A Review on Monoclonal Antibodies

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Abstract:

Monoclonal antibodies (mAbs), which are defined as a single antibody (Ab) cloned from a single B cell, have been used as immune modulators for transplantation, autoimmune disorders, and cancer for decades. To protect itself from many invading diseases, the vertebrate immune system is continually changing. The vertebrate immune system responds to a foreign pathogen by producing complementary antibody (Ab) molecules that can bind to all molecular structures of the microbial pathogen (bacteria, viruses, fungi, nematodes, and other parasites) and keep up with an organism's diverse mutations. Antigen is any particle that enters the body and is identified by the vertebrate immune system as a foreign object. As a result, the immune system is attacked in two ways. First, B cells create a variety of antibodies that are specific for a novel antigen (epitope). Second, the antibody's paratope-encoding genes are rapidly altered in order to cope with and attach strongly to the antigen's epitope. As a result, antibodies were generated.

KEY WORDS: Monoclonal Antibody, Hybridoma Technology, Applications, Types, Side effects.

Introduction:

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a result, antibodies were generated. As a result, the antibodies generated are more capable of attaching to the antigen with high affinity and specificity.

**Antibody Structure:**

Antibodies are large glycoproteins that belong to the immunoglobulin (Ig) superfamily and play a role in the immune system by recognizing foreign antigens, neutralizing them, and eliciting a subsequent immunological response. Their basic structure is made up of two heavy and two light chains arranged in a Y pattern. The fragment antigen-binding (Fab) region of the antibody is located at each tip of the Y and is responsible for antigen recognition. The fragment crystallizable (Fc) region at the Y structure's base mediates interactions between the antibody and other immune system members. Fc regions of antibodies are identified by Fc receptors (FcRs) located on a variety of immune cells. Antibodies are classified into five types based on their heavy chain: IgA, IgD, IgE, IgG, and IgM. Because IgGs interact with their associated type of FcR, FcR, found on natural killer (NK) cells as well as neutrophils, monocytes, dendritic cells, and eosinophils to mediate specialized functions such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), IgG is the most commonly used form in antibody therapy. The IgG class can be further split based on the Fc region's capacity to facilitate those functions: IgG1 and IgG3 can trigger ADCC and CDC, whereas IgG2 and IgG4 cannot.

<table>
<thead>
<tr>
<th></th>
<th>IgA</th>
<th>IgD</th>
<th>IgE</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Molecular forms</strong></td>
<td>Monomer or dimer</td>
<td>monomer</td>
<td>monomer</td>
<td>monomer</td>
<td>pentamer</td>
</tr>
<tr>
<td><strong>% total Ig in serum</strong></td>
<td>10-20</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>70-85</td>
<td>10</td>
</tr>
<tr>
<td><strong>Where found in body</strong></td>
<td>Found in bodily secretions</td>
<td>Found on B-cell surface</td>
<td>Attach to basophils and mast cells</td>
<td>Blood &amp; extracellular fluid</td>
<td>Blood &amp; extracellular fluid</td>
</tr>
<tr>
<td><strong>Functions</strong></td>
<td>Protect external openings</td>
<td>Unknown; maybe antigen detection</td>
<td>Allergic response and defend infection by large parasite</td>
<td>Long term Ab that protect the body</td>
<td>Appear earlier in the infection and offer valuable defense during critical stage of the infection</td>
</tr>
<tr>
<td><strong>Trasferrable to offsprings?</strong></td>
<td>Via colostrum &amp; breast milk</td>
<td>No</td>
<td>No</td>
<td>Via placenta</td>
<td>No</td>
</tr>
</tbody>
</table>
Antibodies are immune system proteins known as immunoglobulins. Each antibody is made up of four polypeptides: two heavy chains and two light chains that come together to create a "Y" shaped protein. The amino acid sequence of the "Y" tips differs widely between antibodies. This variable region confers antigen specificity on the antibody. The mechanism employed to degrade antigen is determined by the constant region.

Types of Therapeutic Monoclonal antibodies:

- Murine MAbs
- Humanised MAbs
- Chimeric MAbs
- Fully human
Murine MAbs:

Due to differences in the human and rat immune systems, the use of murine antibodies produced through hybridoma technology in human therapy (clinical medicine) is limited. With the exception of a few rare cases, this usually results in treatment failure. Murine antibodies have minor cytotoxicity stimulation effects. As a result of the development of human anti-mouse antibodies (HAMA), which usually target the supplied murine mAb and trigger allergic response [6-8], their continued administration frequently results in allergic responses and anaphylactic shock. The first therapeutic mAb licensed for clinical use in human medicine was an anti-CD3 mAb of murine origin (OKT-3). However, the mAb failed to alleviate transplant rejection, owing to a significant human anti-mouse antibody (HAMA) response in patients.

Humanized MAbs:

Because of their safety for in vivo activity, human mAbs (HMA) have been termed natural medicines. Human mAbs are now widely used in the treatment of a variety of diseases, as well as in the creation of innovative immunodiagnostics, thanks to advancements in mAb technologies. Over the last few decades, around 20 mAb drugs, including humanized mouse mAbs, have been approved as therapeutic reagents. Other mAbs are in various phases of clinical trials and are being monitored by different research institutions and/or pharmaceutical businesses. Human mAb technologies are valuable not just for strategic research, but also for health economics.

Chimeric antibodies:

Chimeric antibodies are therapeutic antibodies created by combining genetic elements from humans and non-humans (mice). They are created by modifying human constant regions and mouse variable regions. To reduce the potential of adverse reactions to foreign antibodies, these antibodies are composed of around 65% human genetic material. Surprisingly, the Food and Drug Administration has approved some chimeric antibody-based medications for use in human therapy and research. The suffix "ximab" is used to identify chimeric mAbs in the nomenclature.

Fully human MAbs:

Because of the stress involved in maintaining immortalised cell lines and human hybridomas, producing human mAb using traditional hybridoma procedures is relatively difficult. In vivo immunization of humans with numerous distinct antigens is also not possible when compared to the use of animal models. Methods for producing human mAbs, on the other hand, are made possible by the expression of antibody fragments or single cell variable fragments (Fab or ScFv) in bacteria. Similarly, antibody fragments can be exhibited on filamentous bacteriophages for antibody library screening. Fully human mAbs can be generated as an alternative to re-engineering murine mAbs using a supply of low immunogenic therapeutic antibodies.
Monoclonal antibody preparation:

STAGE 1: IMMUNIZATION

Immunogens are substances that cause an immunological reaction in those who are not familiar with them. For the initial immunization of Balb/c mice, protein (50-100 g), cells (1 107), numerous antigenic synthetic peptides, or a short peptide (6-18 amino acids) coupled to a carrier protein (for example, keyhole limpet hemocyanin) can be employed. An immunogen is frequently provided in conjunction with an adjuvant, which is a non-specific immune booster. Freund's complete/incomplete adjuvants and TiterMax are two common examples. Proteins are almost always administered subcutaneously, whereas cells are administered intraperitoneally. To increase a polyclonal response, regular boosting is required, which can be evaluated indirectly via tail bleeds. These excess serum are used to determine the antibody titre to a specific antigen in an assay technique, such as enzyme linked immunosorbent assay (ELISA), which is ultimately required for the monoclonal reagent. The removal of boosting also promotes immunoglobulin class switching and the production of higher unity antibodies via somatic hypermutation. In general, IgG monoclonal antibodies are favored because they are less susceptible to degradation and may be more beneficial as therapeutic reagents. Of course, the eventual goal, especially for in vivo techniques, is to pick an appropriate mouse (usually the best responder from tail bleeds) and extract (aseptically) antigenically responsive B cells from its spleen (or lymph node) to acquire viable cells for hybridisation. Although in vivo immunization (including intrasplenic injection) is the preferred method in many laboratories, there is also the option for in vitro immunization. In this scenario, cultivated splenic cells are stimulated with a small quantity of antigen.

STAGE 2: FUSION AND SELECTION

The fusion of murine splenic B cells with histocompatibility myeloma cells, such as Sp2/0, is fundamental to the hybridisation process. The latter (as well as other myeloma cell lines including NS1, NSO, and X63Ag8) are preselected for a lack of the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT), for example, by culture in media containing 8-azaguanine. Essentially, this enzyme is critical to the post-fusion hybridoma selection process. To grasp this process, keep in mind that cells have two nucleotide biosynthesis pathways: the de novo pathway and the salvage pathway, which employs HGPRT. As a result, HGPRT-negative myeloma cells are unable to employ the salvage or "alternative" pathway for purine manufacture and are thus fully reliant on the new survival way. In the fusion process, splenic B cells are combined with HGPRT negative myeloma cells and a fusing agent such as polyethylene glycol. Hopefully, the mixing and centrifugation procedures result in myeloma-splenic B cell hybridomas. Once these hybrid cells have been generated and plated into tissue culture wells, the focus changes to eliminating unfused myeloma cells. This is required because the latter have the ability to overrun other cells, particularly weakly established hybridomas. This problem is handled by utilizing a selective medium containing hypoxanthine, aminopterin, and thymidine, abbreviated as "HAT." Aminopterin prevents the de novo pathway, which is the only one open to HGPRT negative cells, and as a result, all unfused myeloma cells
die. Of course, freshly generated hybridomas survive this selection process since the salvage pathway enzyme is given by its splenic B cell counterpart. Unfortunately, some hybridomas are unstable and relapse. As a result, hybridomas should be visually examined with an inverted microscope with great care. A record of poorly growing, freshly emerging, or established hybridomas lends credibility to immunoassay screening findings. Once established, a hybridoma colony will continue to grow in culture medium (such as RPMI1640 with antibiotics and fetal bovine serum) and manufacture antibodies. Hybridomas can now be propagated in "HT" medium (hypoxanthine and thymidine only) 20 to 30 days after infusion because aminopterin is no longer necessary.

**STAGE 3: SCREENING**

This stage focuses on discovering and choosing hybridomas that produce antibodies with the desired specificity. Otherwise, multiple unwanted (at least to you!) hybridomas will compete for your time and incur additional money in terms of culture plates and media. A quick "primary" screening approach that screens the hybridoma culture supernatant for antibody reactivity and specificity is invariably used. An Epstein-Barr virus related protein or peptide, for example, can be coated on plastic ELISA plates. A coloured result indicates a positive hybridoma after incubation with hybridoma culture supernatant, secondary enzyme labelled conjugate, and chromogenic substrate. Alternatively, immunocytochemical screening may be preferable. Ultimately, primary screening is required to "weed out" and eliminate non-specific hybridomas as soon as possible. Obviously, screening supernatants with some degree of fairness is necessary, and it may be prudent to evaluate hybridomas when at least three quarters confluent. Unfortunately, because not all hybridomas grow at the same rate, screening has become an almost daily effort. It is worth noting that slow growing (and frequently extremely stable) hybridomas might arise 25-30 days after fusion, but most become established much sooner. Hybridomas can be produced in multiwell plates at first, then expanded to bigger tissue culture flasks once selected. This progression is required not only to keep the hybridomas alive, but also to give viable cells for cryopreservation and supernatant for further research. As a general approximation, culture supernatant can yield anywhere between 1 and 60 g/ml of monoclonal antibody, which should be stored at 20 °C or lower until needed. The amount of hybridomas that can be "taken through" in a particular laboratory requires constant validation. Furthermore, if a fusion has been very successful, some rationalisation of hybridomas will be required, such as selecting just those with an intensive immunocytochemical staining pattern. Of course, less desirable hybridomas can be cryopreserved and studied at a later period. It is critical to remember that the workload in creating hybridomas is often exponential.

**STAGE 4: CHARACTERISATION**

A possible monoclonal antibody generating hybridoma's reactivity, specificity, and crossreactivity can be investigated further using culture supernatant or a pure immunoglobulin preparation. However, because an original colony may contain at least two populations of fused B cells, it is often required to re-clone hybridomas (for example, by limiting dilution) before proceeding with further work. If not handled, this circumstance may result in ambiguous data from antibodies of the di Verint class, specificity, and a Unity. As a result, isotype
determination not only serves to establish the murine immunoglobulin class or subclass, but it also aids in identifying the existence of a single isotype, such as IgG1, or a mixture, such as IgM and IgG2b. Furthermore, understanding the isotype of a monoclonal antibody.

Furthermore, knowing a monoclonal antibody's isotype will help choose the best column purification process for a culture supernatant—for example, protein G for IgG1. A critical part of characterization is monoclonal antibody profiling in di Verint test methods. This is especially important in terms of the antibody's potential as a diagnostic reagent because some monoclonal antibodies operate well in some systems but not others. Assay restriction refers to how an antibody recognizes its target epitope within the context of the assay system used. In this situation, the immobilisation approach used within a specific technique may conceal, denature, or render an essential epitope inaccessible. Characterization also allows a Vord to test against a large panel of similar antigens or tissue preparations, which is very useful if monoclonal antibodies are being targeted for histopathological objectives.

Of course, these efforts and the hand of serendipity may lead to practical applications elsewhere, helping to capitalize on the initial investment of attention, e Vord, and money. Once a hybridoma has been identified, bulk manufacturing of a monoclonal antibody can be accomplished utilizing surface expanded tissue culture flasks or hollow fiber methods like Technomouse. Although a hybridoma is the fused result of a single B cell and produces a monoclonal antibody with exceptional specificity, this antibody can also crossreact with different antigens or exhibit dual specificity. This corollary occurs when an antibody combining site recognizes more than one antigenic determinant, either due to form or chemical composition similarities. Furthermore, assay system subtleties can bias the exposure of a specific antigenic determinant or epitope. As a result, rigorous examination of a given monoclonal antibody and its target epitope is required, which may include epitope mapping. This method enables for the accurate identification of critical amino acid residues required for antibody recognition and binding. Ynity measurements of antigen–monoclonal antibody interactions using surface plasmon resonance (for example, BIACore or IBIS) could also be used for further characterisation.

STAGE 5: FURTHER DEVELOPMENTS

Once created, monoclonal antibodies can be used as investigative research tools, diagnostic assays, or medicinal medicines. Commercial exploitation of monoclonal antibodies may generate some cash for future research efforts, in addition to potential collaborative prospects. Furthermore, by combining monoclonal antibody epitope mapping with molecular modeling, important antigenic areas on a molecule can be visualized and localized. This knowledge may aid in elucidating the structure-function relationships of proteins, polysaccharides, and other clinically relevant compounds. Of course, one of the ultimate goals of monoclonal experts is to broaden the use of antibodies in clinical treatment of patients. Certain mouse monoclonal antibodies have shown preferential (depending on subclass), however they may eventually produce human antimouse responses. This issue has been solved by either cleaving the immunogenic Fc region of the immunoglobulin molecule or using recombinant methods. These have mostly concentrated on creating chimeric antibodies with a murine antibody recognition unit and a human Fc region, or on using a human IgG molecule and inserting murine complementary determining
residues to keep antibody specificity. Clearly, advancements in so-called magic bullets, whether alone (and relying on the eVector properties of the immunoglobulin isotype) or loaded with radionucleotides or poisons, will surely gain importance.

**Monoclonal Antibody Production**

- **Immunization of mice & isolation of splenocytes**: Mice are immunized with an antigen and later their blood is screened for antibody production. The antibody-producing splenocytes are then isolated for *in vitro* hybridoma production.
- **Preparation of myeloma cells**: Myeloma cells are immortalized cells that, once fused with spleen cells, can result in a hybridoma capable of unlimited growth. Myeloma cells are prepared for fusion.
- **Fusion**: Myeloma cells and isolated splenocytes are fused together to form hybridomas in the presence of polyethylene glycol (PEG), which causes cell membranes to fuse.
- **Clone screening and picking**: Clones are screened and selected on the basis of antigen specificity and immunoglobulin class.
- **Functional characterization**: Confirm, validate, and characterize (e.g., ELISA) each potentially high-producing colony.
- **Scale up and wash**: Scale up clones producing desired antibodies and wash off selection agent(s).
- **Expansion**: Expand clones producing desired antibodies (e.g., bioreactors or large flasks).

**Applications of Monoclonal Antibodies**:

Because of their excellent specificity, MAbs have shown to be particularly beneficial for fundamental immunological and molecular research. They are used in human therapy, commercial protein purification, suppressing immune response, disease diagnosis, cancer therapy, allergy diagnosis, hormone test, purification of complex mixtures, cell membrane structure, identification of specialized cells, vaccine preparation, and increasing the effectiveness of medical substances.

**Diagnostic Application:**

- Biochemical Analysis Tools for Disease Diagnosis Imaging
- MAbs are used in RIA and ELISA in the lab to evaluate the circulation levels of hormones, antigens, interferons, and other substances.
- For cancer
To detect certain tumors at an early stage by using mAb's exclusion When tagged mAbs specific to breast cancer cells are supplied to patients, they identify the spread of cancer, which other scanning techniques cannot.

- Estimation of plasma carcinombryogenic Ag in colorectal and prostate cancer.
- Pregnancy Detection of urine levels of human chorionic gonadotropin Hormonal diseases.
- Thyroxin, triiodothyronine, and thyroid stimulating hormones were studied for thyroid diseases.
- Infectious illnesses.
- To detect the presence of Ags specific to infectious pathogens in the bloodstream.
- MAbs used in diagnostic imaging

**MAB’s in diagnostic imaging:**

- Radiolabelled MAbs are given intravenously into patients and used in disease diagnostic imaging
- Immunoscintigraphic
- Commonly used radioisotopes: Iodine 131 with technetium 99
- Cardiovascular illnesses include myocardial infection and deep vein thrombosis.
- Radiolabelled antimyosin mAbs are utilized to identify myosin and the location of myocardial infection.
- Deepvein thrombosis (DVT) is the formation of blood clots within blood veins. MAbs against fibrin or platelets might be employed.

The MAbs are utilized to boost the host's immune system while causing little toxicity to target organs.

1. In the destruction of disease-causing organisms
2. In cancer treatment, against cancer cell surface Ag's
3. In organ transplant immunosuppression
4. In AIDS treatment
5. autoimmune illness treatment

Toxins, medicines, and radioisotopes can be coupled to tissue-specific MAbs and delivered to target tissues for effective activity.

1. MAbs as immunotoxin (toxins combined with MAbs).
2. Mab is a medication delivery system.
3. MAbs employed in blood clot separation
4. Radioimmune treatment for cancer cells
Protein purification:

Immobilized MAbs are utilized in the immunological affinity approach of protein purification.

Advantages:

Binding of highly specific MAbs to a particular protein

Efficient chromatographic column illusion.

High level of purification.

Miscellaneous Application:

Catalytic MAbs (abzymes) are antibody enzymes that are employed in the research of different processes.

Autoimmune fingerprinting:

Individual specific (IS) autoantibodies are created shortly after birth and reach their peak by the age of two.

MAbs generated against IS-auto Ab's can be utilized to detect and identify individuals and criminals.

Side-Effects and Limitations of MAbs:

When contrast to chemotherapy, intravenous MAbs often have moderate adverse effects. When the medicine is first administered, a minor allergic reaction (rash) may develop. Fever, headache, weakness, chills, nausea with vomiting and diarrhoea, and low blood pressure are all common adverse effects. Other MAb adverse effects are connected to the antigens that are being targeted. Bevacizumab (a drug used to prevent tumour blood vessel formation) can cause kidney damage, high blood pressure, bleeding with poor wound healing, and blood clots. The FDA-approved medicine raxibacumab (MAb) injection is used to treat infected inhalational anthrax when other therapies have failed, according to Mahroof et al. European Journal of Biomedical and Pharmaceutical Sciences www.ejbps.com. The most common adverse effects are a rash with itching, intense pain, and sleepiness.
References:


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