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Review Paper

Production of Monoclonal Antibodies: An Overview

Samaneh Reiszadeh Jahromi, Sareh Raeiszadeh Jahromi and Mohammad Haddadi
Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore, INDIA

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Abstract

On the account of evolutionary aspects, adaptation to new environmental changes made almost all organisms including pathogens to undergo de novo mutagenesis. Human immunodeficiency virus is clear cut substantiation for this statement. In addition, due to maltreatment of antibiotics increases risk of resistance in the next generations. Consequently, production of monoclonal antibodies with precise detection efficiency is indispensable. In this context, new technologies should be developed to generate specific accurate antibodies at maximum possible economical rate to attend the demand of academic and clinical researches. Making use of plants and microorganisms seems to be more efficient rather conventional methods.

Keywords: Monoclonal antibody; Immune response; Hybridoma technique..

Introduction

When the immune system encounters substances foreign to the body, known as antigens it is usually processed to result in immune responses. Immune cells *viz.,* B and T lymphocytes are involved in both humoral and cell-mediated immune responses, respectively[1]. These immune cells do not interact with, or recognize, an entire immunogen (figure 1). In fact, lymphocytes recognize epitopes or antigenic determinants which are discrete immunologically active sites on an immunogen and bind to antigen-specific membrane receptors of B lymphocytes (BCRs) or to paratopes of specific antibodies.

Lymphocytes may interact with different epitopes on the same antigen. An epitope on an antigenic protein may involve elements of the primary, secondary, tertiary and even quaternary structure of the protein. In polysaccharides, multiple branches may contribute to the confirmation of epitopes.

Antibodies are the Y-shaped antigen-binding proteins (figure 2) present on the cell membrane of B lymphocytes. Structure of an antibody is illustrated in figure 3.

Antigen: any substance foreigner to the body that binds specifically to major histocompatibility complex (MHC) or a T-cell receptor is an antigen.

Immunogen: a stimulus that is able to induce humoral or cell-mediated immune response by its own once enters to the body.

All immunogens are antigens but not all antigens are immunogens.

Figure-1
Definition of antigen and immunogen

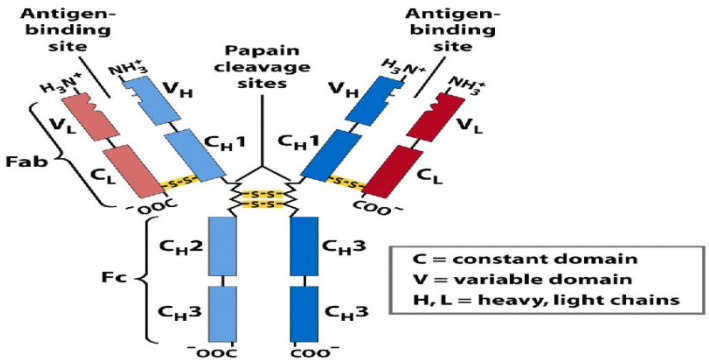

Figure-2
Schematic structure of an antibody Lehninger principles of biochemistry, fifth edition

Fab (fragment, antigen binding) region: The arms of the Y contain the sites that can bind to antigens and, therefore, recognize specific foreign objects and composed of one constant and one variable domain from each heavy and light chain of the antibody.

paratope is formed at the N-terminal of the antibody monomer by the variable domains from the heavy and light chains.

The variable domain is also referred to as the $\mathbf{F_V}$ region and is the most important region for binding to antigens. Variability of Fv region is concentrated in segments called **complementarity determining regions (CDRs)** or hypervariable regions.

The CDRs line the walls of the antigen-binding

Sites in antibody molecules.

Fc (Fragment, crystallizable) region composed of two heavy chains that contribute two or three constant domains depending on the class of the antibody. The base of the Y plays a role in modulating immune cell activity.

Figure-3
A brief illustration of antibody components

The B cells can recognize an epitope on its own and trigger humoral immune response. The T cells can recognize an epitope only when it is associated with an MHC (Major Histocompatibility Complex) molecule on the surface of a self-cell and induce cell-mediated immune response.

This recognition is antigen-specific and specificity of each T and B cell is determined before its contact with antigen by random gene rearrangements during maturation in the thymus or bone marrow respectively. The role of antigen becomes critical when it binds with specific receptor on the antigenetically matched lymphocytes. Such an interaction causes the cell to proliferate and differentiate to population of effector cells with an antigenic specificity as the original parent cells[2].

Humoral immune response results in production of one group of effector B cells called Plasma Cells live for few days, synthesize, and secrete enormous amount of antibody (more than 2000 molecules per second).

Monoclonal Antibodies and their importance

As noted previously, most antigens offer multiple epitopes and therefore induce proliferation and differentiation of various clones of B cells. Therefore, the serum contains a mixture of diverse antibodies resulted from different specific antigen-antibody recognition processes. For most research, diagnostic and therapeutic purposes antibodies against a single antigenic determinant produced by a single clone of B cell are preferable and known as monoclonal antibodies. In order to produce monoclonal antibody, it is necessary to have a single purified antigen so they can attack only one specific target. Monoclonal antibodies are important reagents used in biomedical research, in two major fields, namely diagnosis of diseases and treatment of diseases such as infections and cancer. Given such a diversity of uses for these disease-fighting substances, their production in pure quantities has long been the focus of scientific investigation

Production of MAbs (Monoclonal Antibodies) by Hybridoma Method

Georges Kolher and Cesar Milstein have proposed and developed the hybridoma technique in 1975 and were award the Nobel Prize. Myeloma cells and the B cells are the main components of this technique. Myeloma cells are genetically modified neoplasm that is immortal and capable of continuous multiplication[3]. They are not able to synthesize antibodies and also lack the ability to synthesize hypoxanthine-guanine phosphoribosyl transferase (HGPRT) and thymidine kinase (TK) enzymes. The normal B cells with functional HGPRT and TK enzymes are obtained from the mouse, that is been immunized with a particular antigen of interest. These B cells are producing antibodies but they cannot survive for more than two weeks. Hybridoma technique offers fusion of both these cells by virtue of physical stimuli[4]. Therefore, resulting hybrid cells that called as hybridoma posses the characteristics of both the cell types.

The first step in order to monoclonal antibodies production is immunization of animal by an antigen. Mouse is the most common animal, but rat, hamster or goat can be used too[5]. After repeated immunization with a specific antigen, the blood sample of the animal is collected and tested for antibodies raised against the antigen used in immunization. Once it is confirmed that sufficient antibodies are produced by the animal, it will be sacrificed and its spleen will be dissected out. Since, spleen is B cell- rich organ it may contain B cell clones specific to the target antigen among other varieties of B cell clones. By the help of some basic molecular labeling techniques those B-cell clones are selected and cultured. Then they will be mixed with myeloma cells and process for fusions by the aid of polyethylene glycol (PEG). Not all the cells presented in culture are fused. There are un-fused B cells and myeloma cells along with the hybridoma cells. The next step is separation of hybridoma cells by providing a selective culture medium.

***de novo* and salvage pathway**

Normal growing cells need to synthesize their own DNA. Most of the cells produce nucleotides by *de novo* pathway wherein ribonucleotides are synthesized from precursor molecules. If the *de novo* pathway is disrupted, cells utilize the alternate pathway, salvage pathway that uses the previously made nucleotides. The enzymes which are involved in catalyzing the salvage pathway are hypoxanthine-guanine phosphorribosyl transferase (HGPRT) and thymidine kinase (TK).

HGPRT is a member of transferase family and catalyzes conversion of hypoxanthine to inosine monophosphate and guanine to guanosine monophosphate. In this reaction, 5-phosphoribosyl group transfers from 5-phosphoribosyl 1-pyrophosphate molecule to the purine. HGPRT has a major role in generation of purine nucleotides by means of purine salvage pathway.

Thymidine kinase is a phosphotransferase and plays a key function in DNA synthesis during cell division. It involves in a part of the distinctive reaction chain wherein deoxythymidine is introduced into the DNA. Deoxythymidine is mainly present in body fluids owing to degradation of DNA molecules of up taken nutrients and dead cells.

Figure 4
Mechanism of de novo and salvage pathways in synthesis of nucleotides

Clonal Selection

The selective medium, hypoxanthine-aminopterin-thymidine (HAT) medium contains aminopterin, hypoxanthine and thymidine[6]. Amiopterin inhibits the de-novo pathway and presence of hypoxanthine and thymidine facilitates salvage pathway of nucleotide synthesis. De novo and salvage pathway of nucleotide synthesis are illustrated in brief in figure 4.

The elemental function of selective culture medium is based on *de-novo* and salvage pathway. Un-fused B cells are able to produce DNA by applying salvage pathway since they have functional HGPRT and TK enzymes but not by means of *de-novo* pathway owing to existence of aminopterin. However, as they are mortal, they die following few multiplications. In case of myeloma cells, aminopterin blocks *de-novo* pathway in un-fused ones. They are not capable of utilizing the salvage pathway for the reason that they are deficient in HGPRT and TK enzymes. Even though being immortal, these cells die too. Thus, only hybridoma cells that attain genes encoding functional enzymes from the normal B cells and immortality feature from myeloma neoplasm cells can employ salvage pathway for synthesis of DNA and be immortal. Each individual surviving hybridoma cells could generate antibodies against diverse epitopes presented on the target antigen. The next pace is to pick the monoclonal hybridoma cells that are producing an antibody against the single individual preferred antigen. The cultures are finely diluted to that extent to facilitate transferring of only a single cell to the microtiter plate wells. The cells are then allowed to grow and multiply. The targeted antibody can be willingly detected in the supernatant fluids of these cells with ELISA (Enzyme-linked Immunosorbent Assay) or RIA (Radio Immuno Assay) tests. Those individual clones, which are able to produce antibody of our choice, are allowed moving to the next step of monoclonal antibody production. These selected hybridoma cells can be lyophilized, cultured *in vitro* or inject intra-peritoneally into an animal and ultimately monoclonal antibodies rose whenever required.

Drawbacks and disadvantages of Hybridoma technique

The major drawback of hybridoma method is that hybridomas produce murine antibodies, which can provoke the human immune system as non-self particles. Patients who have received infusions of such monoclonals have experienced a phenomenon so-called HAMA response, stands for the Human Anti-Mouse Antibodies. The HAMA response includes joint swelling, rashes and kidney failure and can be life threatening. It destroys the antibodies as well[7].

The other remarkable disadvantages of monoclonal antibodies (mAbs) are their cost and quantity. Million of dollars are required for productions of a very low quantity of monoclonal antibodies and beside there are high demand for mAbs especially for disease diagnosis and treatments.

Overview of overcomes

Scientists have developed a diversity of techniques in order to avoid the HAMA response and also the problem of premature inactivation of murine antibodies by immune system, in which murine antibodies are being more humanized. As before described antibody is a Y-shaped molecule that binds to antigen through its arms (FAb regions) of that Y. The stalk of the Y (the Fc region), interact with the immune system cells. The Fc region is predominantly important in eradication of bacteria. In fact once antibodies cover a bacterium and bind to it by means of their FAb regions, the Fc regions draw microbe-engulfing cells, macrophages, to obliterate it. Humanization of mAbs implicates genetic engineering to substitute the components of murine antibodies with human proteins at maximum possible level. One approach involves replacement of all components excluding the antigen binding region of murine mAbs with human counterparts.

Some investigators are trying to produce monoclonal antibodies without the aid of mice. Cambridge Antibody Technology in England and MorphoSys AG in Munich are applying a technique called phage display that does mass-produce monoclonals and also helps to find the most specific monoclonals against a particular antigen.

Phage display basis laid on a virus called a filamentous phage which infects bacteria. DNA can be isolated from human B lymphocytes and insert into bacteria such as Escherichia coli and then allow filamentous phages to infect the bacteria. As long as phages are multiplying, they transcribe and translate the antibody genes of the B lymphocytes and the synthesized protein presents on the surfaces of newly forming phage particles. those phages containing the gene for the most specific antibody to the special antigen such as a receptor on cancer cells can be easily fish out and then achievement to mass production of specific antibody can be meet either by transfection of more bacteria by one phage or insertion of the antibody gene into cultured cells[8].

Modern emerging issues in production of Monoclonal Antibodies

The other technique that can be used to overcome on drawbacks of mAbs, is transgenic animals and plants, those organisms genetically engineered to carry genes for specific antibodies. Transgenic mammals that secrete monoclonals in their milk can generate one gram of antibody for one third the cost of traditional production methods. Companies like Centocor and Johnson are looking into producing Remicade using transgenic goats, and Infigen in DeForest, Wis., intends to make monoclonals in cow's milk.

Epicyte Company in San Diego, aim plants as the answer to the mAbs production demand. It is obvious that the existing production facilities cannot accomplish the demand for the most extremely heeded molecules. Plants have the benefits of being inexpensive and simply scalable to every level of demand: they are able to produce metric masses of monoclonal products. However, purification evils remain to be solved.

Epicyte constructed corn plants which are capable to generate monoclonal antibodies for treatment of gastrointestinal or respiratory infectious diseases which will be provide in the form of gel for mucosal surfaces or as edible drugs. Clinical trials for corn-produced monoclonals are begun by them in order to prevent the transmission of herpes simplex virus during childbirth. Further, this company has developed mAbs that can bind to sperms and act as a possible contraceptive. Likewise, antibodies that may protect against human papilloma virus which is cause of genital warts and cervical cancer have been developed by Epicyte. Many mAbs produced by these new techniques, should attain the FDA (Food and Drug administration) approval for commercialization[8].

Types of MAbs

Monoclonal antibodies those are useful for detection of viruses in viral infectious diseases.

In case of HIV (Human Immunodeficiency Virus) detection, in order to recognize which cells have viral antigens, monoclonal antibodies against OKT4 and HIV p17 core antigen are being used to perform indirect immunofluorescence assays in genital secretion of suspected women patients with leucorrhoea. Since lymphocytes are the major source of HIV in cervicovaginal secretions of affected women, these assays are carrying out in order to examine the genital fluid lymphocytes and cervicovaginal epithelial cells.

Abzymes: Some antibodies that show enzymatic activity are known as abzymes or catalytic antibodies. In biochemical points of view, formation of an antibody-antigen complex is similar in many ways to the binding of an enzyme to its substrate *viz.,* involvement of non-covalent bounds, high specificity and often high affinity. Enzyme catalytic activity leads to a chemical change in its substrate while antibody does not alter the antigen. However, some antibodies can act like enzymes and chemically stabilize the transition and unstable state of a bound macromolecule and thereby reduce the activation energy for chemical modification of the substrate[9]. For example when spleen cells from mice immunized with a synthetic hapten-carrier complex in which the hapten is in the transition state of an ester undergoing hydrolysis, and then fused with myeloma cells, result in production of anti-hapten monoclonal antibodies. Incubation of these monoclonal antibodies with an ester substrate boosted the hydrolysis reaction by about 1000-fold. It shows that these mAbs acted as an enzyme and catalyzed the hydrolysis of substrate.

Immunotoxins: Immunotoxins are the complex of antibodies and toxins and/or radioisotopes. Toxins are linked to Fc region of immunoglobulin. Immunotoxins are mainly used to target tumors. When an immunotoxin is injected, it reaches the target cancerous cell by the help of antigen-antibody binding specificity. After their attachment to tumor cells' receptors, it enters into tumor cells through endocytosis and disrupts the cellular metabolism with the help of toxins or radioisotopes and cells died. Chimeric immunotoxins are modified version of immunotoxins and unlike Immunotoxins, toxin replaces one of the constant region domain of Ig especially carboxyl terminal domain of H chain. Due to lack of Fc region in chimeric Immunotoxins, they won't activate complements and consequent inflammatory reaction after binding to the antigens.

For your attention name and other properties of few commercially available Monoclonal Antibodies in the market are given in table 1. The action sites, applications and statement of FDA approval of each individual mAb in addition to their commercial terminology are summarized below.

Table-1
List of few Monoclonal Antibodies available in market

Sl No.	Commercial name	Toxin/Radio isotope	Target	Application	FDA approval
1.	Muromonab-CD3 (OKT3)	Nil	Surface CD3 molecule of T cells	Prevention of organ acute rejection e.g., kidney transplants	Approved
2.	Infliximab (Remicade®)	Nil	Tumor necrosis factor-alpha (TNF-α)	Inhibition of Th1 cells activity and action against inflammatory diseases such as rheumatoid arthritis	Approved
3.	Omalizumab (Xolair®)	Nil	IgE	Prevention of IgE binding to mast cells. Shows assure against allergic asthma	Approved
4.	Daclizumab (Zenapax®)	Nil	Surface part of the IL-2 receptor presented in activated T cells	Prevention of acute rejection of transplanted kidneys. It is also expected to act against T-cell lymphoma	In phase II of clinical trials
5.	Rituxan®	Nil	CD20 molecules on B-cells	Treatment of B-cell lymphomas	Approved
6.	Zevalin®	yttrium-90 or indium-111	CD20 molecules on B-cells	Treatment for lymphoma	Approved
7.	Bexxar®	radionuclide iodine-131	CD20 molecules on B-cells	Treatment for lymphoma	Approved
8.	Herceptin®	Nil	Binds to HER2, a receptor for epidermal growth factor (EGF) that is found on some breast cancers and lymphomas tumor cells	To act against solid tumors	Approved
9.	Erbitux®	Nil	Blocks HER1, another epidermal growth factor (EGF) receptor	Treatment of breast cancers and lymphomas	Approved
10.	Mylotarg®	Calicheamicins	Binds CD33 a cell surface molecular marker for acute myelogenous leukemia cells	To treat acute myelogenous leukemia (AML) without affecting the normal stem cells	In 2000 was approved but due to severe side effects being withdrawn in 2010
11.	LymphoCide	Nil	Binds to CD22 molecules on B cells	To treat B-cell leukemias	In phase II of clinical trials
12.	MabCampath®	Nil	Binds to CD52 molecules on WBCs	To treat chronic lymphocytic leukemia	Approved
13.	Oncolym®	Iodine 131	Binds to the HLA-DR-encoded histocompatibility antigen	Treatment of lymphoma	In phase III of clinical trials
14.	Vitaxin	Nil	Binds to a vascular integrin (alpha-v/beta-3)	To block angiogenesis of solid tumors	In phase II of clinical trials
15.	Avastin®	Nil	As a angiogenesis inhibitor binds to vascular endothelial growth factor (VEGF)	Treatment of colorectal cancers	Approved
16.	ReoPro®	Nil	Binds to and inhibits surface glycoprotein IIb/IIIa of platelets that are linked with fibrinogen	Inhibition of the clumping of platelets, Helpful in preventing reclogging of the coronary arteries in patients who have undergone angioplasty	Approved

Conclusion

To finish, we conclude that with regard to evolutionary aspects, adaptation to the new environmental condition made almost all organisms including viral and bacterial pathogens to undergo a rapid changing process and acquire new genes as a result of *de novo* mutagenesis. HIV is a clear cut evidence for this statement. On the other hand due to mistreatment of antibiotics there are major risks of resistance in the next generations[10]. Therefore, syntheses of monoclonal antibodies with accurate detection efficacy which are also inexpensive and able to fulfill the demands are essential. Since, in molecular biology researches need of mAbs for detection of a particular antigenic molecule is indispensable in most of the experiments[11], biotechnology companies should develop new technologies and methods in order to produce specific accurate antibodies at maximum possible economical rate to attend the demand of researchers in all field of molecular biology. In both the cases making use of plants and microorganisms seems to be more efficient rather conventional methods.

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