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Therapeutic Implications of Monoclonal Antibody

Mohammad Shane Alam[1*], Farhana Riyaz Shah[1], Muntser Mohammad Fadoul Alhassen[1], Saif Elden B. Abdalla[1], Abdul Mateen[2], Md. Shakir Ahmad[3]

[1]Department of Medical Laboratory Technology, College of Applied Medical Sciences, Jazan University, Jazan, Saudi Arabia
[2]Ageing Research Laboratory, Department of Zoology, Lalit Narayan Mithila University, Darbhanga, India
[3]Department of Pathology, Darbhanga Medical College, Darbhanga, India
Email: *mshanealam8@gmail.com, fshah@jazanu.edu.sa, montaser77fadol@gmail.com, abohamodi2@gmail.com, abdulmatin81961@gmai.com, shakir4nz@gmail.com

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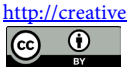

Abstract

Background: The coronavirus disease 2019 (COVID-19) pandemic is a distinct public health issue that calls for the quick development of novel treatments and viral detection. Due to their high specificity and reliability, monoclonal antibodies (mAbs) have emerged as useful diagnostic and therapeutic tools for a variety of diseases. As a result, several scientists have jumped right into developing Ab-based assays for the identification of SARS-CoV-2 and Ab drugs for use as COVID-19 therapy agents. Since the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein is essential for viral infection and has a known precise structure, it has become a key target for the creation of therapeutic antibodies. The use of Ab cocktails is anticipated to be a key component of an efficient COVID-19 treatment plan since SARS-CoV-2 is an RNA virus with a high mutation rate, particularly when subjected to the selection pressure of aggressively applied preventive vaccinations and neutralizing Abs. Furthermore, SARS-CoV-2 infection could provoke an overzealous immune response, leading to a cytokine storm that accelerates the onset of a severe disease. Abs to counteract cytokine storms are also actively being researched as COVID-19 therapies. Abs are now used in SARS-CoV-2 detection assays, including immunoglobulin and antigen tests, in addition to their use as medicines. In order to stop the spread of COVID-19, such Ab-based detection tests are essential surveillance tools. In this article, we'll go over several important ideas related to mAb-based COVID-19 pandemic detection tests and treatments. **Objective:** To understand the role of hybridoma technology in therapeutic implications. 1) To study the basic concepts and options in hybridoma technology; 2) To study the applications of hybridoma technology; 3) To explore how hybridoma technology is applied in diagnostic

histopathology. **Method:** For this method generally there is use of mouse or mammals are transfect with the Ags to find out the formation of antibody afterwards isolate the antibody which has been formed after injecting the antigens for a number of weeks. Following are the steps for mAbs: Step 1: In this step immunization of mouse is done; Step 2: Spleen is used for the isolation of B cells; Step 3: Cultivation of cancerous cells; Step 4: Merging of B cells with Myeloma cells; Step 5: This step cell lines are separated; Step 6: in the next step screening the suitable cell lines; Step 7: observation of multiplication *in vitro* as well as *in vivo*; Step 8: Harvesting. **Discussion:** Now a day there are many diseases which has been cured easily at the mean time it's very difficult to diagnose and get the treatment. Due to advancement of monoclonal antibodies are used in the diagnosis and treatments such as COVID-19, SARS and SARS COV-2. Therefore important part of the monoclonal antibodies are its used in the diagnosis as well as in the treatment tools.

Keywords

Monoclonal Antibody, Cancerous Cell, Receptor-Binding Domain (RBD), Immune System, SARS-CoV-2 and COVID-19

1. Introduction

For a long time monoclonal antibodies universal and highly specific binding proteins were thought to be cure all for diseases and crucial instruments for other biological applications such as diagnosis and research. These applications were only made possible with the development of techniques that enable the separation of individual abs. The hybridoma technology is a pioneer in this regard the technique truly revolutionized the therapeutic. Monoclonal antibodies are not only accurate but also have specific binding proteins which effectively fight against diseases, it is the tools through which we can start the uses of monoclonal antibody for the welfare of mankind like uses of this technology in biology for diagnosis of disease as well other advancement in the research area [1]. Research landscape and the Nobel prize in physiology and medicine was awarded to it in 1984 [2] other methods have been created for the same objective and in this article we examine the hybridoma technologies relevance today how it has changed over time. The benefits and drawbacks in comparison to other approaches this is because the number of patients with covid-19 is increasing globally. Regarding this approach the information provided although it is exceedingly challenging to locate and keep track of patients. For diagnostic evaluation MAb tests are most likely to be able to distinguish Sars-Cov-2 from other viruses exhibiting common symptoms [3]. According to Dillman R O (1987) monoclonal antibodies now a day is part of the rich tapestry of biological knowledge for over a decade. Although enormous advances in the understanding of these molecules have occurred in that time, their strategic application still continues a

major growth area in biological, biotechnological and clinical science. In 1986 Kohler and Milstein were awarded the Nobel Prize in recognition of the importance of their contribution in the field of development and production of monoclonal antibodies. But their true prize must be the realization that their pioneering work has led to an explosive improvement in the understanding of immunology and has produced new possibilities for the investigation, diagnosis and treatment of many hitherto poorly understood diseases. Monoclonal antibodies are not only accurate but also have specific binding proteins which effectively fight against diseases, it is the tools through which we can start the uses of monoclonal antibody for the welfare of mankind like uses of this technology in biology for diagnosis of disease as well other advancement in the research area. [4]. When applied this technology we are going to first isolate and effective implication of that individuals antibodies using different methodology of hybridoma technology. This technology makes a revolution in the field of therapeutic treatment of some form of cancer as monoclonal antibodies activate and destroy the outer wall of the cancer cells with response to the immune system. In this review we make strategies to overcome the situation as recently seen the destruction of corona virus which started from Wuhan and spread all over the world in very short time [2]. As per the information provided number of patients with COVID-19 grows worldwide it is very difficult to find and monitor to evaluate patients with diagnostic tools but most likely monoclonal antibody tests may differentiate SARS-CoV-2 from other viruses causing common symptoms [5].

2. Procedure for the Hybridoma Technology

The hybridoma technology makes possible to immortalize those cells which has been formed by Abs immunized host fused with the myeloma cell that is responsible for the production of clones which is capable to generate single, homogeneous Ab [3]. After the use of MAb the remaining hybridoma cells can be frozen, this frozen hybridoma cells then grown for mass culture or otherwise injected into the animal in order to form tumors which have capacity to generate Abs in large amounts. By this process the frozen cells has capacity to store all the information which makes possible to store the cells for long term. This technology is the one which makes to produce monospecific Abs to the antigens that are impure and also poorly characterized [6]. In addition to these, availability of MAbs let the Abs selection with specific functional characteristics and affinities. Hybridoma technology has several scientific benefits and it provides several opportunities for the examination of clinical and fundamental questions that with the MAbs, are the antibody proteins which have a target antigen (molecule) at antigenic site (one specific site) [7]. MAbs are acting as a key in order to develop new kinds of vaccines. Following **Figure 1** illustrates the hybridoma technology in order to generate monoclonal antibodies.

Today, many monoclonal antibodies are used in the clinical trials as a treatment for many diseases. Monoclonal antibodies are mainly utilized in several

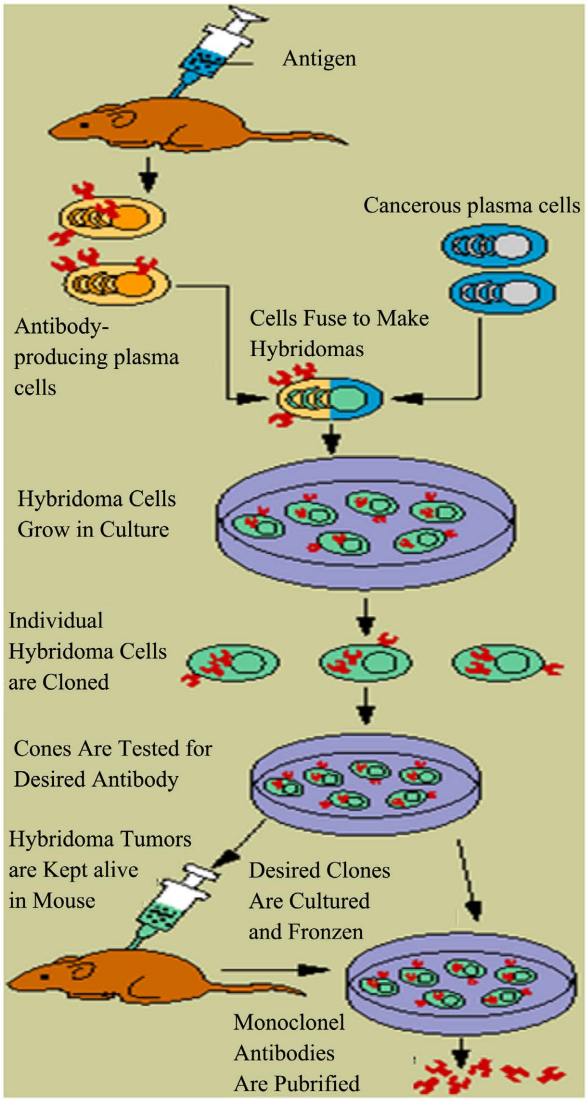

Figure 1. Tell about the production of monoclonal antibody by using hybridoma technology

diagnostic procedures which include: identifying infectious agents, identifying tumorantigens and also auto-antibodies, identifying leukemia and lymphomas, measuring drug levels and proteins in serum, identifying and quantifying the hormones and typing tissue and blood, and identifying the cells that involved in the immuneresponse. Kohler *et al.* in 1975 developed a hybridoma method for producing mabs. The robustness of antibody-producing cells when fused with tumor cells makes this method popular and widely used today. So far, hybridoma technology has been considered a key innovation that enables unlimited production of specific antibody molecules. Kohler *et al.* The 1984 Nobel Prize in Physiology or Medicine was awarded for the discovery of the theory of specificity in the development and regulation of the immune system and the principle of monoclonal antibodies production. Today, monoclonal antibody are established an important research products and its therapeutic application requires further

development, especially with respect to humanization and recombinant production protocols of murine antibodies. Figure 2 shows the primary production of mouse mAbs by hybridomas. Figure 3 shows the different types and uses of mAbs for diagnostic and therapeutic purposes. Traditionally, hybridoma cells have been prepared by cell fusion of spleenocytes (source of B cells) and myeloma cell lines by chemical fusion techniques using, for example, polyethylene glycol (PEG). A recent publication, however, describes another cell fusion technique based on electrofusion [4].

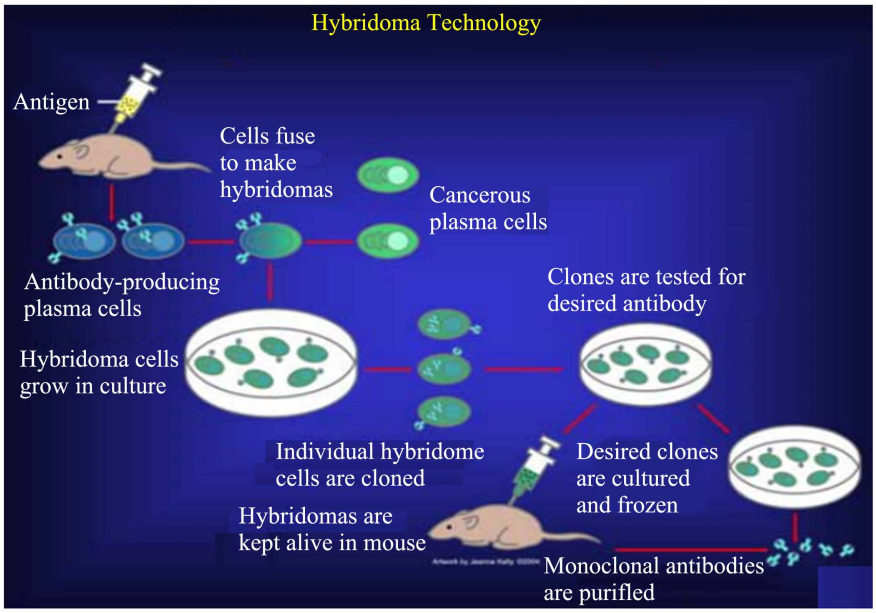

Figure 2. A detailed scheme of the steps involved in hybridoma production.

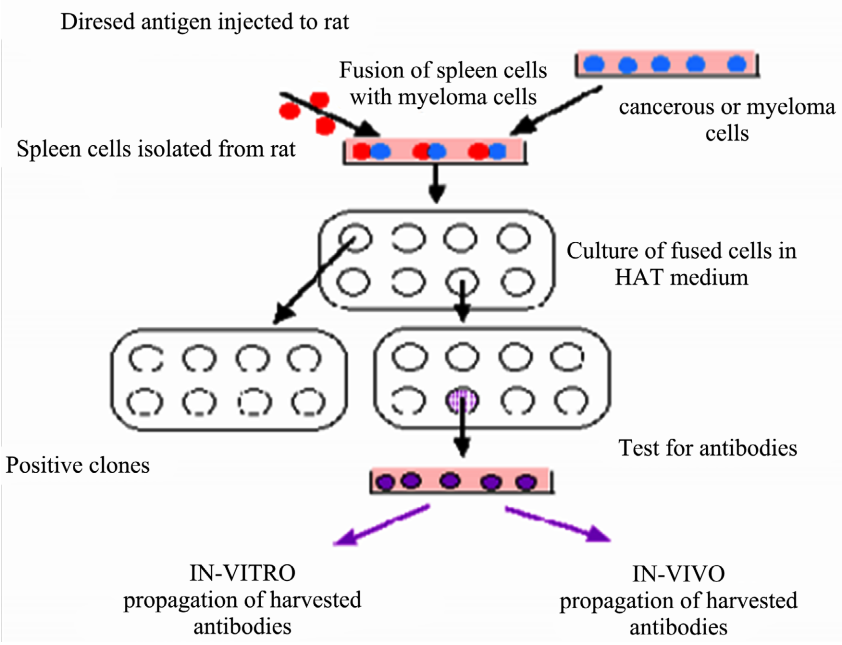

Figure 3. Procedure for hybridoma technique

This technique is superior to the PEG method due to its higher fusion efficiency. [8] reported another approach in which he uses CpG oligodeoxynucleotides (CpG ODNs) to activate cells prior to electro fusion. Kato *et al.* reported that CpG ODN stimulation not only improved fusion efficiency, but also increased the number of Ab producing cells and the number of positive clones. Rat and rabbit His mAbs can be generated by hybridoma technology using rat and rabbit spleen cells respectively. In a recent study rat-His hybridoma clones were generated by cell fusion of immunized rat splenocytes with mouse myeloma SP2/0 cells and generated using recombinant mouse CXCL4 and rhCXCL4. Ab tested. In addition, Zhang *et al.* [9] rabbit hybridomas generate highly sensitive rabbit mAbs that target novel cell surfaces of mesothelioma and other solid tumors that is mesothelin-expressing cancers mouse hybridoma technology for B lymphocyte extraction. This is a multi-step process that has the advantage of producing MAbs that are specific and fully functional on the host which includes the development and optimization of specific immunogenic antigens. After optimization, host animals are immunized by injecting antigen along the peritoneal cavity of mice, followed by administration of antigen for several weeks sera from immunized animals are tested for reactivity and specificity to the immunizing antigen. Certain antigens produce Abs through the production of B lymphocytes, increasing the likelihood of obtaining clonal or hybridoma cells.

On the other hand, animals with high titers of binding antibodies are further selected for splenocyte isolation [10]. Spleen cells are fused with immortalized myeloma cells in the presence of fusogenic agents such as viruses, chemicals, or electrical pulses. The most common myeloma fusion lines are his X63-Ag 8.6539 [11], and Sp2/0-Ag 1410 [12] in BALB/c mice. Fused cells are selected in hypoxanthine-aminopterin-thymidine (HAT) medium. Myeloma cells lack the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) gene required for nucleotide synthesis via a de novo or salvage pathway and are therefore sensitive to HAT medium, whereas unfused B cells are lifelong remain stable. You die because you're small only hybrids (B-cell myeloma) survive. This is because it contains a functional HGPRT gene from B cells. However, hybrid cells retain two of her properties: the antibody-secreting properties of B cells and the continued proliferation (immortality) of myeloma cells. The fused or hybrid cells are then screened by limiting dilution cloning methods or semi-solid selective media to select only hybridomas producing antibodies with the appropriate specificity.

Hybrid cells are formed when b lymphocytes mix with myeloma cells there are several criteria when myeloma cells do not produce abs and contain gene manufacturers such as HGPRT and myeloma cells are fused with abs to create b lymphocytes cell population results show a mixture of cell populations such as B lymphocytes myeloma cells and hybrid cells the cell population mixture is cultured in a selective medium known as hat medium containing the active ingredient aminopterin.

2.1. Hybridoma Technology

A potential advantage of using Abs is immunity to infection for targeting tumors and identifying damaged tissue. Heterogeneity of immune responses and potential and technical ethical issues associated with the generation of human Abs have hindered the use of natural products of the human humeral immune system. The hybridoma technology allows the moralization of antibodies by fusing them with myeloma cells during the generation of cells from an immunized host to create a single homogeneous antibody-producing clone. Hybridoma cells can be frozen and grown in large-scale cultures or injected into animals to form tumors capable of producing large amounts of antibody. Cells can be frozen for long-term storage, so they can be used indefinitely and yield the same antibodies essential for conducting clinical trials. The diagram below (Figure 4) shows the stages of Milstein and Kohler's hybridoma technology development.

Hybridoma technology allows the production of monospecific Abs to antigens, but these are poorly classified and impure. The availability of MAbs allows the selection of Abs with functional properties and specific affinities. For example, issues such as identification and characterization of neutralizing anti-viral Abs, as well as other issues such as purification with high-affinity Abs, are addressed. Column chromatography is used for purification of macromolecules and these are options for low affinity Abs. The advantages of hybridoma technology offer more possibilities for basic and clinical problems even MAbs are used to characterize human Abs produced in autoimmunity [13] [14]. These MAbs are then analyze with complex cellular antigens, which are then localize and purify them [15]. The greatest impact of the identified antibody affects T4,

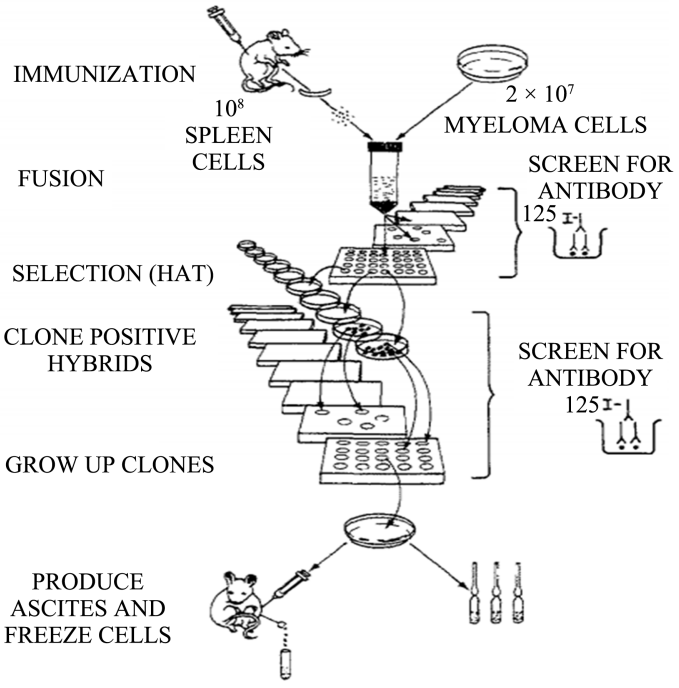

Figure 4. Procedure for Hybridoma preparation.

T8, and other subsets of T cells. These antibodies are used to study immune diseases and characterize lymphoid malignancies [16]. MAbs give the ability to discover about the oncofetal antigens and also to perform about the immmuno histological studies on the various tissues [17].

2.2. Problems Identified

Hybridoma technology has not changed since 1975, when it was developed by Milstein and Kohler. The question here is why it hasn't changed? MAb production is labor intensive and inefficient to obtain sufficient cells, desired Abs and lymph nodes, Abs are used to generate cells from the spleen or peripheral blood of the immunized individual or sometimes the animal of the immunized animal. Fuse these B lymphocytes using myeloma cells and seed the hybrids in small wells (40 plates of 96 wells each) these wells are checked for Abs. Hybridoma cells from positive wells are then cloned, antigens may be weakly immunogenic. In this case, only small amounts (impurities) are available, very few antigenproducing B cells, and only a few positive hybridoma cells are identified. This method is inefficient because 1 in 20,000 or 1 in 200,000 B cells can form viable hybridomas. These processes are currently performed on antigens while hybridoma requires the cells to fuse within 2 - 3 days. This inefficiency makes it very difficult to generate MAbs against weak immunogens with hybridoma technology. Many antigens do not stimulate her B cells, and the Abs that provoke them cannot be obtained like MAbs. It is important to find ways to enhance the immune response to antigens which can be prepared for immunogenicity by chemical modification and conjugation of the antigen to protein carriers [18]. Using a different animal species or strain may also be helpful if antigen testing shows similarity to endogenous epitopes.

2.3. Advancements in the Hybridoma Technology

Great efforts made by many scientist to improve MAbs using hybridoma technology incorporation with chemical fusion promoter replace the Sendai virus used to drive fusion. It is used because myeloma does not secrete Abs as well does not interfere with necessary Ab production. A continuous cell line is used as a fusion partner to generate her B cells with Abs. The feeder layer can consist of additional cells to nourish newly formed hybridomas which are used for any growth and hybridoma production [19]. The FDA has issued Emergency Use Authorizations (EUAs) for three anti-SARS-CoV-2 MAb products for the treatment of mild to moderate COVID-19 infection among non-hospitalized individuals with SARS-CoV-2 infection verified in the lab [20], such as:

Bamlanivimab plus etesevimab: together these are neutralizing mabs that bind to distinct but overlapping epitopes in the SARS-COV-2 spike protein RBD used to treat mild to moderate covid-19 in case of emergency [21] [22] casirivimab plus imdevimab it is human MAbs that bind to non-overlapping epitopes of the SARS-COV-2 spike protein rbd it is also used as a post-exposure prophylac-

tic for those who are at high risk of contracting sars-cov-2 as well as covid-19 [23] [24] sotrovimab this monoclonal antibody was discovered in a SARS-COV-2 survivor in 2003 it binds to an epitope in the spike proteins RBD that is shared by SARS-CO and SARS-COV-2 [25] (**Table 1**).

On the other hand, anti-SARS-CoV-2 MAbs are not now approved for use in patients suffering from severe COVID-19 not yet the patients who have not developed an Ab response or who are not expected to mount an effective immune response to SARS-CoV-2 infection may be eligible for expanded access programs [22].

The feeder tier consists of: Some mouse peritoneal cells there may be extra non-immune splenocytes macrophages are those of rats, guinea pigs, or mice human thymocytes, human peripheral blood monocytes or human fibroblasts these are some of the efforts that have been made with hybridoma. Hybridoma technology is a routine application in the world this technology has been modified many times since it was first published. (PEG) is an agent for inducing cell fusion and form PEG-induced fusion is partially controllable, the success of fusion depends on details such as cell shape and size, shaking strength, and is difficult to standardize. The diagram below (**Figure 5**) shows the generation of Ab-producing hybridoma cells.

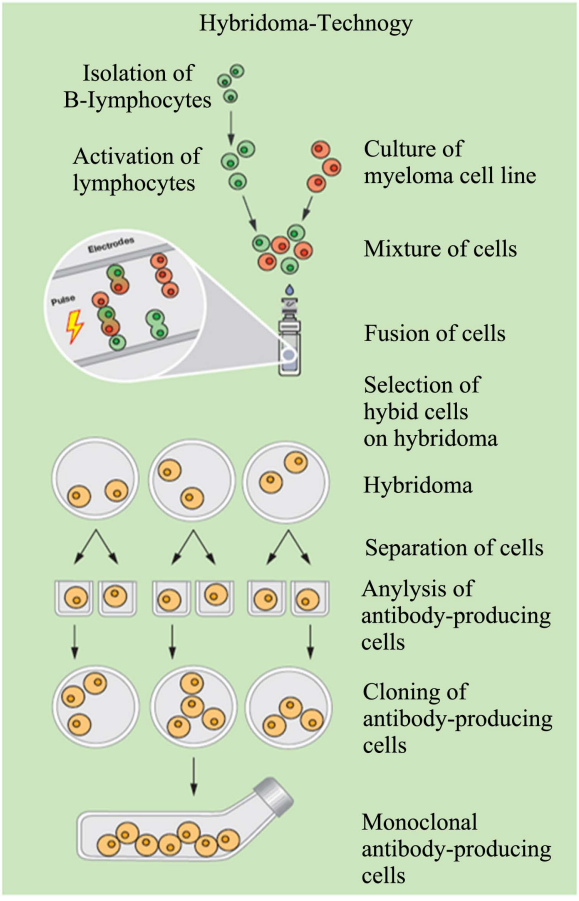

Figure 5. Generation of antibody producing hybridoma cells.

Table 1. Various monoclonal antibodies used in health benefits.

Monoclonal Antibody	Sources	Treatment	References
Bamlanivimab plus etesevimab	Derived from the blood of one of the first U.S. patients who recovered from COVID-19.	SARS-CoV-2 and mild to moderate covid-19 patient.	[21] [22]
Casirivimab plus imdevimab	Type of protein derived from Monoclonal antibody	For treatment and prevention of Covid-19. SARS CoV & SARS Cov-2	[23] [24]
Sotrovimab	Chinese hamster ovary cells	COVID-19 in adults & pediatric patients 12 years of age and older weighing at least 40 kg with positive results of direct SARS-CoV-2 viral testing	[25]

Abbreviations: COVID-19: Corona virus disease of 2019; SARS: severe acute respiratory syndrome; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

3. Methodology

For this method generally there is use of mouse or mammals are transfect with the Ags to find out the formation of antibody afterwards isolate the antibody which has been formed after injecting the antigens for a number of weeks. Following are the steps for mAbs:

Step 1: In this step immunization of mouse is done.
Step 2: Spleen is used for the isolation of B cells.
Step 3: Cultivation of cancerous cells.
Step 4: Merging of B cells with Myeloma cells.
Step 5: This step cell lines are separated.
Step 6: in the next step screening the suitable cell lines.
Step 7: observation of multiplication *in vitro* as well as *in vivo*.
Step 8: Harvesting.

3.1. Improvements in the Hybridoma Technology

To improve inefficiency regarding the hybridoma technology is to advancement the ways which is making Abs of the interest. This reduces the chances of screening and make possible to start with the cells in larger population and immortalize the rare B cells. In mouse this process is done by repopulating the irradiated species incorporation to the lymphocytes from the immunized spleen well boosting the concern species with the Ags to produce the specific Abs and also fusing the cells from the recipient (repopulated) spleen [26].

3.2. Proliferation of Hybridoma Cells (Figure 6)

B cells make the Abs to be enriched by using the *in vitro* immunization when

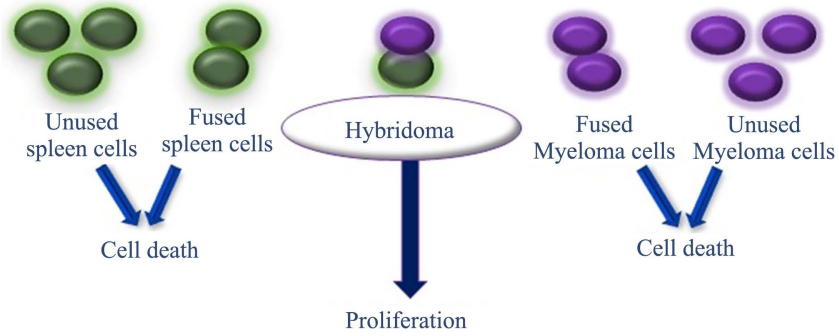

Figure 6. Proliferation of hybridoma cells.

Abs are grown in culture then most of the B cells may die but they are stimulated by the Ags proliferate may overgrow in rest of the populations. When the antibodies are generated *in vitro* are IgM with low affinity which lead the techniques are being developed to solve this problems [27]. Antigen focused fusion technique that is used to enrichment in this technique, the immune cells are incubated with the antigen biotin, conjugates then mixed with the biotin coated myeloma cells [8] [28] [29]. Avidin is joined to bring fusion partner and B cells together and they are fused by the electrofusion in antibodies, the average affinity produced by the hybrids is very high [30].

3.3. Generation of Mutant Monoclonal Antibodies

Now a day many mabs are used in the clinical trials and it was made in the new technology where many of the cells are with the low affinity for the clinical purposes, it is highly desirable which required specific antibodies in the identification and screening of the repertoire of the antibody producing cells. When the desirable specificities are successfully identified then the antibody are not be the subclass or class. By using this process may apply to both the human and mouse mAbs but there is a problem with the human mAbs. To make the human monoclonal antibodies most of the investigators use EBV (Epstein Barr Virus) transformation, in this technique 1 in every 50 B cells are transformed [31]. If the desired Epstein Barr Virus transformed clone is identified then it is possible to fuse with the myeloma cell to improve the stability and also the up regulate production [32]. This type of Epstein Barr Virus transformed cells produces the IgM antibodies which has the low affinity. To generate the more effective MAbs, the hybridoma technology must be improved at the same time, many investigators have also attempted to modify the existing mAbs structure to enhance their effectiveness. Hybridomas are stable in the culture and it must be continue to generate the same Abs indefinitely. The rare mutant clones have arisen that produce or generate the antibody that has changed its affinity, specificity, subclass or class. By studying these type of mutants will make us to know or obtain the better MAbs.

Following **Figure 7** illustrates the graphic depiction of the ELISA assay which allows to screening for the higher binding mutants.

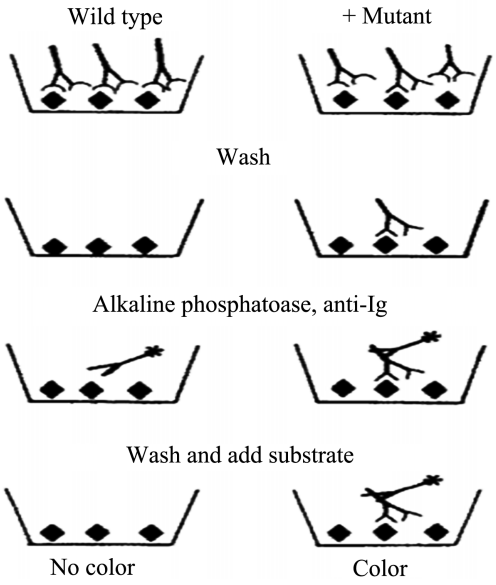

Figure 7. Low density ELISA for high binding mutants hybridoma cells.

If the low density of the antigen is adsorbed to ELISA plate and then the low avidity of IgM Abs are also to be added, after addition the Abs could not able to reach the across to another antigen which will allow the attachment on the binding sites [33]. After fusions the Abs have the affinities of 10^5 or 10^6 M^{-1} this will not bound to the antigens it will allow the bounding at one side only. These types of Abs are then washed when anti-Ig is added and it will be the negative reaction. There is presence of high affinity Abs which will bind to the antigen if it bounds on only one side [34]. A high avidity IgA or IgM Abs with the binding sides per molecule will reach the low density of the antigen and also it may attach multiple combining sites hence these types of Abs are less likely to wash away this can be able to detect when the anti-Ig reagent is added into it.

This ELISA technique combined with the sib selection technique to screen the hybridoma cells in large numbers for the variant clones and also it generates the higher binding antibodies [35]. This approach is starting with the low affinity mouse with IgGI antibody which binds the hapten p-azophenylarsonate (Ars). Following **Figure 8** illustrates the sib selection for higher binding mutants and it also represents how the ELISA technique is combined with the sib selection technique.

In this technique, it is able to plate 250 - 500 cells and 40 wells and 96 well dishes and these allowed the cells to grow in the half confluence and also it allows the screened supernatant by using the low density ELISA. Here, the reconstructions experiment is shown if 1 of the 500 cells are plated in the 96 well dishes and that had the 20 fold higher affinity than the others, then the supernatant from the well will provide 2 to 3 fold to the higher signal in the ELISA. Thus, it is able to screen 2×10^6 hybridoma cells for the higher binding mutants. Following **Figure 9** ELISA with the parental 36 to 65 IgG1 antibody and two control antibodies, 45 to 223 and 36 to 71, which binds with the 20- and 200-fold

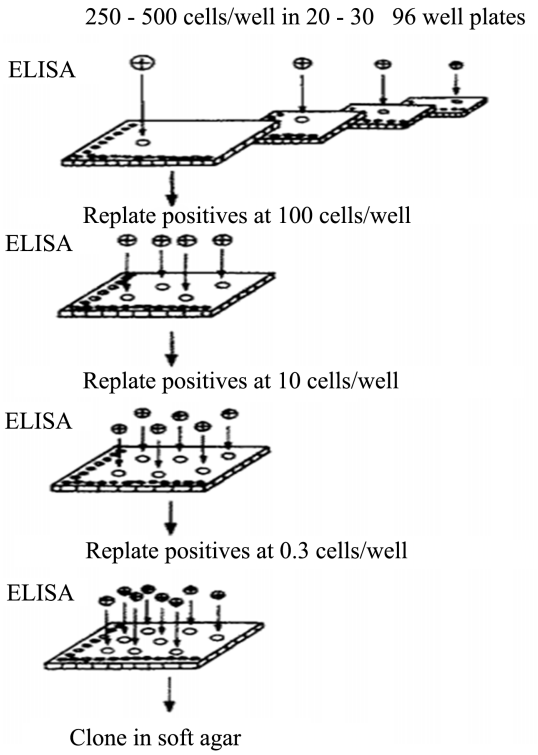

Figure 8. Sib selection for higher binding mutants.

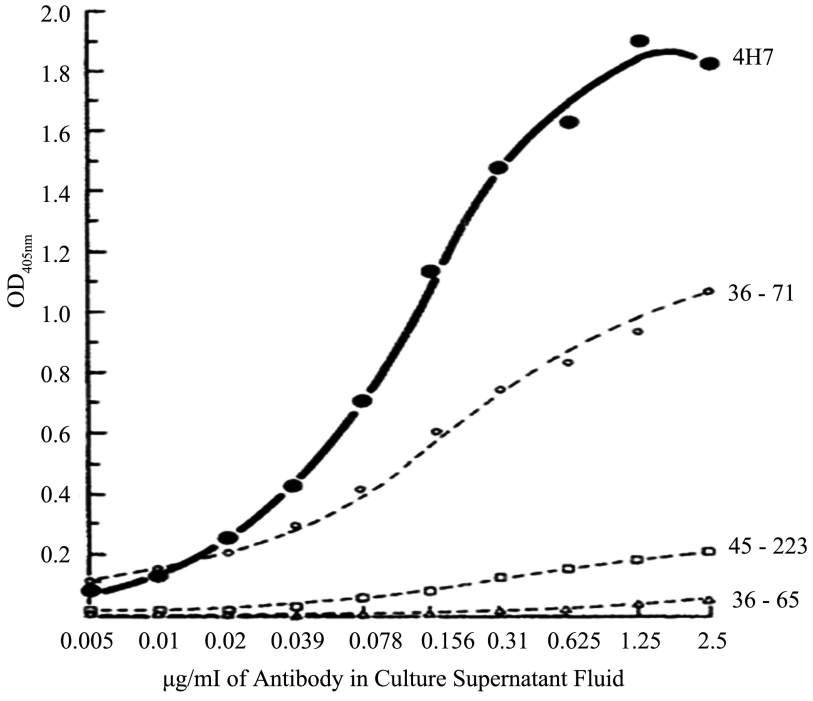

Figure 9. ELISA shows a higher binding mutant.

with higher affinity and also 4H7 is one of the higher binding variants and that have the isolated and characterized (**Figure 10**, ELISA of a higher binding mutant).

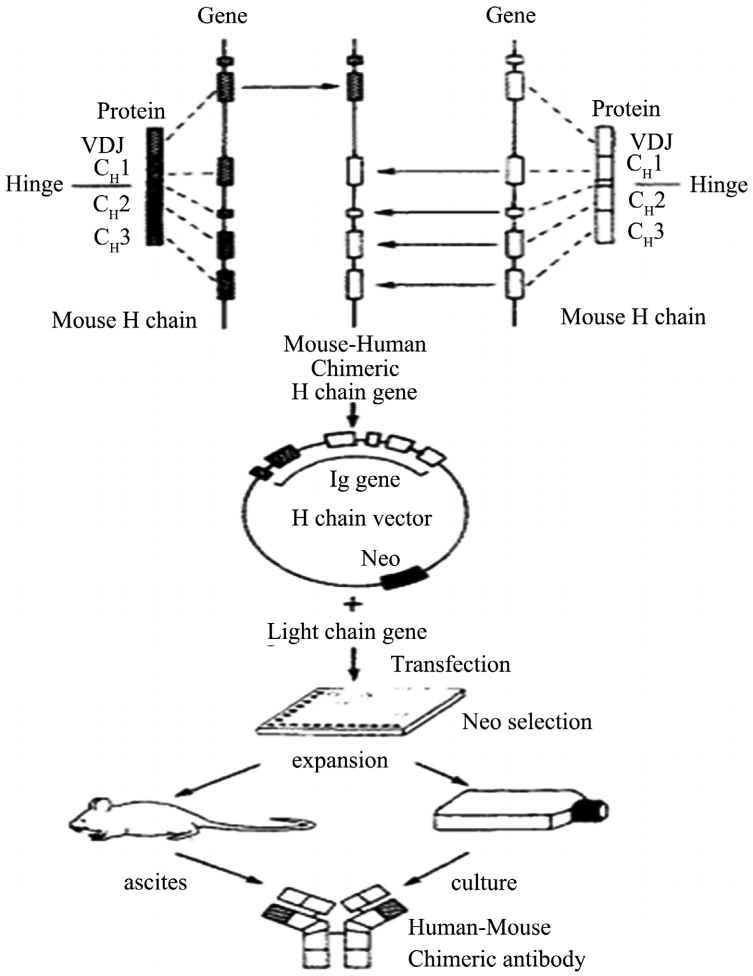

Figure 10. Shows the production of human-mouse chimeric antibodies.

The fourth variant well bound than the high affinity 36 to 71 Abs, which is encoded by germ line variable (V) region genetic elements 36 to 65 but it is heavy and light chain V region somatic mutations *in vivo* to generate 200-fold that increase in the affinity. The higher binding mutants from the 36 to 71 are identical to 36 to 65 in the heavy and light chain V region sequences and it has the same affinity as 36 to 65 [35]. All the deletions or mutations in the constant (C) regions are resulted in the increase of avidity rather than the affinity by polymerization of the H2L2 molecule or by inducing the aggregation.

Another problem in the hybridoma technology is many of the MAbs do not express the desired isotype for the specific effectors functions. The antibody that maintains the binding characteristics was produced by the sib selection technique to identifying the sub clones that has the switched in the tissue culture. [36]. The genetic techniques of the somatic cell are used to identify the mutants that are useful and also suggest for the new approaches, and the engineering of the MAbs for the clinical use and it is accomplished with the rDNA technology. There are some methods for heavy and light chain V regions which are used for encoding the antigen binding site. These genetic elements may attach to the dif-

ferent C regions and these are expressed in the non-producing myeloma to produce the chimeric antibodies.

The following figure illustrates the production of the chimeric antibodies of both human and mouse.

At first, the immunoglobulin gene is cloned with the mouse MAb of the interest the V region may encode the antigen and then the binding site is isolated and also attached to the human C region genes. The constructs may contain both heavy as well as light chains transferred into the nonimmunoglobulin that produces the recipient cell. The cells are screened to the Ab production that allow the designated Ab with these cells known astransfectomas it produces the human chimeric Abs. This makes possible to attach the antigen binding site to different perpetual regions. This may be attached to the nonimmunoglobulin effector functions that are provided by the drugs, enzymes, or the hormones to the antibody binding sites. The recombinant DNA technologies also generates the circumventing the problem for the currently available antibodies are rat or mouse that origin and these are immunogenic in the man. The immunosuppressed patients make the Abs that react with both the C regions and V antigen combining site of the mouse MAbs.

3.4. Problem Identified

MAbs is tool in through which gaining lots of biological knowledge for over a decades, although enormous advances in the understanding of these molecules have occurred in that time, their strategic application still continues a major growth area in biology, biotechnological and clinical science. In 1986 Kohler and Milstein were awarded the Nobel Prize in recognition of the importance of their contribution in the field of MAbs production and development. But their true prize must be the realization that their pioneering work has led to an explosive improvement in the understanding of immunology and has produced new possibilities for the investigation, diagnosis and treatment of many hitherto poorly understood diseases. The modern routine histopathology laboratory although having benefited from a degree of automation and continually evolving enhancements to its standards equipment remains an area that is sensitive to increases in workload activity. Spare capacity relates directly to processing capability and the number of staff available to perform the associated tasks required to produce stained histopathological sections for subsequent microscopial examination and diagnosis by a pathologist. Many modern laboratories have experienced significant increases in workload activity over the past few years as a result of waiting list initiatives and mergers without a directly proportional rise in staffing levels. It is inevitable that these laboratories had to streamline their methodologies and repertories in order to exist in today's high throughput working environment and still continue to provide an efficient and effective quality service. In hybridoma technology a methodology has been developed based on the chain shuffling of VB genes and selection on antigen to convert rodent antibodies into completely human antibodies with similar binding characteristics. This

research discusses in detail the role of hybridoma technology in therapeutic implications of monoclonal antibody.

4. The Production of Human Monoclonal Antibodies

The difficulties in the human MAbs there is a need for the Ab forming B cells and the major problem is that it cannot be immunized with the tumor antigens as the cancer patients Abs are contrary to their tumor antigens, only B cells may produce the Abs against these antigens in the lymph nodes that draining in the tumors. Here, the better fission is needed to make clear human MAbs in this approach, one of the methods is making the human MAbs to create a mice which express the human Ab genes. The Mice with SCID (serve combined immune deficiency) that are engrafted with human peripheral blood or it may completely reconstitute with the bone marrow, human thymus and fetal lymph nodes.

Improvement in Monoclonal Antibody

Monoclonal antibodies are produced by introducing an antigen to a mouse and then fusing polyclonal B cells from the mouse's spleen to myeloma cells. In the subsequent identification step, the culture supernatants of all hybridoma cells are screened weekly for the production of the antibody of interest. Still more efforts are needed to improve the Mabs for the traditional treatment like apart from cancer, COVID-19, SARS, and SARS CoV-2 go for advance treatment. One may easily see how sophisticated formats were developed in response to challenges posed by therapeutic indications.

5. Objectives of the Study

The following are the primary and secondary objectives of the study.

5.1. Primary Objective

To understand the role of hybridoma technology in diagnostic histopathology.

5.2. Secondary Objective

- To study the basic concepts and options in hybridoma technology.
- To study the applications of hybridoma technology.
- To explore how hybridoma technology is applied in diagnostic histopathology.

6. Limitations of the Study

- This study concentrates hybridoma technology and not any other technology.
- This study concentrates on role of hybridoma technology in diagnostic histopathology and does not consider any other application of hybridoma technology.
- This results of the experiments of this study are specific to findings made in a

single biotechnology company in India and results may vary in other labs under different experimental conditions.

7. Conclusion

Now a day there are many diseases which has been cured easily at the mean time it's very difficult to diagnose and get the treatment. Due to advancement of monoclonal antibodies are used in the diagnosis and treatments such as COVID-19, SARS and SARS COV-2. Therefore important part of the monoclonal antibodies is used in the diagnosis as well as in the treatment tools. However, there is still significant growth potential for the therapeutic antibody field. Traditionally, antibodies have been used for the treatment of cancer, autoimmune diseases, and infectious diseases. If the molecular mechanisms of a specific disease can be clearly elucidated and the specific proteins or molecules involved in pathogenesis can be identified, antibodies may provide an effective therapeutic option.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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