

REVIEW ARTICLE

Monoclonal Antibody As Anti Cancer Agents

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ABSTRACT

The recent clinical and commercial success of anticancer antibodies such as rituximab and trastuzumab has created great interest in antibody-based therapeutics for hematopoietic malignant neoplasms and solid tumors. In the recent years of immunologic research, we are witnessing development in the fields of antigen screening and protein engineering in order to create specific anticancer remedies. The developments in the field of recombinant DNA, protein engineering and cancer biology have let us gain insight into many cancer-related mechanisms. Moreover, novel techniques have facilitated tools allowing unique distinction between malignantly transformed cells, and regular ones. This understanding has paved the way for the rational design of a new age of pharmaceuticals: monoclonal antibodies and their fragments. Antibodies can select antigens on both a specific and a high-affinity account, and further implementation of these qualities is used to target cancer cells by specifically identifying exogenous antigens of cancer cell populations. The structure of the antibody provides plasticity resonating from its functional sites. This review will spot the light on some of the novel antibodies and antibody-based approaches that are being currently developed for the new generation of anticancer agents.

Key words: Antibody, cancer immunotherapy, tumor antigens, anti tumor mechanisms, targeted therapy, antibody fusions.

INTRODUCTION

Cancer is a disease in which there is uncontrolled multiplication and spread within the body of abnormal forms of the body's own cells [6].

Types of cancer:-

There are two types of cancer:

- Benign Tumors
- Malignant Tumors

Benign tumors

They arising from fibroblastic cells are called a fibroma, a cartilaginous tumor is a chondroma, and a tumor of osteoblasts is an osteoma. Benign tumors of epithelial that arise from renal tubular cells growing in the form of numerous tightly clustered small glands would be termed as adenoma. Benign epithelial tumor producing microscopically or macroscopically visible finger like projection from epithelial surface are referred to as papillomas.



Fig 1:- Finger like projection from Benign Tumor [2]

Malignant Tumor

These are arising in mesenchymal tissue are usually called sarcomas because they have little connective tissues stroma. Malignant tumor of epithelium derived from any of the three germ layers, are called carcinomas. Thus tumor arising in the epidermis of ectodermal origin is a carcinoma [2].

CHARACTERISTICS OF CANCER CELL

There are different characteristics of cancer cell which are as follows:

- ❖ Uncontrolled proliferation
- ❖ Dedifferentiation and loss of functions
- ❖ Invasiveness
- ❖ Metastasis

Uncontrolled proliferation

Inactivation of tumor suppressor genes or transformation of proto-oncogenes into oncogenes can confer autonomy of growth on a cell and thus result in uncontrolled proliferation. The proliferation of cancer cells is not controlled by the processes that normally regulate cell division and tissue growth. Uncontrolled proliferation is produced by changes in:

- Growth factors and their receptors
- The growth factor pathways- the cytosolic and nuclear transducers
- The cell cycle transducers
- Apoptotic mechanisms
- Telomerase expression
- Local blood vessels

Dedifferentiation and loss of function

The multiplication of normal cells involves division of the stem cells in a particular tissue to give rise to daughter cells. These daughter cells eventually differentiate to become mature cells of the relevant tissue and carry out their programmed function. One of the main characteristics of cancer cells is that they dedifferentiate to a varying degree in different tumors.

Invasiveness

Normal cells are found outside their designated tissue of origin: for ex. Liver cells are found in the bladder. This is during differentiation and during the growth of tissue and organs, normal cell develop certain spatial relationship with respect to each other. These relationships are maintained by various tissue specific survival factors anti-apoptotic factors. Any cells that escape accidentally lose these signals and undergo apoptosis. A cancer of the rectal mucosa invades the tissues in the other pelvic organs. Cancer cells have not only lost the restraint that acts on normal cells, they are also particularly adept at secreting enzyme that break down the extra cellular matrix, enabling the cancer cells to slip through.

Metastases

These are secondary tumors formed by cells that have been released from the initial or primary tumor and have reached other sites through blood vessels. Tumor induced growth of new blood vessels locally makes metastases easier. Secondary tumors occur more frequently in some tissues than others. For e.g. the metastases of mammary tumors are found in lung, bone and brain^[2].

CELL CYCLE

The cell cycle is an ordered series of events consisting of several sequential phases: G1, S, G2, and M

- M is the phase of mitosis
- S is the phase of DNA synthesis
- G1 (presynthetic) is the gap between the mitosis that gave rise to the cell and the S phase; during G1 the cell is preparing for the DNA synthesis
- G2 (premitotic) is the gap between S phase and the mitosis that will gave rise to two daughter

cells; during G2 the cell is preparing for the mitotic division into two daughter cells.

Cell division requires the controlled timing of two critical events of the cell cycle: S phase (DNA replication) and M phase (mitosis). Entry in to each of these phases is carefully regulated this gives rise to two check points (restriction points) in the cycle: one at the start of M. DNA damage results in the cycle being stopped at one or other of these. The integrity of the check points is critical for the maintenance of genetic stability. Neurons and the skeletal muscle cells spend all their lifetime in G0: bone marrow cells and the lining cells of the gastrointestinal tract divide daily. Quiescent cells can be activated into G1 by chemical stimuli associated with damage; for ex, a quiescent skin cells can be stimulated by a wound into dividing and repairing the lesion^[6].

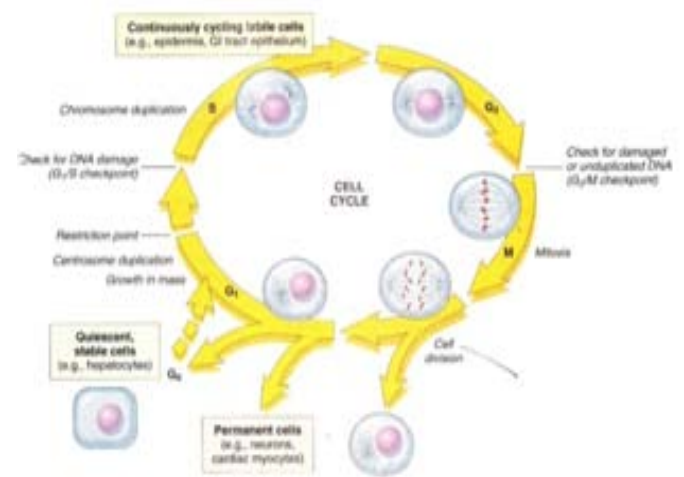


Fig 2:- Cell Cycle^[2]

CELL CYCLE REGULATORS

Growth factors (Cell regulator) stimulate the production of signal transducers of two types:

- ❖ Positive regulator
- ❖ Negative regulators

The maintenance of normal cells numbers in tissues and organs requires that there be a balance between the positive regulators forces and the negative regulatory forces. Apoptosis also has a roll in the control of cell numbers.

Positive regulators

The cell cycle is initiated when a growth factors act on quiescent cell growth factors is to stimulate production of the cell cycle regulators, which are coded for by the delayed response genes. The main components of the controlled system that determine progress through the cycle are two families of proteins: cyclins and cyclin dependent kinases (cdks). The cdks phosphorylate various proteins (ex. enzymes) - activating some and inhibiting others-to coordinate their activities. Each cdk is inactive until it binds to a cyclins, the

binding enabling the cdk to phosphorylate the protein necessary for a particular step in the cycle. It is the cyclin that determines which protein is phosphorylated. After the phosphorylation event has taken place, the cyclin is degraded by the ubiquitination / protease system. There are eight main groups of cyclins. These important in the control of the cell cycle are cyclins A, B, D, E. Each cyclin associated with and activates particular cdk. Cyclin A activities cdks 1 and 2; cyclin B, cdk1; cyclin D, cdks 4 and 6; cyclin E, cdk2. Precise timing of each activity essential and many cycles' proteins are degraded after they have carried out their function. The activity of these cyclin/ckds complexes is modulated by various negative regulatory forces most of which acts at one or other of the two check points.

- Cells in G₀:-

In quiescent G₀ cells, cyclin D is present in low concentration and an impotent regulatory protein- the Rb protein – is hypophosphorylated.

Hypophosphorylated Rb holds the cell cycle in check at check point by inhibiting the expression of several proteins critical for cell cycle progression. The Rb protein accomplishes this by binding to the E2F transcription factors, which control the expression of the genes that code for cyclin E and A, for DNA polymerase, for thymidine kinase, for dihydrofolate reductase, etc.- all essential for NA replication during S phase.

- Phase G₁:-

It is the phase in which the cell is preparing for S phase by synthesizing the mRNA and proteins needed for DNA replication. During G₁, the concentration of cyclin D increases and the cyclin D/ckd complex phosphorylate and activate the necessary protein. In mid G₁, the cyclin D/ckd complex phosphorylates the Rb protein, releasing transcription factor E2F; this then activates the genes for the components specified above that that are essential for the next phase – DNA synthesis – namely cyclins E and A, DNA polymerase.

The action of cyclin E/ckd complexes is necessary for transition from G₁ to S phase, i.e. past check point 1. Once past check point 1, the processes that have been set in motion cannot be reversed and the cell is committed to continue with DNA replication and mitosis.

- S phases:-

Cyclin E/ckd and A/ckd regulate progress through S phase, phosphorylating and thus activating proteins involved in DNA synthesis.

- G₂ phase:-

In G₂ phase, the cell, which now has double the number of chromosomes, must duplicate all other cellular components for allocation to the two daughter cells. Synthesis of the necessary mRNA and proteins occurs. Cyclin A/ckd and B/ckd complexes are active during G₂ phase and are necessary for entry into M phase, i.e. for passing check point 2. The presence of cyclin B/ckd complexes in the nucleus is required for mitosis to commence.

- Mitosis

Mitosis is a continuous process but can be considered to consist of four stages.

1. *Prophase*

The duplication chromosomes condense each now consisting of two daughter chromatids. These are released into cytoplasm as the nuclear membrane disintegrates.

2. *Metaphase*

The chromosomes are aligned at the equator.

3. *Anaphase*

Amitotic apparatus capture the chromosomes. And draw them to opposite poles of the dividing cells.

4. *Telophase*

A nuclear membrane forms round each set of chromosomes. Finally the cytoplasm dividing between the two forming daughter cells. Each daughter cell will be in G₀ phase and will remain there unless stimulating in to G₁ phase. During metaphase, the cyclin A and B complexes phosphorylate cytoskeletal proteins, histone and possibly components of the spindle.

Negative regulators

One of the main negative regulators is the Rb protein that holds the cycle in check while it is hypophosphorylated. Another negative regulatory mechanism is the action of the inhibitors of the cdks. These bind to and inhibit the action of the complexes, their main action being at check point 1.

There are two families of inhibitors:-

- The CIP family (cdk inhibitory proteins: also termed KIP or kinase inhibitory proteins): p21, p27, p57.
- The Ink family (inhibitors of kinases): p16, p19, p15.

Protein p21 is under the control of the p53 gene- a particularly important negative regulator that operates at check point 1.

Inhibition of the cycle at check point 1

The p53 gene has been called the guardian of the genome. It codes for a protein transcription factor-

the p53 protein. In healthy cells, the steady state concentration of p53 protein is low. But when there is DNA damage, the protein accumulates and activates the transcription of several genes, one of which codes for p21. Protein p21 inactivates cyclin/cdk complexes, thus preventing Rb phosphorylation, which means the cycle, is arrested at check point 1. This allows for DNA repair. If the repair is successful, the cycle proceeds past check point 1 into S phase, if the repair is unsuccessful, the p53 gene triggers apoptosis-cell suicide.

Inhibition of the cycle at check point 2

There is evidence that DNA damage can result in the cycle being stopped at check point 2 but the mechanisms involved are less clear than those at check point 1. Inhibition of the accumulation of cyclinB/cdk complex in the nucleus seems to be a factor^[6].

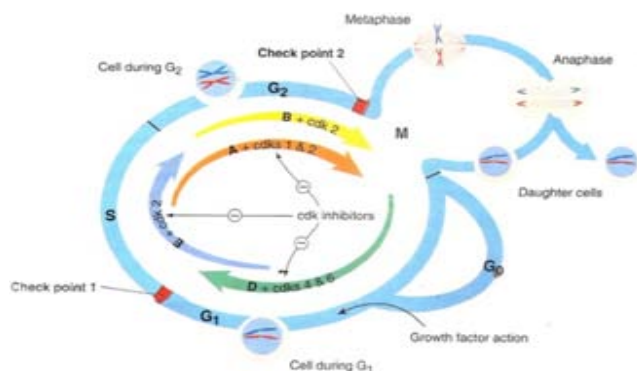


Fig 3:- Cell Cycle Regulators^[6]

CLASSIFICATION OF ANTICANCER DRUGS

Classification

Table 1: Drugs acting directly on cells (Cytotoxic drugs)^[7]

1. Alkylating agents	
a. Nitrogen mustard	Mechlorethamine,
b. Ethylenimine	Cyclophosphamide,
c. Alkyl sulfonate	Chlorambucil, Melphalan
d. Nitrosourea	Thio-TEPA
e. Triazine	Busulfan
	Carmustine, Lomustine
	Dacarbazine
2. Antimetabolites	
a. Folate antagonist	Methotrexate
b. Purine antagonist	6-Mercaptopurine, 6-Thioguanine, Azathioprine
c. Pyrimidine antagonist	5-Fluorouracil, Cytarabine
3. Vinca Alkaloids	Vincristine, Vinblastine
4. Taxanes	Paclitaxel, Docetaxel
5. Epidodophyllotoxin	Etoposide
6. Camptothecin analogue	Topotecan, Irinotecan
7. Antibiotics	Actinomycin-D, Doxorubicin, Daunorubicin, Mitomycin-C
8. Miscellaneous	Hydroxyurea, Procarbazine, Cisplatin

Table 2: Drugs altering hormonal milieu^[7]

1. Glucocorticoids	Prednisolone
2. Estrogens	Fosfestrol, Ethinylestradiol
3. Selective Estrogen Receptor Modulators	Tamoxifen, Toremifene
4. Selective Estrogen Receptor Down Regulators	Fulvestrant
5. Aromatase Inhibitors	Letrozole, Anastrozole
6. Antiandrogen	Flutamide, Bicalutamide
7. 5- α Reductase Inhibitors	Finasteride, Dutasteride
8. GnRH analogues	Nafarelin, Triptorelin,
9. Progestin	Hydroxyprogesterone acetate

Table 3: Monoclonal Antibodies^[9]

Alemtuzumab
Rituximab
Ibritumomab
Tositumomab
Trastuzumab

MONOCLONAL ANTIBODIES

Monoclonal antibodies are the antibody with a single antigenic specificity. The specificity of antibodies, that makes monoclonal antibody technology so valuable.

Types of monoclonal antibodies:-

- Mouse Monoclonal Antibodies
- Chimeric Monoclonal Antibodies
- Humanized Monoclonal Antibodies
- Human Monoclonal antibodies

Mouse (Murine) Monoclonal Antibodies

These antibodies were produced only by mouse (Murine) cells. Example- Muromonab, Capromab, Altumomab etc. the immune system of patients reacted against the foreign mouse proteins, leading to rashes, swelling and even occasional kidney failure, also the destruction of monoclonal antibodies.

Chimeric Monoclonal Antibodies

Use genetically manipulated mice to make a human- Murine hybrid. The variable part of the antibody molecule including the antigen binding site, is murine and the remainder constant region of antibody molecule has been derived from a human source. These mabs are about 66% human. e.g.- Rituximab, Infiximab

Humanized Antibodies

Murine portion is limited to the antigen binding sites. The balance of the variable region and the entire constant region are derived from Human sources. Such mabs are about 90% human. E.g. Herceptin, Alemtuzumab, Xolair.

Human monoclonal Antibodies

Human Monoclonal Antibodies are used to prevent immunological cross-reactivity and sensitization of the patient during antibody

therapy. For production of Human Monoclonal Antibodies one approach is use genetically engineer mice to contain Human antibody genes. These mice would able to produce antibodies that are fully human in some cases. But difficult to produce them in sufficient volumes. Several potential solutions to this problem are under investigation. E.g. Raxibacumab. Etc^[3]

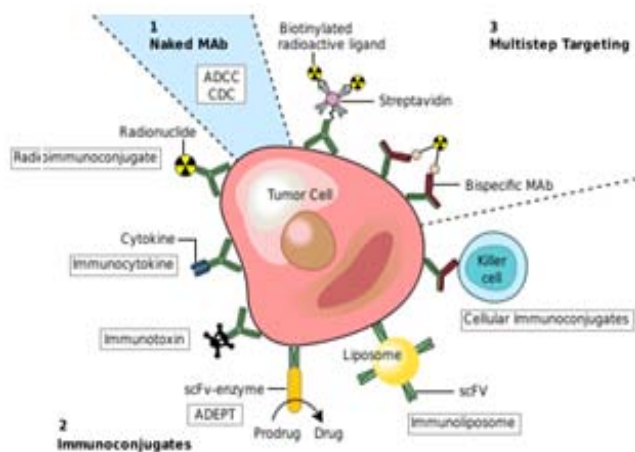


Fig 4:- structure of mabs^[15]

MECHANISM, CHARACTERISTICS, STRUCTURE OF MABS

Mechanism of action of Mabs

The mechanics by which MAbs achieve therapeutic effect is not very clear. Potential mechanisms include:

- Blocking or steric hindrance of the function of target antigen i.e., T-Lymphocytes, β Lymphocytes, tumor necrosis factor- α (TNF α) and interleukin (IL) which are capable of transducing intracellular signals.
- Cytotoxicity to the cell expressing target AG by ADCC (Antibody dependent cell cytotoxicity) or CDC.
- Inhibition of growth factors: - Epidermal growth factor receptor (EGFR) is a cell surface receptor involved in regulation of cell proliferation and survival. Also new vessels grow to feed the cancer cells through this factor. These factors can be inhibited to arrest growth of cancer cells e.g. Cetuximab act as EGFR inhibitor^[1].
- **Character of antibody**
 - They are extremely specific
 - Antibody once activated by the occurrence of disease, continue to confer resistant against that disease^[4].

Structure and function of antibodies

An antibody molecule (immunoglobulin) consists of two identical light (L) chain and two identical heavy (H) chains, held together by both hydrogen

bonding and localized disulfide linkage. The N terminal region of the L and H Chains together form the antigen recognition site of each antibody. The sites that recognize and bind with antigen consisting of three complementary- determining regions (CDR) that lies within the variable (vh and VL) region at the N- terminal ends of the two H and L chains. The L chain contains identical sequence of about 200 amino acids. They are common to all the antibodies and are of two type's kappa (κ) and lambda (λ). A given immunoglobulin may contain either identical kappa or lambda chains.

The H contains 400-500 identical amino acid residues. There are five antigenically distinct isotypes of H-chains are Gamma (γ), Alpha (α), Mu (μ), Delta (δ), Epsilon (ϵ) based on structural difference in carboxy terminal protein of H-chains. Human immunoglobulins are divided into five classes depending on the type of H- chains. They are

- IgM- contains μ chain
- IgG- contains γ chain
- IgA- contains α chain
- IgD- contains δ chain
- IgE- contains ϵ chain

The CDRs are the part of antibody with the greatest variability in amino acid sequence. In addition to the variable regions, each L- chains have one constant region, or domain (C1), and each H chain has three constant regions, (C111, C112, C113).

When antibodies are digested with proteolytic enzyme. Three fragments are released two identical (Fab) fragments, each of which contains an intact L chain linked by disulfide bond of VL and Chi region of the H- chain and one Fc fragments, which consists of two H-chains fragments, each containing the Ch2 and Ch3 domains and jointed by a disulfide bond.

The Fab fragment retains the antigens – binding activity. In fact the N- terminal of the Fab – fragments called Fv fragments, contain the entire antigen binding activity of the intact antibody molecules.

In an intact antibody molecule, the Fc portion elicits several immunological responses after antigen antibody binding occurs.

- The complement cascade is activated. They breakdown cell membranes activate, and generate signals to mobilize other components of the immunological response system.

- Antibody depended cell mediated cytotoxicity (ADCC). ADCC – effected cell release

substances that lye the foreign cells.

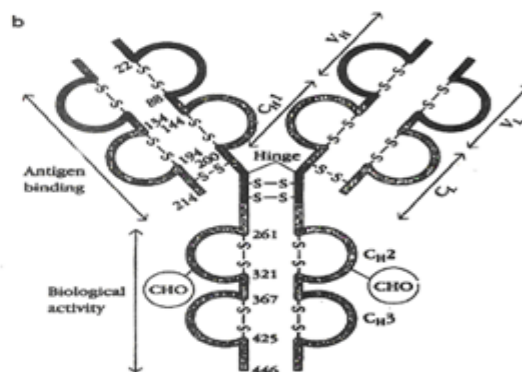
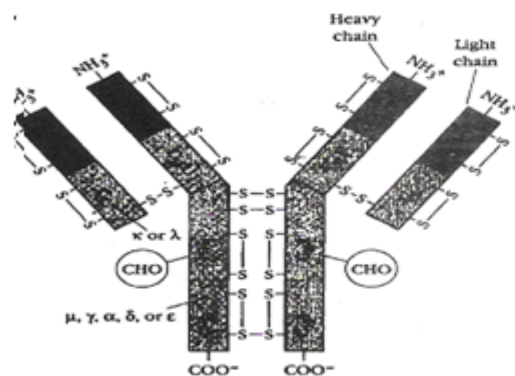


Fig 5:- Primary and Secondary structure of mAbs⁸

- After Fab region binds to a soluble antigen, the Fc portion of an antibody can be bound to Fc – receptor of phagocytic cells, which engulf and destroy the antibody- antigen complex.⁵

cell clones which produce antibodies for a number of different epitopes of that antigen. But unfortunately, these cells can not be maintained in culture and thus prohibit production of monoclonal antibodies.

Production of monoclonal antibody

Principle of production of monoclonal antibodies

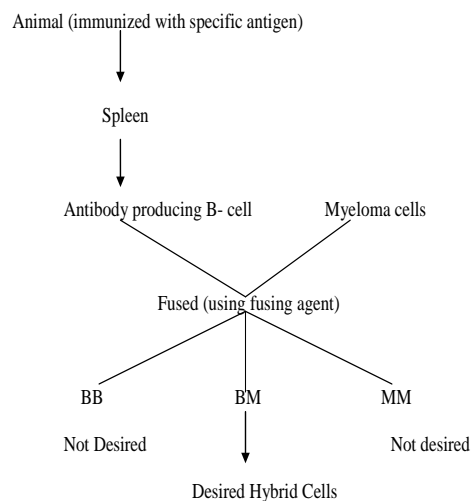


Fig 6:- Production of mabs^[8]

Production of Monoclonal Antibodies

These are produced by two techniques:-

Hybridoma Technology

- Immunization
- Cell fusion
- Selection
- Cloning
- Characterization and storage

Recombinant Technology

Hybridoma Technology

According to clonal selection theory one B- cell can produce an antibody of single specificity. Thus, immunization of an animal with and antigen can stimulate the production of plasma cells or B

De novo pathway

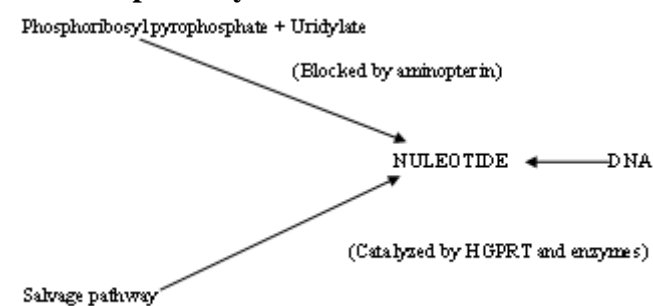


Fig 7. Pathway for Hybridoma technique^[8]

Like other body cells, an antibody secreting plasma cell can undergo malignant transformation. Such cell can proliferate on artificial medium and is called myeloma cell or plasma cytoma cell. Establishment of such plasma cytoma cell lines in culture can overcome the problem of death of plasma cells in the culture, but it is not possible to control which of these cells become malignant. Thus, generally the antibodies produced by them are useless. If the specificity of normal plasma cells (produced after immunization of animal) can be combined with proliferative capacity of malignant plasma cells, a powerful immunochemical technology can be developed for the continuous production of monoclonal antibodies.

The problem was solved by the production of cell hybrids formed by the fusion of normal and malignant cells. These hybrids were called as heterokaryons. The fusion was accomplished by Sendai virus, but later, polyethylene glycol (PEG) was found to be a more efficient fusion.

Littlefield developed excellent method for ensuring that all other cells would die in the medium except the fused hybrids. This method was based on biosynthesis of nucleotides. The de-novo synthesis of nucleotides requires tetrahydrofolate derivatives in three places. Two carbons in the purine ring are provided by N10-formyltetrahydrofolate. Methylation of d-UMP (de-oxy uridine mono phosphate) to form thymidine monophosphate (dTMP) requires N5, n10- methylene tetrahydrofolate. The tetrahydrofolate is obtained by an enzyme dihydrofolate reductase. This enzyme can be blocked by the folic acid analogue aminopterin.

The blocked of de novo synthesis can be overcome by salvage pathway, which utilizes the bases and nucleosides produced by degradation of nucleic acids. Here, an important enzyme is Hypoxanthine- guanine phosphoribosyltransferase (HGPRT). It catalyzes the conversion of guanine to guanosine monophosphate (GMP) and hypoxanthine to inosine monophosphate (IMP). This IMP can be converted in to both adenosine monophosphate (AMP) and GMP. The inhibition of TMP synthesis by aminopterin can be overcome by providing thymidine - which will be converted to TMP by thymidine kinase.

Littlefield, added to 6-thioguanine or 8-azaguanine to the medium. These were incorporated into nucleotides by HGPRT and ultimately the cells died. Only the cells which did not contain an active HGPRT (that is, HGPRT negative) were survived. If these cells were further cultured in a medium containing Hypoxanthine, Aminopterin, Thymidine (HAT medium), they die since they cannot overcome the blocked of nucleoside synthesis by aminopterin if these cells are fused with HGPRT positive cells, the fused cells can proliferate on HAT medium. Another problem arises here. The HGPRT +ive cells have a capacity to survive alone on HAT medium. To overcome this problem, Littlefield the selected the HGPRT +ive cell lines which were deficient in thymidine kinase. Thus, cells from each line (that is HGPRT +ive cells and HGPRT -ive cells) would compliment the deficiency of other and only fused cells can survive on HAT medium. Such fused cells are called as Hybridomas, and if one of the cell line is B cell, the hybridoma can produce monoclonal antibodies continuously. Hence, the technique is known as hybridoma technology.

• Immunization

The serum of animals assay for the antibodies of desired specificity is done. When

antibody concentration is optimum, the animal is scarified and the spleen is taken and is dissociated into single spleenocytes by using enzyme or mechanical methods.

• Cell fusion

Spleenocytes are then mixed with plasmacytoma cells in an appropriate medium exposed to a high concentration of polyethylene glycol (PEG) that is, 50% and fusion is allowed to take place over a period of time. This will form the hybridoma. Mouse is used in the production of monoclonal antibodies. The use of HGPRT cells (that cannot grow in HAT medium) assured that only hybridomas (hybrid myeloma spleen cells) are selected. After 7-10 days of culture in the HAT medium most of the wells contain dead cells, but a few wells contain small cluster of viable cells. Each clusters represent clonal expansion of a hybridoma, the secreted antibody. Wells containing viable clusters are then secreted for antibody production and antibody positive clones are subculture at low cell densities, again to ensure clonal purity in each micro well.

• Selection and screening

Selection of hybridomas in HAT medium is followed by screening of hybridomas for secretion of antibodies of desired specificity. After fusion, the cells are transferred to HAT medium and incubated. The viable cells are the hybridomas. They are removed from the medium and transferred to regular culture medium and the aliquots are distributed among the wells of 96-wells plastic culture plates. The medium in each well is tested periodically for desired antibody reactivity. The most common screening assay is the ELISA. The antigen is adsorbed to the bottom of 96-well plates. Sample to be tested are then incubated in the wells for an appropriate period of time. If sample contain the desired antibody, it will bind to the antigen and remain in the well as the unbound material is washed off. This antibody is then detected by an unconjugate that contains two covalently linked components. One component is an antibody. The second component is an enzyme such as alkaline phosphate or horse radish peroxidase. If antibody from the first incubation is immobilized in the well, the immunoconjugate is retained.

After another washing, a colorless substrate of enzyme is added to the wells. This will be converted to a colored product by the enzyme. For ex. Alkaline phosphate converts p-nitro phenyl phosphate into the yellow colored compound p-nitro phenol (max=405nm). After incubation, the

enzyme action is stopped and the optical density of product is measured at its max using a special colorimeter called plate reader or ELISA reader.

• **Cloning**

Single cell secreting the desired antibody are then isolated from positive culture and propagated into cell lines. The cloning techniques used are limiting dilution method and soft agar method.

Into the limiting dilution methods, cells in the culture are enumerated, diluted and aliquoted into new wells so that each well contains only one cell. Cells are allowed to regrow and procedure is repeated many times to ensure that all cells in a given well are monoclonal.

The soft agar method exploits the fact that many malignant cells will proliferate in a semisolid medium containing low amount of agar to form spherical colonies. If the culture can be reliably dispersed into single cells and the cell concentration such that the colonies will be spaced, then the colonies picked out of agar are most likely to be monoclonal.

In most cases, both methods are combined, since the clones require rescreening. The positive cultures after initial screening are grown by using several cycle of limiting dilution. The medium from each well is screened using ELISA and positive culture is dispersed in soft agar. The individual colonies are then cultured in new wells and transferred to larger vessels successively until seed stocks can be frozen and enough material is available for characterization.

• **Characterization and storage**

The characterization is the process which establishes the monoclonality of the antibody. This requires the biochemical and biophysical characterization of the antibody. Spectrometric,

electrophoretic and chromatographic methods are used for this purpose. Suitability of the antibody for intended use is also tested. For e.g. Therapeutic and diagnostic; coupled to a drug, or radioisotope, or enzyme; associated with a target cell population after injecting into an animal. The affinity of the antibody towards a particular antigen is also characterized for which it is specific, and the number of binding sites it possesses.

The stability of antibody and the cell line is also an important aspect. The ability of the cell to withstand freezing, storage for various time periods and chemical stability of the antibody during storage and use for its intended purpose must be determined. The cell line secreting the antibody must be frozen in liquid nitrogen at several stages of culture to avoid the destruction of clones. Seeds stock should also contain maximum number of clones secreting the antibodies of desired specificity, so that alternatives are available if the desired use of the antibody is changed or if the antibody are proved to be unstable^[8].



Fig 8:- production technique^[10]

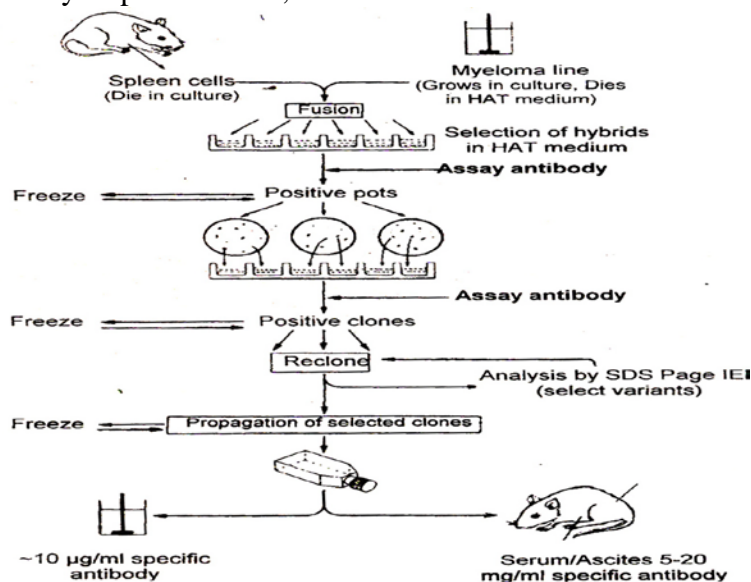


Fig 8:- Hybridoma technology^[8]

Recombinant Technology

In this use of viruses or yeast to create antibodies. This technique is used to enhance the specificity of antibody.

Commercial production of Monoclonal Antibodies

Commercially these are preparing by two methods:-

- ❖ Ascites production in mice
- ❖ In vitro fermentation

Ascites production in mice

In this method hybridoma cells are injected in to peritoneal cavity of histocompatible mice. The mice are pretreated by i.p. injection of pristine to irritate the peritoneal cavity and to establish a conditioned environment that facilitates the growth of ascites tumor. The fluid produced can contain a high concentration of secreted monoclonal antibodies 3-15mg/ml, and 3-5 ml or more can be harvested per mouse.

The method has the following disadvantages:-

1. It is very costly.
2. It is not reliable; product is contaminated with low level of normal mouse immunoglobulin as well as other mouse protein.
3. Viruses can be introduced.
4. Human monoclonal antibodies are very difficult and expensive to produce as ascites in the mice.
5. The human hybridoma may require special immuno deficient mice.
6. The antibody yields in ascites is lower than murine antibodies.

In vitro fermentation

Fermentation is widely accepted method for the production of monoclonal antibodies. Potent steps of fermentation based manufacturing process are following:-

Preparation of cell banks

- Master cell banks
- Manufacturer's work cell banks (MWCB)

Fermentation

- Preculture of MWCB
- Fermentation
- Harvest of culture medium
- Clarification and concentration

Downstream Processing

- Initial purification
- Digestion of pepsin, papain
- Further purification
- Conjugation
- Final purification

Pharmaceutical manufacturing

- Formulation
- Vialating
- Lyophilization

Advantages of this method:-

1. There are no contaminations with normal mouse immunoglobulin.
2. The process can be cost effective.
3. It is reliable.
4. It can be directly scaled up from small, pilot bioreactor to large production scale.
5. There is very less contamination.
6. Human monoclonal antibodies can be produced but lower levels (01-05mg/ml).

The major problem in this is the contamination of product with serum or protein based growth factors and other constituents^[8].

Application of Monoclonal Antibodies In cancer: - (FDA-approved)



Fig 9: Monoclonal Antibody (VEGF – vascular Endothelial growth Factor)^[12]

There are a number of obstacles to successful therapy with monoclonal antibodies. The antigen distribution of malignant cells is highly heterogeneous, so some cells may express tumor antigens while others do not. Antigen density can vary as well, with antigens expressed in concentrations too low for monoclonal antibodies to be effective. Tumor blood flow is not always optimal. If monoclonal antibodies need to be delivered via the blood, it may be difficult to reliably get the therapy to the site.

High interstitial pressure within the tumor can prevent the passive monoclonal antibodies from binding. Sometimes the tumor antigen is even released, so the antibody binds to a free-floating antigen and not the tumor cell. Since monoclonal antibodies are derived from mouse cell lines, the possibility of an immune response to the antibodies exists. This response not only decreases the efficacy of monoclonal antibody therapy, but also eliminates the possibility of re-treatment. Very rarely do we see cross-reactivity with normal tissue antigens – in general target antigens that are

not cross reactive with normal tissue antigens are chosen. Despite these obstacles, there has been tremendous success in the clinical application of monoclonal antibodies in hematologic malignancies and solid tumours^[13].

Table 4 .Antibodies & Application^[9]

Antibody	Application
1) Alemtuzumab (Campath)	Chronic lymphocytic leukemia
2) Bevacizumab (Avastin)	Breast cancer, Colon cancer, Lung cancer
3) Cetuximab (Erbix)	Colon cancer, Head and Neck cancers
4) Gemtuzumab (Mylotarg)	Acute myelogenous leukemia
5) Ibritumomab (Zevalin)	Non-Hodgkin's lymphoma
6) Panitumumab (Vectibix)	Colon cancer
7) Rituximab (Rituxan)	Non-Hodgkin's lymphoma
8) Tositumomab (Bexxar)	Non-Hodgkin's lymphoma
9) Trastuzumab (Herceptin)	Breast cancer
10) Edrecolomab	Breast cancer (rectal cancer)
11) Cytogen (mab)	Prostate cancer
12) Pan carcinoma (mab)	Prostate cancer

Anti-cancer drug passes pre-clinical trials (Recently on 21-Sep-2011)

Moscow-based biotech company OncoMax has recently completed reportedly successful pre-clinical trials of its cutting-edge humanized monoclonal antibody drug. According to OncoMax, their drug target (also known as drug design), designated OM-RCA-01, works by blocking fibroblasts' growth factor receptor, thus inhibiting progression of renal cell carcinoma or Grawitz' tumor^[11]

Selected Antibodies Designed for Hematologic Malignant Neoplasms in Advanced-Stage Clinical Trials

Epratuzumab (LymphoCide). Epratuzumab is a chimeric monoclonal antibody that binds to the CD22 antigen and is in phase 3 clinical trials for the treatment of NHL. This novel therapeutic agent also is being evaluated for combination therapy for lymphoma

Lintuzumab (Zamyl). This humanized antibody targets the CD33 receptor on myeloid blasts and is in phase 3 clinical trials for the treatment of AML and myelodysplastic syndromes.

Iodine 131 Tositumomab (Bexxar). Iodine 131 (¹³¹I)-tositumomab is a radiolabeled anti-CD20 murine monoclonal antibody in phase 3 clinical trials for the treatment of relapsed and refractory follicular or low-grade and transformed NHL. Treatment with ¹³¹I-tositumomab in clinical trials has produced high response rates and durable complete remissions in patients who have received previous chemotherapy or rituximab. Complications related to treatment with ¹³¹I-tositumomab include myelosuppression, secondary acute leukemia, myelodysplasia, and

hypothyroidism. The FDA approval of ¹³¹I-tositumomab is pending over safety issues associated with the relatively long half-life of the ¹³¹I isotope.

Antibodies Designed for Hematologic Malignant Neoplasms in Early-Stage Clinical Trials

A substantial number of monoclonal antibodies designed to treat hematologic malignant neoplasms are in early-stage clinical trials. Popular targets for these antileukemia and antilymphoma drugs include CD20, CD22, CD33, HLA-DR, and ferritin^[14].

Monoclonal antibody treatment for cancer may cause side effects, which are following:

1. Allergic reactions, such as hives or itching
2. Flu-like symptoms, including chills, fatigue, fever, and muscle aches and pain

3. Nausea

4. Diarrhea

5. Skin rashes

More serious side effects of monoclonal antibody.

1. Infusion reactions

2. Dangerously low blood cell counts

3. Heart problems

4. Skin problem

5. Bleeding^[9]

CONCLUSION

Antibodies are the glycoprotein molecules present in the serum. They are produced in response to antigens which are either protein molecules which may be foreign to the body.

The first production of monoclonal antibodies represent the convergence of three areas of basic medical research: immunochemistry, in vitro cultivation of cancer cells and the molecular biology of malignant transformation. This was done by fusing a normal β cell (plasma cell) with a myeloma cell (a cancerous plasma cell) called a hybridoma or heterokaryon, that possessed the proliferating growth properties of the myeloma cell but secreted the antibody product of the β cell. These monoclonal antibodies are of exceptionally high quality; they represent only one molecular species and which may be obtained virtually in a homogeneous state. This can be used for diagnostic purpose in many cardiac diseases like myocardial infarction, deep vein thrombosis.

It is also advantageous in cancer therapy. It possesses therapeutic application also. Use of monoclonal antibody in transplantation is also an important advantage. Thus monoclonal antibodies have promising application in clinical diagnosis and therapy of many diseases. The technical gains and knowledge of Mabs acquire can lead to many

improvements in clinical application in the near future.

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