HYBRIDOMA TECHNOLOGY

MONOCLONAL ANTIBODY (MAb)

What is Hybridoma technology?

Hybridoma technology is a well-established method to produce monoclonal antibodies (mAbs) specific to antigens of interest. Hybridoma cell lines are formed via fusion between a short-lived antibody-producing B cell and an immortal myeloma cell. Each hybridoma constitutively expresses a large amount of one specific mAb, and favoured hybridoma cell lines can be cryopreserved for long-lasting mAb production.

What is mAb?

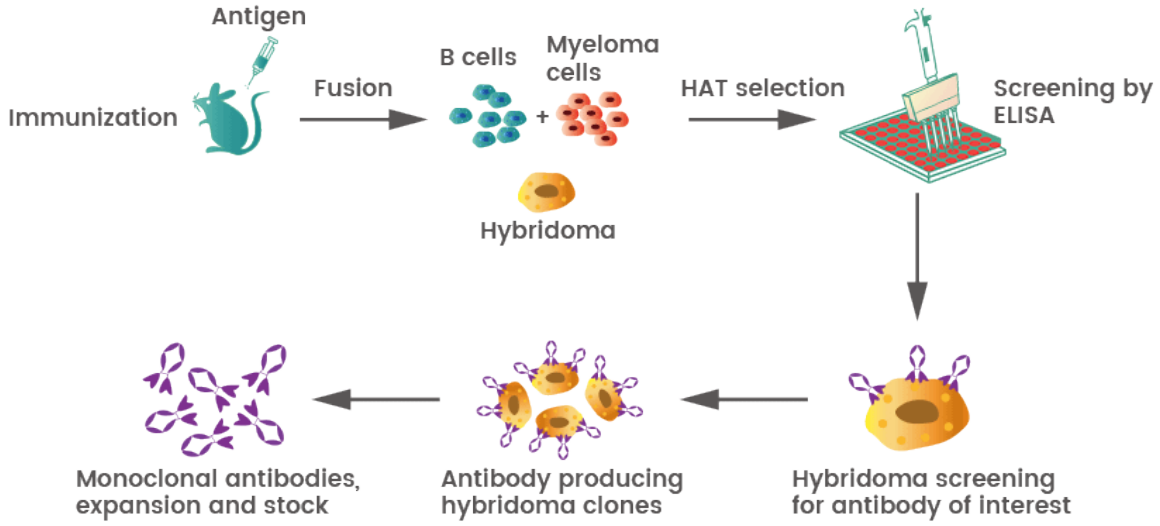
Antibody against a specific epitope is call monoclonal antibody (mAb). The are made by identical immune cells that are all clones of a unique parent cell.

Production of Monoclonal Antibody

In 1975 George Kohler and Casar Milstein (Nobel Prize- 1984) developed a technique to produced antibody specific against an epitope from a hybrid cell that grows continuously.

Principle:

Normal B lymphocyte produced antibody of a single specificity but can't grow indefinitely. So it is necessary to immortalize B cells that produce a specific antibody. This is achieved by cell fusion of somatic cell hybridization between a normal antibody producing B cell and a myolema cell. Such fusion derived immortalized antibody producing cell lines are called hybridomas and the antibodies they produced are monoclonal antibodies.



Steps:

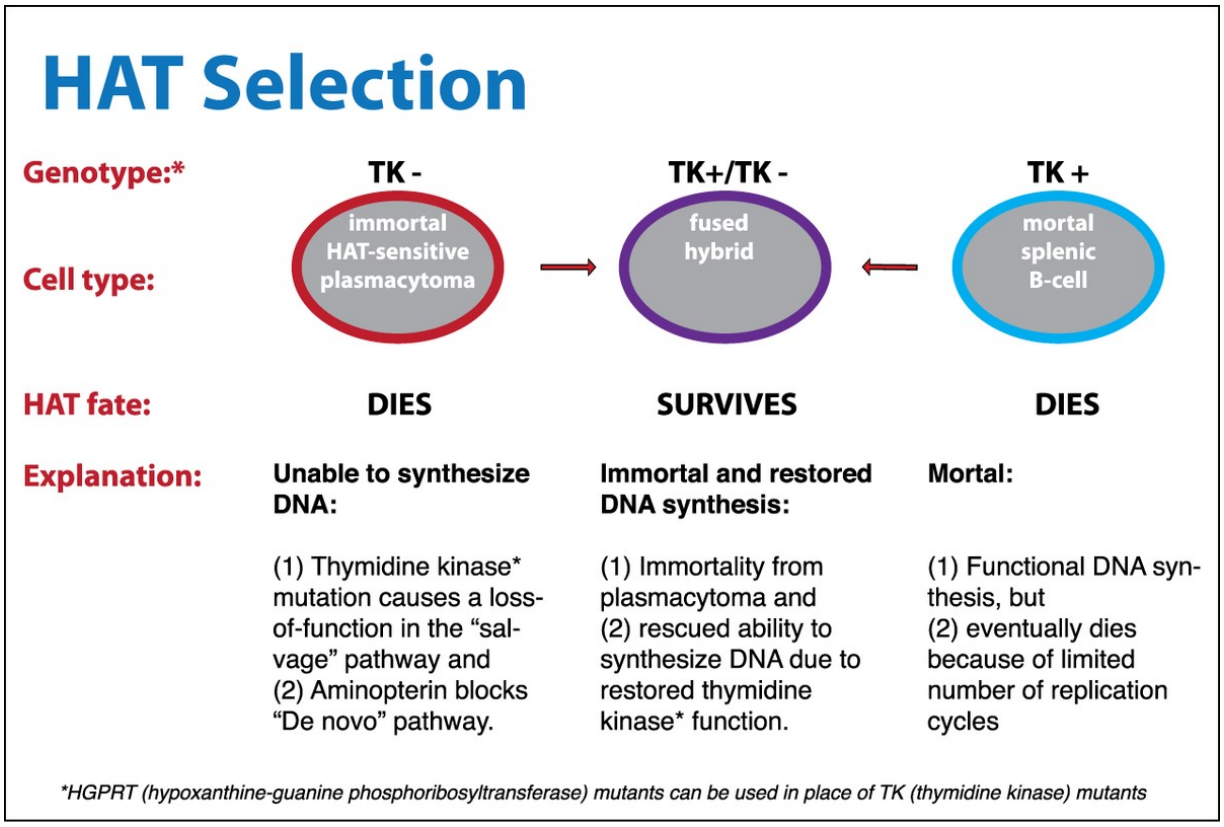
1. Fusion between myeloma cells, which are immortal, do not produced hypoxanthine-guanine phosphoribosyl transferase ($HGPRT^-$) and spleen cell, which are $HGPRT^+$, mortal and secretes antibody.
2. Fusion is achieved by incubating a suspension of two cell types with fusogenic agent like inactivated sendai virus or polyethelyne glycol (PEG).
3. In the process of cell fusion, first the cell membrane and cytoplasm of the two cells become confluent keeping the two nuclei intact known as heterokaryons. Some heterokaryons become hybrid upon fusion of nuclei.
4. Only a small number of cells actually fused. So in the culture mixture of unfused cells (B cell, Myeloma cells), fused with like cell (B cell – B cell, myolema - myolema) and B Cell-myeloma fusion product obtained.
5. The cells are transferred into HAT medium (hypoxanthine, aminopterin and thymidine) where only fused B cell-myeloma cells are survived. This antibody secreting cells are immortal for the continuous production of monoclonal antibody in cell culture.

Selection of Clone Choice:

From Ab-secreting hybridomas, pure clones must be screened for desired Ab-specificity. But there are some clones which produce Ab of unwanted specificities. ELISA & RIA techniques are used for screening, which clone is producing desired antibody.

Propagation of the Desired Clone:

i. Tissue culture: MAb is cultured for indefinite period in culture medium. Ab is secreted into the medium into the medium in a low concentration (10-100 micro gram/ml).
ii. Mice: Hybridoma cells propagated in the peritoneal cavity of histocompatible mice. It secrete Ab in higher concentration (1-25 mg/ml).



Application of Monoclonal Antibody

In Vitro Diagnosis:

1. Diagnosis of much systemic and infectious disease release on the detection of particular antigen or antibody in circulation or in tissue by use of MAbs in immunoassays.
2. These are also used in some other regular clinical diagnosis like blood grouping, pregnancy tests, measuring the level of various drugs etc.

In Vivo Diagnosis:

3. Radio labelled MAbs can be used in vivo for location of tumor antigen.
   e.g.- MAbs to breast Cancer cells are level with I^{131} and introduced into blood to detect the spread of tumor.

Therapy:

4. It helps to identify MHC gene. Micro-organisms are identified in clinical diagnosis.