition is associated with a lytic attack on the target cell exposed by effector cells.

**iii. Complement dependent cytotoxicity (CDC):** CDC occurs due to cytolytic pathway mediated by complement proteins, leading to lysis of the antibody bound cells.

**iv. Neutralization of soluble ligand**: Antibodies binding certain circulating proteins can interfere with their finding targets to facilitate the proliferation of tumors. Bevacizumab, fully human mAbs binds, inactivating the biological activity of VEGF-A, restricting angiogenesis, proliferation and tumor growth.

**v. Delivery of Cytotoxic drug**: Monoclonal antibodies targeted for a tumor are linked to Cytotoxic drugs to deliver them specifically to the tumor cells with 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**

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| --- | --- | --- | --- |
| 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 |

**b). Infectious diseases:** Palivizumab, the monoclonal antibody available currently for the treatment of infectious diseases, was developed as a prophylactic treatment for Respiratory Syncytial Virus (RSV). The relative clearance of a viral infection is typically related to T-cell mediated adaptive immunity. CD8+ T cells primarilly act, by killing virus infected cells, thereby preventing viral replication and subsequently reducing the viral load. Additionally, antibodies can kill infected cells expressing viral specific proteins on their surface through the activation of natural killer (NK) cells that promote ADCC, along with their viral neutralization properties. Inmazeb (atoltivimab, maftivimab, and odesivimab), a three mAbs mixture is the first FDA approved for treatment of Zaire-ebolavirus (Ebola virus) infection in paediatric and adult 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 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 persis. 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.

**d). Autoimmune diseases:** Monoclonal antibodies can be used potentially to suppress the immune response after transplant of tissues or organs. OKT3, a monoclonal antobody to human CD3, and antibodies to CD25 (IL-2 receptor) were being used to reduce allograft rejection. Daclizumab, a humanized monoclonal antibody that targets the anti-IL-2 receptor, reduce the acute rejection of a kidney transplant and also decrease CMV infections among transplant recipients. The inflammatory bowel disorder i.e. Crohn’s disease has been treated with monoclonal antibody therapy. Infliximab (Remicade), a chimeric IgG1k antibody, acts by interacting with the soluble and transmembrane tumor necrosis factor-alpha(TNF-alpha), preventing it from binding to its receptors on activated macrophages. The anti-TNF-alpha antibody provide relief to patients with moderate to severe Crohn’s disease. The monoclonal antibody, infliximab is recently approved for the treatment of rheumatoid arthritis in combination with methotrexate.

**Table 2: Monoclonal antibodies used in autoimmune diseases**

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| --- | --- | --- | --- |
| 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 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 reduced the risk of allergy and increased the effectiveness of mAbs 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 an issue globally such as cancer, AIDS, immune diseases and other bacterial and viral 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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