

Monoclonal antibodies and its impact on modern health services- A Review

Das S^{*1} and Sehgal VK²

¹Post Graduate Resident, Department of Pharmacology, Govt Medical College Patiala, Punjab, India

²Professor, Department of Pharmacology, Govt Medical College Patiala, Punjab, India

QR Code



*Correspondence Info:

Dr. Supratik Das,
Post Graduate Resident,
Department of Pharmacology,
Govt Medical College Patiala, Punjab, India

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Abstract

Recent developments in the ability to manipulate immunoglobulin genes have led to development of monoclonal antibodies directed against therapeutic targets. Nowadays, Monoclonal Antibodies act as useful therapies in case of diseases like Cancer, Autoimmune diseases, Infectious diseases and also in conditions like Transplant rejections. The dream of Monoclonal antibodies has been achieved due to advent of Hybridoma technology in 1975.

Monoclonal antibodies are one of the most upcoming fields in biopharmaceutical sciences. The role they have played has brought a revolution in the sector of health services. Not only as a therapeutic tool, has its role-play also touched the topics of analysis, purification, and enrichment and also in mediation of physiological responses. Today, it is continuing to become a befitting reply to various notorious diseases, for which, health professionals had waited for a long time. The purpose of this manuscript is to bring forward, in brief, the role played by Monoclonal antibodies in health sector, various studies related to this marvellous therapeutic tool and the scope; it carries in the near future, and for many years to come.

Keywords: Monoclonal antibodies, Hybridoma technology, Immunoglobulin, Human mAbs, Cell Engineering.

1. Introduction

Monoclonal antibodies are immunoglobulins of a single molecular type which react with target proteins of desired antigens.[1] Monoclonal antibodies are generally of 2 types: Murine Monoclonal antibodies and Chimeric Monoclonal antibodies.[2] Monoclonal antibodies bind to the same epitope and are produced from a single clones of B-lymphocyte cells. They were first generated in mice in 1975 using a technique known as hybridoma. [3]

Hybridoma technology was discovered in 1975 by Georges Kohler of West Germany and Cesar Milstein of Argentina, who along with Niels Jerne of Denmark were recipients of Noble prize for Physiology and Medicine in 1984[4].

Monoclonal antibodies are nowadays used for many diagnostic and therapeutic applications. The popularity in this field has led to the development of large-

scale manufacturing processes, with productivity improvements and their optimization.[5]

Due to the significant specificity shown by them, Monoclonal antibodies have played an important role in analysis in the field of medical research, diagnosis, therapy, and basic science thus, achieving marked successes in clinical settings [6]. Monoclonal antibodies has brought a revolution in field of diagnostic science with their specificity towards specific antigen groups and showing an almost unlimited production. They can be produced against any antigen and are completely a homogeneous populations, entailing fewer problems of cross reactivity.[7]

According to US Food and Drug Administration, more than 20 mAbs has been approved, and about more than 150 mAbs are currently undergoing clinical trials.[8] Thus, we can say that their role in diagnostic assays and in pharmacotherapeutic field has made a significant impact in the improvement of health in both humans and animals.[9]

2. How are monoclonal antibodies produced?

Monoclonal antibodies are of 2 types: Murine and Chimeric. Generally Chimeric Monoclonal antibodies are used as they show half human half mouse characteristics thus, showing less immunogenicity, whereas Murine monoclonal antibodies may induce a Human-Antimouse allergic response. Monoclonal antibodies are produced by fusing immortal myeloma cells with the B lymphocytes which produces antibody against a desired antigen.[2] A selective medium in which only fused cells can grow is used. That medium is called HAT medium as it contains Hypoxanthine, Aminopterin, and Thymidine. This medium is selective for fused hybridoma cells.[10] Polyethylene Glycol is utilised in order to fuse adjacent plasma membranes[11]

Unfused myeloma cells fail to grow in HAT medium as they are deficient in Hypoxanthine Guanosine Phosphoribosyl Transferase (HGPRT), and thus unable to make DNA. Free B lymphocytes show growth failure due to their short life span. Only fused hybrid cells can grow in HAT medium because partnering B lymphocytes produce HGPRT.[12] Selection of hybridomas secreting desired antibodies is quite lengthy. A screening assay which is rapid, reliable, versatile, sensitive and easy to perform in nature should be chosen. The most commonly used system regarding this, is the enzyme linked immunosorbent assay (ELISA). Antibodies can also be detected by radio immunoassay (RIA), immune fluorescence and haemolytic plaque assays.[13]

3. Drawbacks of early monoclonal antibodies

Orthoclone OKT3 i.e. muromonab-CD3 was the first licensed monoclonal antibody, which was approved in 1986 for use in preventing kidney transplant rejection.[14] Its use was limited to acute cases because of adverse effects.[15] Production of early monoclonal antibodies was limited accounting to availability of a suitable myeloma cell line for which, animals like mouse or rats acted as appropriate sources. Hybridomas were also found to be low yielding or unstable.[16] Expression systems regarding monoclonal antibodies have been tested, which revealed to have been possessing contrasting effects. *E. coli* was most efficient system for expressing antibody fragments like single-chain variable fragments and antigen-binding fragments.[17] The transformation efficiency, and purity of humanised monoclonal antibodies, has been low as the animals which were chosen for this purpose were transgenic.[18] With time, a concept involving use of animal species regarding production of humanised antibodies was initiated.[19]

4. Improvements made in the field of hybridoma technology

Efforts have been initiated in order to upgrade Hybridoma technology, thus enhancing the rate of production of Monoclonal antibodies, these points comprises of the following:

- Chemical fusion promoter such as Polyethylene Glycol was substituted for Sendai virus to fuse adjacent cell membranes.
- Myeloma cells that do not secrete their own antibodies were selected for this task as they do not interfere with the production of the desired antibody.[4]
- The ability of an Antigen to bind with such type of Antibodies can be improved by using phage display libraries which enables us to select antibodies, possessing high affinities for the antigen. Many times often, antibodies having lower affinity for the antigen may be chosen in hopes of better penetration of a tumour [20]

5. Purification

Monoclonal antibodies are purified with the help of 2 methods:

- Ion-exchange chromatography
- Antigen affinity chromatography[4]

5.1 Serum free media for bulk culture of hybridoma cells

Use of serum makes purification of antibodies quite tedious. Also, such an expensive technology for large scale production of hybridoma cells for industrial production of monoclonal antibodies makes use of serum free media for culturing hybridoma cells.

Serum free media has got following advantages

- Increased purity and absence of contaminating immunoglobulin.
- Diminished variability of culture medium.
- Reduced risk of infection.
- Fewer variables for quality control/assurance.
- Enhanced control over bioreactor conditions.
- Potential for enhanced antibody secretion and improved efficiency
- Minimal dependence on animals.
- Pocket friendly.[21]

However some disadvantages are also present:

- Serum free media is not applicable to all cell lines.
- Cells may not grow to as high densities and also may be more fragile than cells in serum
- Media may be cumbersome to prepare[22]

5.2 Basic structure of a Monoclonal antibody molecule and nomenclature related to its origin:

Monoclonal antibodies, like any other conventional Antibodies are made of basic structural components, which consists of 2 large heavy chains and 2 small light chains. Numerous antibody heavy chains define 5 different types of crystallisable fragments (Fc).

They are globular proteins having sugar chains which are added to amino acid residues.

The heavy chains and the light chains are connected with the help of Disulfide bonds.

Apart from Heavy chains and Light chains, there also presence essential components embedded in them. They consist of scFv, Fab, Fv, Diabody[6]

5.3 These components serve a specific function and each provides a unique contribution to immunological sciences.

- scFv:** scFv fragments ie variable domains of heavy and light chains are joined by a flexible linker, which were first explained as small fragments and, which are able to retain the binding of IgG molecule. This moiety has a short half life in serum (2hr).[17]
- Diabodies:** They constitute a class of bispecific antibody fragments. Their size promotes penetration of tumors and its exit from the serum. The fragments are derived from Hybridomas and provide a source of antibody fragments for medical and industrial reasons [23] Diabodies provide unfavourable Pharmacokinetic properties due to their small size [24].
- Fab unit:** It's composed of light chain and N terminal half of heavy chain, and it acts as antibody combining

site. N terminal half has sequences which varies in various immunoglobulins whereas C terminal of half has similar sequences for the parts which are obtained from same subclass.[25]

- Fc unit:** This region is responsible for binding to Fc receptors of invading pathogens/cells thus inducing an immunological response.[26]

5.4 Regarding the nomenclature of Monoclonal antibodies, it depends upon the percentage or amount of characteristics inferred upon that antibody, whether its murine or human. Special suffix added during nomenclature, determine the traits, the given Monoclonal antibodies will possess.

- Murine:** There are no human traits present in that antibody, moreover 100% mouse related trait predominate. (Suffix: Omab)
- Chimeric:** 65% humanised traits predominate (Suffix: Ximab)
- Humanised:** Human related traits are 90% dominant. (Suffix: Zumab)
- Fully Human:** As the name suggests, the antibody is derived fully from human sources (Suffix: Umab)[6]

An important point to consider is that more these antibodies carry murine traits, they are more likely to be immunogenic.

Examples of some Organizations who market Monoclonal antibody technology include:

- Abgenix:** This marketed Xenomouse technology. Abgenix was bought by Amgen in April 2006 [41]
- Regeneron's VelocImmune technology.[42]
- Kymab:-** who market their Kymouse technology.[43]

6. Uses of different monoclonal antibodies

Nowadays, numerous such monoclonal antibodies having appropriate nomenclature according to their origin of hybridisation and suffixes are being used in various notorious diseases.

Table 1: Uses of different monoclonal antibodies

Generic Name	Trade Name	Antibody origin	Antigen	Approved in:	FDA approval
Muromomab	Orthoclone	Murine, IgG2a	CD3	Allograft rejection in allogeneic renal transplantation	19/6/86
Abciximab	ReoPro	Chimeric, IgG1	GPIIb/IIIa r	Maintenance of coronary patency	22/12/94
Rituximab	Mabthera	Chimeric, IgG1	CD20	CD20-positive B-cell non-Hodgkin's lymphoma	26/11/97
Daclizumab	Zenapax	Humanized, IgG1	CD25	Allograft rejection	10/12/97
Basiliximab	Simulect	Chimeric, IgG1	CD25	Allograft rejection	12/5/98
Palivizumab	Synagis	Humanized, IgG1	Protein F	Respiratory syncytial virus (RSV inhibitor) in children	19/6/98
Infliximab	Remicade	Chimeric, IgG1	TNFa	Crohn's disease and rheumatoid arthritis	24/8/98
Trastuzumab	Herceptin	Humanized, IgG1	HER2/Neu	Metastatic breast cancer	25/9/98
Etanercept	Enbrel	huFcγ1/TNFr	TNFa and b	Autoimmune diseases such as ankylosing spondylitis	2/11/98
Gemtuzumab	Mylotarg	Humanized, IgG4	CD33	CD33-positive acute myeloid leukemia	17/5/00
Alemtuzumab	Mabcampath	Humanized, IgG1	CD52	B-cell chronic lymphocytic leukemia	7/5/01
Ibritomomab	Zevalin	Mouse, IgG1	CD20	B-cell non-Hodgkin's lymphoma	19/2/02
Adalimumab	Trudexa	Human, IgG1	TNFa	Crohn's disease and rheumatoid arthritis	31/12/02

Antibody	Trade Name	Target	Indication	Approval Date
Alefacept	Amevive	huFcγ1/LFA-3	Chronic plaque psoriasis	30/1/03
Omalizumab	Xolair	Humanized, IgG1	Treatment of asthma	20/6/03
Tositumomab	Bexxar	Murine, IgG2a	CD20-positive B-cell non-Hodgkin's lymphoma	27/6/03
Efalizumab	Raptiva	Humanized, IgG1	Moderate to severe plaque psoriasis	27/10/03
Cetuximab	Erbix	Chimeric, IgG1	Metastatic colorectal and head and neck carcinoma	12/2/04
Bevacizumab	Avastin	Humanized, IgG1	Metastatic colorectal and non-small cell lung carcinoma	26/2/04
Natalizumab	Tysabri	Humanized, IgG4	Multiple sclerosis	23/11/04
Ranibizumab	Lucentis	Humanized, IgG1	Wet-type age-related macular degeneration	30/6/06
Panitumumab	Vectibis	Human, IgG2	Metastatic colorectal carcinoma	27/9/06
Eculizumab	Soliris	Humanized, IgG2/4	Paroxysmal nocturnal haemoglobinuria	16/3/07
Certolizumab	Cimzia	Humanized, IgG1	Crohn's disease	18/4/08

Drugs like Etanercept, Alefacept are fusions between the IgG1 Fc portion and a receptor. Black Box Warning had been issued by FDA against Etanercept on 2nd May 08[17]

7. Adverse/harmful effects associated with use of monoclonal antibodies

Administration of mAbs leads to effects such as acute anaphylaxis, serum sickness. There are numerous adverse effects that are related to their specific targets, such as Infections, cancer, autoimmune disease, and Cardiotoxicity[8] Certain adverse reactions caused by Antibodies have been described in brief:

a) Immunological reactions: Acute reactions following their infusion can lead to type 1 Hypersensitivity reactions/ Anaphylaxis/ Anaphylactic shock or anaphylactoid reactions against the antibodies. Ex: Cetuximab. Reactions like Tumour lysis Syndrome, Cytokine Release Syndrome may take place due to drugs like Rituximab[27]

b) Infections:

Tuberculosis reactivation: Therapy against pro-inflammatory cytokine TNF α has tendency to induce latent Tuberculosis.[28] Increased risk of tuberculosis were found in patients of inflammatory bowel disease treated with TNF-specific mAbs[29]

Progressive Multifocal Leukoencephalopathy: Humanized CD11a-specific mAb Efalizumab has been associated with this disorder in patients of chronic plaque psoriasis.[30] Other drugs which may cause PML are Rituximab and Natalizumab[31, 32]

c) Haematological disorders: Monoclonal Antibodies such as Infliximab, Efalizumab, Rituximab has potential to cause acute, severe, self-limiting thrombocytopenia[8] Also drugs like Abciximab, Alemtuzumab may also lead to fall in platelet count and lymphocyte count respectively.[33,34] Bevacizumab has been found to show Thromboembolism[35]

d) Autoimmune disorders: Use of Monoclonal antibodies has been associated with rise of autoimmune diseases like Lupus like syndrome, Autoimmune Hyperthyroidism, Autoimmune Colitis etc.[8]

e) Cardiovascular adverse effects: Drugs like bevacizumab, trastuzumab, pertuzumab, ofatumumab, rituximab are responsible for adverse effects on cardiovascular system and it may comprise of:

- Hypertension
- Arterial/Venous thromboembolism
- Congestive Heart failure[36]

f) Pulmonary adverse effects: Adverse effects can be organised into 4 main categories i.e. interstitial pneumonitis and fibrosis, acute respiratory distress syndrome, bronchiolitis obliterans organizing pneumonia, and hypersensitivity reactions. Signs, symptoms, include dyspnea, cough, fatigue, and pulmonary opacities.[36]

g) Proteinuria: Drugs like Bevacizumab leads to proteinuria[37] Microscopy shows thrombotic microangiopathy, collapsing glomerulopathy, and incidences of cryoglobulinemic and immune complex glomerulonephritis.[38]

h)Enterotoxigenicity: Enterocolitis, colitis, gastrointestinal perforation are common gastrointestinal adverse effects.[39]

i) Dermatological: It comprises of Papulopustular Acneiform Eruption, Paronychia Inflammation, Mucositis.[36]

8. Future of monoclonal antibodies

Monoclonal antibody therapy has become a burning issue for clinical treatment procedure regarding various ailments which ranges from inflammatory diseases, cancer, cardiovascular diseases, transplant rejections to infectious, metabolic and neurodegenerative diseases. The advent and development of hybridoma technology has led to production of large scale highly specific antibodies against broad spectrum of diseases. At the same time, they play crucial roles in diagnosis, disease monitoring, prognostic markers identification and Pharmacotherapy.

Nowadays, Monoclonal antibody therapy has been found to show a significant impact on malignancies of solid tissues and haematological origin. In today's era, combination therapy is a well-accepted tactic in tumor therapy, and they are increasingly emerging as an effective component of many therapeutic protocols.[40]

In near future, there is a strong prediction that, the market will be filled by these latest generation antibiotics having new class of medications and biopharmaceuticals. These can be aimed toward the treatment of important and notorious diseases such as cancer, infections and conditions like sepsis, transplant rejection, AIDS and autoimmune diseases [6].

MABs today leads the development of a multi-billion dollar biotechnological research industry. Many leading pharmaceutical brands have entered or invested in this field, due to a faster developmental rate and a pocket friendly developmental cost. Thus, ensuring higher success rates, medium pricing, and a potentially reduced threat from generics in the coming generations. [12]

9. Conclusion

Monoclonal antibodies have indeed been a marvel in the field of science. Who could have wondered that now mankind now has a befitting reply to most of the notorious diseases in the world. Nowadays, both clinicians and patients have a ray of hope that ailments like cancer, infections, and autoimmune diseases can now be put into control. The topic of Monoclonal antibodies is not only a burning issue for researchers but also in the field of pharmaceutical and biopharmaceutical industries. Several advantages like reduction in development costs, reduction in time taken for manufacture, improved results have encouraged a lot to make progress in this field. It is hoped that in near future, these Monoclonal antibodies will have a profound impact on the treatment of these difficult ailments, provided if the adverse effects are also limited. Targets for improving antibody efficacy consist of areas like immunogenicity, antigen-binding affinity, effector functions and pharmacokinetics. Immunogenicity limits non-human sequences by creating chimeric, humanised versions of the antibodies with as few T-lymphocyte epitopes as possible. Antibody fragments are less immunogenic due to a lack of Fc domain. Antigen-binding affinity can be improved by using phage display libraries with an aim to isolate antibodies with strong affinities for the antigen.

Thanks to their specificity and flexibility Monoclonal antibodies now are lucrative options regarding development of new therapies and molecular drug targets against a wide variety of diseases. Factors like, method of production, avidity, effector function and delivery to target

tissue, play a pivotal role in choosing types of monoclonal antibodies, e.g. a smaller scFv has a high probability to penetrate a tumour more effectively than a full-sized antibody. However, despite its drawbacks, there is still major interest and a ray of hope from pharmaceutical companies to develop monoclonal antibodies for both clinical and diagnostic use and has full calibre to brighten the future of treatment and management of the most toughest of ailments from a clinical and economical point of view.

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