International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 7(2): March-April 2017

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences **Review Paper……!!!**

Received: 16-01-2017; Revised: 18-03-2017; Accepted: 19-03-2017

MONOCLONAL ANTIBODIES: AN OVERVIEW

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Keywords:

Monoclonal antibodies, applications, characterization

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ABSTRACT

Monoclonal antibodies are protein molecules made in the laboratory from hybridoma cells (stable cell lines derived by fusing antibody-producing cells from immunised animals with cells that confer immortality and high-yield antibody production) or by recombinant deoxyribonucleic acid (DNA) technology. They are originally derived from the immune system and have a number of unique properties that have stimulated their use in medicine, including the ability to bind specifically and with high affinity to almost any molecular structure. They are made in a homogeneous and reproducible form that allows comparisons across laboratories for diagnosis as well as therapeutics. Current major therapeutic applications of monoclonal antibodies include cancer, chronic inflammatory disease, and infection and they constitute the largest and fastest growing sector of the biological pharmaceutical industry. In this review Production, applications, characterization and regulatory issues of monoclonal antibodies are discussed.

INTRODUCTION

Monoclonal antibodies are the mono-specific antibodies that are the same because they are made by identical immune cells that are all clone of a unique parent cell or also An antibody is called ‘‘monoclonal’’ when each immunoglobulin is produced by a single clone of cells and hence is identical to every other molecule in the preparation, in terms of heavy as well as light chain structure. Thus they are highly specific and offer more consistent efficacy and predictable toxicity in vivo than the polyclonal counterparts.

Immuno-conjugates:

For MABs targeted drug delivery, a drug is bound covalently to an antibody that is chosen to target it to the desired site of action. The resulting immuno-conjugate may contain a spacer between the drug and antibody, or a polymer to increase the number of drug molecules that can be bound to each antibody. Another possibility is a radio immuno-conjugate, which is designed to be concentrated at the target site by the targeting antibody, allowing the radiation from the bound radioisotope to exert its cytotoxic affect[1]. Alternatively, the drug can be incorporated non-covalently into a liposome or microsphere to which the targeting antibody is bound to the surface, yielding an immune-liposome or immune-microsphere, respectively.

Advantages of Monoclonal antibodies

- **Homogeneity**: Monoclonal antibody represents single antibody molecule that binds to antigens with the same affinity and promote the same effector functions.
- **Specificity**: The product of a single hybridoma reacts with the same epitope on antigens.
- **Selection**: It is possible to select for specific epitope specificities.
- **Antibody Production**: Unlimited quantities of a single well-defined mono-specific antibody can be generated.

Disadvantages of Monoclonal antibodies

- **Affinity**: Average affinity of monoclonal antibodies is generally lower than polyclonal antibodies.
- **Cross-reactions**: Antibodies sometimes display unexpected cross-reactions with unrelated antigens.
- **Time and effort commitment**: Very large.

Nomenclature of Monoclonal antibodies

Table no 1 nomenclature of monoclonal antibodies

Prefix	Target substem			Source substem		Stem
	Old	**New**	**Meaning**		**Meaning**	
Variable	-anibi-	-	Angiogenesis(inhibitor)	-a-	rat	-mab
Variable	-ba(c)-	-b(a)-	Bacterium	-e-	hamster	-mab
Variable	-ci(r)-	-c(i)-	Circulatory system	-i-	primate	-mab
Variable	-fung-	-f(u)-	Fungus	-o-	Mouse	-mab
Variable	-ki(n)-	-k(i)-	Interleukin	-u-	human	-mab
Variable	-les-	-	Inflammatory lesions	-xi-	Chimeric	-mab
Variable	-li(m)-	-l(i)-	Immune system	-zu-	humanized	-mab

For example, the drug Daclizumab, '***mab***' indicates monoclonal antibodies, '***zu***' indicates source sub-stem that antibodies are humanized, while '***li***' indicates target sub-stem immune system[2].

Preparation methods of Monoclonal antibodies

Monoclonal antibodies can be prepared by following methods[3]

- Hybridoma technology
- Large scale production
- Encapsulated hybridoma cells

Hybridoma technology

The establishment of hybridoma and production of MABs involves the following steps:-

1. Immunization
2. Cell fusion
3. Selection of hybridomas
4. Screening the Products
5. Cloning and propagation
6. Characterization and storage.

1. **Immunization:** The very first step in hybridoma technology is to immunize an animal (usually a mouse), with appropriate antigen. The antigen, along with an adjuvant like Freund's complete or incomplete adjuvant is injected subcutaneously (adjuvants are non-specific potentiators of specific immune responses). The injections at multiple sites are repeated several times. This enables increased stimulation of B-lymphocytes which are responding to the antigen. Three days prior to killing of the animal, a final dose of antigen is intravenously administered. The immune-stimulated cells for synthesis of antibodies

have grown maximally by this approach. The concentration of the desired antibodies is assayed in the serum of the animal at frequent intervals during the course of immunization.

When the serum concentration of the antibodies is optimal, the animal is sacrificed. The spleen is aseptically removed and disrupted by mechanical or enzymatic methods to release the cells. The lymphocytes of the spleen are separated from the rest of the cells by density gradient centrifugation.

2. **Cell fusion:** Thoroughly washed lymphocytes are mixed with HGPRT defective myeloma cells. The mixture of cells is exposed to polyethylene glycol (PEG) for a short period (a few minutes), since it is toxic. PEG is removed by washing and the cells are composed of a mixture of hybridomas (fused cells), free myeloma cells and free lymphocytes.
3. **Selection of hybridomas:** When the cells are cultured in HAT medium, only the hybridoma cells grow, while the rest will slowly disappear. This happens in 7-10 days of culture. The myeloma cells used in hybridoma technology must not be capable of synthesizing their own antibodies. The selection of hybridoma cells is based on inhibiting the nucleotide (consequently the DNA) synthesizing machinery. The mammalian cells can synthesize nucleotides by two pathways:

- De novo synthesis- The de novo synthesis of nucleotides requires tetrahydrofolate which is formed from dihydrofolate. The formation of tetrahydrofolate (and therefore nucleotide) can be blocked by the inhibitor **Aminopterin.**
- Salvage Pathway- The salvage pathway involves the direct conversion of purines and pyrimidine's into the corresponding nucleotides. *Hypoxanthine guanine phosphoribosyl transferase* (HGPRT) is a key enzyme in the salvage pathway of purines. It converts hypoxanthine and guanine respectively to inosine monophosphate and guanosine monophosphate. **Thymidine kinase (TK),** involved in the salvage pathway of pyrimidine's converts thymidine to thymidine monophosphate (TMP). Any mutation in either of the enzymes (HGPRT or TK) blocks the salvage pathway. When cells deficient (mutated cells) in HGPRT are grown in a medium containing **H**ypoxanthine **A**minopterin and **T**hymidine (**HATmedium**), they cannot survive due to inhibition of de novo synthesis of purine nucleotides (Note: Salvage pathway is not operative due to lack of HGPRT). Thus, cells lacking HGPRT, grown in HAT medium die. The hybridoma cells possess the ability of myeloma cells to grow *in-vitro* with a functional HGPRT gene obtained from lymphocytes (with which myeloma cells are fused). Thus, only the hybridoma cells can

proliferate in HAT medium, and this procedure is successfully used for their selection. Selection of a single antibody producing hybrid cells is very important. This is possible if the hybridomas are isolated and grown individually. The suspension of hybridomas is so diluted that the individual aliquots contain on an average one cell each. These cells, when grown in a regular culture medium, produce the desired antibody.

4. **Screening the Products:** The hybridomas must be screened for the secretion of the antibody of desired specificity. The culture medium from each hybridoma culture is periodically tested for the desired antibody specificity. The two techniques namely ELISA and RIA are most commonly used for this purpose. In both the assays, the antibody binds to the specific antigen (usually coated to plastic plates) and the unbound antibody and other components of the medium can be washed off. Thus, the hybridoma cells producing the desired antibody can be identified by screening. The antibody secreted by the hybrid cells is referred to as monoclonal antibody.
5. **Cloning and Propagation:** The single hybrid cells producing the desired antibody are isolated are cloned. Two techniques are commonly employed for cloning hybrid cells- limiting dilution method and soft agar method.
6. **Characterization and storage:** The monoclonal antibody has to be subjected to biochemical and biophysical characterization for the desired specificity. It is also important to elucidate the MAB for the immunoglobulin class or sub-class, the epitope for which it is specific and the number of binding sites it possesses.The stability of the cell lines and the MABs are important. The cells (and MABs) must be characterized for their ability to withstand freezing, and thawing. The desired cell lines are frozen in liquid nitrogen at several stages of cloning and culture.

Advantages

- Hybridoma serves as an immortal source of monoclonal antibody.
- Same quality of the antibody is maintained amongst the different production batches.
- Highly reproducible and scalable, unlimited production source.
- Can produce antibodies when needed.
- Selection helps to identify the right clones against the specific antigen.

Disadvantages

- Time consuming project
- Very expensive and needs considerable effort to produce them.
- Hybridoma culture may be subject to contamination.

- System is only well developed for mouse and rat and not for other animals.
- More than 99% of the cells do not survive during the fusion process – reducing the range of useful antibodies that can be produced against an antigen.

Large Scale Production

The production MABs in the culture bottles is rather low (5-10µg/ml). The yield can be increased by growing the hybrid cells as ascites in the peritoneal cavity of mice. The ascitic fluid contains about 5-20 mg of MABs/ml. This is for superior than the *in vitro* cultivation techniques. But collection of MABs from ascitic fluid is associated with the heavy risk of contamination by pathogenic organism of the animal. In addition, several animals have to be sacrificed to produce MABs. Hence, many workers prefer *in vitro* techniques rather than the use of animals[4].

Encapsulated Hybridoma Cells

The yield of MAB production can be substantially increased by increasing the hybridoma cell density in suspension culture. This can be done by encapsulating the hybridomas in alginate gels and using a coating solution containing poly-lysine. These gels allow the nutrients to enter in and antibodies to come out. By this approach, a much higher concentration of MAB production (10-100µg/ml) can be achieved. Damon Biotech Company and Cell-Tech use encapsulated hybridoma cells for large-scale production of MABs. They employ 100-liter fermenters to yield about 100g of MABs in about 2 weeks period.

Human Monoclonal Antibodies

- For ethical reasons, humans cannot be immunized against antigens.
- The fused human lymphocyte-mouse myeloma cells are very unstable.
- There are no suitable myeloma cells in humans that can replace mouse myeloma cells.

Applications of Monoclonal Antibodies

The applications of MABs are broadly grouped into 4 types:

Direct use of MABs as therapeutic agents- MABs are used in treatment of

1. Cancer
2. Transplantation of bone marrow and organs
3. Autoimmune diseases
4. Cardiovascular diseases
5. Platelet aggregation

Table no 2 applications of monoclonal antibodies

Generic name	Trade name	Type of MoAb	Applications
Rituximab	Rituxan(IDEC/Genentech)	Chimeric anti-CD20	Non-Hodgkins lymphoma
Trasuzumab	Herceptin (Genentech)	Humanized anti-HER2	Metastatic breast cancer
Muromonab-CD3	Orthoclone OKT3	Murine ant-CD3	Immunosuppressant
Daclizumab	Zenapax	Humanized anti-CD25	Immunosuppressant
Basiliximab	Simulect(Novartis)	Chimeric anti-CD25	Immunosuppressant

Although MABs have many potential uses for tumour therapy, there are inherent problems associated with this approach:

i) Cancer cells are heterogeneous, so those cells that are not recognized by the MABs can escape and proliferate[5].

ii) Some tumours contain semi dead cores with poor circulation and thus cannot be reached by monoclonals[6].

iii) MABs can interact with circulating target antigens before reaching their target[7].

iv) Patients can experience possible immunogenic reactions. For these reasons, it has frequently proven more effective to combine MABs treatment with standard chemotherapeutic agents[8].

- **MABs as targeting agents**- An important part of the design of an antibody directed drug delivery system is the type of linkage and coupling method between antibody and drug. The drug can be covalently bound to the MABs directly or through a short spacer, or the two can be conjugated through a linker such as a water-soluble polymer[9]. Alternatively, a carrier such as a liposome or a polymeric microsphere can be used, wherein the drug is entrapped in or bound to the carrier, and the MABs is bound to the surface of the carrier. Characteristics that would comprise an ideal antibody directed delivery system could include, preparation by a method that has high efficiency and yield, and is capable of scale up; high stability of the conjugate, both under shelf storage conditions and in the circulation after injection; and retention of antigen-binding ability of the antibody while it is carrying the drug to the target tissue. Finally, upon reaching the target, either the immune-conjugate itself should have the desired pharmacological effect equivalent to the free drug, or must release free drug or a derivative that is fully efficacious. Although such a system is probably impossible to achieve for most therapeutic

applications, a variety of coupling reagents are fortunately available that aid in optimizing the properties of an immune-conjugate. Amino, sulfhydryl, and carboxyl groups are the most common functional groups on the antibody, carrier, and drug molecules used for coupling. If the drug lacks the desired group, it may be possible to introduce it[10].

Drug delivery- MABs, against the antigens on the surface of cancer, are useful for the treatment of cancer. The antibodies bind to the cancer cells and destroy them. This is brought out by antibody-dependent cell-mediated cytotoxicity, complement-mediated cytotoxicity and phagocytosis of cancer cells (coated with MABs) by reticulo-endothelial system. The patients suffering from leukaemia, colorectal cancer, lymphoma and melanoma have been treated with MABS[11]. However, there was a wide variation in the success rate. A monoclonal antibody specific to the cells of leukaemia is used to destroy the residual leukaemia cells without affecting other cells. MABs are used *in vitro* to remove the residual tumour cells prior to autologous bone marrow transplantation (transplantation of the patient's own bone marrow cells, due to non-availability of suitable donor).

Dissolution of clot- A monoclonal antibody directed against fibrin can be coupled to tissue plasminogen activator (tPA) and used for degradation of blood clots. MAB-tPA complex due to a high affinity gets attached to fibrin. Due to the concentration of tPA at the target spots, there is more efficient conversion of plasminogen to plasmin which is in turn dissolves blood clot (fibrin) [12]. Good success of the clot lysis has been reported by using MABs-tPA complex in experimental animals.

Immuno-liposome- Generally, the antigens expressed by tumor cells are not specific but are merely present in higher ratio than on the normal cells. Hence, systems such as immune-liposomes have been developed to exploit these opportunities, as they are expected to bind to a greater extent to high antigen density tumor cells than to low antigen density normal cells. In immune-liposomes, the number of antibody molecules per liposome can be varied by as much as two orders of magnitude[13]. Using egg phosphatidylcholine, cholesterol, phosphatidylserine, and N-4-nitrobenzo-2-oxa-1-1, 3-diazole phosphatidylethanolamine in molar ratio of 56: 33: 10: 1, unilamellar liposomes with 12–55 antibody molecules per vesicle have been investigated for binding with RDM-4 lymphoma cells with varying antigen density. The increase in the valency of liposomes (i.e., number of antibody molecules per liposome) increased their binding with low as well as high antigen density cells, and thus the low valency immune-liposomes were found to allow better discrimination between target and normal cells. An additional advantage of immune-liposomes is that a relatively high drug loading can potentially be accommodated, with the result

that a small number of antibody molecules conjugated to the surface of an immune-liposome can deliver many more drug molecules to the target than is otherwise possible. Once the drug is released into the target cell, no further transformation is needed, because the entrapment process does not involve any chemical modification of the drug[14].

Immuno-microspheres- In view of the availability of a wide variety of biocompatible and biodegradable polymers, and the ease of preparation of stable microparticles with predictable physicochemical characteristics, antibodies have been conjugated to polymeric microparticles for controlling their *in vivo* deposition[15]. Although a few in vitro studies have demonstrated promising results with immune-microspheres, limited information has been published on the *in vivo* efficacy of immune-microspheres for drug delivery. In one case, following promising in vitro results, an *in vivo* study was conducted in mice bearing human tumor xenografts, using 14C-polyhexylcyanoacrylate nanoparticles with adsorbed anti-osteogenic sarcoma MABs 971T/36. However, the particles were found to deposit predominantly in liver and spleen, and hence the study failed to demonstrate any appreciable improvements in drug delivery due to the immune-carrier. Lack of optimal particle size and/or tumor tissue permeability, lack of expression of sufficient Fab portions on the surface of particles, particle opsonisation leading to a secondary non-interactive coating, distribution of specific antigens in the liver, and competitive displacement of the adsorbed MABs by serum components were suggested as possible reasons for this undesirable in vivo distribution of the immune-particles. Another study has evaluated the in vivo drug delivery potential of albumin immune-microspheres in mice. The microspheres bearing Lewis lung carcinoma MABs demonstrated slightly higher localization in lung carcinoma at 24 h after its administration.

MABs in radio immunotherapy- The radioisotopes can be coupled to MABs that are directed against tumor cells. This allows the concentration of radioactivity at the desired sites and a very efficient killing of target cells (tumor cells) [16]. The advantage with radio immunotherapy is that conjugated complex need not penetrate the cells, as is required in immunotoxin therapy. The limitation is that the neighbouring normal cells may also get damaged or killed. This can be minimized by using radioisotopes with short half-lives. Ytrium-90 with a half-life of 64 hours is a suitable isotope to be employed in RAIT. Due to shortage in the supply of Ytrium-90, indium-111 is more commonly used.

- Diagnostic use- Monoclonal antibodies have revolutionized the laboratory diagnosis of various diseases. For this purpose, MABs may be employed as diagnostic reagents for Biochemical analysis or as tools for Diagnostic imaging of diseases[17].

a. Biochemical analysis:

Diagnosis of pregnancy,

Cancers,

Hormonal disorders,

Infectious diseases.

b. Diagnostic imaging (immune scintigraphy):

Myocardial infraction,

Deep vein thrombosis,

Atherosclerosis,

Cancers,

Bacterial infections.

Characterization of Monoclonal Antibodies

- **Bio-chemical / bio-physical:**

Class, subclass (when appropriate) and light-chain composition, molecular weight and either N- and C-terminal amino acid sequence, secondary and tertiary structure[18].

- **Immunological:**

antigenic specificity including the characterisation of the epitope the antibody recognises, binding capacity, ability for complement binding and activation, cytotoxic properties[18].

- **Specificity and cross reactivity:**

determination of unintentional reactivity with or cytotoxicity for human tissues distinct from the intended target, and cross-reactivity with a range of human tissues[18].

Marketed preparations[19]

Ofatumumab	Arzerra	2009	Human	CD20	Chronic lymphatic leukaemia
Tocilizumab	Actemra and RoActemra	2010	Humanised	Anti-IL-6R	Rheumatoid arthritis
Belimumab	Benlysta	2011	Human	Inhibition of B-cell activating factor	Systemic lupus erythematosus
Lpilimumab	Yervoy	2011	Human	Blocks CTLA-4	Melanoma

Regulatory Issues[20]

- Exhaustive characterization of the origin of cell lines
- Characterization of production procedures
- Product purification and characterization
- Quality control
- Validation of processes involved during production and testing

CONCLUSION

Antibodies are proteins synthesized in blood against specific antigens just to combat and give immunity in blood. They can be collected from the blood serum of an animal. Such antibodies are heterogeneous and contain a mixture of antibodies (i.e., monoclonal antibodies). Therefore, they do not have characteristics of specificity. If a specific lymphocyte, after the isolation and culture *in vitro*, becomes capable of producing a single type of antibody which bears specificity against specific antigen. It is known as 'monoclonal antibodies'. Due to the presence of desired immunity, monoclonal antibodies are used in the diagnosis of diseases. The increasing importance of mAbs in therapeutic applications,occurring in recent years, has led to the rapid development oftechniques/ strategies for their large-scale production. If the current know-how is not expanded, success will strongly depend on trial-and-errorexperiments, and progress in this field will be dependent on newdiscoveries and their application.

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