Hybridoma Technology

Course Code: ZOOL-2023; Course Title: Immunology
Programme: B.Sc. Zoology (Hons.)

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Introduction

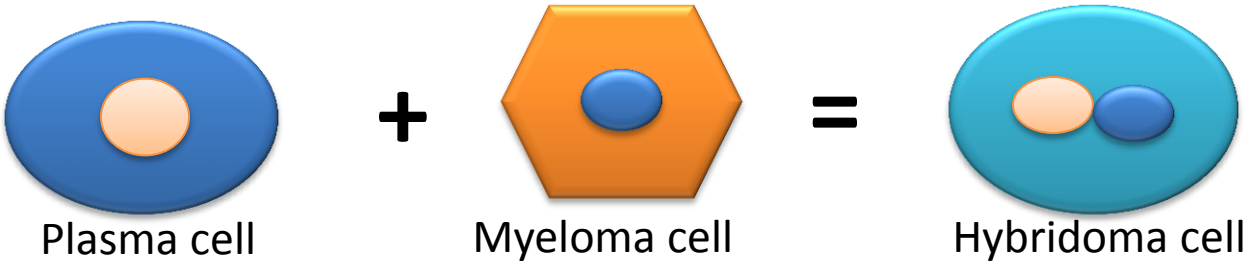
- In hybridoma technology, **monoclonal antibodies** are made out side the body by hybrid cell cultures known as **hybridomas**.
- Hybridomas are cells formed via fusion between a short-lived antibody-producing **Plasma cell** and an immortal **myeloma cell** (Cancer cell).

Plasma cell

- A naive B-lymphocyte immediately differentiate after encounters with an antigen into memory B-cell and effector B-cells called Plasma cell.
- Plasma cell produce secretory antibody.
- It live for only a few days.
- A single plasma cell can secrete more than 2000 molecules of antibody per second.

Myeloma cell

- Myeloma is a form of cancer that begins in the blood's plasma cells.
- Plasma cell transformed into malignant cell known as myeloma cell.
- Grow and spread uncontrollably.
- Immortal in character.
- Myeloma cells are also produce secretory antibody but impaired.

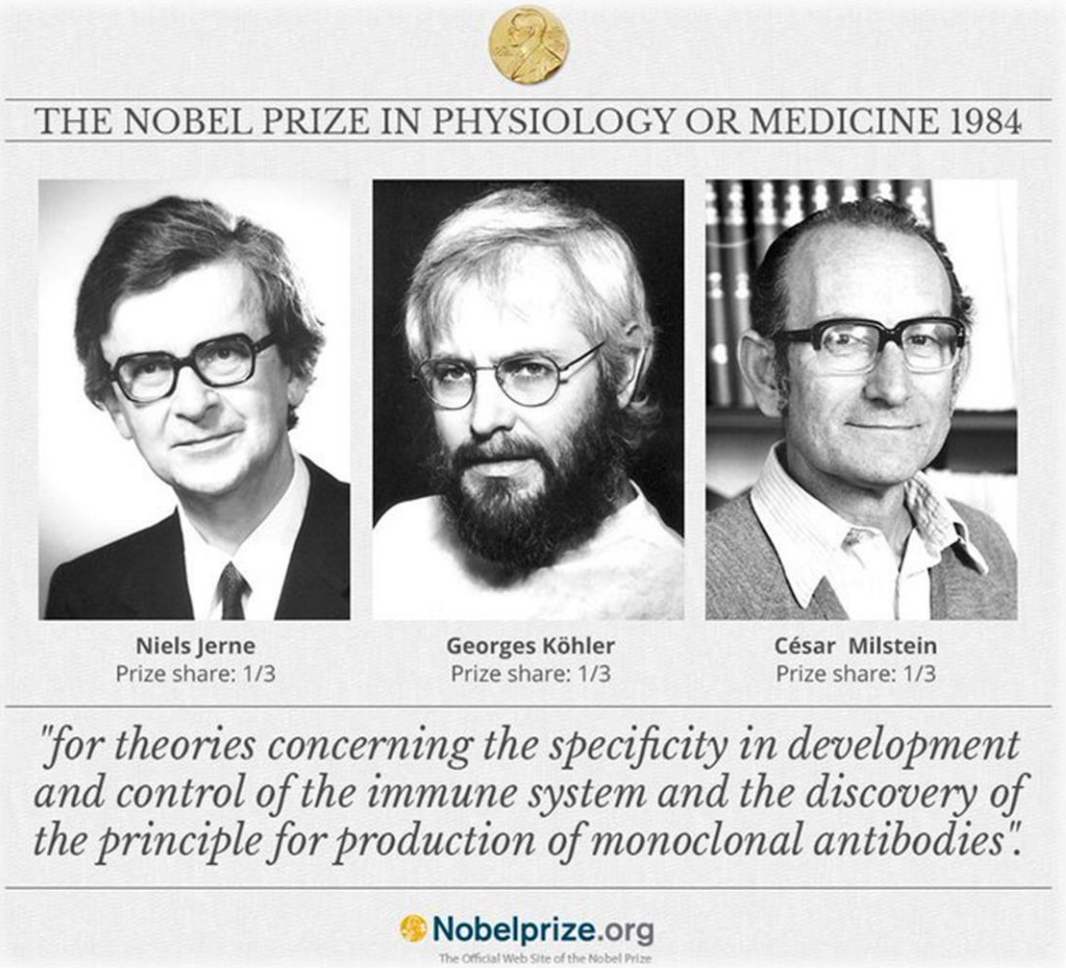


Brief History

- **In 1973**, Jeriod Schwaber and Ed Cohen (from Liribida institute, University of Chicago) – First to produce hybridoma cell through fusion of human B-lymphocyte cells and myeloma cells .

- **In 1975**, Georges Kohler and Cesar Milstein (from Medical Research Council Laboratory, Cambridge, UK)- construct a continuous cell lines, which secrete monoclonal antibodies of a desired specificity.

- **In 1984**, Kohler and Milstein awarded **Nobel Prize** jointly with Niels Jerne in Physiology and Medicine.



Principles

- The hybridoma technique involves the fusion of plasma cells (Effector B-Lymphocyte cells) harvested from spleen of mice, already immunized with immunogen, with a myeloma cell lines, which is capable of grow in animal cell culture medium.
- Fusogens (Electrofusion, PEG etc.) are used for fusion of cells.
- The spleen cells die in course of time in animal cell culture medium.
- The myeloma cells used for fusion are defective in HGPRT.
- In the presence of purine killer analogue 8-azaguanine, the HGPRT defective myeloma cells are selected for resistance.
- In the presence of HAT medium, the myeloma cells (defective HGPRT) die as aminopterin blocks the DNA synthesis pathway.
- Only hybrid cells (hybrid of plasma and myeloma cells) survive in HAT selection medium, since the myeloma cells provide the ability to grow in animal cell culture medium and plasma cells contribute the functional HGPRT enzyme necessary to overcome the aminopterin blocks.

PEG: Polyethylene glycol; **HGPRT**: Hypoxanthine Guanine Phosphoribosyl Transferase enzyme; **HAT**: Hypoxanthine, aminopterine and Thymidine

Basics of monoclonal and Polyclonal antibody

- Monoclonal antibodies are epitope specific and produced by the cloned plasma cells. However, the polyclonal antibodies are capable to bind different epitopes present on the single antigen.

- Monoclonal antibodies are homologous in population (mixture of similar antibodies), while polyclonal antibodies are heterogeneous in population (mixture of different antibodies).

- Monoclonal antibodies are produced through sophisticated hybridoma technology , while the polyclonal antibodies can be harvested directly from the serum of immunised animal.

- Production of monoclonal antibodies are time consuming, need trained manpower and costly, while polyclonal antibodies production is less time consuming and cheaper than monoclonal antibody.

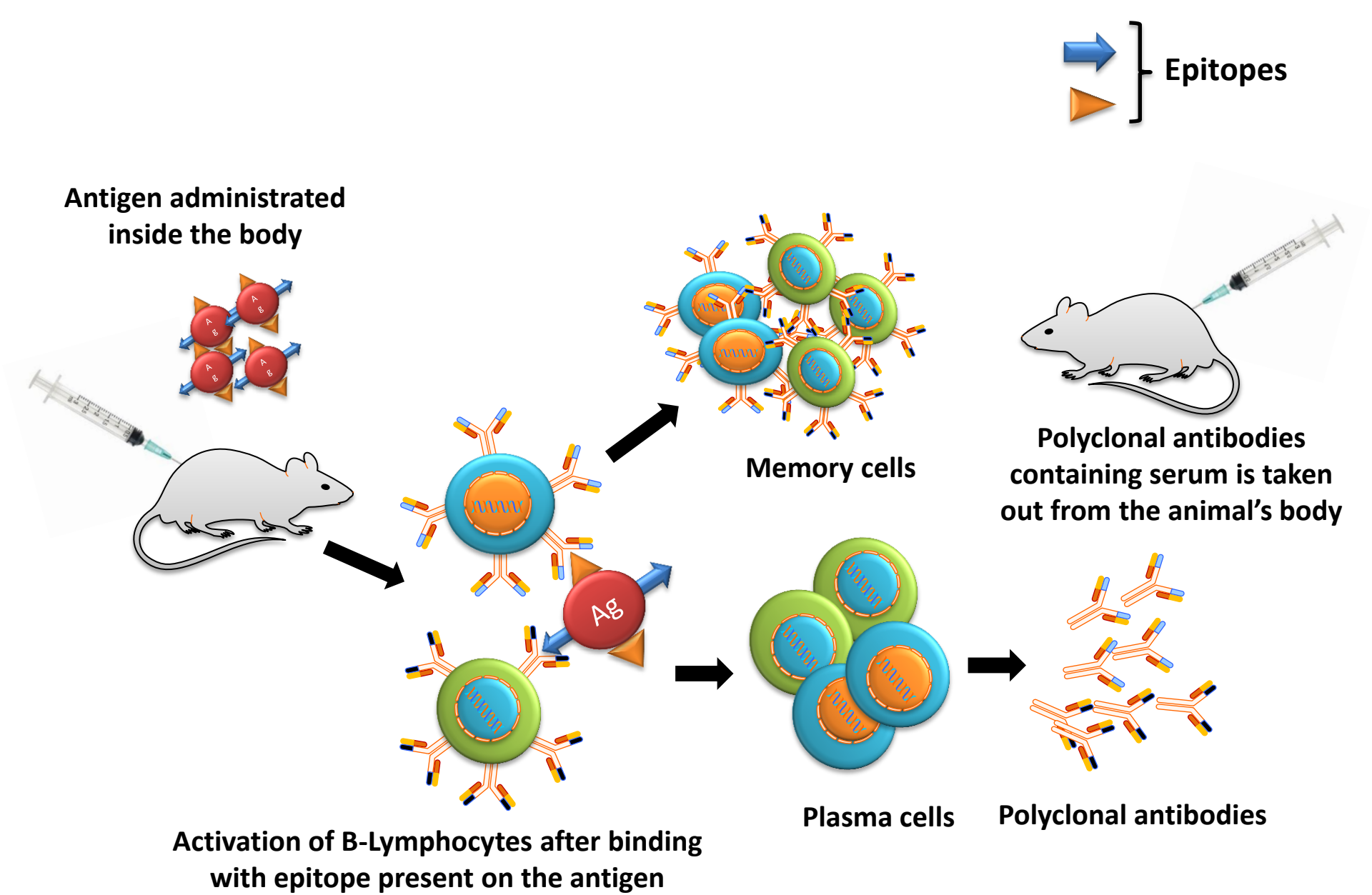


Diagram showing the production polyclonal antibodies.

General steps of hybridoma technology

1. Specific antigens (immunogens) are administrated into the mice and dissect spleen within 3-4 days of antigen administration.
2. Prepare free cell suspension from spleen.
3. Culture of splenocytes with pre-selected myeloma cell lines for cell fusion.
4. Selection of hybrid cells in HAT medium.
5. Screening of hybridoma monoclonal antibody with the help of ELISA, RIA, Immunofluorescence, flow cytometry etc.
6. Cloned hybrid cells are further expand in large quantity.
7. Production of monoclonal antibodies in large quantity.

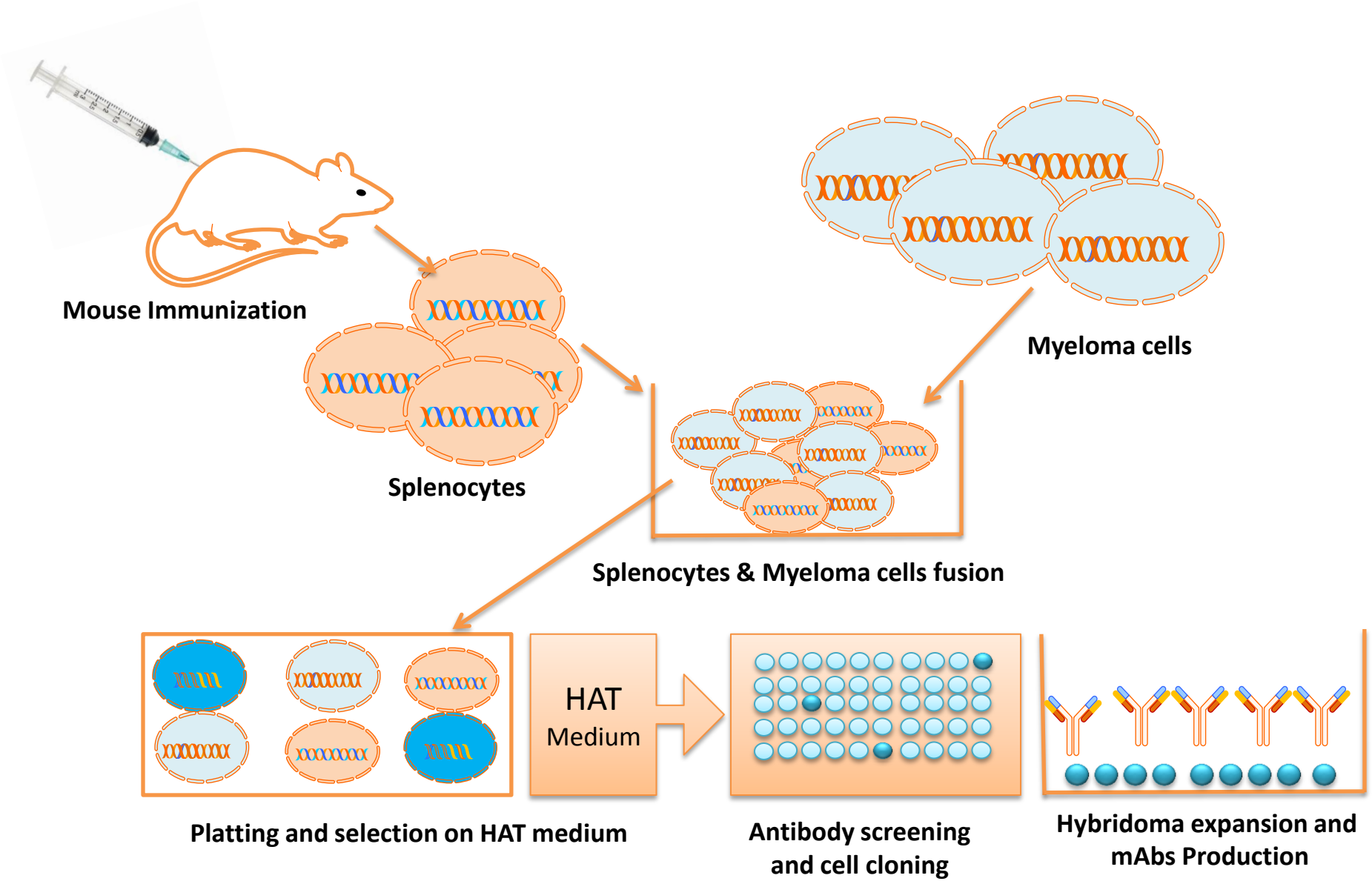


Diagram showing the steps of monoclonal antibody production using hybridoma technology

Basic concepts of HAT selection

- HAT medium consists of **H**ypoxanthine, **A**minopterine and **T**hymidine.
- Splenocytes (Plasma cells) are die in the HAT medium due to limited life span.
- Myeloma cells are also die in the HAT medium due to lack of HGPRT.
- Hybridoma cells are survive in the HAT medium because immortal character gain from myeloma cells and HGPRT positive character gain from splenocytes.
- HGPRT negative cells (Myeloma cells) are unable to use salvage pathway to synthesize purines.
- Myeloma cells cultured in HAT medium containing aninopterin that blocks de novo pathway of purine and pyrimidines biosynthesis.
- Due to HGPRT negative and presence aminopetrin in the HAT medium, myeloma cells are unable to synthesis purine and pyrimidines through de novo pathways as well as salvage pathway and finally dead during HAT selection.
- Splenocyte cells express HGPRT and TK (thymidine kinase) enzymes and able to synthesis purine and pyrimidine through salvage pathway due to presence of hypoxanthine and thymidine in HAT medium. However, the de novo pathways is blocked due presence of aminopterine in splenocyte.
- Hybridoma cells survive in the HAT medium because the synthesis purine and pyrimidine through salvage pathway and immortal in nature.

Applications of Monoclonal antibodies

- **Immunotherapy of cancer:** FDA (US) approved more than 25 mAb approved for the treatment of various type of cancer such as rituximab, cetuximab, pertuzumab etc.
- Extensively used in disease diagnosis and testing kit such as pregnancy etc.
- Molecular farming
- Used in vaccine production.
- Used in protein purification and other biomolecules.
- Drug targeting
- Used as enzyme (abzyme) and many more.

Thanks