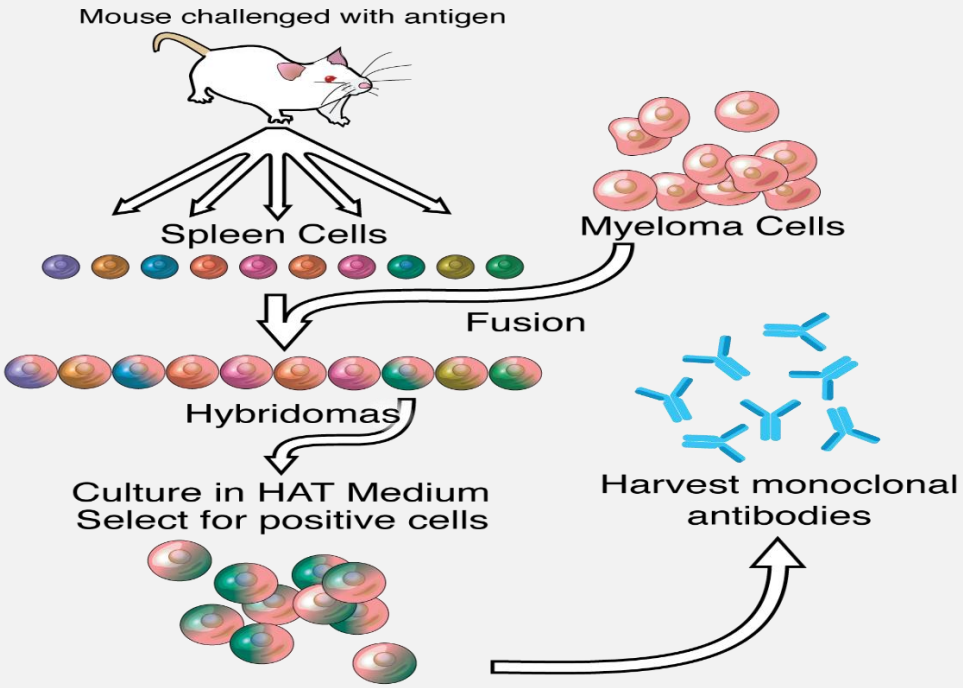
MONOCLONAL ANTIBODIES

- Monoclonal antibodies are lab-made proteins designed to target and bind to a single, specific antigen, such as those found on cancer cells or viruses.
- They are produced from a single clone of a B cell, which allows for the creation of identical antibodies with a high degree of specificity.
- These antibodies can be used as a type of targeted therapy to block signals, deliver harmful substances, or help the immune system recognize and destroy disease-causing cells.



1. Definition

- **Monoclonal antibodies (mAbs)** are **identical immunoglobulin molecules** produced by a single clone of B lymphocytes (plasma cells) that recognize and bind to **one specific epitope** on an antigen.
- Each monoclonal antibody is **monospecific**, **homogenous**, and **highly reproducible**.

2. Background

- **Discovered by:** Georges Köhler and César Milstein (1975).
- **Nobel Prize in Physiology or Medicine (1984)** awarded to Köhler, Milstein, and Niels Jerne.

3. Basic Concept

When an antigen enters the body, multiple B-cell clones respond — producing **polyclonal antibodies** (mixture). However, by isolating a **single B-cell clone** that produces one specific antibody and fusing it with a **myeloma (cancer) cell**, we obtain **hybridoma** cells that:

- Live indefinitely (immortal)
- Continuously secrete one specific antibody

4. Properties of Monoclonal Antibodies

Property	Description
Specificity	Recognize a single epitope on an antigen
Homogeneity	Identical antibody molecules
Reproducibility	Can be produced indefinitely from hybridoma line
Purity	Free from contamination by other antibodies
Applications	Diagnosis, therapy, immunoassays, research

5. Production of Monoclonal Antibodies (Hybridoma Technology)

A. Principle

Fuse a **specific antibody-producing B cell** with a **myeloma cell** (immortal cell line) → form a **hybridoma** → clone and select for desired antibody production.

B. Steps in Production

Step 1: Immunization

- Inject a **mouse (or other animal)** with the desired **antigen**.
- Wait for immune response → spleen rich in **B cells** producing antibodies.

Step 2: Cell Fusion

- Isolate **spleen cells** (antibody-producing B cells).
- Fuse with **myeloma cells** (cancerous plasma cells lacking HGPRT enzyme) using **polyethylene glycol (PEG)**.

Step 3: Selection in HAT Medium

- Culture fused cells in **HAT (Hypoxanthine–Aminopterin–Thymidine) medium**:
  - **Aminopterin** blocks de novo DNA synthesis.
  - Only hybridoma cells survive (use salvage pathway from B cells; immortality from myeloma cells).

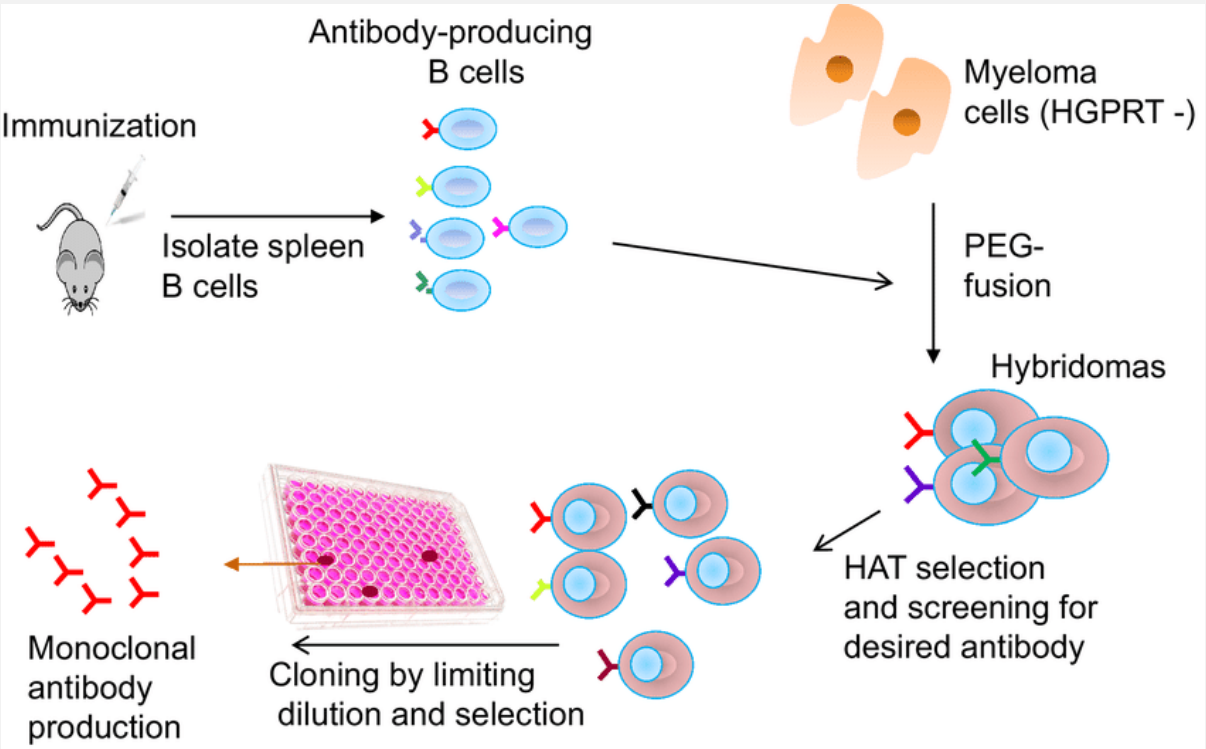
Step 4: Screening & Cloning

- Screen supernatants for desired antibody using **ELISA or RIA**.
- Clone positive hybridomas by **limiting dilution**.

Step 5: Large-Scale Production

- Grow selected hybridoma clones in:
  - Tissue culture (in vitro)
  - Ascitic fluid (in vivo)
- Purify antibody using **affinity chromatography**.

C. Overview Diagram (Text Summary)



6. Applications

A. Diagnostic

- Detection of hormones, enzymes, pathogens, or tumor markers:
  - ELISA, Western blot, Immunofluorescence, Immunohistochemistry.
  - Example: Pregnancy test (hCG detection), Troponin assays.

B. Therapeutic

- **Cancer therapy:** Rituximab (CD20), Trastuzumab (HER2), Bevacizumab (VEGF)
- **Autoimmune diseases:** Infliximab (anti-TNF), Adalimumab (anti-TNF), Natalizumab (anti-integrin)
- **Transplant rejection:** Basiliximab (anti-IL-2R)
- **Infectious diseases:** Palivizumab (anti-RSV)
- **COVID-19:** Casirivimab + Imdevimab (anti–SARS-CoV-2)

C. Research

- Cell typing (flow cytometry, immunophenotyping)
- Receptor localization
- Protein purification (affinity columns)

7. Recent Advances

- **Bispecific antibodies:** Bind two different antigens (e.g., Blinatumomab — targets CD3 and CD19).
- **Antibody-drug conjugates (ADCs):** mAb linked to cytotoxic drug (e.g., Brentuximab vedotin).
- **Nanobodies:** Single-domain antibodies from camelids — smaller, more stable.
- **CAR-T cell therapy:** T cells engineered with antibody-derived antigen-recognition domains.