WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 17, 502-510. Review Article ISSN 2277– 7105

HYBRIDOMA TECHNOLOGY: A NEW INNOVATION AND APPLICATION

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Article Received on
05 August 2018,
Revised on 26 August 2018,
Accepted on 16 Sept. 2018
DOI: 10.20959/wjpr201817-13390

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ABSTRACT

The Term Hybridoma was coined by Leonard Herzenberg in the laboratory of Cesar Milstein in 1976-1977. Hybridoma Technology is the method in which large quantity of identical antibodies are produced which are also known as monoclonal antibodies. It is done by the administration of antigen in mouse which produces an immune response. The B-cells producing antibodies are then harvested from the injected mouse. The harvested B-cells are then fused with B cancer cells which remain immortal. This produces hybrid cell line called hybridoma which possesses the antibody-producing ability of the B-cell. The hybridomas can be grown in culture with one viable cell which produces cultures having genetically indentical hybridomas. It produces monoclonal antibodies. It retains the ability to grow in tissue culture and do not possess antibody producing capability.

KEYWRODS: Term Hybridoma and Leonard Herzenberg.

INTRODUCTION

Biochemistry is an experimental rather than a theoretical science. It is no exaggeration to state that the foundations for the present knowledge of biochemistry are based on the laboratory tools employed for biochemical experiment.[1] Hybridoma technology is a method for producing large numbers of identical antibodies (also called monoclonal antibodies). This process starts by injecting a mouse (or other mammal) with an antigen that provokes an immune response. A type of white blood cell, the B cell that produces antibodies that bind to the antigen are then harvested from the mouse. These isolated B cells are in turn fused with immortal B cell cancer cells, a myeloma, to produce a hybrid cell line called a hybridoma.

The production of monoclonal antibodies was invented by César Milstein and Georges J. F. Köhler in 1975. They shared the Nobel Prize of 1984 for Medicine and Physiology with Niels Kaj Jerne, who made other contributions to immunology. The term **hybridoma** was coined by Leonard Herzenberg during his sabbatical in César Milstein's laboratory in 1976–1977.[2]

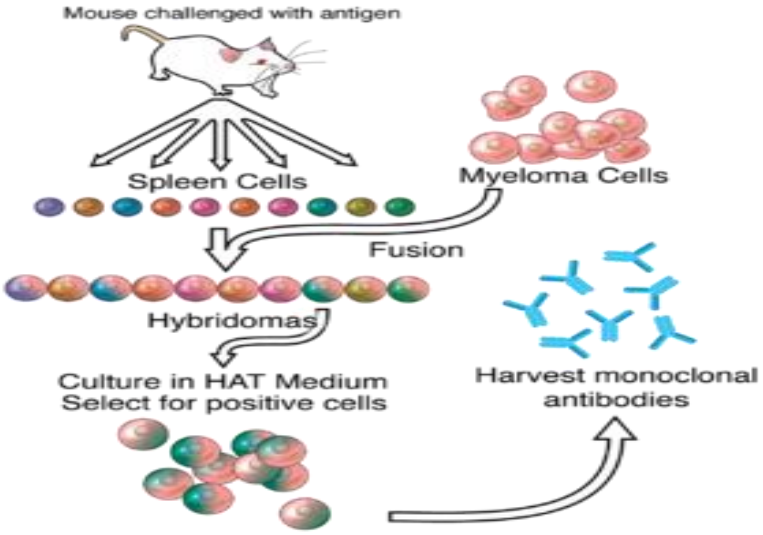

Fig.1: Formation of mAb.

Procedure

Laboratory animals like mice are administered with a series of injections of the test antigen for several weeks against which the antibody is to be generated. The splenocytes are isolated from the mouse spleen and the B cells are fused with immortalised myeloma cells by the process of electrofusion. Alternatively, chemical reagent like polyethylene glycol is used for the fusion of B-cells and myelomas. The myeloma cells are selected which lack the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) gene, making them sensitive to the HAT medium.

The fused cells are incubated in HAT medium (hypoxanthine-aminopterin-thymidine medium) for about 10 to 14 days. The unfused myeloma cells are removed as they can outgrow other cells, especially weakly established hybridomas. Unfused B cells die as they have a short life span. By this method the B cell-myeloma hybrids survive. Then the dilution of the incubated medium is carried out in 96-well plates. The B-cells produce the antibodies and are directed towards the same epitope, and are thus monoclonal antibodies.[3]

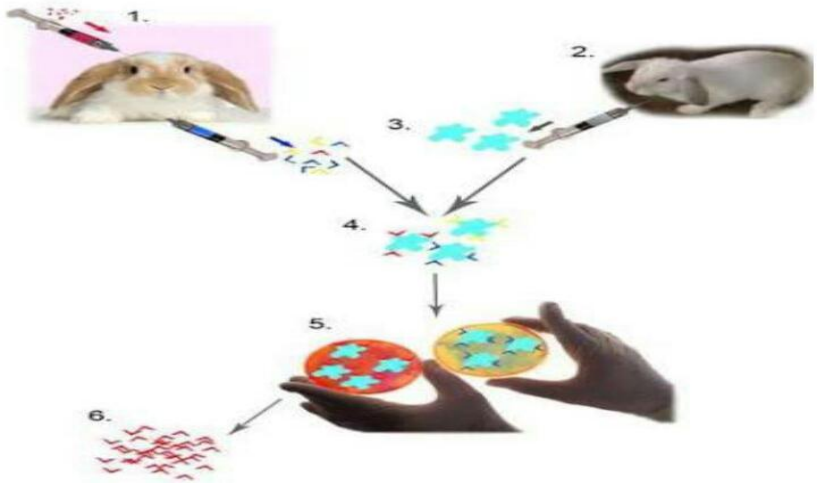

Fig. 2: Formation of Monoclonal Antibodies.

Growth Medium

Many identical daughter clones can also be produced from the B cell that produces the desired antibodies. The use of interleukin-6 is required in the supplementary media for this mechanism. The hybridoma colony is then subjected to grow in culture medium like RPMI-1640 (with added antibiotics and fetal bovine serum) thereby producing the antibodies.

Hybridomas are grown in multiwell plates and then followed by their growth in larger tissue culture flasks. This maintains the hybridomas for long period and also suffices its cryopreservation at -20oC or at even lower temperature. The culture supernatant can yield about 60 µg/ml of monoclonal antibody. The culture supernatant or a purified immunoglobulin preparation can be used for potential monoclonal in terms of specificity, reactivity and cross-reactivity.[3]

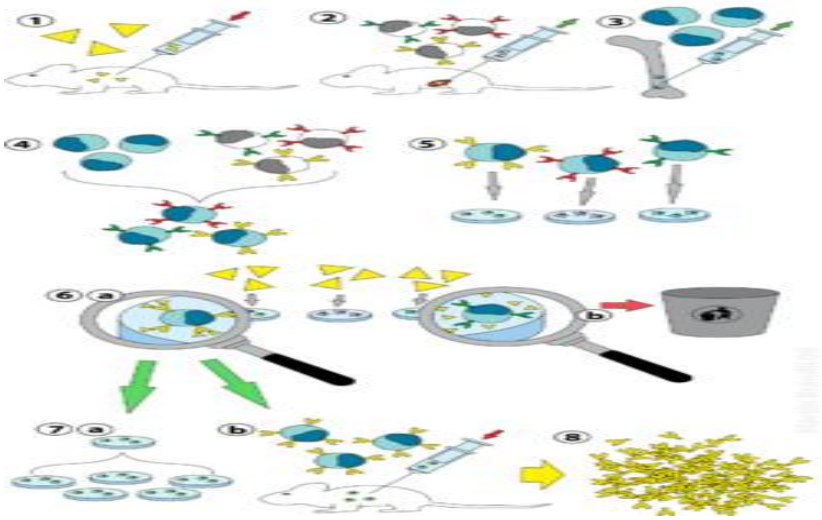

Fig.3: Duplication of Antibodies.

In vitro

Advantages

- In vitro methods reduce the use of mice at the antibody-production stage but can use mice as a source of feeder cells when antibody generation is under way).
- In vitro methods are usually the methods of choice for large-scale production by the pharmaceutical industry because of the ease of culture for production, compared with use of animals, and because of economic considerations.
- In vitro methods avoid the need to submit animal protocols to IACUCs.
- In vitro methods avoid or decrease the need for laboratory personnel experienced in animal handling.
- In vitro methods using semipermeable-membrane-based systems produce mAb in concentrations often as high as those found in ascitic fluid and are free of mouse ascitic fluid contaminants.[3]

Disadvantages

- It should be noted that each of the items below pertains to only a fraction (3–5%) of hybridomas, but they indicate some of the difficulties associated with in vitro methods.
- Some hybridomas do not grow well in culture or are lost in culture.
- In vitro methods generally require the use of FBS, which limits some antibody uses. The use of in vitro methods for mAb production generally requires the use of FBS, which is a concern from the animal-welfare perspective.
- The loss of proper glycosylation of the antibody (in contrast with in vivo production) might make the antibody product unsuitable for in vivo experiments because of increased immunogenicity, reduced binding affinity, changes in biologic functions, or accelerated clearance in vivo.
- In general, batch-culture supernatants contain less mAb (typically 0.002-0.01) per milliliter of medium than the mouse ascites method. Note that semipermeable-membrane-based systems have been developed that can produce concentrations of mAb comparable with concentrations observed in mouse ascites fluid.[3]
- In batch tissue-culture methods, mAb concentration tends to be low in the supernatant; this necessitates concentrating steps that can change antibody affinity, denature the antibody, and add time and expense. Adequate concentrations of mAb might be obtained in semi permeable-membrane-based systems.

- Most batches of mAb produced by membrane-based in vitro methods are contaminated with dead hybridoma cells and dead hybridoma-cell products, thus requiring early and expensive purification before study.
- mAb produced in vitro might yield poorer binding affinity than those obtained by the ascites method.
- In vitro culture methods are generally more expensive than the ascites method for small-scale or medium-scale production of mAb (Hendriksen and de Leeuw 1998; Jackson and others 1996; Peterson Peavey 1998; Marx 1998; Lipman 1997).
- The number of mAb produced by in vitro methods is limited by the amount of equipment that it is practical to have available.
- The Food and Drug Administration (FDA) estimates that proving the equivalence of an mAb produced by in vitro methods to an mAb previously produced by the mouse ascites method would cost the sponsor $2–10 million (Stein 1998; Maxim 1998).[4]

In vivo method

Advantages

- The mouse ascites method usually produces very high mAb concentrations that often do not require further concentration procedures that can denature antibody and decrease effectiveness.
- The high concentration of the desired mAb in mouse ascites fluid avoids the effects of contaminants in in vitro batch-culture fluid when comparable quantities of mAb are used.
- The mouse ascites method avoids the need to teach the antibody producer tissue-culture methods.[3]

Disadvantages

- The mouse ascites method involves the continued use of mice requiring daily observation.
- MAb produced by in vivo methods can contain various mouse proteins and other contaminants that might require purification.
- The mouse ascites method can be expensive if immunodeficient mice in a barrier facility must be used.
- In vivo methods can cause significant pain or distress in mice.[3]

APPLICATIONS OF HYBRIDOMA TECHNOLOGY

- **Diagnostic Applications**

Biosensors & Microarrays

- **Therapeutic Applications**

Transplant rejection Muronomab-CD3

Cardiovascular disease Abciximab

Cancer Rituximab

Infectious Diseases Palivizumab

Inflammatory disease Infliximab

- **Clinical Applications**

Purification of drugs, Imaging the target

- **Future Applications**

Fight against Bioterrorism[5]

Diagnostic Applications

- Antibodies are used in several diagnostic tests to detect small amounts of drugs, toxins or hormones.
- Human Monoclonal antibodies to Human chorionic Gonadotropin (HCG) are used in pregnancy test kits.
- Another diagnostic, uses of antibodies is the diagnosis of AIDS by the ELISA test.
- Once monoclonal antibodies for a given substance have been produced, they can be used to detect the presence of this substance.
- The Western blot test and Immuno dot blot tests detect the protein on a membrane.
- Useful in immunohistochemistry, which detect antigen in fixed tissue sections and Immunofluorescence test, which detect the substance in a frozen tissue section or in live cells.[6]

Pregnancy Test

- A pregnant woman has the hormone Human Chorionic Gonadotrophin (HCG) in her urine.
- Monoclonal antibodies to HCG have been produced. These have been attached to enzymes which can later interact with a dye molecule and produce a colour change.[5]

Diagnosis of HIV

The test of HIV infection is based on detecting the presence of HIV antibody in the patient's blood serum.

- HIV antigen is attached plate.
- Patients serum passed over the plate. Any HIV antibody in the patients serum will attached to the antigen already on the plate.
- A second antibody which is specific to the HIV antibody is passed over the plate. This antibody will attach to the concentrated HIV antibody on the plate. This second antibody has an enzyme attached to its structure.
- Chromagen dye is passed over the complex of concentrated HIV antibody/conjugated antibody.
- The enzyme will turn the chromagen to a more intense colour. The more intense the colour, the greater the HIV antibody level. This would be the positive result for a HIV test.[7]

Clinical Applications of Monoclonal Antibodies

Imaging the target organ

- Monoclonal antibodies directed against tumour-associated antigens labelled with radioisotopes localize specifically into tumour after intravenous injection. This property is used for diagnostic tumour imaging by immunoscintigraphy.
- The radio-labeled antibody-isotope conjugate is injected into the patient and allowed to localize to the target over a 2- to 7-day period. The patient then undergoes imaging with a nuclear medicine gamma camera, and radioisotope counts are analyzed.
- used in colorectal & prostate cancer.[8]

ADEPT (Antibody Directed Enzyme Pro-drug Therapy)

- Involves the application of cancer associated monoclonal antibodies which are linked to a drug-activating enzyme.
- Subsequent systemic administration of a non-toxic agent results in its conversion to a toxic drug, and resulting in a cytotoxic effect which can be targeted at malignant cells.[8]

Clinical Importance of In Disease Diagnosis

The hybridoma cells are utilized in diagnostic histopathology. Monoclonal antibodies increase the sensitivity in detecting even small quantities of invasive or metastatic cells. The

technique is used in the precise diagnosis of breast cancer, pleural and peritoneal mesothelioma, adenocarcinoma and metastases. It finds its usage along with immunecytochemistry using tumor associated monoclonal antibodies for the detection of neoplastic cells.[9]

Future Use in Bioterrorism

Raxibacumab

- It is a human monoclonal antibody
- Antibody *against Bacillus anthracis* protective antigen
- Intended for the prophylaxis and treatment of inhaled anthrax.
- Its efficacy has been proved in rabbits and monkeys.
- As of January 2011, the drug has reached stage three clinical testing in humans
- On December 14th 2012 the FDA approved Raxibacumab for the treatment of inhalation anthrax.[10] [11] [12]

REFERENCES

1. U. Satyanarayana, U. Chakrapani; Biochemistry; Elsevier Publications; Page No 4th Edition 2013; 719.
2. Milstein, C. "The hybridoma revolution: an offshoot of basic research". BioEssays, 1999; 21(11): 966–73.
3. Nelson PN, Reynolds GM, Waldron EE, Ward E, Giannopoulos K, Murray PG. Demystified: Monoclonal antibodies. Molecular pathology, 2000; 53(3): 111–7.
4. Generation of Hybridomas: Permanent Cell Lines Secreting Monoclonal Antibodies. https://www.nap.edu/read/9450/chapter/4
5. Large-Scale Production of Monoclonal Antibodies. https://www.nap.edu/read/9450/chapter/8
6. Bretton PR, Melamed MR, Fair WR, Cote RJ. Detection of occult micrometastases in the bone marrow of patients with prostate carcinoma. Prostate, 1994; 25(2): 108–14.
7. Scientific Needs for Mouse Ascites Production of mAb Link:- https://www.nap.edu/read/9450/chapter/6
8. Ghosh AK, Spriggs AI, Taylor-Papadimitriou J, Mason DY. Immunocytochemical staining of cells in pleural and peritoneal effusions with a panel of monoclonal antibodies. J Clin Pathol, 1983b; 36(10): 1154–64.
9. Katzung Basic & clinical Pharmacology 11th Edition.

10. Harrison's Internal Medicine 17th Edition.
11. Dipiro's pathophysiologic Approach 8th Edition.
12. Jancie, M Recheit, etal. Nature biotechnology, Sep, 2005; 23(9). Stamatis-Nick C. J Allergy Clin. Immunol, Oct. 2005.