HYBRIDOMA TECHNOLOGY

This technology is used for the production of monoclonal antibodies. These are antibodies which are produced by a single clone of lymphoid cells either invitro or invivo. Monoclonal antibodies exhibit high affinity for a specific Ag and therefore can be used as therapeutic agents and also in diagnostic kits. B lymphocytes under invitro conditions unfortunately cannot produce antibodies indefinitely. To make it happen the clone is usually derived by fusing a sensitized spleen cell and a continuously growing myeloma cell. This hybrid cell is called as hybridoma and the technique is called as hybridoma technology. This technique involves somatic hybridization of the spleen cell and myeloma cell using polyethylene glycol (PEG). Some fraction of cells fuse and progress to nuclear fusion and a proportion of these fused cells progress through mitosis, such that both sets of chromosomes replicate together and a hybrid is formed. Insome interspecific hybrids Eg: human-mouse, one set of chromosomes is gradually lost and genetic recombination's is possible invitro leading to a functional hybridoma cell. These hybrids retain attributes characters of both cancerous cells and B-cells based on which they may be selected.

Procedure: Committed B-cells are isolated form mice spleen. These mice are repeatedly injected with Ag of interest for about 2-3 weeks, their serum is tested for the presence of Ab's and then spleen B-cells are isolated from them.

A cell suspension of both myeloma cells and B-cells is made and mixed with 35% PEG for few minutes and then transferred to a growth medium containing Hypoxanthine, Aminopterin and Thymidine (HAT medium). Polyethylene Glycol promotes cell fusion, but the fusion events are rare and random. Therefore in the mixture there will be myeloma cell, spleen cells, myeloma-spleen fusion cells, myeloma-myeloma fusion cells and spleen-spleen fusion cells. HAT medium however promotes growth of Myeloma-Spleen fusion cells.

Proliferation of spleen-spleen fusion cells is inhibits by aminopterin which blocks endogenous synthesis of purines and pyrimidines. Hence they are unable to synthesize DNA and therefore die.

Myeloma cells and myeloma-myeloma fusion cells which are $HGPRT^-$ die as they cannot synthesize guanine and adenine from its precursor hypoxanthine.

The spleen-myeloma fusion cells survive in HAT medium because B-cells contribute to functional HGPRT, which can utilize hypoxanthine present in the medium. Though aminopterin Blocks dihydrofolate reductase required for purine synthesis the salvage pathway is functional and purines are synthesized from hypoxanthine. These hybrids utilize thymidine and start dividing. These are left for 10-14 days on the HAT medium. They colonies formed are then transferred to microtitre plates and cultured using complete medium.

Screening: To identify the wells having functional hybridomas, medium is collected form wells and transferred to another microtitre plate whose wells are coated with Ag. Then a labeled anti-Ab is added and analysed for positive results. The well which showed positive results is identified , the cells are isolated and then cultured.

Screening can also be done by replica palting method. Some of these cells are used for production, some are prepared as stocks and stored inliquid nitrogen. These may be introduced into mice where they settle in spleen or some secondary lymphoid organs and produce antibodies indefinitely.

Applications of Monoclonal antibodies:

1. Preventing rejection of transplanted organs: These are aimed at diminishing circulating lymphocytes which can act on the transplanted organs. Example: OKT3 monoclonal antibodies are used for suppressing T-cell population. These Ab's bind to CD3 cell surface receptors on T cells and suppress their activity.

2. Treatment of bacterial blood infections: Bacteremia caused by gram –ve bacteria were treated with antibodies. The toxicity of gram –ve bacteria is due to release of an endotoxin (lipopolysaccharide) component of their cell wall. The soluble form of endotoxin triggers a set of immune responses that results in fever, cell lysis, increased heart beat and organ failure. Usage of monoclonal antibodies can be a promising approach to control deaths. According to economic initial cost, dose and year was estimated to be at $3,750/dose and 2.3 billion dollars/year.

3. Genetically engineered immunotherapeutic agents

a) Chemically linked mAb's: These are used to clear blocks of coronary artery by linking antifebrin Ab to a Plasminogen activator.

Usage of mAb's specifically causes lysis of blood clot and does not effect circulating fibrinogen concentration.

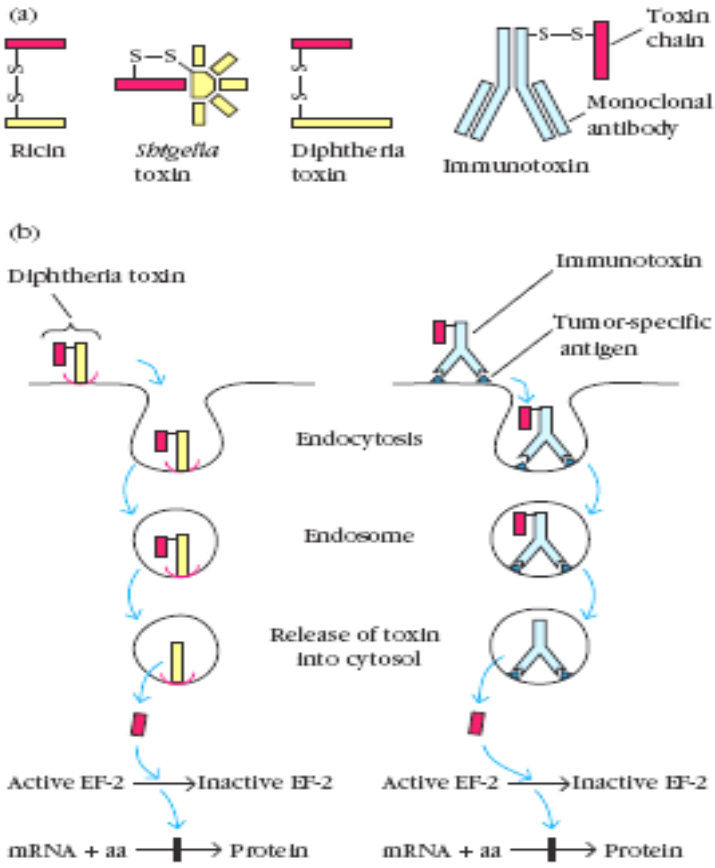
b) HIV therapeutic agents: The target cell for HIV viruses is T_H cells. They bind to CD4 receptors via their gp120. if mAb's are raised against CD4, it is lethal. Therefore proteins which can bind to gp120 were identified. Pseudomonas toxin is also used.

Pesudomonas Exotoxin A (66KDa protein) has 3 domains, first domain can bind to cell surface of the host, second domain helps translocation of the protein into cells and the

third domain is for ADP ribosylation. It inhibits protein synthesis by binding to EF-2 (Elongation factor 2).

If the first domain sequence is replaced with CD4 domain coding sequences, then the exotoxin will specifically bind to gp120 present on infected cells. This change at DNA level will express a CD4-toxin fusion protein. A plasmid is constructed to express this endotoxin in E.coli. The resulting fusion protein when introduced binds to HIV infected cells, the toxin enters the cells and inhibits protein synthesis which inturn will block HIV replication.

c) **Immunotoxins:** composed of tumor-specific monoclonal antibodies coupled to lethal toxins are potentially valuable therapeutic reagents. The toxins used in preparing immunotoxins include ricin, *Shigella* toxin, and diphtheria toxin, all of which inhibit protein synthesis. These toxins are so potent that a single molecule has been shown to kill a cell. Each of these toxins consists of two types of functionally distinct polypeptide components, an inhibitory (toxin) chain and one or more binding chains,which interact with receptors on cell surfaces; without the binding polypeptide(s) the toxin cannot get into cells and therefore is harmless. An immunotoxin is prepared by replacing the binding polypeptide with a monoclonal antibody that is specific for a particular tumor cell. In theory, the attached monoclonal antibody will deliver the toxin chain specifically to tumor cells, where it will cause death by inhibiting protein synthesis. The initial clinical responses to such immunotoxins in patients with leukemia, lymphoma, and some other types of cancer have shown promise, and research to develop and demonstrate their safety and effectiveness is underway.



4) Other applications:

a) Diagnostics- In RIA, ELISA, Western blotting, immunohistochemistry etc.

b) Protein purification: Affinity chromatography.

c) Diagnostic imaging.

d) Tissue typing and blood grouping.

e) In the treatment of allergies and asthma.

Question: Write a detained account on hybridoma technology and mentioned some of its applications. -14Marks