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Review article

Monoclonal Antibodies: Production, Techniques, and Global Marketing

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Abstract

Monoclonal antibodies are becoming increasingly important for molecular immunology research. It has also become key components in a wide variety of clinical laboratory diagnostic tests. The wide applications of serum analytes in the detection and identification of cell markers and pathogenic agents have arisen in large part due to the excellent specificity of these unique reagents. Furthermore, continuous culture of hybridoma cells producing these antibodies offers the potential for an unlimited source of reagents. In essence, the continuous supply feature provides standardization of both reagent and assay technique, compared to the very limited supply of polyclonal antibody reagents. Clearly, polyclonal and monoclonal antibodies have advantages and disadvantages in terms of production, cost, and general applications. As a result, monoclonal antibodies are produced only when necessary because their production is a time-consuming and laborious process, although highly rewarding. In this article, the production and application of monoclonal antibodies are illuminated to provide better understanding and formulate new ideas for clinicians and scientists alike.

Key words: *Monoclonal antibody, hybridoma, phage display*

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1. Introduction

Information on the structure and functions of antibodies as we know them today began to become clear only after the 1950s. When we look at the information obtained from 1950 until today, antibodies are generally known as the most effective components of humoral immunity. There are 5 types of antibodies in humans. These are called immunoglobulin A (alpha), D (delta), E (epsilon), G (gamma), M (mu).(1).

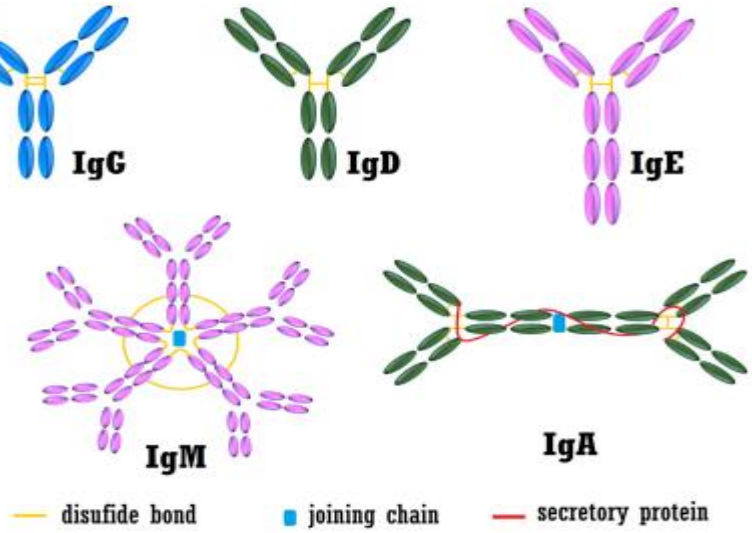

Figure 1: Types of immunoglobulins (2).

Antibodies generated from a single clone of B cells are called monoclonal antibodies. Monoclonal antibodies (mAbs) only react against one epitope. Lymphoid or myeloid cells are responsible for generating the immunological response. These cells within the lymphoid cells are called “B lymphocytes”. Antibodies that occur as a result of stimulation of B cells in the body and have different activities are called polyclonal antibodies. If the resulting antibody consists of a single cell and its cloning, it is called a monoclonal antibody (3, 4). mAbs are antibodies with high specificity for a single antigen or a single epitope on the antigen (5). mAbs were first produced in 1975 by scientists named Köhler and Milstein using hybridoma technology. To date, a large number of mAbs and their derivatives have been used in many clinical applications (6).

2. Uses, Advantages, and Disadvantages of Monoclonal Antibodies

mAbs find a wide variety of uses due to their high specificity. It is used in laboratory tests such as the treatment of diseases, protein purification, suppression of immune response, some allergy tests, identification of special cells, hormone testing, for diagnostic purposes, and basic scientific research. (7).

The diseases in which antibodies are used for therapeutic purposes are increasing day by day. It finds use in cancer, prevention of organ transplant rejection, cardiovascular diseases, inflammatory diseases, and autoimmune diseases. This number is predicted to increase in the future (8, 9).

mAbs are also used effectively and widely to detect various agents threatening human health that can be found in food, water, and soil. There are various mAb-based diagnostic systems for the detection of aflatoxin, which can be found in high

amounts in traditional foods and causes significant harm (10, 11). In addition, monoclonal antibodies are used in the diagnosis of *Bacillus anthracis*, which can be found in water and soil and can cause human death (12, 13).

Oncology and Hematology Treatment

mAbs are preferred because of their high target-specific binding properties. (3). The main purpose of cancer immunotherapy is to reactivate the immune system that has been silenced by the tumor cell in various ways and to make the tumor cells recognizable. The first of the three basic approaches in this regard is monoclonal antibody-mediated cell death targeting tumor-related antigens. Others are immune system control inhibitors and cancer vaccines. (14).

Although there are many effective drugs in cancer treatment, their use is limited due to their strong side effects. Compared to conventional chemotherapeutic drugs, mAbs stand out because they can distinguish antigenic differences between normal and malignant tissues and have minimal effects on healthy tissues. (15).

mAbs show their effects with various mechanisms of action in cancer treatment. These mechanisms show their effects by inhibition of cell signaling and apoptosis, antibody-dependent cellular cytotoxicity (16), apoptosis dependent on modulation of the receptor by signal inhibition (15), as well as tumor-associated antigens blocking the proliferation of tumor cells.

Autoimmune Diseases

mAbs act in the prevention of autoimmune reactions by targeting the basic cells of the immune system such as T and B lymphocytes. The movements and functions of mAb-activated cells are inhibited, the levels of proinflammatory cytokines are reduced, and thus the pathological effects are alleviated by suppressing the excessive immune response. In the first studies conducted in autoimmune diseases, it was determined that in the treatment of rheumatoid arthritis and Crohn's disease, it binds to tumor necrosis factor (TNF) and by neutralizing it, pathologies related to these diseases are prevented. (17). There are mAbs developed against many autoimmune diseases (Table 1). In the coming years, it is expected that new antibodies will be discovered in the treatment of different autoimmune diseases.

Infectious Diseases

The number of bacteria that develop resistance to antibiotics is increasing over time. Especially coping with hospital infections has become important problems. The development of resistance to existing antimicrobials and the inability to develop new antimicrobial drugs have increased the interest in mAbs in this field. mAbs with antimicrobial activity neutralize bacterial toxins and alleviate the pathological activities of bacteria. It is stated that the possibility of developing resistance against antibodies is low because mAbs show their effects by different mechanisms than classical antibiotics (18). Monoclonal antibodies such as Raxibacumab and Obiltoxaximab are used in the treatment of anthrax of bacterial origin (*Bacillus anthracis*). These mAbs prevent the binding of protective antigens in bacteria to cell receptors, thereby preventing edema and the formation of deadly toxins (19, 20).

Infusion Reactions

Side effects that may occur during the infusion of mAbs may occur locally or systemically (21). Common symptoms include fever, flushing, chills, chest discomfort, abdominal pain, nausea,

vomiting, diarrhea and skin rash. Rarely, anaphylaxis may occur. Side effects due to mAb infusion may occur within the first two hours, but may be delayed up to 14 days after treatment. The period when the risk of side effects is highest is during the first or second application, but the risk may decrease with repeated applications. (22). Side effects such as anaphylactic reactions to the mAb, serum sickness, tumor lysis syndrome (TLS), and cytokine release syndrome (CRS) may occur after infusion of mAb (23).

3. Techniques used in the production of monoclonal antibodies

The use of antibodies for diagnostic and therapeutic purposes dates back to the first years when their existence was proven (24). For this purpose, the collection and purification of polyclonal antibody-containing serum of an animal immunized with a specific antigen and its use for the specified purpose have been tried. However, this situation has led to the development of a monoclonal antibody molecule gaining importance due to the drawbacks in the use of polyclonal antibodies (25). First, Köhler and Milstein, in 1975, "The foundations of hybridoma technology were laid by obtaining continuous cell lines of fused cells that secrete specific antibodies for a particular antigen (26). Molecules that can bind specifically to a certain antigen and have a uniform (homogeneous) immunoglobulin (IgG) structure are called “monoclonal antibodies” (Mabs) (Costa et al., 2010). Since the formation and production of hybridomas, there have been significant developments in the biotechnology industry (27). The main field of use of mAbs is diagnostics and immunotherapy. After the first experiments with mouse Mabs, with the advances in technique and the emergence of new fusion mates, hybrids between rat, hamster, human and other species dec also be obtained. In addition, technologies have been developed that allow the production of important immunological T-cell hybridomas that secrete single reproductive factors (28-30). Hybridoma technology is based on fairly clear and unambiguous foundations, but difficulties may arise during the acquisition of antibodies and clones of the desired characteristics and biological character. The procedures take a lot of time and require intensive laboratory labor (31).

The basis of hybridoma technology is “creation of cells capable of indefinite reproduction and antibody formation *in-vitro*”. However, antibody-producing B-lymphocytes usually die within a few weeks in cell culture (in-vitro) (32). Therefore, antibody-producing B-lymphocytes must be modified to live in cell culture for a long time. For this reason, lymphocyte tumor cells (myeloma) that are capable of proliferating indefinitely are used. The fusion of antibody-producing B-lymphocytes and myeloma cells, which are capable of endless reproduction, is carried out (33). Immortal cell lines formed as a result of the fusion of B-cell and myeloma are hybrid in nature and are called "hybridoma". Hybridoma cell lines have characteristics of both fusion partners (34). The basic process steps have changed little since Köhler and Milstein. The critical steps of the process are the achievement of efficient and efficient cell fusion and then selection of clones of appropriate character.

The steps of the method can be listed as follows:

- An effective immunization (in-vivo or in-vitro).
- Acquisition of immune B-lymphocytes.
- Preparation of myeloma cells.
- Hybridization (cell fusion) and obtaining hybridoma.
- Control of immunoglobulin synthesis.
- Cloning of hybridomas.
- Production of hybridomas on a large scale.
- Purification of monoclonal antibodies.

Phage Display Technique

This technique stands out as the most popular of the available monoclonal antibody development methods. It has many advantages, such as the fact that it allows the development of recombinant antibodies with different properties and their production using protein chains of human origin. It is based mainly on the principle of directed evolution. Accordingly, as a result of somatic mutation, the antibody has become specific to the antigen to be developed and the best-binding M13 bacterial phage has been selected, and the protein chain that provides binding is produced using recombinant DNA technology methods (35-37). Within the scope of the technique, firstly, the gene framework encoding the VH and VL protein chains present in the human B-cell is amplified by PCR (polymerase chain reaction). The transcribed gene region is integrated in front of the pIII surface protein gene region so that the recombinant antibody (in the form of scFv or VH) can be synthesized to coincide with the pIII surface antigen of the M13 bacterial phage (38-40). Escherichia coli strain is infected with the bacterial phage prepared in this way and the phages are reproduced. Replicated phages are superimposed on antigens attached to the surface of the chip or 96-well plate for the first selection step. At this stage, phages that can bind to the antigen are bound, while those that cannot bind are removed by washing. In the next step, the surface of the well is treated with a secondary antibody with a phage-specific and luminescent conjugate. After the last washing process, the presence of the bound antibodies can be detected spectrophotometrically, so that the conjugate bound to the secondary antibody is irradiated and the measurement is made immediately afterwards. According to the results obtained, the phages in the wells determined to be positive are collected by trypsinization and propagated in E.coli (41, 42).

For the phages collected from the positive wells obtained as a result of the first selection, the selection process is performed two more times in the same way. The secondary and tertiary selection steps performed in this way are performed to mature the affinity of the antibody to be obtained. Thus, it is ensured that the antibody to be obtained will have a high binding ability (43-45).

After the relevant gene chain of the antibody obtained by the binding site phage display technique is reached, the gene chain in question is cloned into a plasmid vector with the appropriate expression cassette. The resulting plasmid is transferred into bacterial (often E.coli), yeast (often Pichia pastoris) or mammalian (often CHO) cells, depending on the final antibody form designed (holistic, mini-structure, scFv, etc.). Thus, the antibody developed to have high specificity and

affinity is produced for use for diagnosis and/or treatment purposes (46-48).

4. Global market for monoclonal antibodies

The first chimeric mAbs were generated in the late 1990s. Subsequently, there was a significant increase in the sales of mAbs in 2013 in parallel with the increase in the speed of approval of humanized and fully human mAbs. In 2013, annual worldwide sales of monoclonal antibodies amounted to approximately $75 billion, accounting for almost half of the biopharmaceutical product market (49).

The last 20 years have seen rapid growth in therapeutics in the monoclonal antibody class. Today, there are over 300 clinically developed monoclonal antibodies (50). The demand for monoclonal antibodies on a global scale is increasing. Global sales revenue for monoclonal antibodies in 2018 reached $115.2 billion (51). It is thought that this sector will be a 300 billion dollar industry by 2025 (52).

5. Conclusions

Recently, depending on the difference in the results obtained in personal treatment, the concept of personalized treatment has begun to gain more and more importance. With this, the importance of monoclonal antibodies in the treatment has begun to increase. The ability of monoclonal antibodies to bind to a single epitope is one of the main reasons for the increased interest in these molecules. The molecular and cellular biology of cancer will be better understood when preclinical studies are completed in many areas such as cancer formation, progression, metastasis, the mechanisms of cancer to render immune system elements unresponsive, to secrete factors that increase tumor growth, and to develop resistance to chemotherapy. The effect of cancer cells at the receptor level, especially in cancerous tissue, has led to an increase in studies and clinical uses in this field. Today, monoclonal antibodies are one of the groups with the largest market share among all pharmaceutical products. With the increasing interest and studies on monoclonal antibodies, it is expected that even more monoclonal antibodies will be available in the coming years.

6. Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

9. Acknowledgement

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Table 1: List of Monoclonal antibodies approved for clinical use in the United States (53).

Cancer				
Generic Name Brand Name	Type Antigenic Target	Approval	Likelihood Score	Major Uses
Alemtuzumab Campath	Humanized CD52	2001	C	Chronic lymphocytic leukemia
Atezolizumab Tecentriq	Humanized PD-L1	2016	D	Urothelial carcinoma Non-small cell lung cancer
Avelumab	Human	2017	E*	Merkel cell carcinoma

Bavencio	PD-L1			Urothelial carcinoma
Bevacizumab	Humanized	2004	E	Colorectal cancer
Avastin	VEGF	2006		Non-small cell lung cancer
		2009		Macular degeneration (off lbl) Renal
		2018		cancer
				Ovarian cancer
Blinatumomab	Mouse	2014	E*	Acute lymphoblastic leukemia
Blincyto	CD3, CD19			
Brentuximab	Chimeric	2011	E*	Hodgkin lymphoma
Adcetris	CD30	2018		Peripheral T-cell lymphoma
Cemiplimab	Human	2018	C	Squamous cell carcinoma
Libtayo	PD-1			
Cetuximab	Chimeric	2004	E	Head and neck cancer
Erbitux	EGFR			Colorectal cancer
Daratumumab	Human	2015	E	Multiple myeloma
Darzalex	CD38			
Dinutuximab	Chimeric	2015	E*	Neuroblastoma
Unituxin	GD2			
Durvalumab	Human	2017	C	Urothelial carcinoma
Imfinzi	PD-L1			
Elotuzumab	Humanized	2015	D	Multiple myeloma
Empliciti	SLAMF7			
Gemtuzumab	Humanized	2000	A	Acute myelogenous leukemia
Mylotarg	CD33	2017		
Inotuzumab	Humanized	2017	B	Acute lymphoblastic leukemia
Besponsa	CD22			
Ipilimumab	Human	2011	A	Malignant melanoma
Yervoy	CTLA4			
Mogamulizumab	Humanized	2018	C	Mycosis fungoides
Poteligeo	CCR4			Sézary syndrome
Moxetumomab	Mouse	2018	E*	Hairy cell leukemia
Lumoxiti	CD22			
Necitumumab	Human	2015	E	Non-small cell lung cancer
Portrazza	EGFR			
Nivolumab	Human	2015	E*	Malignant melanoma
Opdivo	PD-1	2018		Metastatic small cell lung cancer
Ofatumumab	Human	2009	E*	Chronic lymphocytic leukemia
Arzerra	CD20			
Olaratumab	Human	2016	E	Soft tissue sarcoma
Lartruvo	PDGF			
Panitumumab	Human	2006	E	Colorectal cancer
Vectibix	EGFR			
Pembrolizumab	Humanized	2014	E*	Malignant melanoma
Keytruda	PD-1	2015		Non-small cell lung cancer
		2018		Advanced cervical cancer
Pertuzumab	Humanized	2012	E	Breast cancer
Perjeta	HER2			
Ramucirumab	Human	2014	E*	Gastric, non-small cell lung cancer
Cyramza	VEGF	2015		Colorectal cancer
Rituximab	Chimeric	1997	A	Chronic lymphocytic leukemia
Rituxan	CD20			Non-Hodgkin lymphoma
				Rheumatoid arthritis
Tositumomab	Mouse	2003	E*	Non-Hodgkin lymphoma
Bexxar	CD20	Withdrawn		
Trastuzumab	Humanized	1998	D	Breast and gastric cancer
Herceptin	HER2			
Autoimmune Diseases				
Adalimumab	Human	2002	B	Inflammatory bowel disease
Humira	TNFα			Rheumatoid, Psoriatic arthritis
				Severe psoriasis

Alemtuzumab Lemtrada	Humanized CD52	2014	C	Multiple sclerosis
Belimumab Benlysta	Human B cell activity factor	2011 2020	E	Systemic lupus erythematosus Lupus nephritis
Brodalumab Siliq	Human IL-17A	2017	E	Plaque psoriasis
Canakinumab Ilaris	Human IL1β	2009	E*	Autoinflammatory diseases
Certolizumab Cimzia	Humanized TNFα	2008	E*	Inflammatory bowel disease Rheumatoid arthritis
Daclizumab Zinbryta	Humanized CD25	2016	C	Multiple sclerosis
Dupilumab Dupixent	Human IL-4α	2017	E	Atopic dermatitis
Efalizumab Raptiva	Humanized CD11a	2003 Withdrawn	D	Plaque psoriasis
Golimumab Simponi	Human TNFα	2009	E*	Inflammatory bowel disease Rheumatoid, psoriatic arthritis
Guselkumab Tremfya	Human IL-23	2017	E*	Plaque psoriasis
Infliximab Remicade	Chimeric TNFα	1998	A	Inflammatory bowel disease Rheumatoid arthritis Severe psoriasis
Ixekizumab Taltz	Humanized IL-17A	2016	E	Plaque psoriasis Psoriatic arthritis
Ocrelizumab Ocrevus	Humanized CD20	2017	D	Multiple sclerosis
Omalizumab Xolair	Humanized IgE	2003 2014	E	Eosinophilic asthma Chronic idiopathic urticaria
Risankizumab Skyrizi	Humanized IL-23	2019	E	Plaque psoriasis
Rituximab Rituxan	Chimeric CD20	1997	A	Chronic lymphocytic leukemia Non-Hodgkin lymphoma Rheumatoid arthritis
Sarilumab Kevzara	Human IL6R	2017	E*	Rheumatoid arthritis
Secukinumab Cosentyx	Human IL-17A	2015 2016 2018 2020	E*	Plaque psoriasis Psoriatic arthritis, Ankly. Spondylitis Scalp psoriasis Axial spondylarthritis
Siltuximab Sylvant	Chimeric IL6	2014	E	Castleman disease
Tildrakizumab Ilumya	Humanized IL-23	2018	E*	Plaque psoriasis
Tocilizumab Actemra	Humanized IL6R	2010 2011 2017	C	Rheumatoid arthritis Juvenile idiopathic arthritis Giant cell arteritis
Ustekinumab Stelara	Human IL-12, IL-23	2010	E*	Plaque psoriasis Psoriatic arthritis
Vedolizumab Entyvio	Humanized Integrin α4β7	2014	D	Inflammatory bowel disease
Liver Transplantation				
Daclizumab Zenapax	Humanized IL-2	1997 Withdrawn	C	Prevention of transplant rejection
Muromonab-CD3 OKT3	Mouse CD3 T cells	1985 Withdrawn	E	Prevention of transplant rejection
Basiliximab Simulect	Chimeric IL-2Rα	1998	E	Prevention of transplant rejectio

Various mAbs				
Abciximab Reopro	Chimeric GpIIb/IIIa	1993		Inhibition of platelet aggregation
Aducanumab Aduhelm	Human Amyloid β	2021	E	Alzheimer disease
Alirocumab Praluent	Human PCSK9	2015	E	Hypercholesterolemia
Benralizumab Fasenra	Humanized IL5	2017	E	Eosinophilic asthma
Bezlotoxumab Zinplava	Human C. difficile toxin B	2016	E	Prevention of recurrence of C. difficile infection
Burosumab Crysvita	Human FGF 23	2018	E	X-linked Hypophosphatemia
Caplacizumab Cablivi	Humanized vWF	2019	E	Acquired thrombotic thrombocytopenic purpura
Crizanlizumab Adakveo	Humanized P-selectin	2019	E	Sickle cell disease
Denosumab Prolia, Zgeva	Human RANKL	2010	E*	Osteoporosis Bone metastases
Eculizumab Soliris	Humanized C5	2007 2011	D	Paroxysmal nocturnal hemoglobinuria
Emapalumab Gamifant	Human Interferon Gamma	2018	E	Hemophagocytic lymphohistiocytosis
Emicizumab Hemlibra	Humanized Factor IXa & X	2017	E	Hemophilia A
Eptinezumab Vyepti	Humanized CGRP	2019	E	Migraine headache
Erenumab Aimovig	Human CGRP	2018	E	Migraine headache
Evinacumab Evkeeza	Human ANGPTL3	2021	E	Hypercholesterolemia
Evolocumab Repatha	Human PCSK9	2015	E	Hypercholesterolemia
Fremanezumab Ajovy	Humanized CGRP	2018	E	Migraine headache
Galcanezumab Emgality	Humanized CGRP	2018	E	Migraine headache
Ibalizumab Trogarzo	Humanized CD4	2018	E	HIV infection
Lanadelumab Takhzyro	Human Kallikrein	2018	E	Hereditary angioneurotic edema
Mepolizumab Nucala	Humanized IL15	2015	E	Eosinophilic asthma Hypereosinophilic syndrome
Natalizumab Tysabri	Humanized Integrin α4β7	2004	B	Multiple sclerosis Inflammatory bowel disease
Obiltoxaximab Anthim	Chimeric Anthrax toxin	2016	E	Inhalational anthrax
Omalizumab Xolair	Humanized IgE	2003 2014	E	Eosinophilic asthma Chronic idiopathic urticaria
Palivizumab Synagis	Humanized RSV fusion protein	1998	E	Respiratory syncytial virus infection
Ranibizumab	Humanized	2006	E	Macular degeneration

Lucentis	VEGF-A			
Ravulizumab Ultomiris	Humanized Complement C5	2018	E*	Paroxysmal noctural hemoglobinuria
Raxibacumab	Human Anthrax toxin	2012	E	Inhalational anthrax
Reslizumab Cinqair	Humanized IL5	2016	E	Eosinophilic asthma
Romosozumab Evenity	Humanized Sclerostin	2019	E	Osteoporosis
Teprotumumab Tepezza	Human IGF1R	2019	E	Graves ophthalmopathy

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