A.S.D.GOVERNMENT DEGREE COLLEGE FOR WOMEN (AUTONOMOUS) KAKINADA

DEPARTMENT OF MICROBIOLOGY

Monoclonal Antibodies-Production &Applications

II BSc CBMB SEM IV

BY

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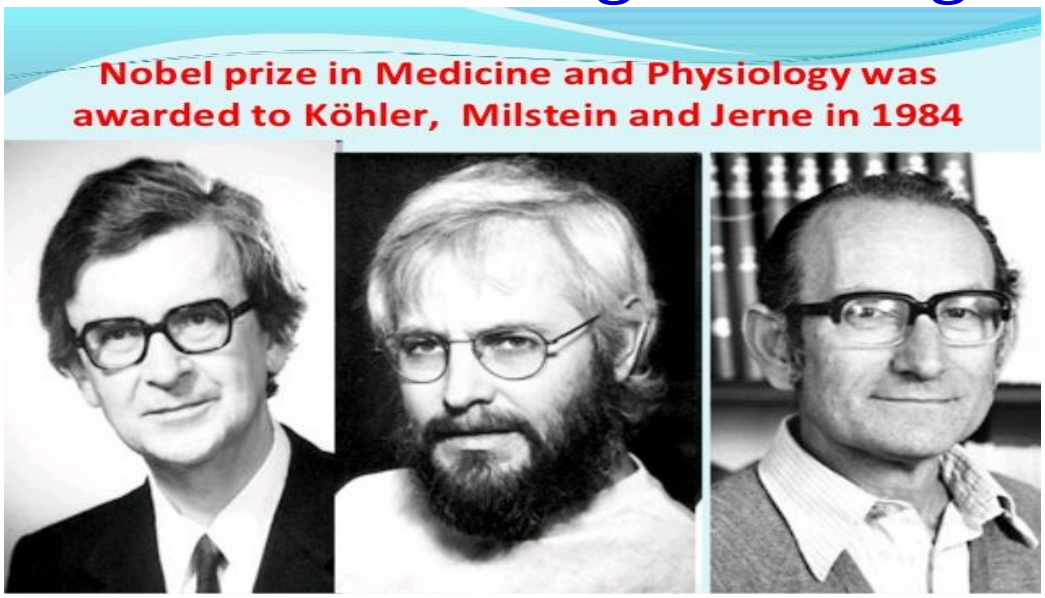
Hybridoma Technology

In 1975, hybridoma technology developed by Georges J.F.Kohler and Cesar Milstein.

- **And in 1984, they shared a Nobel prize for this discovery.**
- **They make a hybrid cell that will make a numbers of monoclonal antibodies against antigen .**



PRINCIPLE of Hybridoma Technology

The hybrid cell has the capacity of antibody production derived from B-cells (spleen cell)

At the same time it can divide continuously by the quality derived from myeloma cell.

By combining the desired qualities of both the cells, the technology ensures large, antibody production of single specificity.

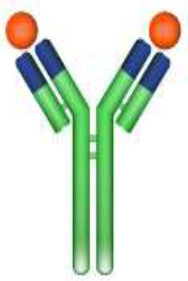
Specific hybridomas (spleen cell and myeloma cell) obtain monoclonal antibodies in artificial media, this technology called as Hybridoma technology

MONOCLONAL ANTIBODY

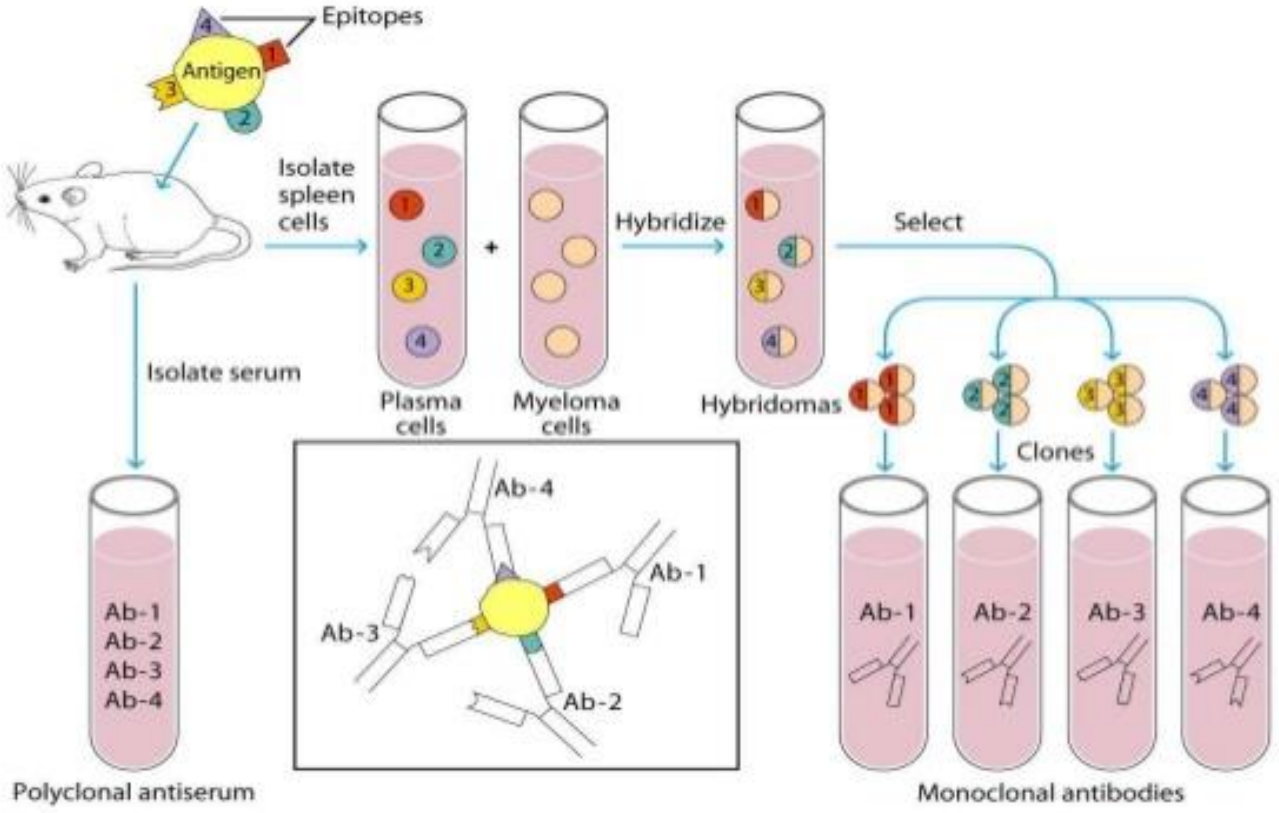
Monoclonal antibodies (mAb) are antibodies that are identical because they are produced by one type of immune cell, all clones of a single parent cell.

Basically produced by white blood cell

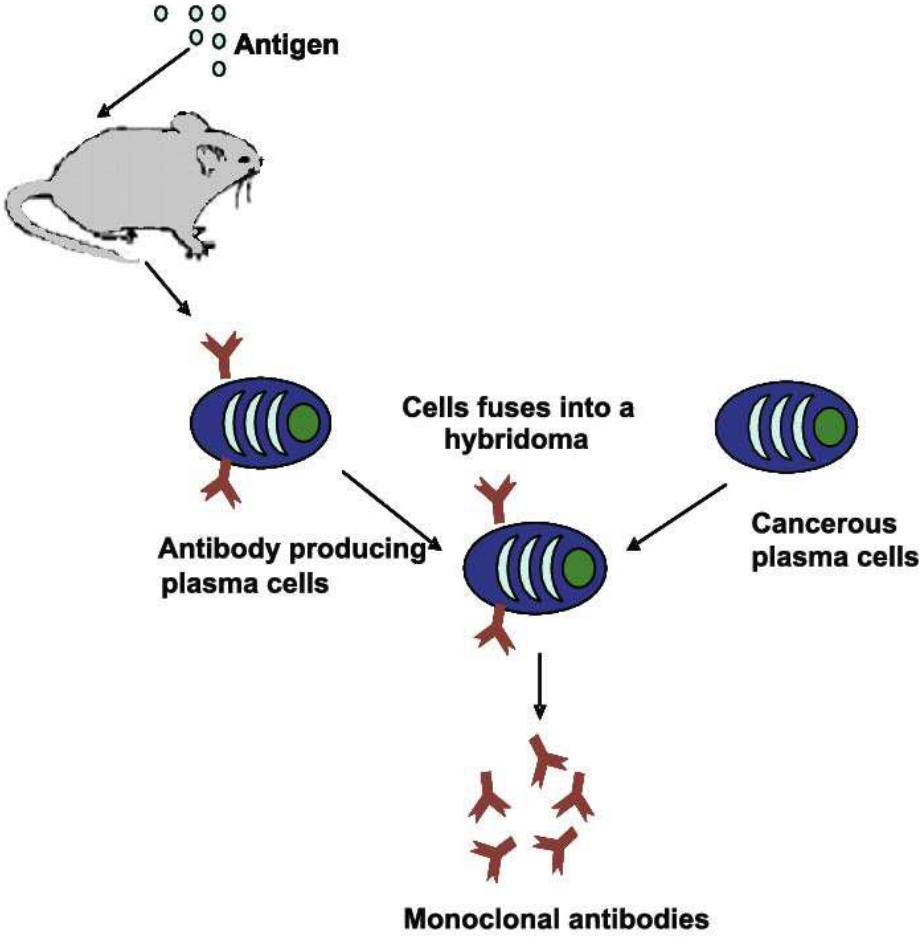
which is called as plasma cell. Is used for treatment of cancerous cells and as anti-venom(anti snake venom)



Polyclonal and Monoclonal antibodies



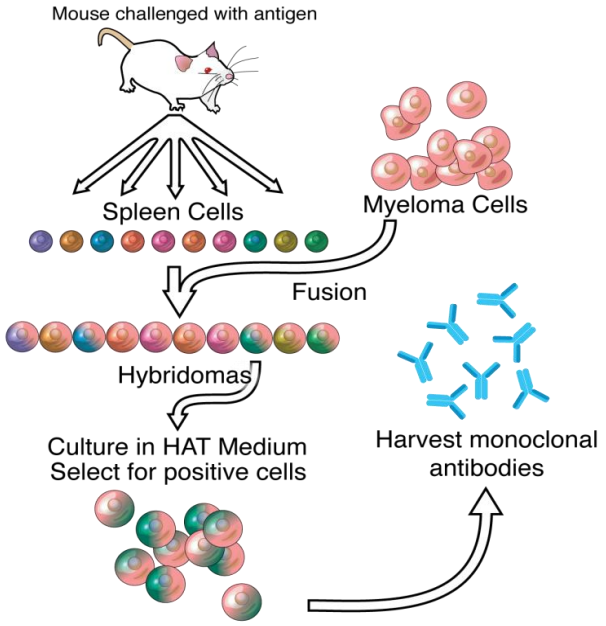
Production of Monoclonal Antibodies



Making Hybridoma

Procedure

1. **Immunization of specific animal with specific Antigen**
2. **Isolation of myeloma cells.**
3. **Fusion between separated spleen cell and myeloma cell.**
4. **Selection of Hybridoma Cells**
5. **Isolation of hybridoma cell.**



1. Immunization of specific animal

- An antigen immunized to an animal (like mice) via intravenously(directly to blood) by injection.
- Where in spleen it activate B-cell which produce plasma cell (spleen cell).
- Plasma cell to produce monoclonal antibodies
- Isolation of plasma cell from spleen of animal.

The final dose of the antigen is given intravenously three days before the animal is killed.
Spleen of the killed animal is removed and lymphocytes are separated from the spleen fluid.

2. Isolation of myeloma cells

Myeloma cells are Cancerous cells which is isolated from bone-marrow

Myeloma cells are generally **immortal in** nature (that which never dies) and has multiplication property.

Myeloma call line not capable of synthesizing antibodies.

HPRT–Negative Cell Lines are selected by growing them in the presence of 8-azaguanine.

Most of the cells killed by this technique but a few survive.

The surviving myeloma lymphocytes are resistant to 8-azaguanine as they have a defect in Hypoxanthine Phosphorisyl transferase (HPRT).

3. Fusion of spleen cell and myeloma cell

It requires PEG (poly ethylene glycol)medium for fusion

It can also done by electro fusion.

Fusion between spleen cell and myeloma cell produced

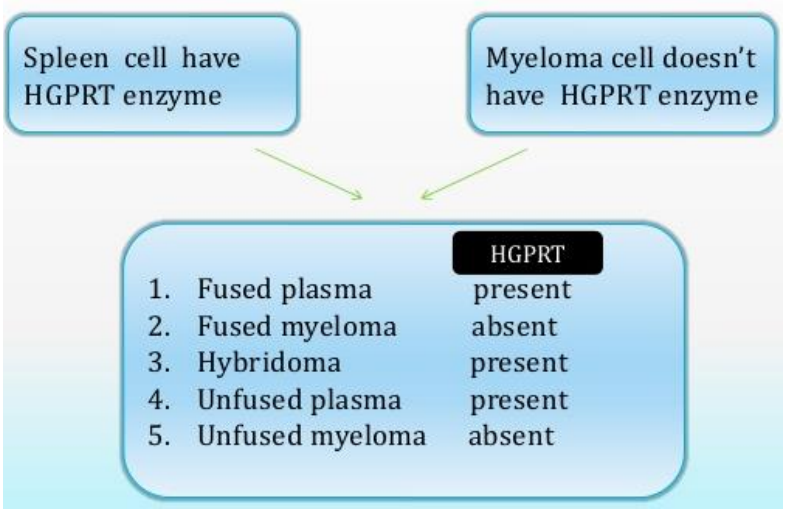
Five different types of cells.

1.Fused Plasma Cells
2.Fused myeloma Cells
3.Hybridoma Cells
4.Unfused plasma Cells
5. Unfused myeloma Cells

So in HAT medium, Cells not synthesized by De-novo synthesis due to presence of Aminopterin in HAT medium which blocks **Di-hydro follate enzyme which is** necessary for these synthesis.

For synthesis in salvage pathway it must requires **HGPRT enzyme (Hypoxanthine** Guanine Phospho-Ribosyl Transferase).Where hypoxanthine and thymidine are used as precursors.

4. Selection of hybridoma cell



From the mixture of Cells, the Hybrid cells to be selected by using the HAT- medium for growth.

HAT –medium represents the mixture of Hypoxanthine, Aminopterin and thymidine.

The unfused normal lymphocytes and the unfused myeloma lymphocytes fail to grow .

The Hybridoma Cells grow in the HAT-medium

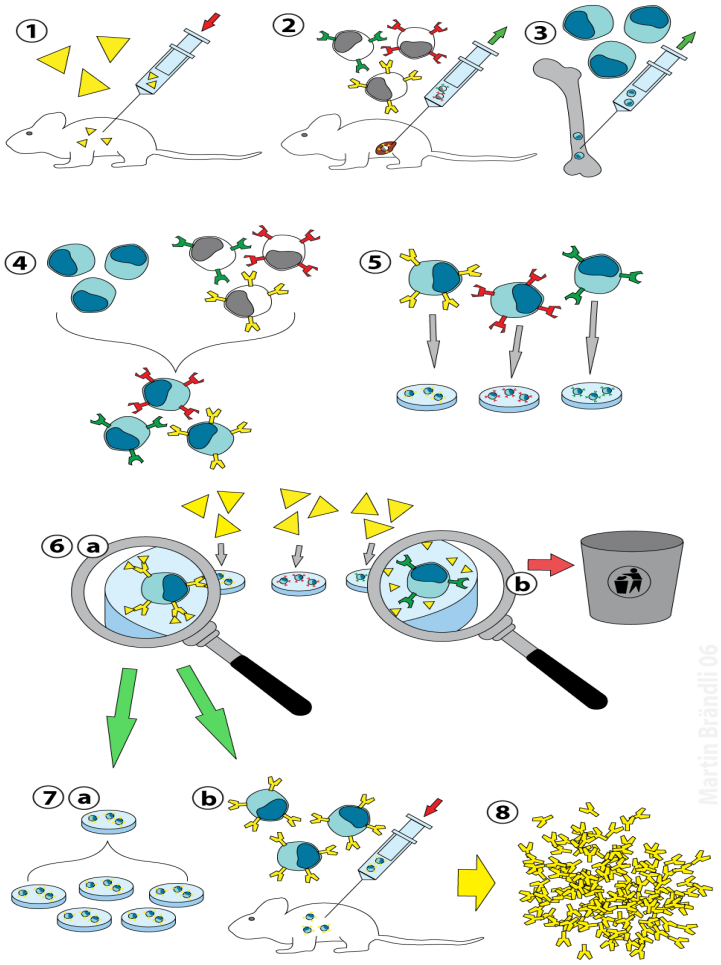
5. Isolation of a Hybridoam Cells

This is done by diluting a suspension of hybridoma Cells

The suspension of hybridoma cells is so diluted that the individual aliquots contain on an average one cell each. These cells , When grown culture media, produce the desire antibodies.

Mass of hydride cells are produced from a single parent hybridoma cell is Now examined to determine whether it produce the desired Monoclonal antibodies

Production of Monoclonal Antibodies



(1) Immunisation of a mouse

(2) Isolation of B cells from the spleen

(3) Cultivation of myeloma cells

(4) Fusion of myeloma and B cells

(5) Separation of cell lines

(6) Screening of suitable cell lines

(7) *in vitro* **(a)** or *in vivo* **(b)** multiplication

(8) Harvesting

APPLICATION OF HYBRIDOMA TECHNOLOGY

Serological:

Identification of ABO blood group

Diagnosis:

Detection of pregnancy by assaying of hormones with monoclonal.

Separation of one substance from a mixture of very similar molecules.

Immunopurification:

Purification of individual interferon using monoclonal.

Inactivation of T-lymphocytes responsible for rejection of organ transplants.

Therapy:

Removal of tumour cell from bone marrow.

Treatment of acute renal failure.

Treatment malignant leukemic cells, B cell lymphomas, and a variety of allograft rejections after transplantation.

IMMUNOTOXINS

- Immunotoxins are proteins that contain a toxin along with an antibody that binds specifically to target cells.
- All protein toxins are work by enzymatically inhibiting protein synthesis.
- Various plant & bacterial toxins have been genetically fused/chemically conjugated with the antibodies that bind to cancer cells.
- Plant toxins: ricin,abrin,modecin
- Bacterial toxins: diptheria and pseudomanas aeruginosa toxin A.

Applications of Monoclonal Antibodies

Diagnostic Testing

Once mAbs are produced for a specific substance, they can be then used to test for the presence of that substance in a vessel. This can includetoxins, drugs or hormones.

Pregnancy Testing

MAbs that have been developed to detect human chorionic gonadotropin (HCG) are now present in pregnancy test kits.

Radioimmunodetection (RID) of Cancer

An imaging technique used to detect the presence of cancerous or cancer-specific cells has been developed deploying radio-labelled antibodies, which can be produced as mAbs.

Radioimmunotheraphy (RIT) of Cancer

Similar to RID, RIT uses mAbs to specifically target antigen cells that are associated with tumours, and then blast these with a lethal dose of radiation, whilst minimising the level of radiation absorbed by normal cells.

Treatment of Cancer through Drugs

Many different drugs are being developed in clinical trials with the ultimate hope of being able to treat various strains of cancer. In fact, some of these are already on the market. In 1997, a drug named Ritoxin was approved by the FDA for commercial use which is based on mAb technology.

Viral Disease Treatment

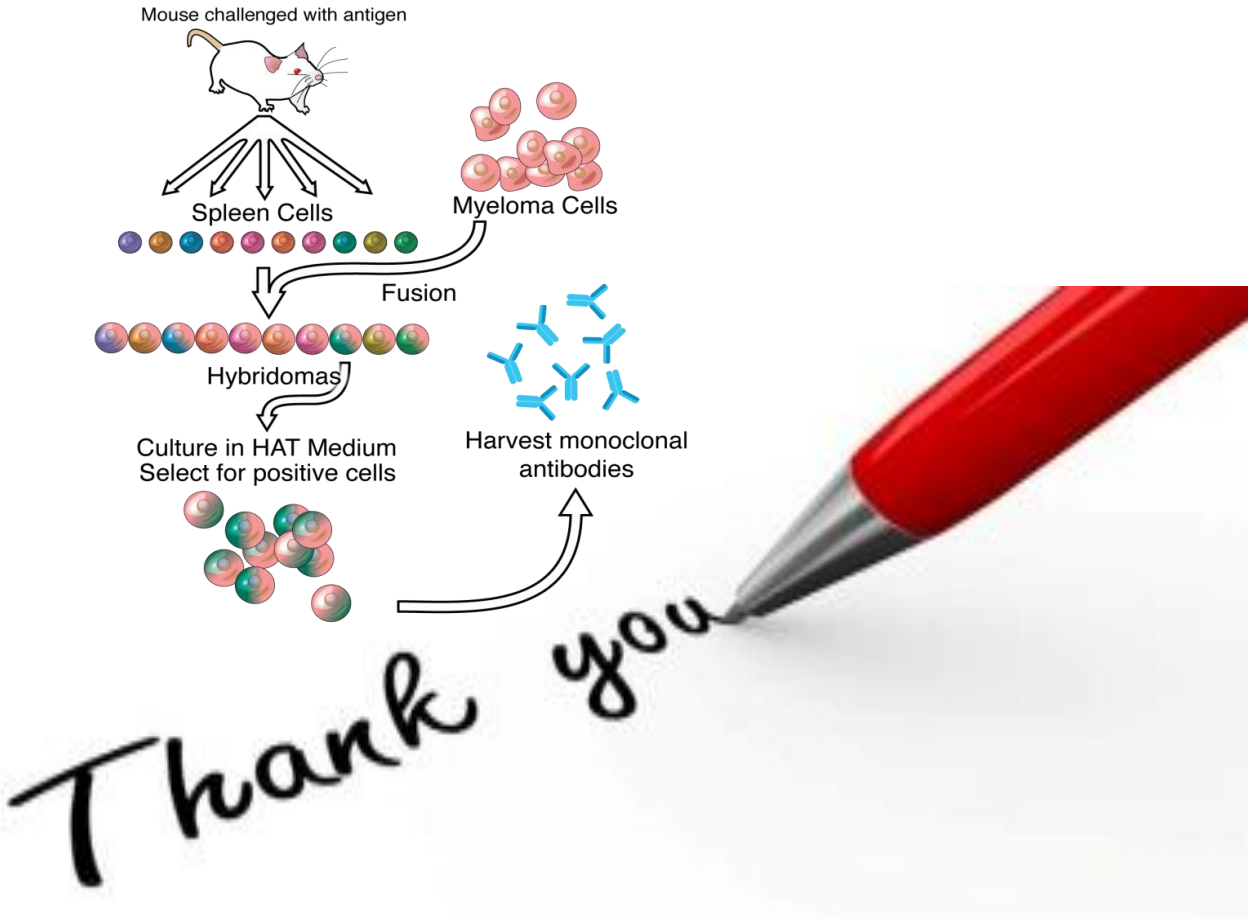
Doctors hope that with further research into mAbs and an increased knowledge of their properties, treatments will become available for diseases previously thought to be incurable, such as AIDS.

Identifying Pathogens

MAbs can now be used to identify strains of a single pathogen, for example *neisseria gonorrhoeae*.

Organ Rejection

A certain mAb named OKT3 (developed as an antibody to the T3 antigen) is able to be used to alleviate the effects and likelihood of organ rejection when transplanting new organs into a subject.


Mouse challenged with antigen
Spleen Cells
Myeloma Cells
Fusion
Hybridomas
Culture in HAT Medium
Select for positive cells
Harvest monoclonal
antibodies
Thank you