MONOCLONAL ANTIBODIES: Preparation & Application

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Basic concepts and Introduction

MONOCLONAL ANTIBODIES

What are antibodies?

- An antibody is a **protein used by immune system to identify and neutralize foreign objects** like bacteria and viruses. Each antibody recognizes a specific antigen unique to its target.
- The **high specificity of antibodies** makes them an excellent tool for detecting and quantifying a broad array of targets, from drugs to serum proteins to microorganisms.
- With *in vitro* assays, antibodies can be used to **precipitate soluble antigens, agglutinate (clump) cells, opsonize and kill bacteria** with the assistance of complement, and neutralize drugs, toxins, and viruses.

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.

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:

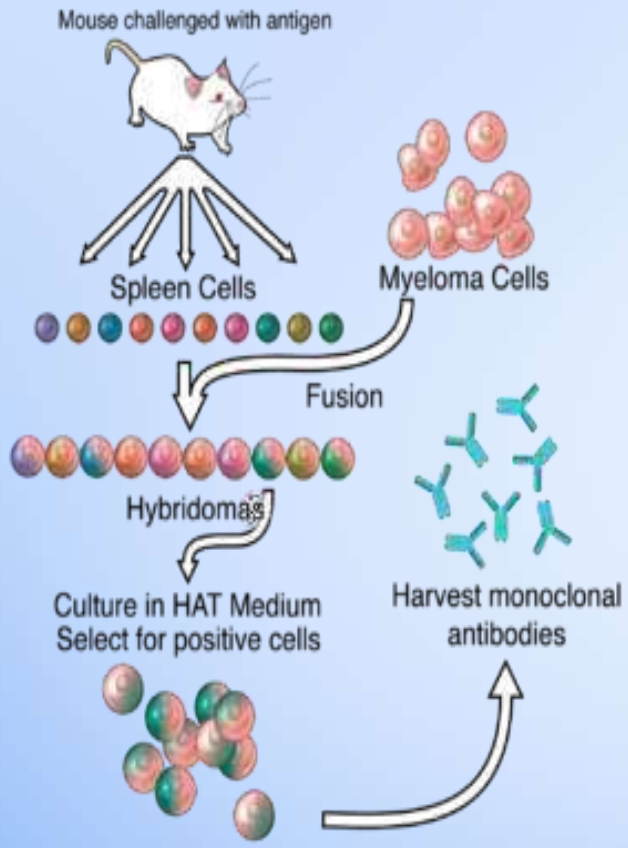
- **Time consuming project** - anwhere between 6 -9 months.
- **Very expensive** and needs considerable effort to produce them.
- Small peptide and fragment antigens may not be good antigens- monoclonal antibody **may not recognize the original antigen**.
- Hybridoma culture may be subject to **contamination.**
- 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
- It is **possibility of generating immunogenicity.**

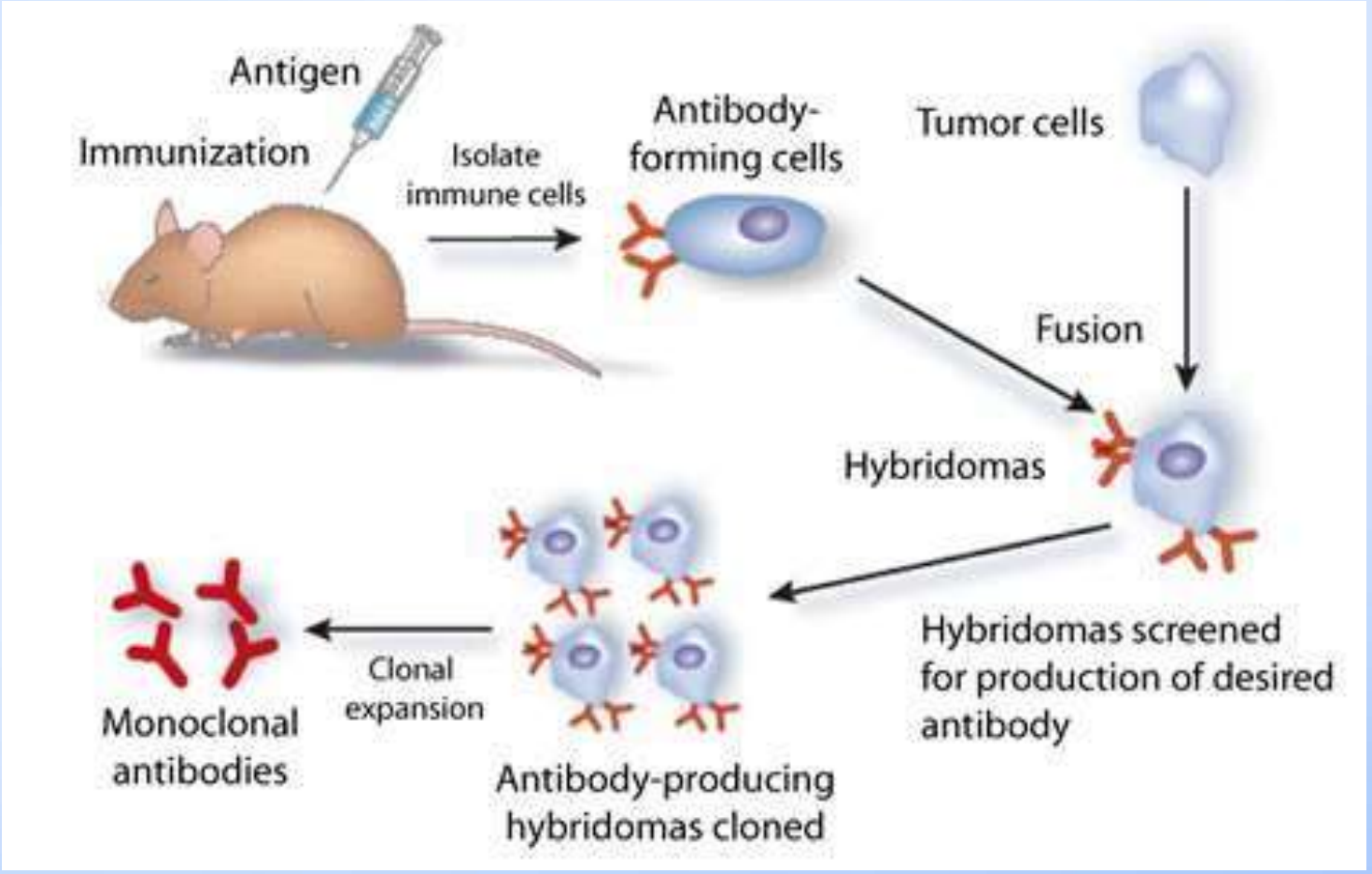
Preparation

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.

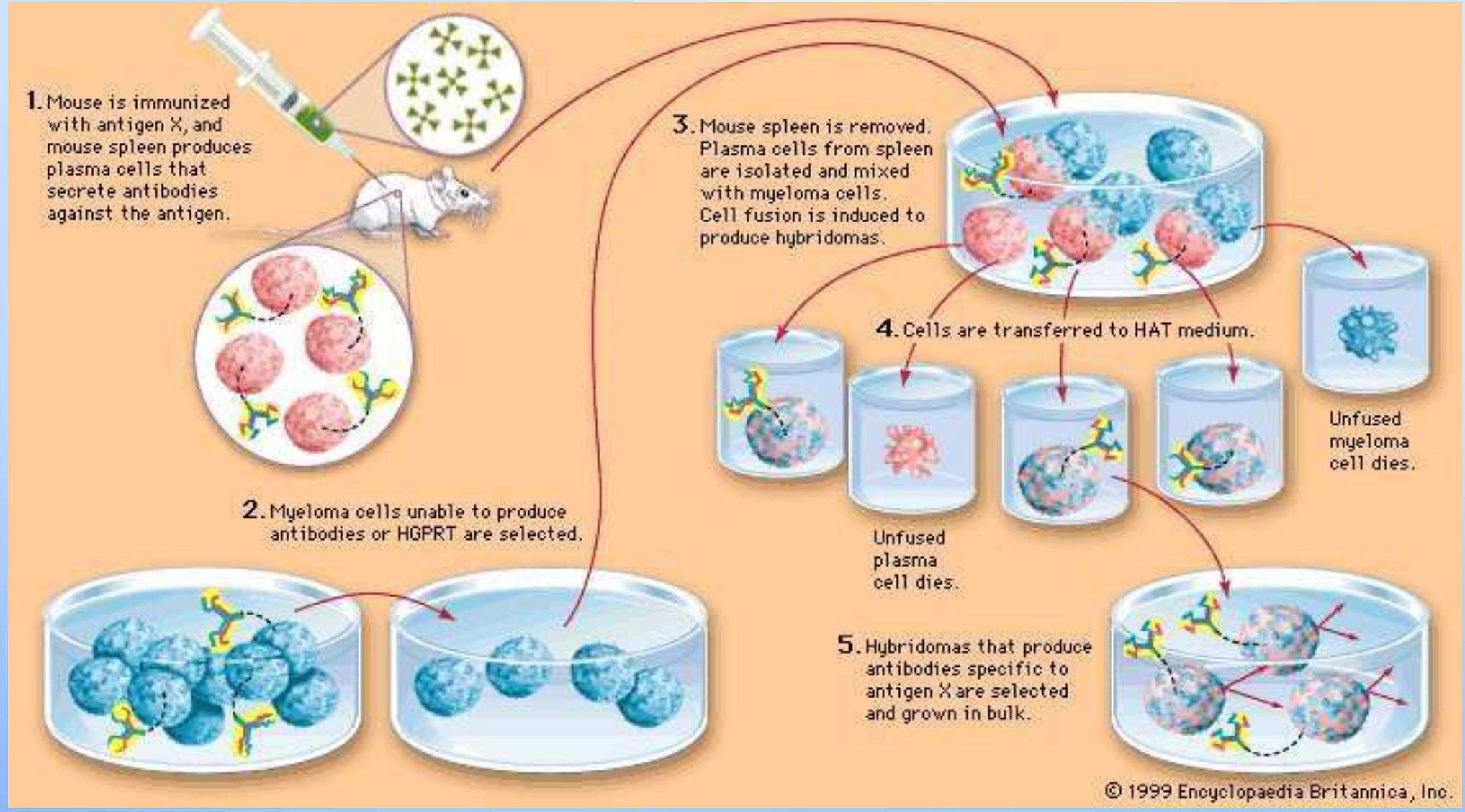




Preparation of Monoclonal Antibodies

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


1. Mouse is immunized with antigen X, and mouse spleen produces plasma cells that secrete antibodies against the antigen.
2. Myeloma cells unable to produce antibodies or HGPRT are selected.
3. Mouse spleen is removed. Plasma cells from spleen are isolated and mixed with myeloma cells. Cell fusion is induced to produce hybridomas.
4. Cells are transferred to HAT medium.
Unfused myeloma cell dies.
Unfused plasma cell dies.
5. Hybridomas that produce antibodies specific to antigen X are selected and grown in bulk.
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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.

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

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.

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Thank
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