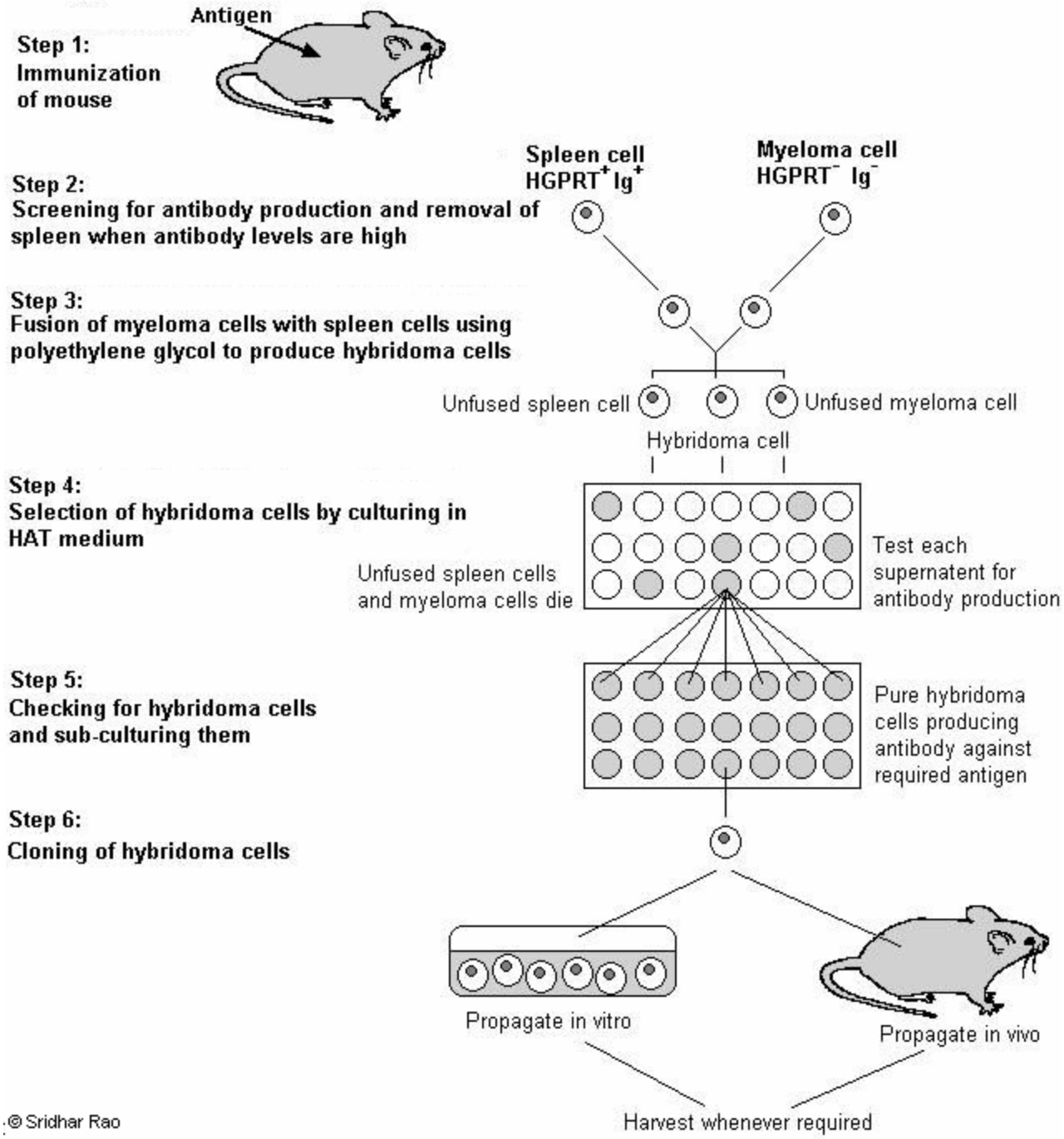
Sridhar Rao P.N
Assistant Professor
Dept. of Microbiology
JJMMC, Davangere
www.microrao.com

MONOCLONAL ANTIBODY (mAb):

Normal B lymphocytes and plasma cells do not survive for long or secrete significant quantities of antibodies in tissue culture. However there is a class of malignant B cell tumours called myelomas that can be propagated indefinitely in tissue culture and will proliferate rapidly, often secreting large quantities of immunoglobulins. Monoclonal antibodies are made by fusing antibody-secreting B cells with myeloma cells. These fused cells now become immortal (they will grow and divide indefinitely) and are called hybridoma. The hybridoma cells will secrete monoclonal antibodies. This technique was introduced by Kohler and Milstein in 1975 and were awarded Nobel Prize.



Procedure:

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 emulsifying the antigen with Freund's adjuvant or other adjuvants. In general, mice are immunized every 2-3 weeks.
Step 2: 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 enzyme-linked immunosorbent assay (ELISA). When the antibody titer is high enough, the mice are euthanized and their spleens removed for in vitro hybridoma cell production.
Step3: Fusion of Myeloma Cells with Immune Spleen Cells
Spleen cells from the immunized mouse are fused with the previously prepared myeloma cells. Fusion is accomplished by a technique called somatic cell hybridization. This is achieved by co-centrifuging freshly harvested spleen cells and myeloma cells in polyethylene glycol, a substance that causes cell membranes to fuse.
Step 4: Selection of hybridoma cells
Those myeloma cells that have lost the ability to synthesize any antibody molecules of their own and lack enzyme hypoxanthine-guanine-phosphoribosyltransferase (HGPRT) are selected. They are both $HGPRT^-$ and Ig^-. This enzyme enables cells to synthesize purines using an extracellular source of hypoxanthine as a precursor. Ordinarily, the absence of HGPRT is not a problem for the cell because cells have an alternate pathway that they can use to synthesize purines. However, when cells are exposed to aminopterin, they are unable to use this other pathway and are now fully dependent on HGPRT for survival. The mouse derived B cells have this enzyme and can produce antibodies too. They are $HGPRT^+$ and Ig^+. The cells are then placed in HAT (hypoxanthine, aminopterin, thymidine) medium. Since the unfused myeloma cells lack HGPRT and aminopterin disallows alternative pathway, they die. Unfused B Cells are mortal and cannot proliferate, so they too die. Only the hybridoma cells that have the ability to multiply immortally and possess HGPRT will survive. The HAT medium allows only the fused cells to survive in culture.
Step 5: Checking for hybridoma cells
The supernatants from each culture are tested to find those producing the desired antibody. Since there may be more than one hybridoma cell in the original cultures, single cells from each antibody-positive culture must be isolated and subcultured. The supernatant of each must be tested for the desired antibodies.
Step 6: Cloning of Hybridoma Cell Lines
There are two methods for growing these cells: injecting them into the peritoneal cavity of a mouse or using in vitro cell-culture techniques. When injected into a mouse, the hybridoma cells multiply and produce fluid (ascites) in its abdomen; this fluid contains a high concentration of antibody. The other alternative is to grow hybridoma cells in a tissue-culture medium. Further processing of the mouse ascitic fluid and of the tissue-culture supernatant might be required to obtain mAb with the required purity and concentration.

Application of monoclonal antibodies:

- Monoclonal antibodies are widely used as diagnostic and research reagents. They are used in diagnostic kits such as ELISA, Immunofluorescence to diagnose various diseases.
- Enumeration of human lymphocyte subpopulations, anti-CD3 identifies all mature T lymphocytes, anti-CD4 identifies helper T lymphocyte subset, anti-CD8 identifies cytotoxic T lymphocyte subset.
- Treatment with a cocktail of anti-CD3 monoclonal antibody and complement kills T cells in human bone marrow before transplantation, thus minimizes graft versus host reaction.
- Immunosuppression: anti-CD3 depresses T cell function and anti-CD4 induces tolerance.
- Passive immunization: High titre antimicrobial human monoclonals can passive protection.
- Blood grouping: anti-A monoclonal provides a more reliable standard reagent than conventional antisera.
- Diagnosis in cancer: Monoclonal anti-T acute lymphocytic leukemia (ALL) allows differentiation from non T-ALL.
- Imaging: Radioactive anti-carcinoembryonic antigen used to localize colonic tumours or secondary metastases by scanning.
- Treatment of cancers: monoclonal antibody is coupled to a strongly-radioactive atom, such as Iodine-131 to aid in killing the target cancer cells.
- Purification of antigen: Isolate antigen from mixtures by monoclonal affinity chromatography.
- Chimeric monoclonal antibodies, which contain human Fc portion, are more useful for human use.