A Review on Monoclonal Antibodies
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A B S T R A C T
monoclonal antibody therapy is the use of monoclonal antibodies to specifically bind to target cells or proteins. MAB’s have increased demand in the diagnostic and therapeutic applications. Some antibodies with few adverse effects are used for treatment of cancer and infectious disease. Large amount of research and development currently being undergone to create monoclonal for numerous serious diseases also .monoclonal antibody technology was quickly adopted by scientists in both industry and academia led to unlimited usefulness of monoclonal antibodies. This review explains about the monoclonal antibodies importance, types and their applications.

KEYWORDS:
Antibodies, Antigen, immunity, B-Lymphocytes, Hybridoma technology

1.INTRODUCTION
Antibodies or immunoglobulins are protein molecules produced by a specialized group of cells called B-lymphocytes.Antibodies are a part of a defence system to protect the body against invading foreign substances namely antigens.In response to an antigen, B-lymphocytes gear up and produce many different antibodies.

Functions Of Antibody Molecule:
Antibody has an important function in recognizing and binding to an epitope on an antigen. The antibody molecule stimulates a useful response to the antigen. The variable regions are responsible for recognizing the epitopes and the constant regions are meant for stimulating a useful response1.

Structure Of Antibody
An antibody is a Y shaped molecule and is a tetramer consisting of four polypeptides (2 heavy chains and 2 light chains). Disulphide bond holds the four chains together .The Polypeptide chains are two identical light chains and two identical heavy chains that fold one around another the light chains are located on the periphery are smaller and thus lighter .the two heavy chains are on the inner side2.

The amino terminal of both light chain and heavy chain contains the variable region of about 100 to 110 amino acids long.This is called as variable region because every b-cell produces a different variable region (i.e. the sequence of amino acids varies with the antibodies specific to different antigens) .the variable region of light chain is denoted as VL and that of heavy chain as as VH.

The variable region is further subdivided into:

a) Hypervariable region: Three HV regions exist in the light and heavy chain comprising of high ratio of different amino acids.

b) Framework region: four FR regions in the light and heavy chains with more stable amino acids sequences.
Monoclonal Antibodies

Monoclonal antibodies are specific antibodies produced in large quantities using genetic engineering techniques, by fusing B-cells derived from a single ancestral b-cell with a tumour cell. These cultures of b cells are called monoclonal as they are derived from a single cell. These cells are used to harvest single kind of antibodies called as monoclonal antibodies.

Importance

Monoclonal antibodies are important reagents used in biomedical research, in diagnosis of diseases and in treatment of such diseases as infections and cancer.

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.

History

The idea of “magic bullets” was first proposed by Paul Ehrlich. Who at the beginning of the 20th century, postulated that if a compound could made that selectively targeted a disease causing organism, then a toxin for that organism could be delivered along with the agent of selectively.

In the 1970’s the B-cell cancer multiple myeloma was known. It was understood that these cancerous B-cells are produce a single type of antibody (paraprotein). This was used to study the structure of antibodies but it was not yet possible to produce identical antibodies specific to a given antigen. In 1975, George Kohler and Cesar Milstein succeeded in making fusions of myeloma cell lines with B-cells to create hybridomas that could produce antibodies specific to known antigen. The hybridomas cells possess the growth and multiplying properties of myeloma cells but secrete antibody of B-lymphocyte.

Advantages

a) Though expensive monoclonal antibodies are cheaper to develop than conventional drugs because it is based on tested technology.

b) Side effects can be treated and reduced by using mice human hybrid cells or by using fractions of antibodies.

c) They bind to specific diseased or damaged cells needing treatment.

d) MAB’s are homogenous and consistent.

e) They can be renewably generated once a suitable hybridoma is developed.

f) The purity and concentration of a specific antibody is higher in MAB’s as compared to polyclonal antibodies.

Disadvantages

a) MAB’s mono specificity also limits their applications.

b) Minor changes in antigen epitope structure affect the function of MAB’s.

c) MAB production should be very specific to the antigen to which it needs to bind.

d) They are not suitable for use in assays such as haemagglutination; slight modifications affect the binding site of the antibody.

2. MATERIAL AND METHODS

Techniques Involved In Preparation Of Monoclonal Antibodies

- Hybridoma technology: Hybridoma technology is a method of producing large number of identical antibodies called monoclonal antibodies.

- It was discovered by G. kohler and C. Milstein in 1975.

- The hybrid cells are produced by fusing B-lymphocyte with myeloma cells or tumour cell

Procedure

i. The mouse is immunised by specific antigen injection against which monoclonal antibodies have to be produced.

ii. After 72 hrs of immunisation spleen is collected from mouse. (Antibody producing B- Cells).

iii. The B-cells are fused with immortalised myeloma cells by polyethylene glycol.

iv. The fused cells are incubated in HAT medium.

v. The hybridoma cells or fused cells are selected using media are called HAT medium.

vi. It contains hypoxanthine, aminopterin, and thymidine.

vii. The unfused B cells will die due to their short life span.

viii. The myeloma cells can synthesize DNA nucleotides using two pathways: DENOVO PATHWAY and SALVAGE PATHWAY. In HAT medium, the myeloma cells are unable to replicate because the Denovo pathway is blocked by aminopterin in the medium.

ix. When denovo pathway is blocked, the cell will utilize Salvage as an alternative pathway.

x. So it is contributed by B cell and is rich in HGPRT+.

xi. The salvage pathway is also inhibited due to the mutation of thymidine kinase, an enzyme that catalyses the phosphorylation reaction.

xii. The resulting clones of hybridoma cells secrets large quantities of monoclonal antibodies.

Phage Display Technology

1. Phage display is a powerful technique in isolation of monoclonal antibodies with high affinity to their target.

2. Phage display was originally invented by GEORGE P. smith in 1985.

3. The steps involved in using phage display:

4. Construction and production of phage libraries

5. Bio panning

6. Binding
Construction and production of phage libraries: This phase involves the animal immunization with the desired antigen and then isolation of B-lymphocytes, mRNA extraction and CDNA synthesis. The synthesized CDNA contain genetic information of antibodies targeting various antigens. CDNA is produced by reverse transcription from B-lymphocyte mRNA and used as a template for PCR.

1. Prepared DNA fragments are inserted in to a suitable vector for cloning and displaying of antibody fragments libraries.
2. So far a phages like phage T4, T7, and M13 have been described for displaying of antibody fragments. These vectors help to the displayed antibodies to maintain their function.
3. The antigen is immobilized on a solid surface.
4. Incubated with antibody phage of the antibody gene library.

Binding: selection of specific clone that recognize the antigen perform by bio panning since the antibody fragments on the surfaces of the phage are functional, the phage carrying specific antibody can be isolated from non-specific phages (due to antigen–antibody binding property). At this step expressed antibodies on the surface of phage based on their ability bind to the target antigen will be enriched through bio panning.

Washing: Unbound phages are washed away leaving only those showing affinity for the receptors.

Elution: Bound phages can be eluted by disrupting the protein binding interactions by changing conditions with
- Acidic buffer
- Basic buffer
- Addition of soluble ligand for receptor

Amplification: Eluted phages showing specificity are used to infect new host cells for amplification. Cycle repeated 2-3 times for stepwise selection of best binding sequence.

Analysis: Soluble monoclonal antibody fragments or

3. RESULTS AND DISCUSSION:

Types Of Monoclonal Antibodies

Based on evolution

Murine antibodies: These murine antibodies derived from the mice, these proteins are purified after immunization with antigens 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.

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

Humanized antibodies: humanized antibodies are produced by grating murine hypervariable region on amino acid domains in to human antibodies this results in a molecule of approximately 95% human origin.

Human monoclonal antibodies: Human monoclonal antibodies are produced by transferring human immunoglobulin genes in to the murine genome after which the transgenic mouse is vaccinated against the derived antigen, leading to the production of monoclonal antibodies.

Based on Design

Naked monoclonal antibody: Naked monoclonal antibodies are those without any drug or radioactive material attached to them. Naked Mab’s are the most commonly used Mab’s at this time. Although they all work by attaching themselves to specific antigens, they can be helpful in different ways.

Functions
- Markers for destruction: Some naked MAbs attach to cancer cells to act as a marker for the body’s immune system to destroy them.
- Attach to receptors: block binding of growth factors.

Conjugated monoclonal antibodies: Conjugated MAbs are monoclonal antibodies that are attached to drugs, toxins, or radioactive substances. The MAbs are used as homing devices to take these substances directly to the cancer cells. The MAb circulates in the body until it can find and hook onto the target antigen. It then delivers the toxic substance where it is needed most.
- MAbs with radioactive particles attached are referred to as radio labeled, and therapy with this type of antibody is known as radio immunotherapy (RIT).
- MAbs with chemotherapy drugs attached are often referred to as chemo labeled.

Immune-toxin monoclonal antibody: An immunotoxin is prepared by replacing the binding polypeptide with a monoclonal antibody that is specific for a particular tumour cell. The attached monoclonal antibody will deliver the toxin chain specifically to tumour cells where it will cause death by inhibiting protein synthesis.

Mechanism Of Monoclonal Antibody

The mechanism by which MAB’s therapeutic effect is not very clear. Potential mechanisms include:
I. Make the cell more visible to the immune system: The immune system attacks foreign invaders in our body, but it doesn't always recognize cancer cells as enemies. A monoclonal antibody can be directed to attach to certain parts of a cancer cell.

II. Block growth signals - cetuximab

III. Deliver radiation to cells: By combining a radioactive particle with a monoclonal antibody, doctors can deliver radiation directly to the cancer cells.

IV. Preventing blood vessel growth: in order for a cancerous tumour to grow and survive it needs a blood supply some monoclonal antibody drugs block protein cell interactions necessary for the development of new blood vessels.

V. Directly attacking cancer cells: certain monoclonal antibodies may attack the cell more directly even though they were designed for another purpose.

VI. Delivering chemotherapy: similarly some monoclonal antibodies are attached to a chemotherapeutic drug in order to deliver the treatment directly to the cancer cells while avoiding healthy cells.

Side Effects

i. Monoclonal antibodies are given intravenously (injected into a vein). Compared with side effects of chemotherapy, the side effects of naked MAbs are usually fairly mild and are often more like an allergic reaction. If they do occur, it is most often while the drug is first being given. Possible side effects can include:
   a. Fever, Chills, Weakness, Headache, Nausea, Vomiting, Diarrhoea, Low blood pressure

ii. Some MAbs also have effects that are specific to the antigens they target. For instance, like most

iii. Chemotherapy drugs, some can affect the bone marrow. This can cause lower levels of blood cells, which can increase the risk of bleeding and infection in some people.

   a. Rare, But More Serious Side Effects Of Monoclonal Antibody Therapy May Include

   iv. Infusion reactions: Severe allergy-like reactions can occur and in very few cases lead to death. You may receive medicine to block an allergic reaction before you begin monoclonal antibody treatment. Infusion reactions usually occur while treatment is being administered or soon after so your health care team will watch you closely for a reaction.

v. Dangerously low blood cell counts: Low levels of red blood cells, white blood cells and platelets may lead to serious complications.

vi. Skin problems: sores and rashes on your skin can lead to serious infections in some cases. Serious sores can also occur on the tissues that lines your cheeks and gums.

   Bleeding: Some of the monoclonal antibody drugs are designed to stop cancer from forming new blood vessels. There have been reports that these medications can cause bleeding.

Diagnostic Application Of Monoclonal Antibodies

a. Monoclonal antibodies are used widely in the diagnostic laboratory.

b. Monoclonal antibodies allow rapid diagnosis of hepatitis, influenza, herpes, and Chlamydia infections.

c. They are also very useful in immunohistochemistry, which detect antigen in fixed tissue sections and immunofluorescence test, which detect the substance in a frozen tissue section or in live cells.

a) Diagnosis of HIV Infection

   • HIV antigen is attached to the plate.
   • Patients serum passed over the plate. Any HIV antibody in the patients serum will attached to the antigen already on the plate.
   • A second antibody which is specific to the HIV antibody is passed over the plate. This antibody will attach to the concentrated HIV antibody on the plate. This second antibody has an enzyme attached to its structure.
   • Chromagen dye is passed over the complex of concentrated HIV antibody/conjugated antibody.
   • The enzyme will turn the chromagen to a more intense colour. The more intense the colour, the greater the HIV antibody level. This would be the a positive result for a HIV test.

b) Pregnancy Tests

   • A breakthrough in Diagnostics a monoclonal antibody can be used to detect pregnancy in only 14 days after conception.
   • A pregnant woman has the hormone human chorionic gonadotrophin (HCG) in her urine.
   • Monoclonal antibodies to HCG have been produced. These have been attached to enzymes which can later interact with a dye molecule and produce a colour change.
c) **Purification Of Proteins**
   - Monoclonal antibodies can also be used to purify a substance with techniques called immuno-precipitation and affinity chromatography.
   - The Western blot test and immune dot blot tests detect the protein on a membrane.

d) **Monoclonal Antibodies In Drug Targeting**
   - The concept of using specific antibodies conjugated with toxic or isotopically labelled materials for specific sites and drug localization is not new.
   - This concept with the development of MAbs and hybridoma technology has gained wide appreciation in their use. The use of monoclonal antibodies to target drugs to specific cell types is a promising approach\(^{11}\).

**Therapeutic Uses**

1) **Immunosuppression**: Normally, tissue and bone marrow transplants are rejected which is mediated primarily by the T-cells in order to prevent the rejection. T-cells present in the circulatory system of the recipient are eliminated before transplanting the organ by using immunosuppressive drugs which include cyclosporine and some steroids these drugs suppress all aspects of the immune system thus making the patient susceptible to all kinds of infectious diseases.

2) **Auto immune diseases**: Autoimmune diseases caused by the attack of immune system on the tissues of the body include myasthenia gravis and multiple sclerosis. Investigations are being done to develop monoclonal antibodies against these immune cells that activate immune responses to treat autoimmune conditions.

3) **Malignancies**: monoclonal antibodies are man-made versions of immune system proteins. Antibodies can be very useful in treating cancer because they can be designed to attack a very specific part of a cancer cell.

4) **Antiplatelet**: Abciximab was the first antagonist to be evaluated. It inhibits the clumping of platelets by binding to surface receptors that normally are linked by fibrinogen. It is helpful in preventing the relogging of the coronary artery.

5) **Infectious disease**: Palvizumab, a humanized MAB directed against respiratory syncytial virus is used for the treatment of premature infants and infants with bronchopulmonary dysplasia. A MAB was also found to be useful to cure west Nile fever in mice.

6) **Asthma**: Omalizumab MAB has shown promise in allergic asthma. It acts by binding to IgE thus preventing IgE from binding to mast cells. Omalizumab has shown to reduce serum IgE levels, reduced inhaled steroid consumption and was also well tolerated by children and adults\(^{12}\).

**Monoclonal Antibodies Use For Cancer Treatment**

Monoclonal antibody therapy is the use of monoclonal antibodies (or MAb) to specifically bind to target cells or proteins. This may then stimulate the patient’s immune system to attack those cells. It is possible to create a MAb specific to almost any extracellular cell surface target, and thus there is a large amount of research and development currently being undergone to create monoclonal for numerous serious diseases (such as rheumatoid arthritis, multiple sclerosis and different types of cancers). There are a number of ways that MAbs can be used for therapy. One possible treatment for cancer involves monoclonal antibodies that bind only to cancer cell-specific antigens and induce an immunological response against the target cancer cell.

**Route Of Antibody Administration**: MAbs for cancer treatment are usually injected to the bloodstream with a carrier although some clinical studies have involved the use of intraperitoneal administration of mAb where the treatment solution is injected to body cavities. Studies in experimental animals and in humans show that direct cavity injection targets smaller peritoneal tumors more efficiently than intravenous antibody treatment.

**Targeted Conditions (Cancer)**\(^{13}\):

- Anti-cancer monoclonal antibodies can be targeted against malignant cells by several mechanisms:
  - **Radio immunotherapy (RIT)** involves the use of radioactively conjugated murine antibodies against cellular antigens. Most research currently involved their application to lymphomas, as these are highly radiosensitive malignancies.
  - **Antibody-directed enzyme prodrug therapy (ADEPT)** involves the application of cancer associated monoclonal antibodies which are linked to a drug-activating enzyme. Subsequent systemic administration of a non-toxic agent results in its conversion to a toxic drug, and resulting in a cytotoxic effect which can be targeted at malignant cells.
  - **Immunoliposomes** are antibody conjugated liposomes. Liposomes can carry drugs or therapeutic nucleotides and when conjugated with monoclonal antibodies, may be directed against malignant cel
FDA-approved monoclonal antibodies for cancer treatment

<table>
<thead>
<tr>
<th>Name</th>
<th>Target Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Alemtuzumab (Campath)</td>
<td>Chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>II. Bevacizumab (Avastin)</td>
<td>Breast cancer, colon cancer, lung cancer</td>
</tr>
<tr>
<td>III. Ibritumomab (Zevalin)</td>
<td>Non-Hodgkin's lymphoma</td>
</tr>
<tr>
<td>IV. Panitumumab (Vectibix)</td>
<td>Colon cancer</td>
</tr>
<tr>
<td>V. Rituximab (Rituxan)</td>
<td>Non-Hodgkin's lymphoma</td>
</tr>
<tr>
<td>VI. Gemtuzumab (Mylotarg)</td>
<td>Acute myelogenous leukemia</td>
</tr>
<tr>
<td>VII. Cetuximab (Erbitux)</td>
<td>Colon cancer, head and neck cancers</td>
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</table>

**CONCLUSION**

MAbs represent an important advance in the treatment of certain hematologic malignancies and solid tumors. Unlike many small molecules, mAbs offer unique target specificity. MAbs are highly specific Abs produced by a clone of single hybrid cells formed by fusion of B cell with the tumor cell. The hybridoma formed yields higher amount of MAbs. MAbs can be produced in vitro and In vivo. Animals are utilized to produce MAbs, but these antibodies are associated with immunogenicity and ethical problems. Recombinant DNA technology, genetic engineering and transgenic animals are used to produce humanized MAbs or pure human MAbs, with fewer ADRs Used for treatment of cancer, autoimmune disorders, graft rejections, infections, asthma.

**Conflicts Of Interest**

The authors do not have any conflict of interest.

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