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Hybridoma technology and its significance

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Abstract
The article explores the ground breaking hybridoma technology, pioneered by Cesar Milstein and Georges Kohler in the mid-1970s. This innovative approach streamlined the creation of monoclonal antibodies tailored to precise antigenic reactivity. Prior to this breakthrough, researchers relied on polyclonal antibodies, which lacked consistency and specificity. Hybridoma technology involves combining immortal myeloma cells with antibody-producing B cells results in the creation of hybridoma cell lines capable of producing monoclonal antibodies (mAbs). The article explains the principle of hybridoma technology, detailing the steps from immunization and cell fusion to antibody production and purification. It highlights the advantages of mAbs over polyclonal antibodies, including high specificity and reproducibility. Applications of mAbs in immunodiagnostics, experimental research, and immunotherapy are explored. The article emphasizes the importance of hybridoma technology in scientific advancements and medical practices, while acknowledging challenges and limitations. Overall, hybridoma technology remains a fundamental tool in antibody generation, contributing significantly to various fields.

Keywords: Hybridoma technology, monoclonal antibodies, antigen detection, immune responses, immunotherapy

Introduction
Before the mid-1970s, scientists in the field of molecular biology relied extensively on polyclonal antibodies obtained from conventional sera to identify and quantify specific proteins within complex biological sample mixtures. Despite their advantageous features, such as a broad range of reactivity, these polyclonal antibodies were unsuitable for in vivo experiments and therapeutic applications. The inconsistency between batches and the elevated background reactivity levels associated with polyclonal antibodies rendered them unsuitable for such purpose (De Almeida *et al.*, 2018; Zaroff and Tan, 2019) [2, 18].

Hence, a prominent concern of that period was the lack of capability to isolate and purify singular antibodies of a well-defined specificity (referred to as monoclonal antibodies) from the myriad of different antibodies produced within the body. This limitation hindered the advancement of targeted research and applications in various fields, including diagnostics and therapeutics. In 1975, Cesar Milstein and George Kohler, in collaboration with Shirley Howe, achieved a significant breakthrough in molecular biology by inventing a method for creating "hybridomas." They accomplished this by fusing myeloma cell line P3-X63-Ag8 with individual clones of B-cells, derived from mouse spleens immunized with sheep red blood cells, resulting in the successful production of large quantities of monoclonal antibodies. This pioneering work introduced hybridoma technology as a transformative advancement in the field of molecular biology. In 1984, Cesar Milstein and George Kohler were jointly awarded the Nobel Prize in Medicine and Physiology, along with Niels Kaj Jerne (as mentioned in Milstein's 1999 work). Additionally, the term "hybridoma" was coined during the years 1976-1977 by Leonard Herzenberg while working in César Milstein's laboratory (Ganguly and Wakchaure, 2016) [4].

Hybridoma technology
The fundamental and pioneering approach for generating monoclonal antibodies (mAbs) tailored to desired antigens is hybridoma technology. This methodology involves the fusion of a transient antibody-producing B cell (typically sourced from the spleen) with an immortal myeloma cell (derived from a plasma cell tumor), resulting in the creation of hybridoma cell lines (Parray *et al.*, 2020) [13].

"It's a method designed to produce a continuous and consistent reservoir of monoclonal antibodies, capitalizing on the unbounded proliferation potential of myeloma cells. This process harnesses the cellular machinery to generate antibodies with a consistent antigenic specificity, mirroring that of the B cell."

Fig 1: Hybridoma technology Inventor: Georges Kohler and Cesar Milstein

Clone: A group of cells originating from a single parent cell.

Monoclonal antibodies

Monoclonal antibodies (mAbs) refer to antibodies with consistent specificity generated by a single clone of B cells targeting a solitary antigenic feature or hapten.

Polyclonal antibodies: Polyclonal antibodies are the collective product of various B cell clones, each reacting to different antigenic elements of a multivalent antigen**.**

Table 1: Monoclonal antibodies vs polyclonal antibodies

Polyclonal Antibodies	Monoclonal Antibodies
Polyclonal antibodies (pAbs) comprise a collection of immunoglobulin molecules originating from various B cell lineages, and they exhibit reactivity towards multiple epitopes of a particular antigen	Monoclonal antibodies exhibit monospecificity as they are generated by identical B cells characterized by a strong affinity and specificity for a solitary epitope of an antigen
Nonspecific antibodies produced	Specific antibodies produced
Mixture of different antibody classes are produced	All of a single antibody class is produced
Potential for cross reactivity is high	Potential for cross reactivity is low
Time scale is short and inexpensive	Time scale is more and is expensive
Can have batch to batch variability	Once a hybridoma is established, it serves as a consistent and sustainable source, ensuring that all subsequent batches remain identical
Less skill and technology needed	More skill and technology needed for production
Hybridoma cell lines are not necessary for polyclonal antibody production, as antibodies can be directly extracted from the serum	The production of monoclonal antibodies requires the production of hybridoma cell lines
Polyclonal antibodies offer several advantages, including their high affinity, resilience to minor variations, and enhanced detection capabilities	Monoclonal antibodies present several benefits, such as an inexhaustible supply, exceptional specificity, and remarkable reproducibility

Principle of hybridoma technology

At the core of hybridoma technology lies the process of conferring immortality upon B lymphocyte cells, which possess the capability to produce antibodies but exhibit limited growth characteristics *in vitro*. These lymphocytes are merged with cells from a myeloma cell line, which does not produce antibodies but maintains continuous and unrestricted growth. This fusion results in hybrid cells that maintain the ability to secrete antibodies, while inheriting the perpetual replication potential characteristic of the parent tumor cell. Thus, Monoclonal Antibodies are the outcome of hybrid cells formed through the fusion of two distinct cell types: one being a myeloma cell, genetically predisposed to indefinite multiplication, and the other harboring a gene encoding the desired antibody. (Kumar *et al.*, 2012) [6].

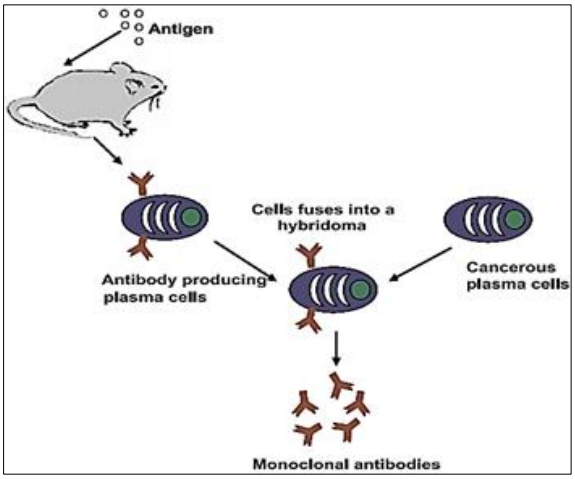


Fig 2: Principle of hybridoma technology

Preparation of monoclonal antibodies (MAB) using hybridoma technology

Hybridoma technology is composed of several technical procedures, including

- Immunization
- Isolation of B lymphocytes
- Preparation of myeloma cell lines
- Cell fusion
- Hybridoma selection
- Screening of hybridoma cells
- Cloning and propagation of hybridoma cell
- Characterization and production of specific antibodies.

Producing monoclonal antibodies (mAbs) using the hybridoma method demands proficiency across diverse disciplines and mastery of adaptable technical proficiencies. This encompasses competencies spanning animal care, immunology, and cellular and molecular biology. The creation and discernment of superior hybridoma clones constitute an exhaustive and time-consuming endeavor, necessitating several months of committed effort from initial immunization to the ultimate pinpointing of specific hybridomas.

Immunization

Monoclonal antibodies possess a monovalent nature, selectively binding to a single epitope, originating from a single clone of B-lymphocytes (Little et al., 2000) [8]. The process initiates with the introduction of specific antigens into laboratory animals, typically mice or rabbits, through a series of injections spanning several weeks. These injections prompt the transformation of B cells into plasma B cells and memory B cells. After sufficient antibody production has occurred in the animal's serum, humane euthanization is carried out. Detection of antibodies in the serum is achieved using methods such as ELISA or flow cytometry. The serum contains activated B lymphocytes responsible for antibody production

Isolation of B lymphocytes

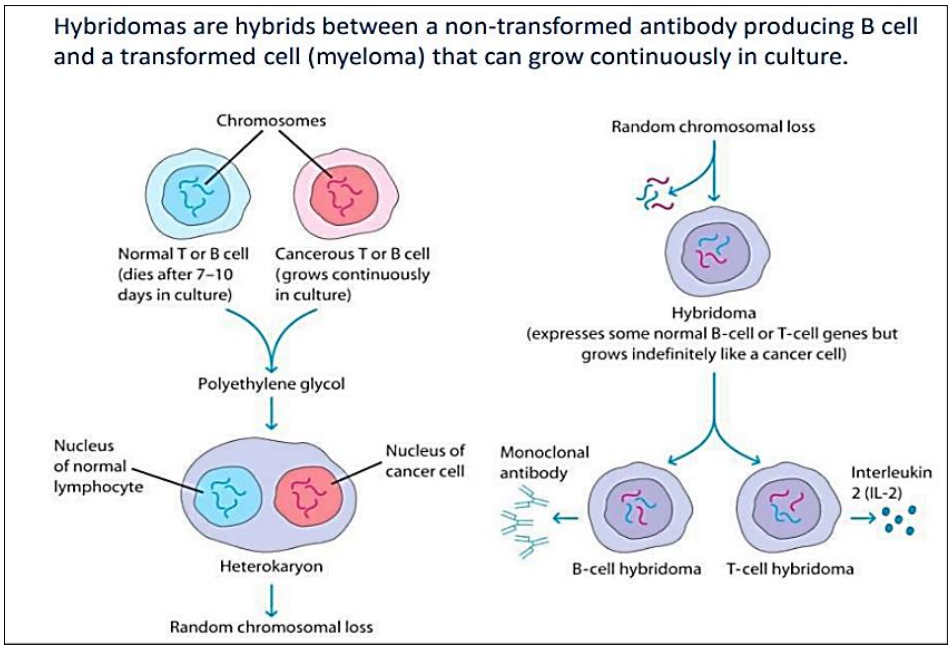
After the animal is euthanized, the spleen is extracted under sterile conditions to isolate the activated B-cells. This isolation process involves utilizing density gradient centrifugation techniques.

Preparation of myeloma cell lines

Several weeks prior to cell fusion, metastatic tumor cells are exposed to an incubation period with 8-azaguanine. This treatment is aimed at rendering the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) genes within the myeloma cells non-functional. Consequently, the disrupted HGPRT function hampers the synthesis of nucleotides via the salvage pathway. As a result, the metastatic tumor cells become susceptible to growth media containing HAT (hypoxanthine, aminopterin, and thymidine) (Pandey, 2010) [10].

Cell fusion

The critical step of cell fusion involves combining activated B lymphocytes with HAT-sensitive myeloma cells. This process is achieved by centrifuging freshly collected activated B cells and HAT-sensitive myeloma cells in a fusion-inducing medium. To facilitate this fusion, polyethylene glycol (PEG) is used, enhancing cell fusion by facilitating the merging of the plasma membranes of the myeloma cells with those of the antibody-producing cells. This results in the formation of cells with multiple nuclei, creating what is referred to as a heterokaryon. An alternative fusion method employs electrofusion, where cells are fused in the presence of an electric field, offering greater efficiency compared to the previous technique



Hybridoma selection

Within the medium containing PEG, cells undergo fusion, resulting in the creation of hybridoma cells. However, even with the most optimized fusion technique, only a fraction of approximately 1 to 2% of hybridoma cells are formed. Additionally, roughly 1 out of every 100 cells constitutes a viable hybrid cell. As a consequence, a significant proportion of cells remain unfused within the medium. This stage facilitates the discrimination and extraction of the fused cells from the entirety of unfused cells, allowing for the isolation of

the desired hybridomas.

This objective is achieved by a two-phase process: an initial incubation phase, followed by subsequent cultivation for 10-14 days in HAT media, which acts as a selective growth environment. The HAT medium is composed of hypoxanthine-aminopterin-thymidine, with aminopterin inhibiting nucleotide synthesis through the de novo pathway. However, cells possessing functional hypoxanthine-guanine phosphoribosyltransferase (HGPRT) genes can survive, thanks to the presence of hypoxanthine and deoxythymidine, which support survival through salvage pathways. As unfused B cells have a limited lifespan, they naturally expire within days.

Unfused malignant neoplastic cells, lacking the HGPRT gene, face demise due to aminopterin's inhibition of nucleotide synthesis through the de novo pathway. Consequently, the remaining viable cells in the medium are exclusively hybrid cells. These hybrid cells have the capacity to propagate and divide within HAT media because they possess the functional HGPRT gene inherited from the B lymphocytes. This genetic characteristic makes them HGPRT positive, allowing them to proliferate unrestricted in significant quantities within HAT media.

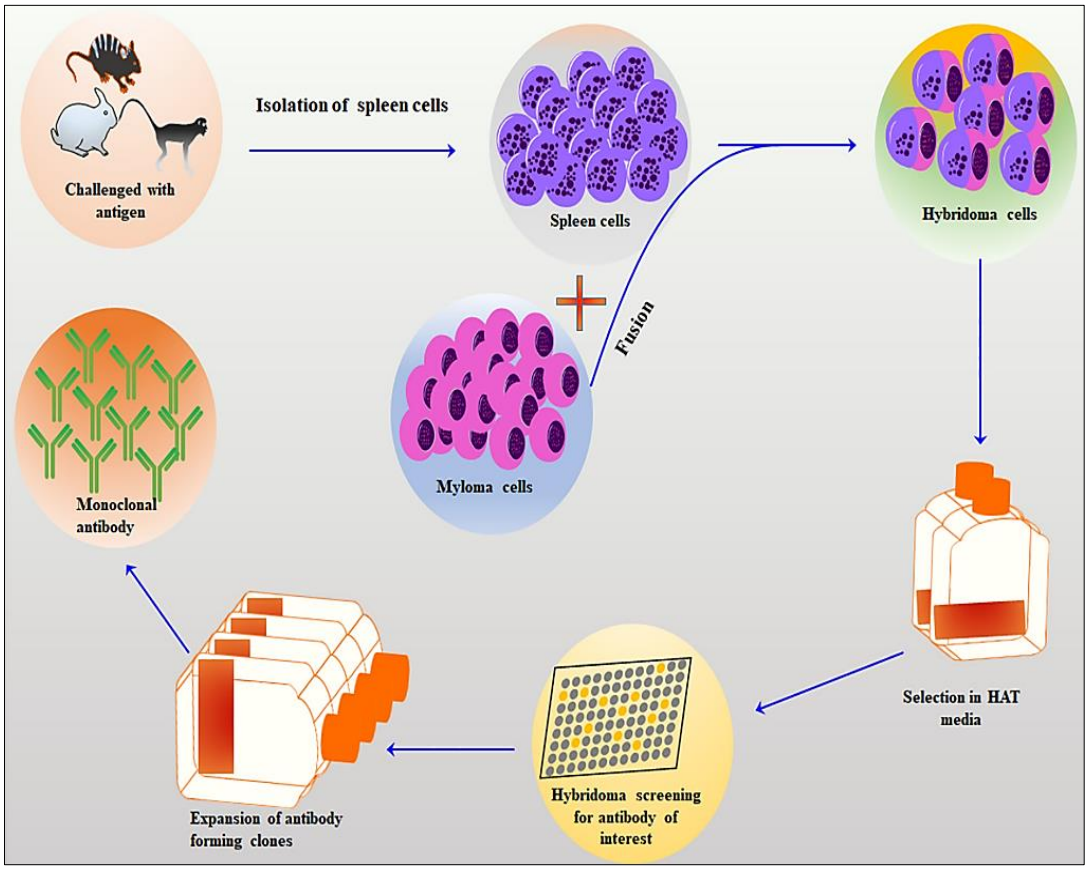
Cloning and propagation of hybridoma cell

The process of cloning and propagating hybridoma cells involves several steps. Once hybridomas producing the desired antibodies are selected, they are transferred into large-scale culture vessels or flasks. These cultured hybridoma cell lines can be maintained and preserved within the culture media to produce monoclonal antibodies.

1. ***In vivo* Approach:** In the in vivo method, mice are used to generate monoclonal antibodies. Approximately 105 to 110 viable hybridoma cells are injected into the peritoneal cavity of mice. After several weeks, ascites fluid is collected from an anesthetized mouse. However, this ascites fluid contains some mouse immunoglobulins, requiring purification to isolate monoclonal antibodies. If ensuring high antibody purity is essential, this technique may present some challenges.
2. ***In vitro* Approach:** Another approach involves the *in vitro* cultivation of hybridoma cells under controlled laboratory conditions. This method includes growing the hybrid cells within a culture medium and then extracting monoclonal antibodies from the cultured media. The *in vitro* method is more suitable for hybrid cell cultivation as it reduces the risk of contamination. Antibody production using *in vitro* techniques results in exceptionally pure antibodies.

Purification of monoclonal antibody

Affinity chromatography utilizes protein-A, an immunoglobulin-binding protein originating from specific Staphylococcus aureus strains, to distinguish various subclasses of mouse IgG. Different buffers are employed to elute distinct classes of mouse immunoglobulins from protein-A Sepharose columns. In more recent times, ion-exchange high-performance liquid chromatography (HPLC) has emerged as a technique for isolating monoclonal antibodies' activity. These chromatographs exhibit remarkable resolution of IgG, effectively separating them from transferrin and albumin, which are the primary contaminants found in mouse ascites fluid. The same methods used for extracting monoclonal antibodies from ascites fluid can also be adapted to the tissue culture supernatants obtained from hybridoma cultures grown in serum-free media



(Parry *et al.*, 2020)

Fig 3: Diagramatic representation of production of monoclonal antibodies by hybridoma technology

Application of hybridoma technology

Monoclonal antibodies find significant utility in both human and veterinary medicine across three primary domains. These domains encompass:

1. **Immunodiagnostic Reagents:** Monoclonal antibodies are pivotal as immunodiagnostic reagents, serving multiple roles. They are employed for the direct detection of causative agents within tissues or bodily fluids. Furthermore, they function as reagents in indirect diagnostic methods, such as serological assessments to identify antibodies against the causative agents.
2. **Experimental Applications:** Monoclonal antibodies are instrumental in experimental contexts, ranging from the detailed analysis of antigenic epitopes to the use of monoclonal anti-idiotype antibodies as vaccines to evoke protective immune responses. This realm facilitates deep molecular understanding and innovative vaccine strategies.
3. **Immunoprophylaxis and Immunotherapeutics:** These antibodies play a pivotal role in immunoprophylaxis and immunotherapeutics. They can be employed against infectious diseases or as carriers to deliver toxic substances, such as targeting tumors. Monoclonal antibodies also serve as tools to precisely locate and identify target tumors within the body. (Kumar *et al*, 2012) [6].

These applications represent critical contributions of monoclonal antibodies to medical science and practice.

Immunodiagnostic Reagents

Antigen Detection of Infectious Agents

Significant advancements have been made in the direct detection of infectious agents or their antigens within tissue or body fluid samples, driven by the development of diverse monoclonal antibodies. These antibodies, customized for distinct specificities, have played a pivotal role in this progress. Various viruses, including rabies, foot-and-mouth disease virus, Newcastle disease virus, feline leukemia virus, bovine leukemia virus, bovine enteric coronavirus, and rotavirus, have been targeted through the creation of monoclonal antibodies**.**

The utility of monoclonal antibodies extends to parasites as well, with documented instances of antibodies developed against entities such as *Trypanosoma cruzi*, *Trichinella spiralis*, *Babesia bovis*, and *Dirofilaria immitis*. In-depth investigations have also been conducted using monoclonal antibodies to study a variety of bacteria. These antibodies have not only found applications in Escherichia coli research but have also proven highly valuable in identifying bacteria that are challenging to cultivate or have prolonged *in vitro* incubation periods, including Mycobacterium and Brucella spp.

Immunodiagnostic Reagents for Antibody Detection

Another application of monoclonal antibodies in disease diagnosis involves the identification of antibodies, primarily through the use of anti-globulin reagents combined with detection systems such as fluorochromes, enzymes, or isotopes in primary binding assays. While radioisotopes have been considered as detection agents, their impracticality in terms of disposal and handling has led to a greater emphasis on enzyme-conjugated monoclonal antibodies. Within the domain of diagnostic test procedures, several indirect assays have emerged. Notable examples include the utilization of lipoarabinomannan from *Mycobacterium paratuberculosis* and smooth lipopolysaccharide from B. abortus as antigens for detecting antibodies in bovine sera against these respective microorganisms.

Immunodiagnostic Reagents: Anti-Idiotype Antibody

A distinct approach in diagnostic serology involves the use of anti-idiotype antibodies to detect antibodies with specificities. This technique entails a sequential process: an animal is immunized with an antigen, resulting in the production of antibody Ab I. Subsequently, using Ab I as an antigen in another animal yields anti-idiotype antibody Ab 2. The application of Ab 2 as an antigen in a third animal leads to the production of anti-anti-idiotype antibody. Monoclonal antibodies can effectively serve as capture agents in primary binding assays. The application of anti-idiotype antibodies has been extended to antibodies targeting various entities, such as E. coli, various bovine pathogens including Staphylococcus spp. and Streptococcus spp., as well as B. abortus**.**

Clinical importance in disease diagnosis

Hybridoma cells hold a critical role in diagnostic histopathology, particularly in enhancing the sensitivity of detecting minute amounts of invasive or metastatic cells. This technique proves invaluable in accurately diagnosing conditions like breast cancer, pleural and peritoneal mesothelioma, adenocarcinoma, and metastases. Additionally, it complements immunocytochemistry, where tumor-associated monoclonal antibodies assist in identifying neoplastic cells (Ghosh et al., 1983) [5].

One example that underscores the utility of this approach is Radioimmunodetection (RID) of cancer.

Functional analysis of cell surface and secreted molecules

For defining the functions of surface molecules, involving receptors for antigens.

Immunopurification

This method has been employed for isolating individual interferons and has the potential for application in purifying proteins and enzymes (Pandey, 2010) [10].

Monoclonal antibody (mAbs) therapeutics

Compared to other biologics, monoclonal antibodies (mAbs) exhibit a remarkable ability to maintain exceptionally high affinity for their specific targets. This heightened affinity and specificity have motivated researchers to explore the therapeutic potential of mAbs in various roles, including metabolic activation, inhibition, and immunomodulation. Their applications encompass a wide range of ailments, spanning from autoimmune disorders to inflammatory conditions and cancer.

An illustrative example involves the successful oral administration of a monoclonal antibody designed to target the K99 pilus antigen of enterotoxigenic Escherichia coli (ETEC). This administration effectively prevented severe and fatal enteric disease in both colostrum-fed and colostrum-deprived calves during experimental challenges with ETEC.

Organ transplantation

The utilization of monoclonal antibodies extends to the neutralization of T-lymphocytes, which contribute to the rejection of transplanted organs. Monoclonal antibodies,

exemplified by OKT3, assume a crucial role by disrupting T-cell function in graft rejection. OKT3, specifically, targets the CD3 receptor, a surface membrane protein on T cells. Nevertheless, this application is merely the tip of the iceberg, as an array of additional applications continues to emerge.

In the context of domestic animals, the present or potential applications of monoclonals can be categorized as follows:

1. **Passive Antibody Administration for Infectious Diseases:** Passive antibody administration serves either prophylactic or therapeutic purposes against infectious diseases.
2. **Passive Antibody Targeting Cell Markers:** Monoclonal antibodies, either individually or linked to cytotoxic agents, are employed as passive antibodies to target specific cell markers.
3. **Passive Antibody for Enhanced Detoxification:** Passive antibody administration enhances the elimination of toxic substances.
4. **Passive Antibody Modulation of Immune Responses:** Monoclonal antibodies are utilized as passive antibodies to influence the cellular or messenger components of in vivo immune responses.
5. **Anti-Idiotypic Antibodies as Immunogens:** Anti-idiotypic antibodies are administered as immunogens to elicit specific immune responses.

The applications of monoclonal antibodies in animals are evolving rapidly, encompassing diverse therapeutic and preventive domains.

Advantages of using hybridoma technology

The hybridoma serves as an enduring reservoir of monoclonal antibodies, providing a consistent and perpetual supply.

- With high reproducibility and scalability, it offers an inexhaustible source of production.
- Hybridoma technology yields antibodies of exceptional purity and specificity.
- It enables the execution of assays with remarkable sensitivity and precision.
- The necessity to maintain animals within the laboratory for antibody production is eliminated.
- In this method, the purity of the antigen or immunogen is not obligatory.
- The selection mechanism aids in pinpointing the suitable clones against a specific antigen.
- This technique is less labor-intensive compared to *in vitro* antibody generation methods requiring immune libraries.
- Reliability of antibodies is a critical aspect of hybridoma technology, ensuring accurate analyses and assay development. Once hybridoma cells attain stability, they enable the continuous production of cost-effective, standardized antibodies.
- An invaluable research tool, applicable across diverse fields like toxicology, animal biotechnology, medicine, and pharmacology. Hybridoma technology's versatile applications encompass research for vaccine production and its utilization in various chemotherapeutic regimens to combat different cancer types.

Challenges of hybridoma technology

Engaging in this method demands a substantial timeframe, spanning from 6 to 9 months.

- The process entails notable expenses and substantial production efforts.
- Generating antibodies against small peptides and fragment antigens is not feasible through this method.
- Hybridoma culture is vulnerable to heightened contamination risks.
- The efficiency of viable cell generation is notably low, with over 99% of cells perishing during the cell fusion phase. This reduced efficiency not only limits the method's effectiveness but also narrows the diversity of potentially beneficial antibodies targeted against specific substances.
- When monoclonal antibodies are targeted at a single antigenic determinant, they do not exhibit cross-reactivity with other determinants. However, it's important to note that within mammalian chromosomes, retroviruses are commonly found. Animals used in monoclonal antibody production, such as mice, may carry various viruses like vesicular stomatitis virus, retrovirus, reovirus, herpesvirus, and thymic virus. This introduces a risk of cross-contamination or potential human infection, which is a significant concern for disease transmission from mice or rats to humans. Despite rigorous purification efforts, it is impossible to provide an absolute guarantee that monoclonal antibodies produced through the hybridoma technique are entirely free of viruses. In the context of human systems, there are no stable myeloma cells suitable for antibody production as an alternative to mouse myeloma cells

Conclusion

In the present day, hybridoma technology stands as the predominant approach for procuring monoclonal antibodies. These antibodies emanate from hybridoma cells, formed through the fusion of a cohort of evanescent antibody-producing B cells with immortal myeloma cells. They serve as a highly valuable resource for scientific exploration and analysis, finding extensive application across diverse disciplines such as biochemistry, immunology, molecular studies, pharmaceuticals, and biotechnology. Renowned for their remarkable specificity, sensitivity, and reliability, monoclonal antibodies contribute significantly to these fields.

While each methodology indeed carries its own set of advantages and limitations, hybridoma technology retains its preeminence as the "senior" approach to antibody generation, even in 2022. It remains the preferred avenue for uncovering monoclonal antibodies tailored for in vivo applications. As new breakthroughs continually enhance the landscape of antibody discovery, the future appears exceedingly promising for the continued adaptation and evolution of hybridoma technology.

Reference

1. Abrams PG, Oldham RK. Monoclonal antibody therapy of solid tumours. In Monoclonal Antibody Therapy of Human Cancer, Jr.; c1985. p. 103-120.
2. De Almeida R, Nakamura CN, de Lima Fontes M, Deffune E, Felisbino SL, *et al*. Enhanced immunization techniques to obtain highly specific monoclonal antibodies. In *MAbs*. Taylor & Francis. 2018;10(1):46-54.
3. Gamble HR. Application of hybridoma technology to problems in the agricultural sciences. Biotechnology for Solving Agricultural Problems; c1986. p. 39-52.
4. Ganguly, Subha, Wakchaure, Rajesh. Hybridoma

technology: a brief review on its diagnostic and clinical significance. Pharmaceutical and Biological Evaluations. 2016;3:554-555.
5. Ghosh AK, Mason DY, Spriggs AI. Immunocytochemical staining with monoclonal antibodies in cytologically negative serous effusions from patients with malignant disease. J Clin Pathol. 1983a;36(10):1150-3.
6. Kumar A, Singh M, Gupta SM. Hybridoma Technology. In: Biotechnology in medicine and agriculture: principles and practices. (eds. Kumar A, Pareek A & Gupta SM) I. K. Intern; c2012.
7. Langone JJ, Van Vunakis H. Hybridoma technology and monoclonal antibodies. (No Title); c1986.
8. Little M, Kipriyanov SM, Le Gall F, Moldenhauer G. Of mice and men: hybridoma and recombinant antibodies. Immunology today. 2000;21(8):364-370.
9. Liu JK. The history of monoclonal antibody development - Progress, remaining challenges and future innovations. Ann Med Surg. (Lond). 2014;3(4):113-116. DOI: 10.1016/j.amsu.2014.09.001. PMID: 25568796; PMCID: PMC4284445.
10. Milstein C. The hybridoma revolution: an offshoot of basic research. Bio. Essays. 1999;21(11):966-973.
11. Nielsen KH, Henning MD, Duncan JR. Monoclonal Antibodies in Veterinary Medicine, Biotechnology and Genetic Engineering Reviews. 1986;4(1):311-354.
12. Pandey, Shivanand. Hybridoma technology for production of monoclonal antibody. Int. J Pharm Sci. Rev; c2010.
13. Parray HA, Shukla S, Samal S, Shrivastava T, Ahmed S, Sharma C, *et al*. Hybridoma technology a versatile method for isolation of monoclonal antibodies, its applicability across species, limitations, advancement and future perspectives. International immunopharmacology. 2020;85:106639.
14. Gupta, Sanjay, Kumar A, Singh M, Gupta SM. Hybridoma Technology. In: Biotechnology in medicine and agriculture: principles and practices, I. K. International publishing house Pvt. Ltd., New Delhi, India; c2012. p. 338-367.
15. Raybould, Applications of Monoclonal Antibodies in Animal Health and Production. Animal Biotechnology; c1989. p. 21-37.
16. Tizard IR. Veterinary immunology. 9. St. Louis: Elsevier Saunders; c2013. p. 156.
17. Babu YH0 Veterinary immunology: Basic concept and applications, Jaypee Brothers medical publisher; c2003.
18. Zaroff S, Tan G. Hybridoma technology: the preferred method for monoclonal antibody generation for in vivo applications. Biotechniques. 2019;67(3):90-92.
19. Zhang C. Hybridoma technology for the generation of monoclonal antibodies. In Antibody methods and protocols. Humana Press, Totowa, NJ; c2012. p. 117-135