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PHARMACEUTICAL BIOTECHNOLOGY

UNIT 3

TOPIC :

- f) Hybridoma technology- Production, Purification and Applications

Hybridoma Technology

- Hybridoma Technology is a technique used for the production of Monoclonal Antibodies (mAbs) by fusing antibody-producing B-lymphocytes with immortal myeloma cells.
- It was developed by Georges Köhler and César Milstein in 1975.
- The technology enables the production of a large quantity of identical antibodies with high specificity.

Monoclonal Antibodies (mAbs)

- Monoclonal antibodies are identical antibodies produced from a single clone of B-lymphocytes.
- They recognize and bind to only one specific antigenic determinant (epitope).

Principle of Hybridoma Technology

- Hybridoma technology is based on combining the properties of two different cells:

B-Lymphocytes

- Produce specific antibodies
- Have limited lifespan
- Cannot survive for long in culture

Myeloma Cells

- Cancerous plasma cells
- Immortal in culture
- Do not produce antibodies

Fusion Result

Fusion of B-cells and myeloma cells produces hybridoma cells that possess:

- Antibody-producing ability of B-cells
- Immortality of myeloma cells

Thus, hybridoma cells continuously produce specific monoclonal antibodies.

Steps in Hybridoma Technology

1. Immunization

Procedure

- A mouse is injected with the target antigen.
- Booster doses are given at regular intervals.
- The immune system produces antigen-specific B-cells.

Purpose

To generate a large number of antibody-producing B-lymphocytes.

2. Isolation of B-Lymphocytes

Procedure

- The immunized mouse is sacrificed.
- Spleen is removed aseptically.
- B-lymphocytes are isolated from the spleen.

Importance

The spleen is rich in antibody-producing B-cells.

3. Preparation of Myeloma Cells

Characteristics

Myeloma cells are:

- Immortal
- HGPRT deficient
- Unable to survive in HAT medium

Purpose

Provide unlimited growth potential.

4. Cell Fusion

Procedure

B-cells and myeloma cells are fused using:

Polyethylene Glycol (PEG)

Fusion Mixture Contains

- Free B-cells
- Free myeloma cells
- Hybridoma cells
- Fused myeloma cells

- Fused lymphocytes

5. Selection of Hybridoma Cells

HAT Medium

Hybridoma cells are selected using:

HAT Medium

HAT =

- Hypoxanthine
- Aminopterin
- Thymidine

Principle of HAT Selection

- Aminopterin blocks de novo DNA synthesis.
- Cells can survive only through the salvage pathway.

Myeloma Cells

- HGPRT absent
- Cannot use salvage pathway
- Die

B-Lymphocytes

- Have HGPRT
- Short lifespan
- Eventually die

Hybridoma Cells

- Receive HGPRT from B-cells
- Receive immortality from myeloma cells
- Survive and multiply

6. Screening

Purpose

To identify hybridomas producing the desired antibody.

Methods

ELISA

(Enzyme Linked Immunosorbent Assay)

RIA

(Radioimmunoassay)

Only hybridomas producing the required antibody are selected.

7. Cloning

Purpose

To obtain a pure population of identical hybridoma cells.

Methods

Limited Dilution Method

Single cells are isolated and cultured separately.

Soft Agar Method

Individual colonies are isolated from agar medium.

8. Mass Production

Selected hybridomas are grown in:

Bioreactors

Large quantities of monoclonal antibodies are produced continuously.

Flow Chart of Hybridoma Technology

Antigen Injection into Mouse

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Formation of Specific B-Cells

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Isolation of Spleen Cells

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Preparation of Myeloma Cells

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Fusion Using PEG

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Hybridoma Formation

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Selection in HAT Medium

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Screening by ELISA/RIA

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Cloning

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Mass Production

↓

Monoclonal Antibodies

Purification of Monoclonal Antibodies

After production, monoclonal antibodies are mixed with contaminants such as:

- Cell debris
- Culture medium proteins
- DNA
- Lipids
- Serum proteins

Purification is necessary to obtain highly pure antibodies.

Objectives of Purification

- High purity
- High specificity
- Stability
- Therapeutic quality

Steps in Purification

1. Clarification

Procedure

Cell debris is removed by:

- Centrifugation
- Filtration

Purpose

Obtain clear antibody-containing solution.

2. Precipitation

Procedure

Antibodies are precipitated using:
Ammonium Sulfate

Purpose

- Increase antibody concentration
- Remove impurities

3. Affinity Chromatography

Most important purification step.

Principle

Antibodies bind specifically to ligands present in the chromatography column.

Advantages

- High purity
- High specificity

4. Ion Exchange Chromatography

Principle

- Separation based on electrical charge differences.

Purpose

- Removal of remaining proteins and impurities.

5. Size Exclusion Chromatography

Principle

Separation based on molecular size.

Purpose

Removal of:

- Aggregates
- Small contaminants

6. Dialysis / Buffer Exchange

Purpose

- Remove salts
- Remove chemicals
- Maintain suitable pH
- Improve stability

7. Sterilization

Procedure

Final filtration through:
0.22 µm membrane filter

Purpose

Ensure sterility of antibody preparation.

Characteristics of Monoclonal Antibodies

- Highly specific
- Bind to a single epitope
- Uniform structure
- Consistent activity
- Produced in unlimited quantities
- High purity
- Reproducible results

Applications of Hybridoma Technology

1. Diagnostic Applications

➢ Monoclonal antibodies are used in diagnostic tests such as:

Disease Detection

- HIV
- Hepatitis
- COVID-19

Pregnancy Testing

Detection of human chorionic gonadotropin (hCG).

ELISA Kits

Used for identification of infectious diseases.

2. Therapeutic Applications

➢ Monoclonal antibodies are widely used in treatment of:

Cancer

Targeted destruction of tumor cells.

Autoimmune Diseases

Examples:

- Rheumatoid arthritis
- Psoriasis
- Crohn's disease

Organ Transplantation

Prevention of transplant rejection.

3. Vaccine Development

Monoclonal antibodies help in:

- Identification of antigenic epitopes
- Vaccine design
- Development of subunit vaccines
- Evaluation of vaccine efficacy

4. Purification of Biomolecules

Used in affinity chromatography for purification of:

- Proteins
- Hormones
- Enzymes
- Vaccines

Advantages of Hybridoma Technology

- Produces highly specific antibodies
- Unlimited antibody production
- Uniform quality
- High purity
- Useful in diagnosis and therapy
- Large-scale production possible