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Hybridoma Technique in Pharmaceutical Science

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Abstract: Hybridomas are the specific cells that have specific character to produce desire antibody. To produce monoclonal antibodies, β cells are removed from the spleen of an animal that has been immunized with the releant antigen. These β cells are then fused with myeloma tumor cells in the presence of PEG. The use of HGPRT cells assured that only hybridomas are selected. The fused hybrid cells (hybridomas), produce large amounts of the desired antibodies. They have to be selected and cloned. Cloning is done after identification of positive primary hybridoma cells. The hybridoma technique to produce monoclonal antibody was first invented by Cesar Milstein, Niels Kaj Jerne and Georges J. F. Köhler in 1975.
Key words: Hybridoma, Myeloma cell, PEG, HGPRT, HAT.

INTRODUCTION:

Monoclonal antibody are specific against single antigen. [1] Hybridomas are the hybrid cells of myeloma (cancer) cells with antibody-producing cells (lymphocytes from, an immunized donor (animal). [2] Hybridoma technique is a method of creating pure and uniform antibodies.The hybrid cell or hybridoma resulting from the fusion between myeloma cell and spleen cell of immunized cell of the donor. The term antibodies can be produced in specialized cells through a technique now popularly known as the hybridoma technology.Term hybridoma is applied to fused cells resulting due to fusion of following two types of cells:

1.An antibody producing lymphocyte cell**.**
2. A single myeloma cell (bone marrow tumour cell).

This technology was discovered in 1975 by two scientists, Georges Kohler of West Germany and Cesal Milstein of Argentina, who jointly with Niels Jerne of Denmark were awarded the 1984 Nobel Prize for Physiology and Medicine.

EXPERIMENT TECHNIQUE:

To produce the monoclonal antibody first of all the mouse immunized with specific antigen then spleen cells of mouse are removed out.Monoclonal antibodies are made by fusing myeloma cells and spleen cells of mouse. Polyethylene glycol (PEG) is used to increase the somatic cell division,only fused cells can grow. This is because myeloma cells are not able to synthesize hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) enzyme.This enzyme necessary for the salvage pathway of nucleic acids. The cells are not affected in the absence of HGPRT unless the de novo synthesis pathway is also disrupted. In the presence of aminopterin the cells are unable to use the de novo pathway and thus these cell become auxotrophic for nucleic acids as supplement to (Hypoxanthine Aminopterin Thymidine medium). In this medium only fused cells will grow. Unfused myeloma cell does not have ability to grow in this HAT medium because they lack HGPRT, and thus the cell are not able to produce the DNA. Unfused spleen cells can not grow because of short life span. Only

fused hybrid cells are called hdybridomas. Hybrid cells have capicity to grow in the HAT medium because the spleen cell partner produce HGPRT. This hybrid cell clones are generatate from single host cells. The antibodies secreted by the different clones are then tested for to check the ability to bind to the antigen (this test known as ELISA).The clone is then selected for future use.[1,2,3,4]

THE BASIC STEPS ARE INVOLVED IN HYBRIDOMA TECHNIQUE:

The basic steps involved are:

1. Immunization.
2. Generation of β cell hybridomas by fusing prime β cells and myeloma cells.
3. Selection and the screening of resulting clones.
4. Cloning by propagating the desire hybridomas.[5]

FUSION TECHNIQUE:

These are the agents which induse the somatic cell fusion. There are following type of agents:

1.PHYSICALTECHNIQUE:

A single-beam gradient force optical trap is combined with a pulsed UV laser microbeam in order to perform laser induced cell fusion.[7]

2.CHEMICAL TECHNIQUE:

Poly ethylene glycol (PEG) is used to induced cell fusions and a high number of cells can be fused in the presence of PEG in a short time.[8]

3.ELECTROCHEMICAL TECHNIQUE:

In this technique electric potential is applied in the fusion medium to induse cell fusion. This is known as electrofusion. These factors included specific resistance and osmotic strength the ionic composition, of the fusion medium and field strength and proteolytic pretreatment of the cells effect the electrofusion.[9,10]

ADVANCEMENT:

The method of generating a hybridoma cell that avoids the use of animals has not been found. Recent development of in vitro techniques allow the production of antigen-binding antibody fragments, but these techniques are still experimental and have an uncertain result. Biological techniques for diagonesis of some specific disease and for detection of pathogen are usually slow process. The immunological and nucleic-acid hybridization-based methodes are being applied for the detection of plant pathogens.[12] Monoclonal antibodies play very important role in the diagnosis of viral diseases. The human murine/human chimeric monoclonal antibodies are applied to clinical use as anticancer therapy like HER-2-specific trastuzumab (Herceptin) against breast cancer, showing increased overall survival. Recent advances have allowed the use of rabbit β-cells. Rabbits are injected with a purified antigen with an adjuvant in 2 to 3 doses and the animal is killed and spleen cells are collected.The rapid progress being made in the commercialization of monoclonal antibodies led to a need to produce these reagents in bulk.[13,15,16]

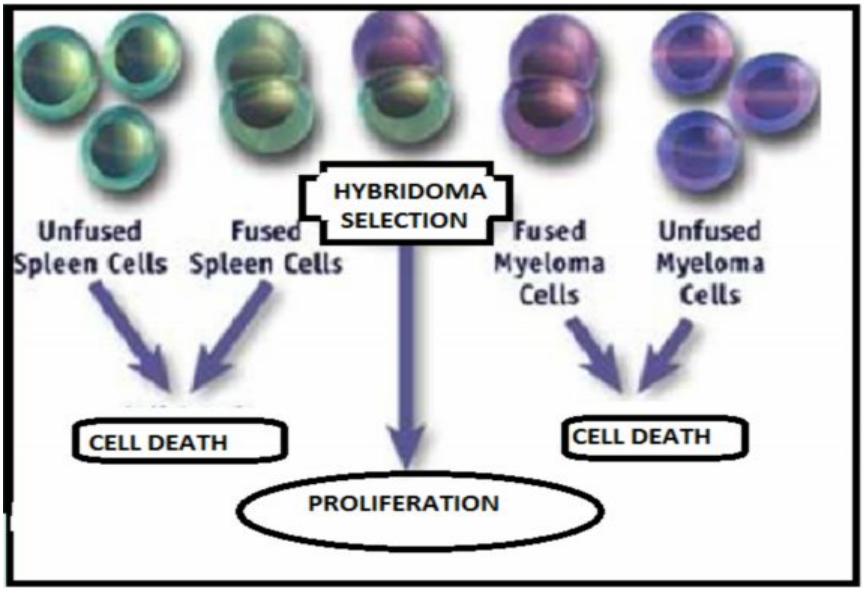

FIGURE 1 FORMATION OF HYBRIDOMA CELL[3]

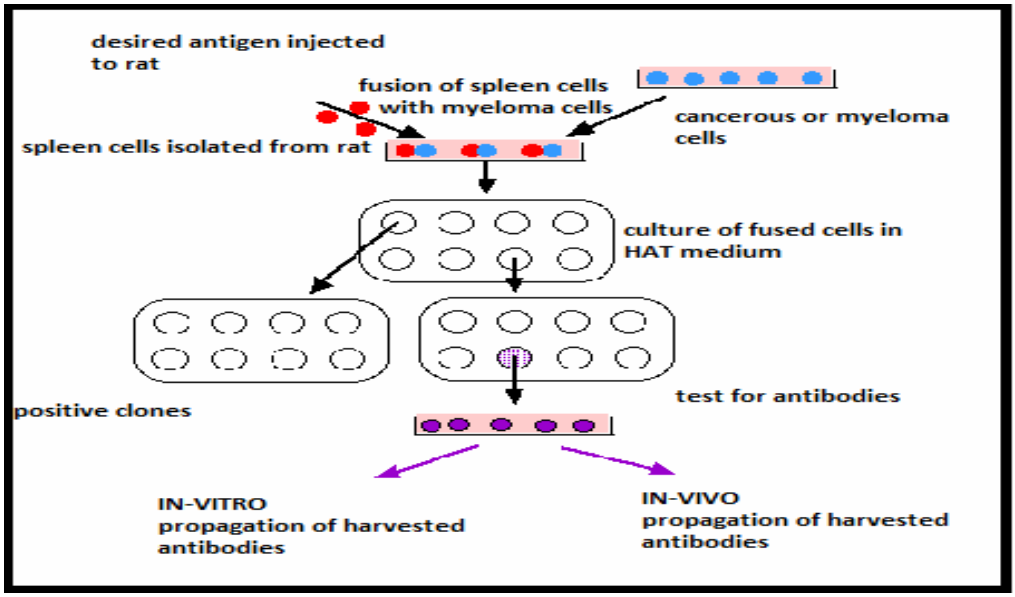

FIGURE-2 PROCEDURE FOR HYBRIDOMA TECHNIQUE[11]

TABLE 1: COMPARISON BETWEEN ANTISERUM POLYCLONAL AND MONOCLONAL ANTIBODIES: [12]

Criteria	Monoclonal anntibody	Antiserum
Spceiricity	Standard, highly specific. Unexpected cross reactions may occur. May be too specific for Requirement.	Variable with animal and breed. Partial eross-reactions with common determinants, seldom too specific
Antigenic deteiniinani/ epitope recognitii	Single.	Several
Affinity	May be selected during Cloning.	Variable with breed
Yield of use antibody	1 ip to IO.ig/inl in tissue cultureUp to 20nig/nil in ascitic Lluid.	Up to 1 ing/ml
Contaminating immuno-globulins	None in cell culture, 10% in ascitic lluid.	Lip to l(K)%
Purity of antigen required	Some degree of antigen purification desirable but not essential.	Pure antigen is required

DIAGNOSTIC USE:
Monoclonal antibodies were first produced in 1975 by Cesar Milstein, Georges J. F. Köhler and Niels Kaj Jerne. Scientists recognized their practical uses, especially in diagnosis of disease and in therapy. Several diagnostic procedures are now available.[5,15]

A BREAKTHROUGH IN DIAGNOSTICS:
A monoclonal antibody can be used to detect pregnancy. Pegnancy can be detected only 14 days after conception. Other Hepatitis can be rapidly diagnosed by the monoclonal antibodies.

KNOWLEDGE ON MONOCLONAL'S ADVANCE:
Hybrid (chimeric monoclonal antibodies) injected in to the human constant regions, the immune system only "sees" a human protein and does not react against them. So, they can be injected many times to kill all of the cells in a tumor.[16]

HELPS IN CRITICAL DIAGNOSTIC DECISIONS:
Monoclonal antibody can be used to detect the viral infections.

CONJUGATED MONOCLONAL ANTIBODY THERAPY:
Radioactive isotopes are bound to the constant region of the MAbs. When monoclonal antibody injected in to the tumorr suffering person. The MAb binds to the surface cells of a tumor the radioactivity will kill the cancer cells and all cells. In this way cancer cells within the tumor will be kill.[17]

MONOCLONAL'S HELPS IN IMMUNODIAGNOSTIC TESTS:
Monoclonal's helps In Immunodiagnostic tests Monoclonal antibodies can also be used to purify a substance with techniques called immunoprecipitation and affinity chromatography.[18]

INVESTIGATIONAL AND ANALYTICAL APPLICATION:
It includes the Lymphocyte phenotyping, purification of protein, radioimmunoassay.[5]

TRANSPLANTATION:
Monoclonal antibody is used for organ and bone marrow transplantation. Daclizumab, an IL-2 receptor antagonist, has been used safely and effectively for over 10 years across different transplant types.[17]

DRUG TARGETING:
It includes the immunotoxins, suppresser deletion and site specific modification.[18]

MONOCLONAL ANTIBODIES FOR CANCER TREATMENT:
Specific monoclonal antibodies when injected in to the cancer suffering person, MAb bind only to cancer cells specific antigen and induce immunological response on the target cancer cell. MAb can be modificated for delivery of radioisotope, cytokine.[3,6] Monoclonal antibodies can be used in the investigation of the sentinel axillary lymph node for metastatic breast cancer.[19]

FDA APPROVES :
FDA approves the example described in class is Herceptin. Certain forms of breast cancer can be treated by using these monoclonal antibodies and have passed clinical trials and been approved for use by the FDA.[20]

FDA APPROVES AND TRAILS ON :
Murine IgG2a CD3 specific transplant rejection drug, OKT3 (muromonab)are the first FDA-approved therapeutic monoclonal antibody.and approved in 1986.

MARKETED PRODUCTS :
Monoclonal antibodies such as infliximab and adalimumab can be used for autoimmune diseases. which are effective in, Crohn's disease, rheumatoid arthriti and ulcerative colitis by their ability to bind to and inhibit TNF-α.. IL-2 on activated T cells are inhibited by basiliximab and daclizumab and there by help preventing acute rejection of kidney transplants.Omalizumab inhibits human immuno - globulin E (IgE) and is useful in moderate-to-severe allergic asthma.[20]

TABLE-2 MARKETED MONOCLONAL ANTIBODIE'S PRODUCT [20]

Activity	Type	Application
Anti-inflammatory	infliximab	Rheumatoid arthritis Crohn's disease Ulcerative Colitis
	Adalimumab	Rheumatoid Arthritis Crohn's disease Ulcerative colitis
	Etanercept	Rheumatoid arthritis
	Basiliximab	Acute rejection of kidney transplants
	Daclizumab	Acute rejection of kidney transplants
	Omalizumab	Moderate-to-severe allergic asthma
Anti-cancer	gemtuzumab	Relapsed Acute Myeloid Leukaemia
	Alemtuzumab	β cell leukemia
	Rituximab	Non-hodgkin's Lymphoma
	Trastuzumab	Breast cancer
	Nimotuzumab	Approved in Squamous Cell Carcinomas, Glioma Clinical trials for other indications underway
	Cetuximab	Approved in Squamous Cell Carcinoma, Colorectal Carcinoma
	Bevacizumab	Anti-angiogenic Cancer Therapy

RESULT AND CONCLUSION:

Hybrid cell can be cloned to produce identical daughter clones. These daughter clones have ability to secrete the immune cell product. Since these momoclonal antibodies come from simillar type of cell (hybridoma cell) they are known as monoclonal antibodies HAT medium is used to produce the monoclonal antibodies. Laboratory animals (eg. mice) are first injected by an antigen to which we are interested in isolating an antibody. Spleen cells are isolated from the mammal, the β cells are fused with myeloma cells. Fused cells are incubated in the HAT medium. Unfused spleen cells can not grow because of their limited life span. Only fused hybrid cells are known as hybridomas. Hybrid cells are able to grow indefinitely in the HAT medium because the spleen cell partner supplies HGPRT. Hence Unfused β cells die as they have a short life span. Only hybridomas are selected.

FUTURE ASPECTS:

Monoclonal antibody can be adsorbed on a carrier like nanoparticle of any drug. Then it can be used for site specific delivery of the drug due to monoclonal antibody are specific for particular antigen**.**

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