

# Immunology

## Lec. 7

### **Monoclonal Antibodies**

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- **Monoclonal antibodies** (MAbs) are an old immunological tool with applications in the fields of immunology, biotechnology, biochemistry, and applied biology. Production of monoclonal antibodies using hybridoma technology was discovered in 1975 by Georges Kohler of West Germany and Cesar Milstein of Argentina.

A monoclonal antibody is the antibody produced by a single clone of B lymphoid cells: all the antibody molecules have the same amino acid sequence and hence the same binding properties. A myeloma is a tumor of antibody-secreting cells, apparently derived from a single cell, and is thus a clone of cells producing a monoclonal antibody.

## **Synthesis and Production of Monoclonal Antibody:-**

- **Step 1: Immunization of Mice and Selection of Mouse Donors for Generation of Hybridoma Cells.**

Mice are immunized with an antigen that is prepared for injection either by antigen with Freund's adjuvant or other. Intact cells, whole membranes, and microorganisms are sometimes used as immunogens. In almost all laboratories, mice are used to produce the desired antibodies.

- **Step 2: Screening of Mice for Antibody Production**

Screening of Mice for Antibody Production After several weeks of immunization, blood samples are obtained from mice for measurement of serum antibodies. Serum antibody titer is determined with various techniques, such as ELISA technique and flow cytometry.

- **Step 3: Preparation of Myeloma Cells**

Fusing antibody-producing spleen cells, which have a limited life span, with cells derived from an immortal tumor of lymphocytes (myeloma) results in a hybridoma that is capable of unlimited growth. Cells must have high viability and rapid growth.

- **Step 4: Fusion of Myeloma Cells with Immune Spleen Cells**
- Single spleen cells from the immunized mouse are fused with the previously prepared myeloma cells. Only fused cells will grow in the special selection medium. Feeder cells are believed to supply growth factors that promote growth of the hybridoma cells.

- **Step 5: Cloning of Hybridoma Cell Lines by “Limiting Dilution” or Expansion and Stabilization of Clones by Ascites Production**

At this step new, small clusters of hybridoma cells from the 96 well plates can be grown in tissue culture followed by selection for antigen binding or grown by the mouse as cites method with cloning at a later time. The hybrid cells clones are generated from single host cells, the antibodies secreted by the differ-ent clones are then tested for their ability to bind to the antigen using an enzyme-linked immunosorbent assay (ELISA).

# Monoclonal Antibody Production

