MONOCLONAL ANTIBODIES: Preparation, evaluation & Application

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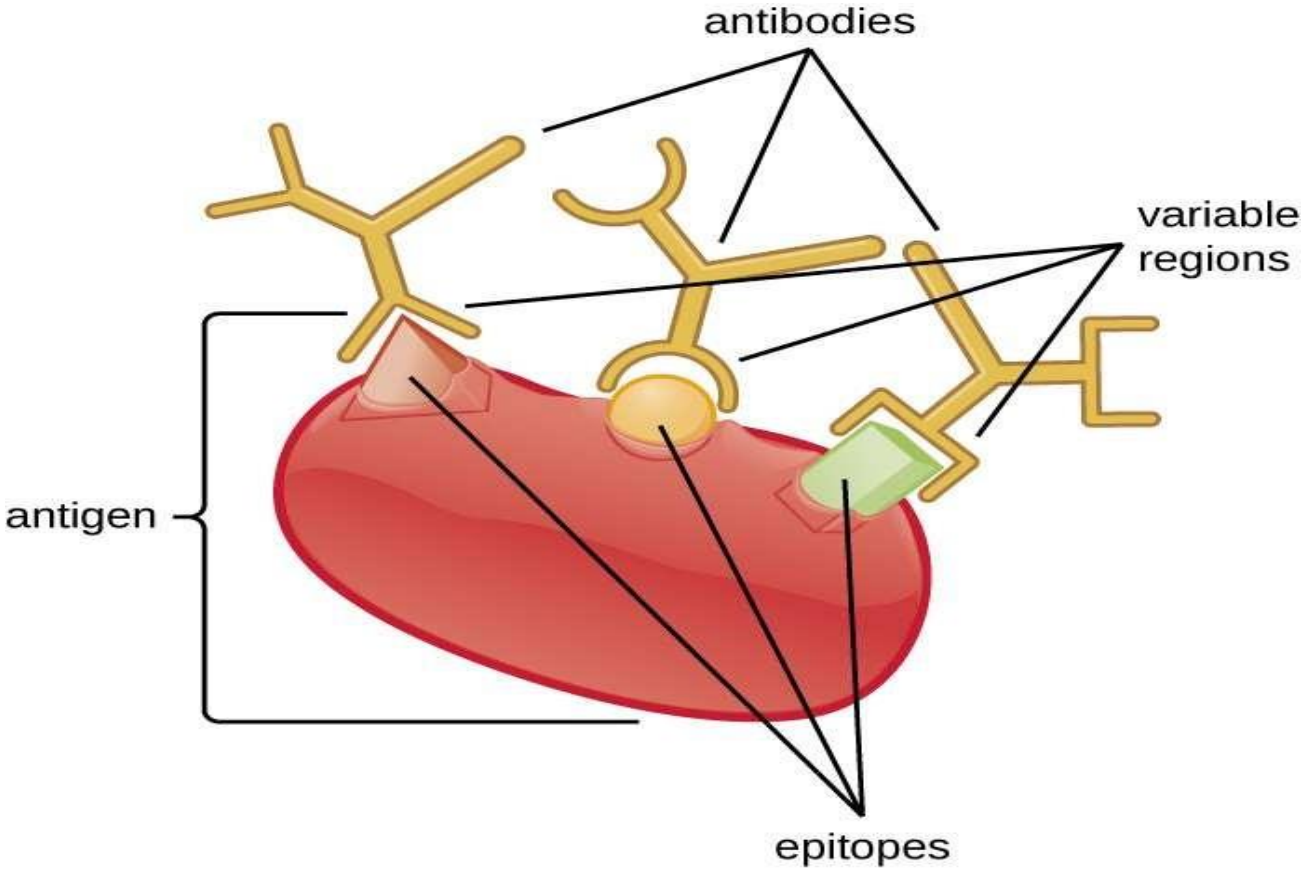
Mpharm 2nd sem

Contents:

- Basic concepts and Introduction
- Monoclonal Antibodies: History and Development
- Preparation of Monoclonal Antibodies
- Evaluation
- Applications

Basic concepts and Introduction

MONOCLONAL ANTIBODIES



An antibody binds to a specific region on an antigen called an epitope. A single antigen can have multiple epitopes for different, specific antibodies.

Monoclonal antibodies

- Monoclonal antibodies are identical immunoglobulins, generated from a single B-cell clone. These antibodies recognize unique epitopes, or binding sites, on a single antigen. **Derivation from a single B-cell clones and subsequent targeting of a single epitope** is what differentiates monoclonal antibodies from polyclonal antibodies.
- Polyclonal antibodies are antibodies that are derived from different cell lines. They differ in amino acid sequences.

Characters of monoclonal Antibodies

- Monoclonal antibodies (mAB) are single type of antibody that are identical and are directed against a specific epitope (antigen, antigenic determinant) and are produced by B-cell clones of a single parent or a single hybridoma cell line.
- A hybridoma cell line is formed by the fusion of one B-cell lymphocyte with a myeloma cell.
- Some myeloma cell synthesize single mAB antibodies naturally.

Differences between polyclonal and Monoclonal antibodies

	Polyclonal antibodies	Monoclonal antibodies
Produced by:	Many B cell clones	A single B cell clone
Binds to:	Multible epitopes of all antigen used in the immunization	A single epitope of a single antigen
Antibody class:	A mixture of different Ab classes (isotypes)	All of a single Ab class
Ag-binding sites:	A mixture of Abs with different antigen-binding sites	All Abs have the same antigen binding sites
Potential for cross-reactivity:	High	Low

Advantages of using Monoclonal Antibodies:

- Though expensive, monoclonal antibodies are **cheaper to develop than conventional drugs** because it is based on tested technology.
- **Side effects can be treated** and reduced by using mice-human hybrid cells or by using fractions of antibodies.
- They **bind to specific diseased or damaged cells** needing treatment.
- They **treat a wide range of conditions.**

Disadvantages of using Monoclonal Antibodies:

- **Time consuming project** - anwhere between 6 -9 months.
- **Very expensive** and needs considerable effort to produce them.
- Small peptide and fragment antigens may not be good antigens- monoclonal antibody **may not recognize the original antigen**.
- Hybridoma culture may be subject to **contamination.**
- System is **only well developed for limited animal** and not for other animals.
- More than **99% of the cells do not survive** during the fusion process – reducing the range of useful antibodies that can be produced against an antigen

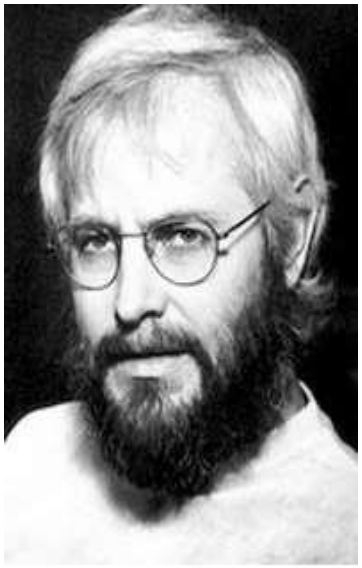
History and Development

MONOCLONAL ANTIBODIES

Monoclonal Antibodies: History and Development

- **Paul Enrlich** at the beginning of 20th century coined the term "magic bullets" and postulated that, **if a compound could be made that selectively targets a disease-causing organism, then a toxin for that organism could be delivered along with the agent of selectivity.**
- In the 1970s, the **B-cell cancer multiple myeloma** was known. It was understood that these cancerous B-cells all produce a single type of antibody (a paraprotein).

- In 1975, **Kohler and Milstein** provided the most outstanding proof of the clonal selection theory by fusion of normal and malignant cells (Hybridoma technology) for which they received Nobel prize in 1984.
- In 1986, first monoclonal antibody was licenced by FDA. Orthoclone OKT3 (muromonab-CD3) which was approved for use in preventing kidney transplant rejection.



Georges J.F. Köhler

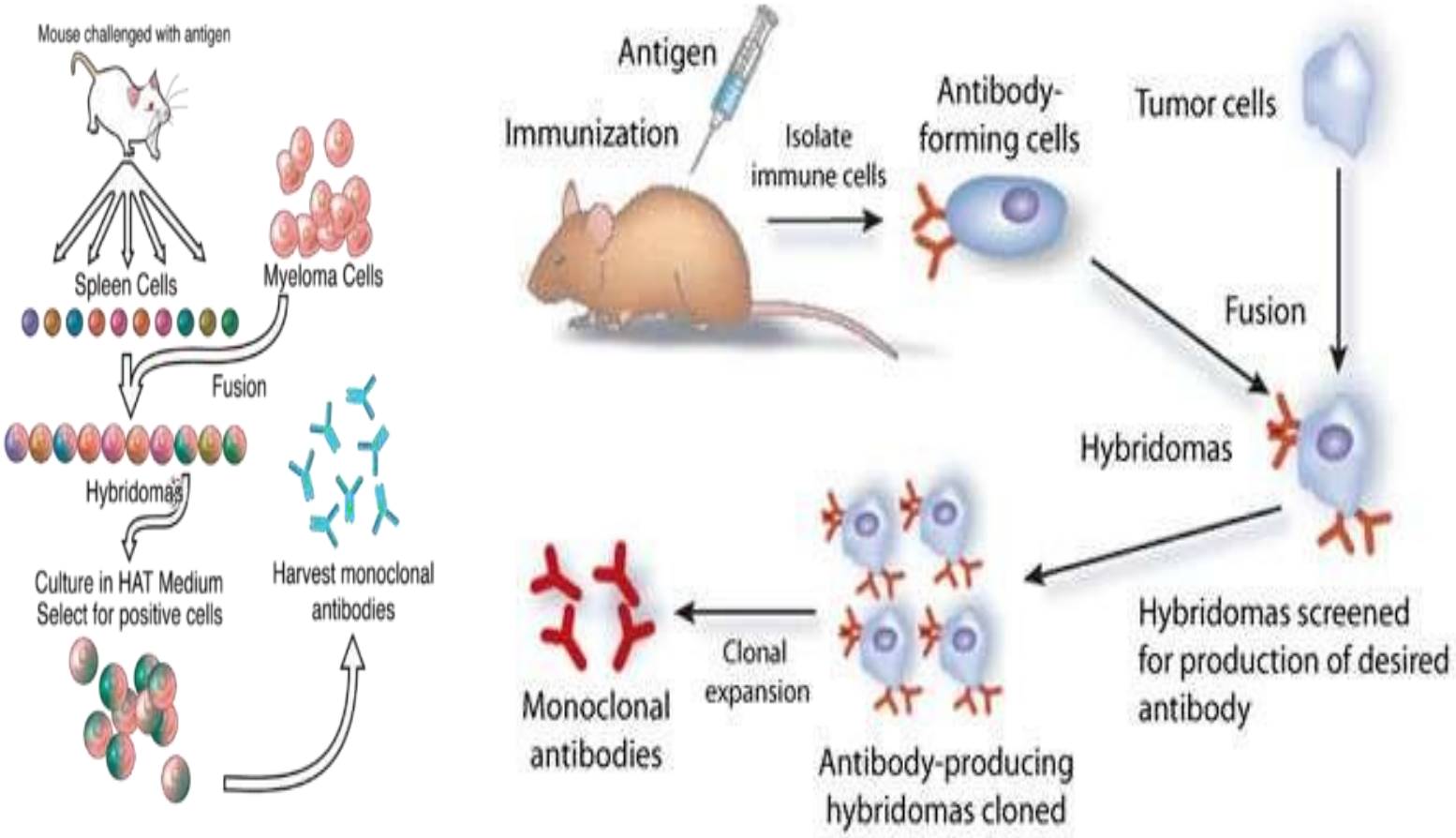
César Milstein

Preparation

MONOCLONAL ANTIBODIES

Preparation of Monoclonal Antibodies

- Monoclonal Antibody production or mAb is produced by cell lines or clones obtained from the immunized animals with the substances to be studied. Cell lines are produced **by fusing B cells from the immunized animal with myeloma cells**.
- To produce the desired mAB, the cells must be grown in either of two ways**:** **by injection into the peritoneal cavity** of a suitably prepared mouse (in vivo method) or by **in vitro tissue culture**.
- The vitro tissue culture is the method used when the cells are placed in culture outside the mouse the mouse's body in flask.



Preparation of Monoclonal Antibodies

Practical steps for production

1. Immunize animal
2. Isolate spleen cells (containing antibody-producing B cell)
3. Fuse spleen cells with myeloma cell (using PEG)
4. Allow unfused B cell to die
5. Add aminopterin to culture and kill unfused myeloma cells
6. Clone remaining cells (place 1 cell/well and allow each cell to grow into a clones of cell)
7. Screen supernatant of each clone for presence of desired antibody
8. Grow chosen clone of cells in tissue culture indefinitely
9. Harvest antibody from the culture.
10. 1000-2000 per mg

Evaluation

MONOCLONAL ANTIBODIES

1. Characterisation of monoclonal antibodies

- **Physicochemical characterisation**
- **Immunological properties**
- **Biological activity**
- **Purity, impurity and contaminants**
- **Quantity**

2. Specifications

- **Identity**
- **Purity and impurities**
- **Potency**
- **Quantity**
- **General tests**

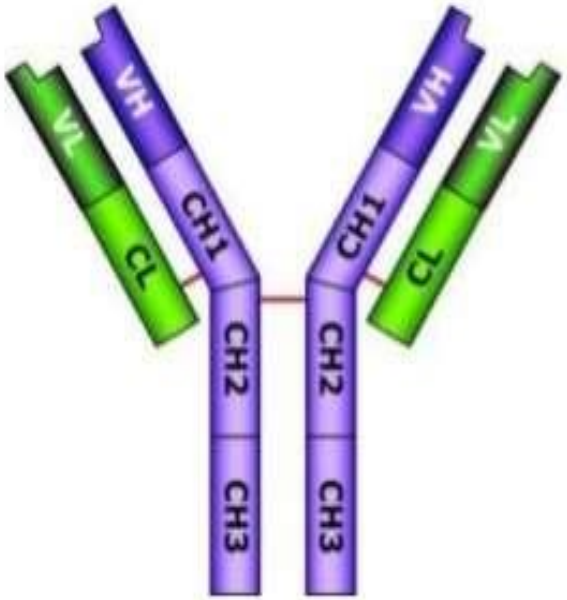
1.1 Physicochemical characterisation

- A physicochemical characterisation program will generally include a determination of the class, subclass, light chain composition and primary structure of the monoclonal antibody.
- The class or subclass of an antibody is defined by its heavy chain. There are five main classes of antibodies: M, G, A, E, and D. The method of antibody purification will differ based on the class.
- The amino acid sequence should be deduced from DNA sequencing and confirmed experimentally by appropriate methods (e.g. peptide mapping, amino acid sequencing, mass spectrometry analysis).
- The variability of N- and C- terminal amino-acid sequences should be analysed (e.g. C-terminal lysine(s)).

Types of Immunoglobulin

Classes based on constant region of heavy chains

- Immunoglobulin A (IgA)
- Immunoglobulin D (IgD)
- Immunoglobulin E (IgE)
- Immunoglobulin G (IgG)
- Immunoglobulin M (IgM)



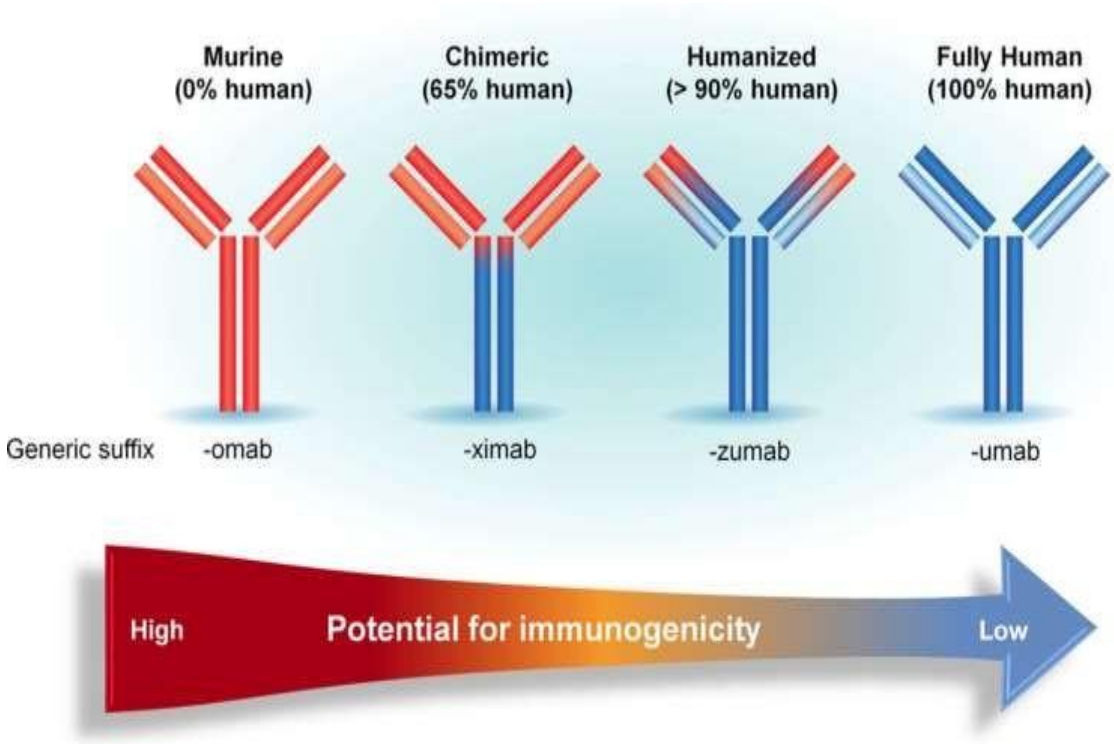
Differentiation of heavy chains

- Length of C region, location of disulfide bonds, hinge region, distribution of carbohydrate

1.2 Immunological properties

- Binding assays of the antibody to purified antigens and defined regions of antigens should be performed, where feasible, to determine affinity, avidity and immunoreactivity (including cross reactivity with other structurally homologous proteins).
- Unintentional reactivity/cytotoxicity for human tissues distinct from the intended target should be documented.
- The epitope and molecule bearing the relevant epitope should be defined. This should include a biochemical identification of these structures (e.g. protein, oligosaccharide, glycoprotein, glycolipid), and relevant characterisation studies (amino acid sequence, carbohydrate structure) to the extent possible.

Immunogenicity potential based on source of Ig



1.3 Biological activity

- The biological activity (i.e. the specific ability or capacity of a product to achieve a defined biological effect) should be assessed by appropriate *in vitro* assay(s).
- Where *in vivo* assays are necessary, the use of such assays should be thoroughly justified.
- The mechanism of action and the importance (or consequences) of the product effector functions with regards to the safety and efficacy of the product should be discussed.

1.4 Purity, impurity and contaminants

- These methods generally include the determination of physicochemical properties such as molecular weight or size, extinction coefficient, electrophoretic profiles, chromatographic data and spectroscopic profiles.
- Potential process-related impurities (e.g. HCP, host cell DNA, cell culture residues, downstream processing residues) should be identified, and evaluated qualitatively and/or quantitatively, using chromatographic technique.
- Contaminants, which include all adventitiously introduced materials not intended to be part of the manufacturing process (e.g. microbial species, endotoxins) should be strictly avoided and/or suitably controlled.

1.5 Quality

- Quantity should be determined using an physicochemical and/or immunochemical assay.
- It should be demonstrated that the quantity values obtained are directly
  related to those derived using the biological assay.
- When this correlation exists, it may be appropriate to use measurement of quantity rather than the measurement of biological activity in the product labelling and manufacturing processes, such as filling.

2. Specifications

- Specifications are one part of a total control strategy designed to ensure product quality and consistency, and when tested, the product should be in compliance with its specification.
- Specifications should be set and take into account relevant quality attributes identified in characterisation studies.
- Selection of tests to be included in the specifications is product specific.
- The rationale used to establish the acceptable range of acceptance criteria should be described.

2.1 Identity

- The identity test(s) should be highly specific and should be based on unique aspects of the product's molecular structure and/or other specific properties (e.g. peptide map, anti-idiotype immunoassay, or other appropriate method).
- Considering the great similarity of the constant domains of different antibodies, more than one test (physicochemical, biological and/or immunochemical) may be necessary to establish identity, and such test(s) should be able to discriminate other antibodies that may be manufactured in the same facility.

2.2 Purity and Impurities

- As noted in the characterisation section, monoclonal antibodies may display a complex purity/impurity profile that should be assessed by a combination of orthogonal methods, and for which individual and/or collective acceptance criteria should be established for relevant product-related variants.
- For example, separation methods based on charge heterogeneity is considered to quantitatively and qualitatively monitor charge variants.
- Considering that glycosylation may have an impact on the pharmacokinetics of the product, and may modulate its immunogenic properties, appropriate acceptance criteria should be considered for this attribute. In addition, such control will further confirm the consistency of the product.

2.3 Potency

- Potency is the quantitative measure of biological activity based on an attribute of the product which is linked to the relevant biological properties. A relevant potency assay should be part of the specifications for drug substance and/or drug product, and should ideally reflect the biological activity in the clinical situation.
- For antibodies for which the clinical activity is only dependent on binding/neutralising properties, a potency assay that measures binding to the target (i.e. binding assay) is acceptable when appropriately justified.

2.4 Quantity

- The quantity of the drug substance, usually based on protein content (mass), is determined chromatographically using reference standard.

2.5 General tests

Appearance, solubility, pH, osmolality, extractable volume,sterility,bacterial endotoxins, stabiliser and water, is assessed where appropriate.

Applications

MONOCLONAL ANTIBODIES

Major Applications:

(1) **Diagnostic Applications**

- Biochemical analysis
- Diagnostic Imaging

(2) **Therapeutic Applications**

- Direct use of MAbs as therapeutic agents
- MAbs as targeting agents.

(3) **Protein Purification**

1a. Biochemical analysis

- Routinely used in **radioimmunoassay** (RIA) and **enzyme-linked immunosorbent assays** (ELISA) in the laboratory.
- These assays **measure the circulating concentrations of hormones** (insulin, human chorionic gonadotropin, growth hormone, progesterone, thyroxine, triiodothyronine, thyroid stimulating hormone) and several **other tissue and cell products** (blood group antigens, blood clotting factors, interferon's, interleukins, tumor markers).

Eg. Pregnancy by detecting the urinary levels of human chorionic gonadotropin.

Hormonal disorders analysis of thyroxine, triiodothyronine.

Cancers estimation of plasma carcinoembryonic antigen in colorectal cancer, and prostate specific antigen for prostate cancer

1b. Diagnostic imaging

- **Radiolabeled**—MAbs are used in the diagnostic imaging of diseases, and this technique is referred to as immunoscintigraphy. The radioisotopes commonly used for labeling MAb are iodine—131 and technetium—99. The MAb tagged with radioisotope are injected intravenously into the patients.
- These MAbs localize at specific sites (say a tumor) which can be detected by imaging the radioactivity. In recent years, single photon emission computed tomography (SPECT) cameras are used to give a more sensitive three dimensional appearance of the spots localized by radiolabeled— MAbs.
- Myocardial infarction, DVT, atherosclorosis etc.

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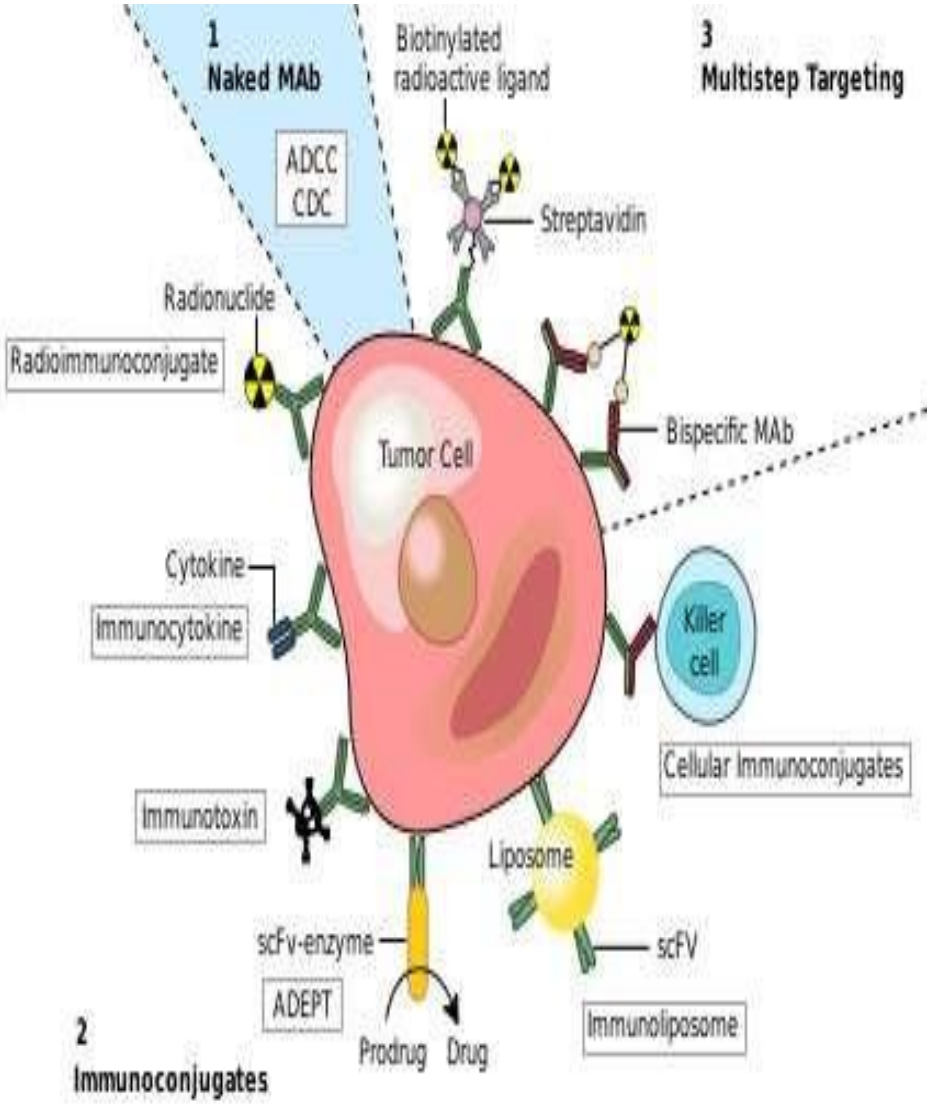
2a. Direct use of MAbs as therapeutic agents

- **In destroying disease-causing organisms:** MAbs promote efficient opsonization of pathogenic organisms (by coating with antibody) and enhance phagocytosis.
- **In the immunosuppression of organ transplantation:** In the normal medical practice, immunosuppressive drugs such as cyclosporin and prednisone are administered to overcome the rejection of organ transplantation. In recent years, MAbs specific to T-lymphocyte surface antigens are being used for this purpose



- **In the treatment of cancer:**
  MAbs, against the antigens on the surface of cancer cells, are useful for the treatment of cancer. The antibodies bind to the cancer cells and destroy them via different pathways.

- **In the treatment of AIDS:** Genetic engineers have been successful to attach Fc portion of mouse monoclonal antibody to human CD_4 molecule. This complex has high affinity to bind to membrane glycoprotein gp120 of virus infected cells. The Fc fragment induces cell-mediated destruction of HIV infected cells.

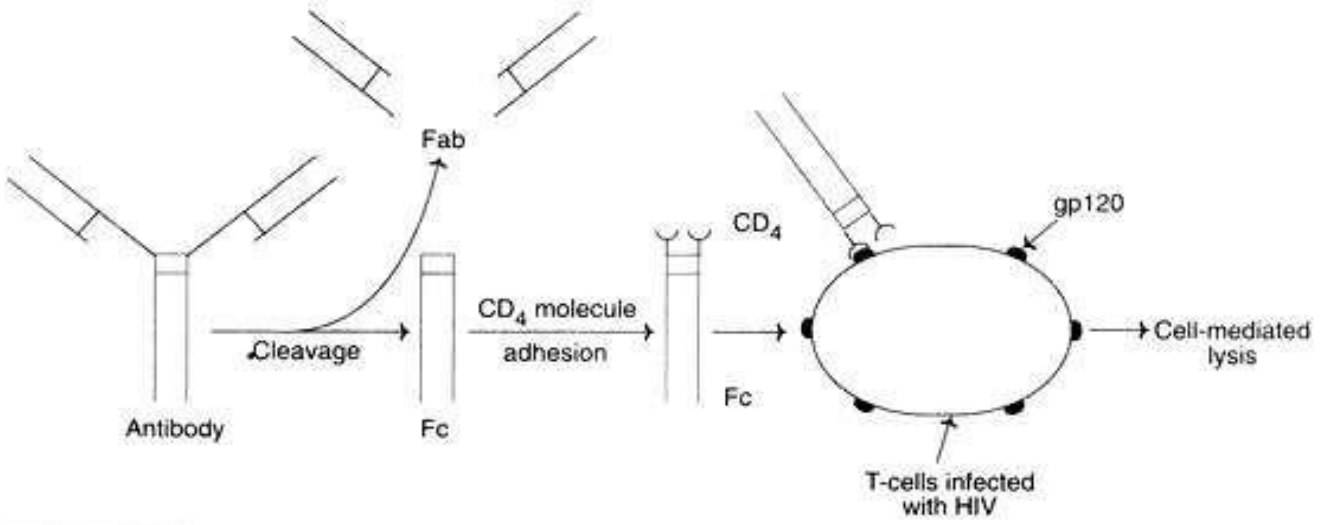


Fig. 17.7 : Modified monoclonal antibody in the treatment of AIDS.

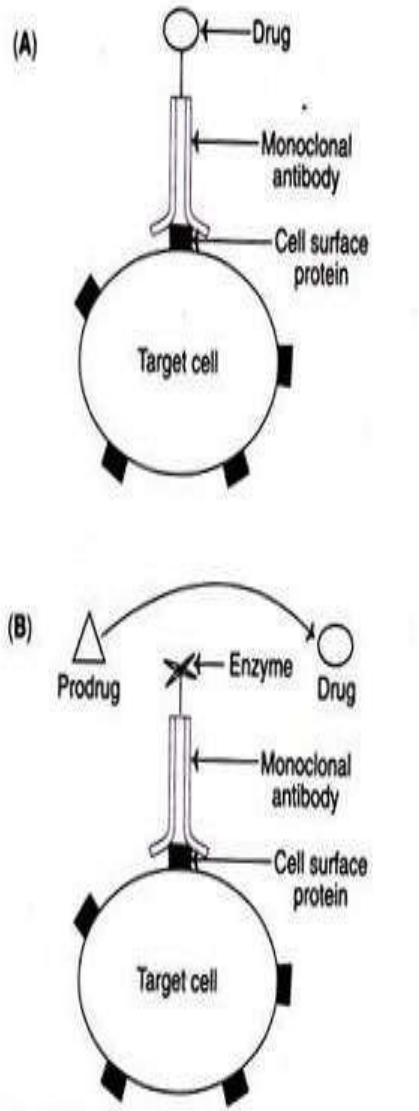


Fig. 17.9 : Monoclonal antibody based drug delivery to the target cells. (A) The drug is bound to MAb (B) The enzyme that converts prodrug to drug is bound to MAb.

2a. MAbs as targeting agents.

- The drugs can be coupled with MAb (directed against a cell surface antigen of the cells, say a tumor) and specifically targeted to reach the site of action.

Eg. **Alkaline phosphatase** for the conversion of phosphate pro-drugs.

Carboxy peptidase for converting inactive carboxyl pro-drugs to active drugs.

Lactamase for hydrolyzing β-lactam ring containing antibiotics.

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- **Drug delivery through liposomes coupled to tissue-specific MAbs:**

✓ Liposomes are sacs or vesicles formed spontaneously when certain lipid molecules are exposed to aqueous environment.

✓ Drug entrapped in liposomes that are coated with MAbs directed against tissue-specific antigens are being tried for drug delivery.

✓ Unfortunately, the progress in this approach has been limited, since such liposomes do not reach the target cells.

✓ They are retained mostly in the liver and spleen (reticuloendothelial cells), and degraded.

3. Protein Purification

- Monoclonal antibodies can be produced for any protein. And the so **produced MAb can be conveniently used for the purification of the protein against which it was raised.**
- MAbs columns can be prepared by coupling them to cyanogen bromide activated Sepharose (chromatographic matrix). The immobilized MAbs in this manner are very useful for the purification of proteins by immunoaffinity method.
- There are certain advantages of using MAbs for protein purification. These include the specificity of the MAb to bind to the desired protein, very efficient elution from the chromatographic column and high degree of purification.

Few Commercially available mAb approved by FDA

Name	Trade name	Target	Use
Abciximab	ReoPro	CD41 (integrin alpha-IIb)	Platelet aggregation Inhibitor
Adalimubab	Humira	TNF-*alpha*	Rheumatoid arthritis, Crohn's Disease, Plaque psoriaris, psoriatic Arthritis
Alirocumab	Praluent	PCSK9	Hypercholesterolemia
Avilumab	Bavencio	PD-L1	Cancer
Benralizumab	Facenra	CD125	Asthma
Daclizumab	Zenapax	CD25	Organ transplant rejection
Daratumubab	Darzalex	CD-38	Multiple Myeloma

Questions

- Describe in brief the preparation ,evaluation and application of MAB ? (10M – 2 Times)
- Explain the preparation and evaluation of MAB ? (5M)
- Explain monoclonal antibodies with their application ?(5m)

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