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Preparation of Synthetic Antibodies and their use in Drug Identification

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ABSTRACT

This research examines antibodies, their overall structure, and the tools used for recognizing diverse antigens. In this study, a review of the historical development of the immune response caused by them, particularly with their main feature, the ability to produce synthetic antibodies that specifically react only with one or a few among an unlimited number of natural or synthetic substances (antigens) is presented. Significant progress has been made over the past centuries in understanding the biological basis for their incredible characteristics. This research explores hypotheses regarding the origin of antibodies, with a structural basis for antibody features and subsequent speculations about possible tools for examining the production of all diverse antibodies. When antibodies are used as antigens, the antibodies generated from them recognize various epitopes on the immunizing antibody. Many early studies on antibody sequences and three-dimensional structures have been conducted with mouse or human myeloma proteins. Furthermore, it will be acknowledged that recently, the identity of antigen receptors on T lymphocytes has been established, receptors that are notable both for their similarities and differences from antibodies or B lymphocyte receptors. This relates to hypotheses about the origin of antibodies, with a structural basis for antibody features and subsequent speculations regarding possible tools related to the production of all diverse antibodies. The extraordinary mechanism will be centered on how a limited number of genes can produce an apparently unlimited number of antibodies.

Keywords: Antibodies, T lymphocytes, B lymphocytes, Proteins

INTRODUCTION

The immune system of vertebrates is one of the most complex vital systems of living organisms, which can be compared to the complexity of the brain. Just as artificial neural networks are inspired by the brain's functions, artificial immune systems are designed using existing knowledge about how the immune system of vertebrates works. The artificial immune system is one of the newest methods in soft computing.

Therapeutic proteins play an important role in the treatment of various diseases [1-3].

Currently, there are over 660 biologists around the world. With recent advances in protein engineering, today it is possible to adjust desirable protein characteristics to achieve an optimal balance between efficacy, safety, stability, and yield. Producing a protein drug is a very complex process that involves about 5000 critical steps [4]. Throughout all stages of development, the stability of a protein drug is a major concern. The choice of formulation can significantly impact the structure, colloidal properties, and chemical stability, all of which must be controlled in the final product. The large number of formulation parameters and conditions that need to be considered require substantial investment of resources and time. Moreover, it has been shown that only 8% of newly researched candidates addressing drug-related issues gain access to approval [5]. Therefore, the use of limited resources and ultimately improving the success rate of drug candidates is of special importance. Nowadays, high-throughput methods are commonly used in the early stages of protein production to select promising candidates and their formulations, which are proposed to conduct forced degradation studies and real-time stability tests.

RESEARCH HISTORY

Many immunologists agree that the start of at least the modern era of their subject is related to the work of a number of researchers in the late 19th century. In fact, if one had to choose a new discovery that accelerated the transformation of this field from a collection of observations into a scientific discipline, the candidate would be the first demonstrations

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by von Behring and Kitasato (1890) that an animal could be made resistant to tetanus toxin by transferring serum from an actively immunized donor - at least in principle, the serum could be fragmented and the biochemical character responsible for resistance identified and described.

Nearly 50 years later, significant advances were made towards achieving this goal. It was at this time that Elie Metchnikoff (1892) showed that cells play a role in the immunity of an animal due to their ability to phagocytize bacteria. These two discoveries initiated a long debate over whether cells or "humoral" factors are the most important weapons of the immune system.

Interestingly, Robert Koch, whose institute in Berlin was a major center advocating the superiority of humoral factors in immunity, discovered the skin test for that year. Although Koch attributed the reaction to the high toxicity of tuberculin in diseased patients, he did not appreciate that this test eventually became a textbook response of cellular immunity mediated by what is now recognized as T lymphocytes.

Other significant discoveries in these years included cell lysis in the presence of immune serum and complement and anaphylaxis. However, perhaps the signaling event that marked the beginning of the modern era in immunology was the delivery, on March 22, 1900, of a lecture to the Royal Society in London by Paul Ehrlich (1900). In this lecture, Ehrlich outlined a theory regarding the origin of antibodies. He suggested that cells contain receptors or side chains that have a normal function in the cell consuming nutrients and that the antigens are recognized by these receptors due to their structural similarity to normal ligands (cross-reactivity). As a result of interaction with the antigen, the cell is stimulated to actually produce more receptors, and the excess is shed to circulate as "Antikorper" (antibody).

Today, Ehrlich's proposal for the origin of specific antibodies can be called a selective theory that contains this principle and is by no means a clear principle that receptors or antibodies existed simply in animals before the introduction of an antigen, thus enhancing the production of specific antibodies that bind to the introduced antigen. Describing the onset of the modern era of immunology as advancements that occurred in the late century does not mean disregarding the important and foundational contributions of early pioneers like Jenner and Pasteur.

However, their observations and experiments, as established, are essential. Practical methods for inducing immunity were not accompanied by an appreciation of their biological basis.

Further discussion on many of the topics addressed can be found in the following sources. Debra Bibel (1988) provides many classic papers by pioneers in the field of immunology, valuable translations that have been translated into English when necessary and with interpretation. Arthur Silverstein's monograph (1989) includes several articles regarding the historical development of key concepts in immunology. Gallagher and colleagues (1995) have compiled a series of writings with a historical tilt by some of the main contributors in the field. These evoke a personal flavor and sense of excitement that can sometimes be absent from the hard literature. The Annual Review of Immunology series (1983-present) contains relevant articles in its own memoirs, often providing a body of work in a personal historical context. The language of immunology can be an obstacle for another reader. Many specialized terms are explained in the text. Additional definitions and details can be found in dictionaries and encyclopedias.

RESEARCH ESSENTIALS

Antibody (in French anticorps:) or antibody (in English antibody:) is a type of protein produced by the immune system in response to the presence of a specific antigen, circulating in the blood or remaining at the production site to attack the antigen (usually foreign bodies such as bacteria and viruses, but sometimes even the body's own tissues or a food substance) and neutralize it.

Antibodies are found in many body fluids, including tears, respiratory secretions, saliva, intestinal contents, and urine. However, their highest concentration is in blood serum, which is why serum antibodies are used for various tests due to ease of obtaining them.

Antibodies are classified based on their physical, chemical, and immunological properties: Antibody A Antibody D Antibody E Antibody M Antibody G **(Figure 1)**.

Monoclonal antibodies are a type of antibody produced in the laboratory that can be used in diagnosis or treatment. They block the growth of blood vessels in tumors, or they may assist the immune system in killing cancer cells. Monoclonal antibody treatment can lead to side effects for patients. Its side effects can include fever, chills, weakness, headache, nausea, vomiting, diarrhea, low blood pressure, and rash **(Figure 2)**.

What is an antibody test and what is its application in the context of the COVID-19 pandemic?

Antibody tests-also known as serology tests-are not used to diagnose active coronavirus infection, but rather examine proteins in the immune system known as antibodies through a blood sample. The presence of these antibodies in the body means that the individual has been exposed to the virus and has developed antibodies against it. This indicates that the individual has at least some immune protection, although experts are still unsure how strong this immunity may be or even how long it will last. The synthesized molecules have two major functions of antibodies-they can recognize their targets and trigger immune responses.

The first group of synthesized molecules created by scientists simultaneously attaches to both sick cells and disease-fighting cells, resulting in a broad immune system response. This type of immune system reaction is similar to

when natural antibodies are stimulated. These molecules are thermally stable and can be taken orally like many medications. These molecules are used to fight cancer, including prostate cancer, by binding to cancer cells through the secretion of specific proteins and destroying them.

The artificial immune system or AIS, short for Artificial Immune System, was first introduced as an algorithm by De Castro and Zuben under the name of the colony algorithm. The main idea in the artificial immune system is derived from the process of cellular replication after the detection of an external agent in the natural immune system. The artificial immune system has been successfully employed for a wide range of issues-from intrusion detection in networks to classification models, data learning, the concept of robotic data clustering, pattern recognition, data mining, and it is also used for initializing weights in neural networks and optimizing multi- dimensional functions.

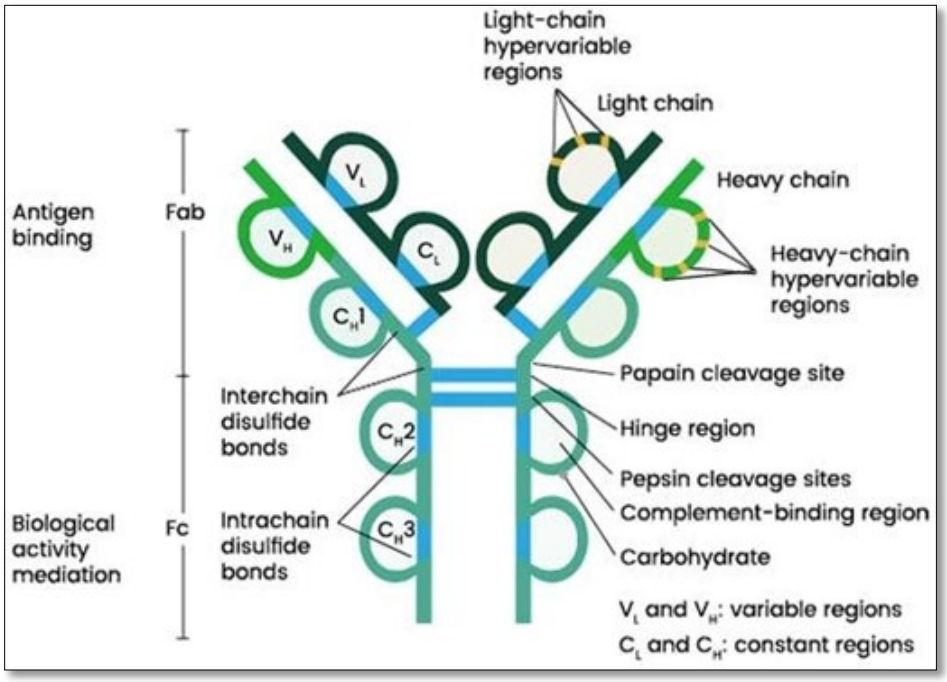

Figure 1. The Arrangement of Antibodies.

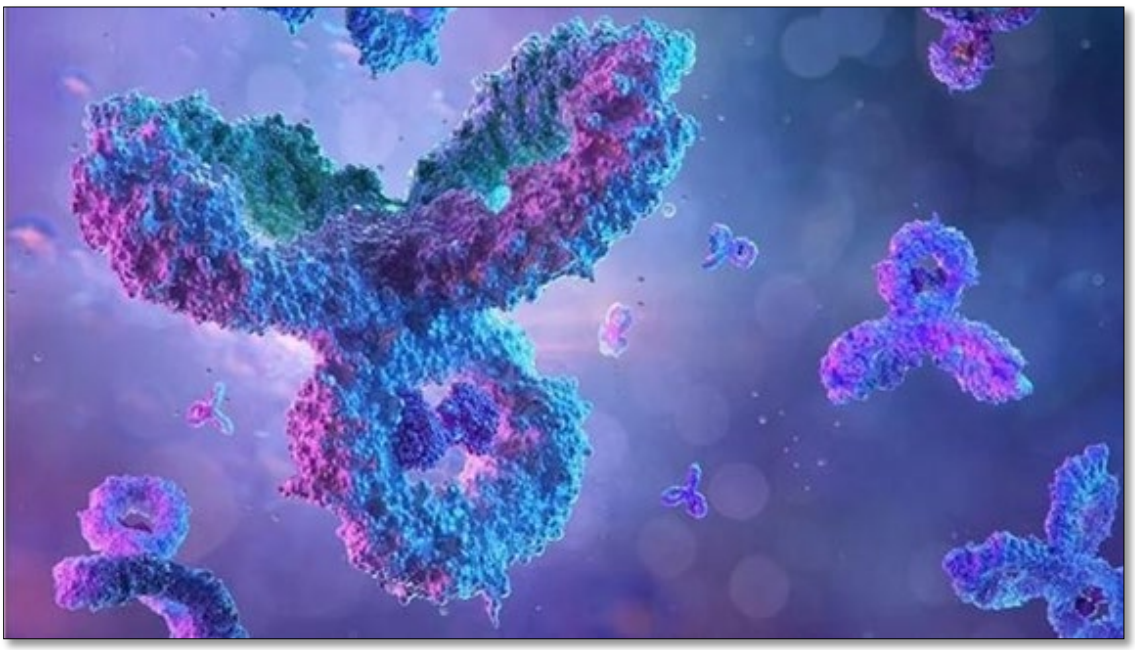

Figure 2. Monoclonal Antibody.

Important characteristics of the artificial immune system are as follows:

1. Use of bit string representation
2. Fixed and identical length for each cell
3. Utilization of a population or a fixed number of members, although in the artificial immune system, by defining length for population cells, a population with variable member numbers can also be utilized.

A new method for creating synthetic vaccines based on protein combinations marks a milestone in the history of vaccine development, as synthetic vaccines represent a significant advancement compared to traditional vaccines based on weakened or inactivated microorganisms. Synthetic vaccines are not only safer than weakened or inactive microorganisms, but also provide the opportunity to design vaccines for specific purposes. The first generation of synthetic vaccines is primarily based on recombinant DNA technology and genetic manipulation. This new generation can sometimes be time-consuming and costly, especially during times of genomic exploration and when facing outbreaks of infectious diseases.

MATERIALS AND METHODS

Antibodies can eliminate cancer. A treatment that uses antibodies helps the body fight cancer, infections, or other diseases; antibodies are proteins produced by the immune system that bind to specific markers in certain cells or tissues. Monoclonal antibody is a type of antibody produced in the laboratory and can be used in diagnosis or treatment.

Monoclonal antibody is a therapy used by the body's immune system to attack cancer cells. Our body produces billions of different types of antibodies that are part of the immune system. They have specific features in the immune system that are targeted, such as pathogens like sick cells or viruses. The monoclonal antibodies used in cancer treatment are designed in the laboratory to target specific antigens in the body that reside on the surface of cancer cells. By targeting these antigens, the antibody can bind to cancer cells and serve as a (call to arms) for other fighters against diseases in the immune system.

More than twelve monoclonal antibodies have been approved by the Food and Drug Administration (FDA) to fight various types of cancer, including breast cancer, liver cancer, bladder cancer, and skin cancer, as well as Hodgkin lymphoma. Initially attempted in advanced melanoma, monoclonal antibodies can keep some patients alive for up to 10 years longer. In cancer treatment, monoclonal antibodies that may directly kill cancer cells, block the growth of tumor blood vessels, or help the immune system kill cancer cells may be used. Naked monoclonal antibodies, or monoclonal antibodies that work on their own, are the most common type of monoclonal antibodies used for cancer treatment. Like all monoclonal antibodies, they bind to specific proteins on cancer cells and mobilize immune cells for their cause. One way they do this is by targeting immune checkpoint inhibitors (immune regulators).

Cancer has grown sufficiently and matured enough to create a set of escape mechanisms to deceive the immune system and ignore it as a threat. This includes impersonating normal body cells and exploiting checkpoint proteins like PD-1 in T cells within the immune system. T cells search for and destroy foreign invaders and cancer cells, but PD-1 helps inhibit T cells so they do not destroy healthy cells. However, many cancer cells wear a protein called PD-L1 that can trick T cells into treating them as normal cells, allowing them to proliferate freely.

That is where naked monoclonal antibodies come in; they can block these molecules and allow T cells to seek out and destroy cancer cells. The naked monoclonal antibody can work by binding to the antigen of the cancer cells and assists in their destruction. An example of this is trastuzumab (Herceptin), which is used to target the HER2 protein that is sometimes found on the surface of breast and stomach cells **(Figure 3)**.

Monoclonal antibodies conjugates: other monoclonal antibodies containing radioactive drugs or toxins that kill cancer cells are identified by the antibody. These are called conjugated monoclonal antibodies. Conjugated antibodies are antibody drugs that have chemotherapy agents attached to them target the surface of cancer cells and deliver the toxic material specifically to that area, this method can eliminate some side effects of chemotherapy that can harm healthy cells when using a single agent. Radioimmunotherapy is a treatment that uses conjugated monoclonal antibodies in combination with radiation. By attaching a monoclonal antibody to a radioactive molecule, this technique can directly deliver the therapeutic amount of radiation to cancer cells.

Monoclonal antibody therapy can lead to side effects for patients, especially when used in combination with other treatments. Its side effects may include fever, chills, weakness, headaches, nausea, vomiting, diarrhea, low blood pressure, and rash. Discuss your cancer treatment options with your doctor. The use of monoclonal antibodies (mAbs) for cancer treatment has achieved remarkable success in recent years. Drug-conjugated antibodies are new treatment options for lymphoma and solid tumors, and immunization antibodies have also recently achieved significant clinical success. The development of therapeutic antibodies requires a deeper understanding of cancer, protein techniques, the engineering of mechanisms of action, resistance, and the interplay between the immune system and cancer cells. This review outlines the fundamental strategies needed to develop antibody therapies for cancer patients through iterative approaches for the targeting and selection of antibodies, extending from preclinical studies to human trials.

Antibody-based cancer therapy has emerged over the past 15 years and is now one of the most successful and important strategies for treating patients with hematologic malignancies and solid tumors. Clinical trial evidence for antibodies in cancer patients has highlighted the importance of iterative approaches for selecting optimal antigen targets and antibodies. Killing cancer cells using monoclonal antibodies (mAbs) can result from the direct action of the antibody mechanism on cell intermediary immune delivery of useful payloads and antibody- specific effects in tumor vasculature and stroma. Tumor antigens that are successfully targeted include epidermal growth factor receptor (ERBB2 EGFR), vascular endothelial growth factor (VEGF), cytotoxic T lymphocyte-associated antigen 4 (CD20 CLA CD30), and CD52. Serological, genomic, proteomic, and bioinformatics databases are also used to identify antigens and receptors that are overexpressed in tumor cell populations or associated with gene mutations linked to cancer cell proliferation such as CTLA4, MET, EGFR vIII, and fibroblast activation protein (FAP).

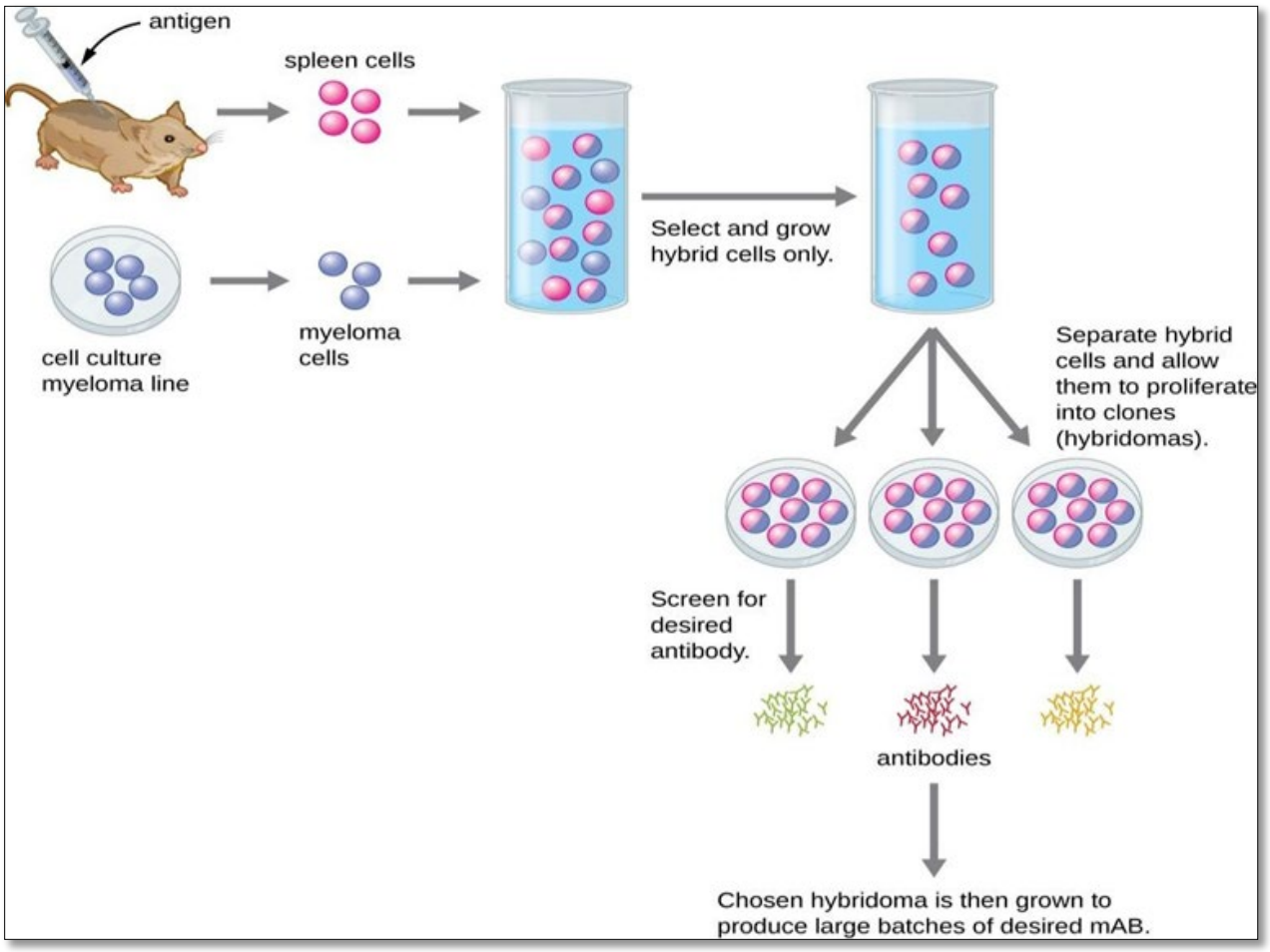

Figure 3. Research and Development in Monoclonal Antibody Production for Cancer Treatment.

The successful development of candidate mAbs involves a complex process of scientific and clinical evaluations that include identifying the physical and chemical characteristics of the antibody; examining the precise and effective functionality of the antibody; analyzing the performance in laboratory conditions and distribution in tumor or syngeneic systems; and witnessing the therapeutic activity of the antibody inside the body. A primary goal for clinical evaluation of mAbs has been to determine the toxicity and therapeutic efficacy of the antibody alone or as a delivery system for radioisotopes with other toxic agents. It is also very important to assess its performance in laboratory conditions through determining the biodistribution in patients and evaluating the level of antibody uptake in the tumor versus normal tissues.

Researchers at the Massachusetts Institute of Technology (MIT) have successfully produced synthetic antibodies using carbon nanotubes and polymers. In this project, the researchers coated the surface of the nanotubes with an amphiphilic polymer (with both hydrophilic and hydrophobic ends), which had different binding sites that could attach to various target molecules. The researchers found that this feature could be used to trap different proteins

such as those related to cancer or diabetes by effectively trapping predetermined proteins using a special polymer; such a structure is a synthetic antibody.

This method is a very interesting strategy that can produce various types of antibodies. On the other hand, this strategy can be used to construct various sensors in such a way that instead of covering carbon nanotubes with natural antibodies, synthetic antibodies can be used. Natural antibodies sometimes break down within cells or tissues and disappear, while synthetic antibodies have higher stability [6, 7]. In a study, a new synthetic antibody for troponin was synthesized through the molecular imprinting (MI) effect on multi-walled carbon nanotubes (MWCNT). This was achieved by binding TnT to the surface of MWCNT and filling the empty spaces by polymerization under mild conditions with acrylamide (monomer), N- Methylene bisacrylamide as a cross-linker, and ammonium persulfate (initiator). After removing the biological template, the resulting material was able to rebind TnT and separate it from other interfering species. The stereochemical recognition of TnT with the capability of non-rebinding by non-imprinted materials (NI) was confirmed using non-imprinted template printing. Surface modification of MWCNT was verified by SEM analysis and FTIR. The ability of this biological material to restore TnT was confirmed by adding it as an electroactive compound in a PVC- plasticizer mix coated with wire made of silver, gold, or titanium. Anionic ranges of 50 millivolts per decade for gold wires coated with MI-based membranes immersed in HEPES buffer were obtained from a pH of 7. The detection limit was 0.16 grams per milliliter. Neither NI-MWCNT nor MWCNT showed the ability to detect this pattern. A good selectivity against creatinine, sucrose, fructose, myoglobin, sodium glutamate, thiamine, and urea were observed. This sensor was successfully tested on serum samples. It is expected that this effect will open new horizons for designing new synthetic antibodies for complex protein structures [8]. A Japanese-American research group is currently working on developing methods for producing polymeric particles sized like proteins that are comparable in binding and functional selectivity to true and natural antibodies. This method combines the creation of molecularly imprinted nanoparticles with a functional monomer optimization strategy. In fact, they have created a plastic antibody that is considered a synthetic version of a real antibody; they also claimed that this antibody functions in the blood of a living animal, and it can now be observed that those synthetic polymer nanoparticles created through an inorganic process in a chemical laboratory can serve as suitable alternatives for biological macromolecules. Today, the applications of these antibodies include antidotes for toxins, protein purification, and therapy. According to the results obtained, it is concluded that polymeric nanoparticles can efficiently absorb cytotoxic peptide metabolites from the blood. The strong and specific affinity of the receptor nanoparticles enables rapid assay and separation of the target peptide in environmental samples.

In a study conducted by Sirid Amy Klerman and colleagues in 2002, the discovery of antibodies using transgenic mice for the production of human monoclonal antibodies for therapy was introduced. This research acknowledged that technical advances in the 1980s and early 1990s led to monoclonal antibodies that are now approved for human treatment. Transgenic mouse strains provide a powerful technological platform for generating fully human monoclonal antibodies as therapies. Ten antibodies have entered clinical trials since 1998, and more are under clinical investigation. Improved transgenic mouse strains provide a powerful technological platform for future human therapies [9].

The first transgenic antibody derived from mice entered human clinical trials in 1998 [10]. Since then, other mAbs have included antibodies against epidermal growth factor receptor [11], CTLA4 (cytotoxic T lymphocyte-associated protein 4), and several cancer-specific target antigens. A range of human antibodies from transgenic mice are currently in clinical trials, and data collected from patients to date indicates that these antibodies meet expectations due to their non-immunogenicity and pharmacokinetic properties. Effectiveness should be evaluated on a case-by-case basis, as a function of the correlation / antibody strength as well as the disease connection of the target antigen [12,13].

In another study, to facilitate and expedite time and resources, artificial neural networks (ANN) were used to predict the biophysical properties of therapeutic monoclonal antibodies, namely melting temperature Tm, the onset aggregation temperature Tags, and interaction parameter KD as a function of pH and salt concentration. Designed ANNs achieved high prediction accuracy with first-stage screening datasets. By using only, the amino acid composition, they were able to keep the ANNs simple, providing possibilities for general use, robustness, and high interpretability. Finally, a novel approach (knowledge transfer) was proposed, which could be easily applied based on the simple algorithm design to understand how the designed ANNs reach their conclusions [14].

According to Ehrlich, nutrients are substances that can enter the protoplasm and are therefore easily absorbed. Toxins (which can induce antibody formation) have hap to for groups that allow the toxin to be recognized by cell sidechains. Antioxidants are nothing more than side chains produced during excessive reconstruction, and thus they come out of protoplasm and therefore exist close to Ehrlich's italic. Another group designated as toxophore is the cause of toxic action. Other substances, alkaloids, aromatic amines, antipyretics, and aniline dyes do not contain hap to for groups and cannot be absorbed **(Figure 4)**.

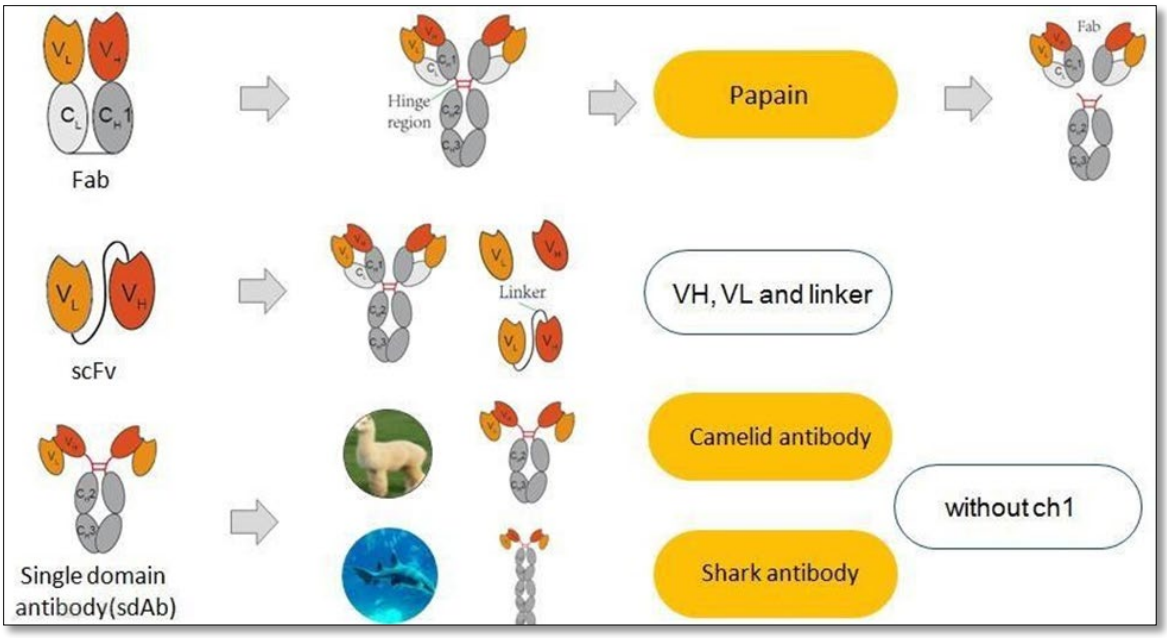

Figure 4. Production and Method of Manufacturing Monoclonal Antibody.

Hence, they do not induce antibodies, a notion that had been held for two decades before Landsteiner's study showed that such groups, if attached to appropriate carrier proteins, could induce specific antibodies. The "relationship of the corresponding groups", that is, the side chains of the antitoxins and the food (toxins) must be specific, compatible with each other, like, for example, male and female screws (Pasteur), or in the form of a lock and key (E. Fischer). In addition, the cells are so-called trained or educated to produce the necessary side chains in increasing amounts [15].

RESULTS

The development of a new protein drug typically begins with the design, expression, and biophysical characterization of many different protein structures. The large number of constructs is initially reduced to a small number of candidates that have the desired biological and physicochemical properties. This process of protein expression and characterization to find the most promising molecules is both costly and time-consuming. As a result, many companies are adopting and implementing philosophies; for example, operating systems for protein expression and computational learning methods, machine learning to save resources and facilitate the production of protein drugs. It is also worth remembering that to stimulate the formation of antibodies, they must be conjugated to immune system carriers (usually proteins), through which an organism can recognize its own cells from other non-self-cells. For this reason, research institutions have used antibodies from the blood of recovered patients to treat severe cases, including patients with COVID-19. This new clinical trial is different from those studies because gemcilumab is an antibody that is made in the lab and can be mass-produced if proven effective. In fact, it is this connection between the production of synthetic antibodies and clinical trials that leads to the identification and development of a variety of preventive drugs.

REFERENCE

1. Gong R, Chen W, Dimitrov DS (2012) Expression, purification, and characterization of engineered antibody CH2 and VH domains. Methods Mol Biol 899: 85-102.
2. Dimitrov DS (2010) Therapeutic antibodies, vaccines and antibodyomes. MAbs 2 (3): 347-356.
3. Elvin JG, Couston RG, van der Walle CF (2013) Therapeutic antibodies: Market considerations, disease targets and bioprocessing, Int J Pharm 440 (1): 83-98.
4. Lagassé HA, Alexaki A, Simhadri VL, Katagiri NH, Jankowski W, et al. (2017) Recent advances in (therapeutic protein) drug development. F1000Res 6: 113.
5. US Department of Health and Human Services. Innovation or stagnation: Challenge and opportunity on the critical path to new medical products.
6. Zhang Y, Zhu C, William M (2002) Pardridge, Antisense Gene Therapy of Brain Cancer with an Artificial Virus Gene Delivery System, Department of Medicine, UCLA School of Medicine, Los Angeles, California 90024, USA.

7. Agasti SS, Rana S, Park M-H, Kim CK, You C-C, et al. (2010) Nanoparticles for Detection and Diagnosis. Adv Drug Deliv Rev 62: 316-328.

8. Moreiraa FTC, Dutrab RAF, Noronhad JPC, Cunhae AL, Salesa MGF (2011) Artificial antibodies for troponin T by its imprinting on the surface of multiwalled carbon nanotubes: Its use as sensory surfaces. Biosens Bioelectron 28: 243-250.

9. Kellermann SA, Green Larry L (2002) Antibody discovery: The uses of transgenic mice to generate human monoclonal antibodies for therapeutic. Curr Opin Biotechnol 13(6): 593-597.

10. Yang XD, Corvalan JR, Wang P, Roy CM, Davis CG (1999) Fully human anti-interleukin-8 monoclonal antibodies: potential therapeutics for the treatment of inflammatory disease states. J Leukoc Biol 66: 401-410.

11. Yang XD, Jia XC, Corvalan JR, Wang P, Davis CG, et al. (1999) Eradication of established tumors by a fully human monoclonal antibody to the epidermal growth factor receptor without concomitant chemotherapy. Cancer Res 59: 1236-1243.

12. Fishwild DM, Hudson DV, Deshpande U, Kung AH (1999) Differential effects of administration of a human anti- CD4 monoclonal antibody, HM6G, in nonhuman primates. Clin Immunol 92: 138-152.

13. Russell ND, Corvalan JR, Gallo ML, Davis CG, Pirofski L (2000) Production of protective human antipneumococcal antibodies by transgenic mice with human immunoglobulin loci. Infect Immun 68: 1820-1826.

14. Gentiluomoa L, Roessnerb D, Augustijnc D, Svilenova H, Kulakovad A, et al. (2019) Application of interpretable artificial neural networks to early monoclonal antibodies development. Eur J Pharm Biopharm 141: 81-89.

15. Steiner LA (1997) Foundations of Modem Biochemistry, Chapter 9 Antibody specificity and diversity: The proteins (Part I). 3: 277-322.