Hybridoma technique

Hybridoma technology is the most favored and efficient method for exploring and **producing monoclonal antibodies (mAbs)**. At present, The majority of the mAbs approved by the US-FDA are manufactured utilizing hybridoma technique. It is introduced by Kohler and Milstein in 1975 and got the Nobel prize in 1984. OKT3 (muromonantibody) against CD-3 is the first mAb approved by FDA in 1985, is used to reduce graft rejection in organ transplantation.

Procedure

1. Immunogenic induction

Rodents are given multiple doses of the desired **antigen** and **adjuvant** mixture. Generally, A primary injection is given to mice, followed by two booster doses after 14 and 28 days. Human and mouse antibodies have **structural similarities**, which is one of the reasons for their high acceptability.

2. Harvesting of splenic B-cells

The mice are euthanized and the spleen is harvested to receive B-cells after 5 to 7 days of the final booster dose.

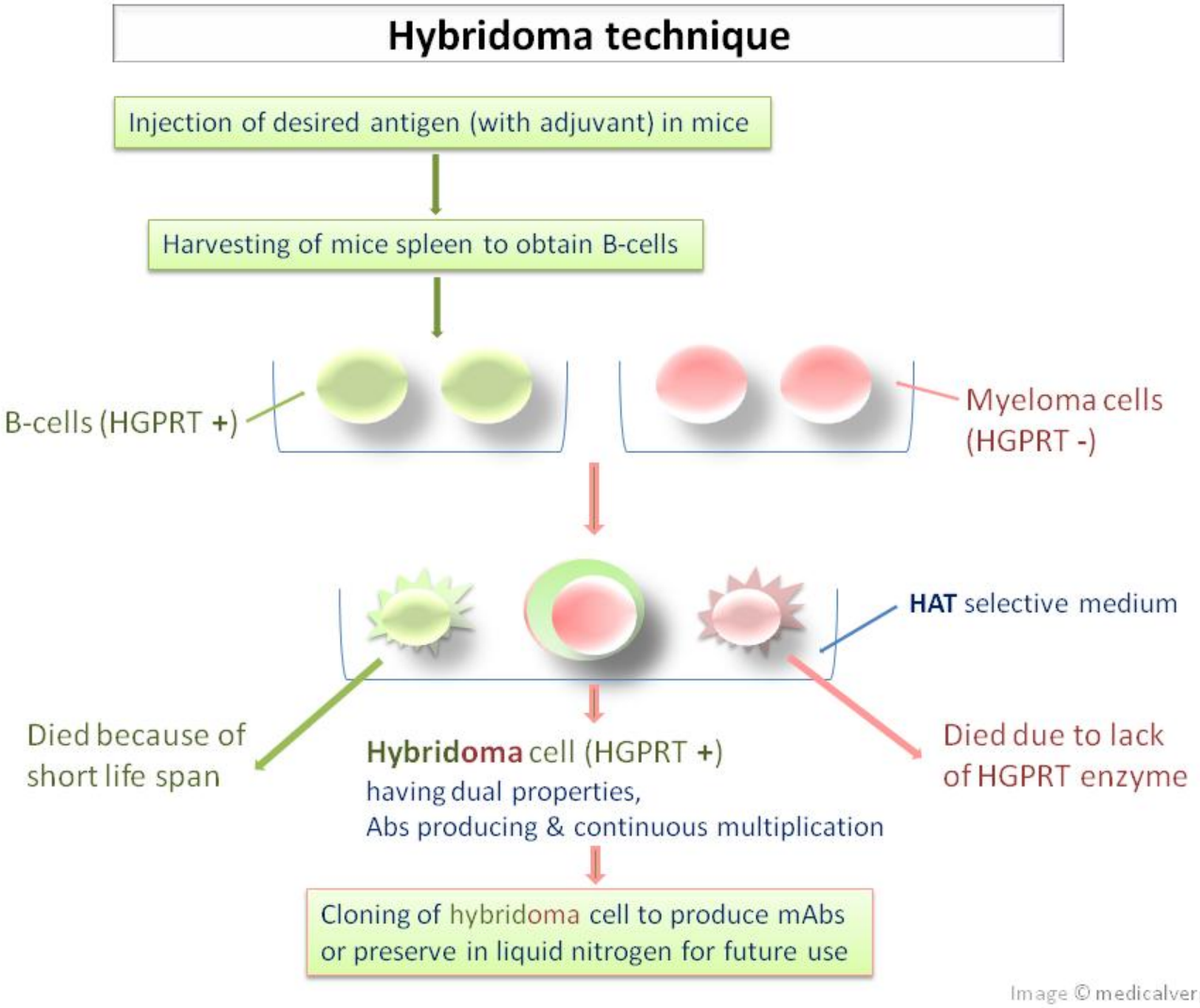
3. Procurement of myeloma cells

HGPRT-negative myeloma cell line of the same species is obtained from the culture collection library, e.g. ATCC (American type culture collection). Most commonly used myeloma cell lines are X63-Ag8.6539 and SP2/0-Ag1410.

4. Fusion of B-cells with myeloma cells

The spleen cells (B-cells) are mixed with the immortalized myeloma cells in the presence of fusogenic agents like viruses, chemicals (e.g. PEG) and electric pulses. During centrifugation,

PEG (polyethylene glycol) or other fusogenic agents fuse the plasma membranes of neighbouring cells, resulting in cells with two or more nuclei. Individual chromosomes are segregated into daughter cells during mitosis and subsequent rounds of division. During this step, chromosomes are often lost, making fusion unsuccessful. Just 1% of starting cells are fused in even the most efficient hybridoma fusions, and only 1 in 10^5 forms viable hybrid.



5. Selection of hybridoma cells (using HAT medium)

Nucleotide synthesis takes place in two ways in cells: de novo and salvage. Any of these pathways will allow any cell to live. **Aminopterine inhibits de novo** nucleotide (both purine and pyrimidine) synthesis in the **HAT** (hypoxanthine Aminopterine Thymidine) selective culture

medium. As a result, cells depend on the **salvage** pathway, which requires the **HGPRT enzyme** (hypoxanthine guanine phospho-ribosyl transferase). Hypoxanthine and thymidine serve as salvage pathway precursors. Unfused myeloma cells die due to a lack of the HGPRT gene, while unfused B cells die due to their short life span. Thus, only hybrid cells survive and preserve dual properties, antibody secreting property of B-cells and continuously dividing property (immortality) of myeloma cells.

6. Screening of hybridoma cells

Screening is done by using the **ELISA** technique, to ensure appropriate **specificity** of antibodies generated by the hybridoma. Non-antibody producing hybrid can overgrow the antibody generating hybrid, which is slower to grow comparatively. Hybrid in positive wells generate specific antibodies against test antigen. After identifying the clone producing the desired antibodies, further expansion of clone is done by '**limited dilution cloning**.'

7. Utilization of hybridoma clone

Hybridoma clones may be used for antibody manufacturing or experimental purposes after further expansion, or they can be preserved in liquid nitrogen for future use. The cells are screened by ELISA after each cloning to ensure that antibodies are still being produced.

Since mAbs are a biological product, there is a high chance of variation in mAbs produced by different manufacturers' laboratories. As a result, the regulatory principle of '**biosimilar**' or 'similar biologics' is used to ensure that a biological product produced by any manufacturer is comparable in terms of quality, safety, and efficacy to a reference product/innovator product authorized by the FDA and ICH.

Issues and progress in human hybridoma

- The human body may **recognize murine mAbs** as foreign elements. As a result, antibodies are produced against mAbs, which leads to **decreased efficacy** and allergy. As a consequence of the antigen-antibody complex deposition of the glomerular basement membrane, complications such as glomerulonephritis can occur.

- Due to a lack of a suitable **myeloma partner**, human hybridoma development is slow, although some lines have been isolated and are now in use.

- Obtaining antigen-primed B-cells from **human spleen** is a challenge. B-cells from peripheral blood have to be used to make hybridomas, which are less suitable for fusion.

- Owing to **ethical concerns**, human volunteers cannot be hyperimmunized to a wide variety of antigens.

The following approaches have been attempted with limited success to meet these challenges:

- The antigen priming of B-cells **in vitro** has been attempted, but it is not only inefficient for mass production of mAbs, but it also produces low affinity IgM only.

- **EBV** (Epstein-Barr virus) **transformed** Ab secreting **cells** were used, but since they are not malignant cells, they cannot multiply indefinitely and are difficult to clone. They are unable to produce substantial amount of mAbs. To overcome this limitation, researchers have tried fusing EBV-transformed cells with mouse myeloma cells as a **hetero-hybridoma**.

- **Transgenic mice**, such as HumAbs mice, were created through genetic engineering, which contain the human **immunoglobulin gene** in place of the corresponding loci of the murine immunoglobulin gene. This is how *canakinumab* was developed.

- The **phage display technique** was recently invented. A **human variable region** sequence is inserted into the **coat** protein of a **bacteriophage**, allowing the phage to display the antigen binding site on its coat. To make a complete human mAb, the desired variable region gene is combined with the gene encoding the human constant region. This is how *adalimumab* was created.