Monoclonal Antibodies in Different Diseases
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Abstract. This article introduces the four development stages of monoclonal antibodies: murine monoclonal antibodies, chimeric monoclonal antibodies, humanized monoclonal antibodies, and fully human monoclonal antibodies. Fully human monoclonal antibodies are among the most advanced and commonly utilized in the world. This article also introduces the disease history, pathogenesis, symptoms, epidemiological history, treatment methods, information on monoclonal antibodies, and the mechanism of action of monoclonal antibodies that can treat hepatitis B virus, trastuzumab and eculizumab. Therapeutic hepatitis B virus monoclonal antibody has not yet entered clinical application, and in-depth research is still needed by scholars. Trastuzumab plays an irreplaceable role in the treatment of HER-2-positive breast cancer, and eculizumab is a monoclonal antibody for the treatment of rare diseases, known as the "most expensive monoclonal antibody", which plays an important role in the treatment of rare diseases such as PNH. This article is concluded that the in-depth research on the selection of appropriate targets for monoclonal antibodies in clinical applications and the innovative and revolutionary research on the way of administration will become one of the research hotspots in the global biomedical field in the future. Biosimilars will also become a research hotspot in the global biomedical field in the future as a result of researchers, companies, and institutions dedicating a great deal of their research energy to this area as the patents on various monoclonal antibodies expire.

Keywords: Monoclonal antibodies; hepatitis B virus; breast cancer.

1. Introduction

Monoclonal antibodies have undergone rapid development for almost 50 years, and today they are one of the primary areas of biomedical research. Because of their high specificity and low defectivity, monoclonal antibody therapies have gradually been licensed for use in the treatment of cancers, immunological illnesses, cardiovascular diseases, and neurological diseases, among other things, with enormous scientific value and market potential. Currently, China's monoclonal antibody medication market is not as large as it could be, and the country invests less in monoclonal antibody research than does the United States and European countries. In contrast to polyclonal antibodies, which are produced by numerous types of B cells, monoclonal antibodies are substances that are produced by only a single kind of immune cell. Monoclonal antibodies are created when cells combine with malignant cells. These fusion cells can divide like tumor cells and make antibodies like immune cells. Hybrid cells following fusion can produce massive quantities of the same antibody. When it is applied in medical care, the little alternations in antigen recognition, if any, serve to help minimize side effects. Antigens stimulate B cells to multiply and develop into plasma cells, which produce glycoproteins known as antibodies. Antibodies attach to specific antigens and are the primary effector molecules that mediate humoral immunity, with the majority of them found in serum. As for the development of Monoclonal antibodies, Molecular biologists Koehler and Milstein collaborated to establish monoclonal antibody (mAb) technology in 1975[5]. The first hybridoma cell line developed through this method can be passaged in vitro indefinitely and secretes anti-sheep erythrocyte-specific antibodies that identify a single epitope. This discovery led to a huge advance in the study of antibodies. Humanized monoclonal antibody, completely human monoclonal antibody, mouse chimeric monoclonal antibody, and murine monoclonal antibody are the four stages of development that mAbs have gone through to date. Among the most developed and popular class of monoclonal antibodies at the moment is the totally human one. About murine monoclonal antibody, Murine monoclonal antibodies are defined as antibodies produced by hybridoma cells, which are created
when B cells that originate from immunized mice fuse with mouse myeloma cells. When the human immune system encounters this antibody, it triggers the severe human anti-mouse antibody response, or HAMA for short. Monoclonal antibodies' half-lives are shortened, their potency is diminished, and severe adverse responses are frequently caused by the HAMA reaction. More research on mAbs has started in an effort to prepare appropriate monoclonal antibodies and address a number of the drawbacks of murine monoclonal antibodies [6]. About human mouse chimeric monoclonal antibody, Chimeric monoclonal antibodies, which are composed of both human and mouse, are the first type of humanized antibodies. This antibody is prepared by recombining the human antibody's constant region gene with the mouse antibody's variable region gene, then introducing the mixture into myeloma cells. The resultant antibody is an expression-based chimeric monoclonal antibody. Nonetheless, this antibody is immunogenic to humans since its variable region is produced from mice genes and preserves roughly 30% of the characteristics of a murine antibody. In clinical settings, patients receiving this antibody treatment may experience HAMA responses. In order to lessen the immunogenicity of the antibody and lower the likelihood of a HAMA reaction, the researchers further humanized the mAb. About humanized monoclonal antibody, Modified, transplanted, and humanized antibodies are alternative names for second-generation humanized antibodies. The complementarity-determining region (CDR) sequence from the murine monoclonal antibody's variable region is substituted for the matching position in the human antibody's variable region, with a humanization rate of more than 90%. This antibody is specific for murine monoclonal antibodies while maintaining affinity for the human body, minimizing the heterologous nature of the antibody. Nevertheless, the antibody is merely a CDR sequence transplant; it does not consider the impact of the antibody's structural alteration on the binding of the antigen. Moreover, the humanized anti-antibody still contains the structure of the murine antibody, so it is inevitable that the HAMA reaction will occur. Meanwhile, humanized antibody manufacture isn't without its challenges, though, including the intricate nature of the process, the requirement for structural design, and the necessity of consistently determining the antibody's affinity for antigens. About fully human monoclonal antibody, of all the therapeutic monoclonals, fully human monoclonals are the most desired. Completely human monoclonal antibodies have the lowest risk of causing hypersensitivity or rejection once they enter the human body since human genes encode the amino acid sequences of the antibody proteins. Currently available methodologies for producing fully human monoclonal antibodies include single B cell, antibody library, and transgenic mice methods [1].

2. Monoclonal Antibody Applications

2.1. Hepatitis B Virus

The hepatitis B virus is the source of hepatitis B, a liver infection. One can have acute (short-term, severe) or chronic (long-term) hepatitis B infection. Hepatitis B patients frequently have an increased risk of liver cancer and cirrhosis. Contact with contaminated body fluids, including blood, saliva, vaginal secretions, and semen, can result in the transmission of hepatitis B. Additionally, childbirth can spread it from mother to child[3].Regarding the pathogenic mechanism of HBV, the mechanism of immunopathological damage generated by HBV typically involves a virus-induced poor immune response, medication resistance caused by viral mutation, antibody-mediated immunopathological damage, and cell-mediated immune disease damage. Hepatitis B virus infections typically cause fatigue, appetite loss, nausea, vomiting, diarrhea, hepatomegaly, liver damage, and in rare cases, jaundice and fever. Some patients encounter upper respiratory tract symptoms, arthralgia, or hives. Liver failure brought on by severe acute hepatitis can result in mortality [2]. Hepatitis B virus epidemiological features include high incidence, wide epidemic areas, complicated modes of transmission, and high level of infectiousness. 30.4 million people (10.5% of the estimated total number of persons living with hepatitis B) knew if they were infected as of 2019, and 6.6 million people (22%) were receiving treatment for those who had been diagnosed. The percentage of children under five who have chronic hepatitis B virus infection has decreased from roughly 5% in the 1980s.
of the 20th centuries to just under 1% in 2019, prior to the availability of vaccines, according to the most recent data from the World Health Organization [3]. Interferons and nucleosides like entecavir and tenofovir disoproxil fumarate are the mainstays of treatment for the hepatitis B virus. However, because the virus produces genetic alterations that lead to drug resistance and lengthy treatment cycles, researchers worldwide are also investigating the use of immunotherapy to treat hepatitis B[4]. For the three hepatitis B virus serotypes (adw, ayw, and adr) S proteins, there are three fully human monoclonal antibodies with neutralizing activity: 1F2, 2A1, and 2H2. The binding of 1F2 and 2A1 antibodies depends on the natural conformation of the antigen protein, and the binding of 2H2 antibody depends on the continuous amino acid sequence, and the binding of 1F2 and 2A1 antibodies depends on the natural conformation of the antigen protein, while the binding of 2H2 antibody depends on the continuous amino acid sequence[4]. In contrast to antibodies that isolated directly from blood, non-serum-derived fully human monoclonal antibodies may be safer and perform better than antibodies extracted straight from blood. However, monoclonal antibodies that can treat hepatitis B virus are still in the experimental stage and have not yet entered clinical trials.

2.2. Her2-Positive Breast Cancer

Breast cancer is a condition when a number of carcinogens cause the proliferation of breast epithelial cells to become uncontrollable. Breast lumps, nipple discharge, and swollen axillary lymph nodes are examples of early cancer symptoms. Later on, advanced cancer cells have the potential to spread to other organs such as the liver, brain, lungs, and bones. When they get to these areas, other cancer-related symptoms including headaches or bone pain could appear [3]. The International Agency for Research on Cancer (IARC) reported that 2.26 million women worldwide were diagnosed with breast cancer in 2020, and 685,000 of those cases resulted in death. This made female breast cancer the most common type of cancer worldwide. Nowadays, among all cancers, breast cancer is now one of the most common worldwide. 15% to 20% of all incidences of breast cancer are positive for the human epidermal growth factor receptor-2 (HER-2). In comparison to other breast cancer subtypes, HER2-positive breast cancer has high invasiveness, easy recurrence and metastasis, and a poor prognosis, making it a research hotspot in the field of breast cancer [8]. Together with HER-1, HER-3, and HER-4, HER2 is a member of the epidermal growth factor receptor (EGFR) family. This receptor family consists of four subunits in its extracellular domain (ECD) (I, II, III, IV), a transmembrane lipophilic domain (containing 19–25 amino acids), and an intracellular tyrosine kinase domain [9]. A receptor for orphans is HER-2. Since its ECD lacks a unique ligand-binding domain, it is unable to interact with ligands directly. HER2 is therefore thought to function as a coreceptor or dimerization partner for the effects of other HER receptors [9]. Nowadays, there are three primary categories of HER-2 targeting medications: antibody conjugate medications, small molecule tyrosine kinase inhibitors, and monoclonal antibodies [10]. The most important of these is the trastuzumab monoclonal antibody that acts on ECD-IV in HER-2. Trastuzumab is an antibody that generated from recombinant DNA and manufactured through suspension culture of mammalian cells (Chinese hamster ovary cells, CHO) in a nutritional medium containing gentamicin. Trastuzumab can activate a number of signaling pathways by binding to the extracellular Her-2 neighboring membrane surface, decreasing the development, reproduction, and survival of Her-2-dependent malignant tumor cells while suppressing the proliferation of normal immune cells. Apoptosis to further maintain the body's immune regulatory balance [11]. Trastuzumab's substantial method of action is to prevent the creation of Her-2 receptor dimers, increase receptor degradation by internal digestion, and decrease extracellular site shedding. Furthermore, it promotes autoantibody-dependent cytotoxic actions, killing tumor cells while inhibiting their activity. [11] In addition, trastuzumab can also downregulate intravascular growth factors and inhibit the multiple growth of tissue blood vessels and cells in breast cancer malignant tumors [11].
2.3. Paroxysmal Nocturnal Hemoglobinuria (PNH)

Paroxysmal nocturnal hemoglobinuria is a nonmalignant clonal disease caused by mutations in the acquired somatic PIG-A gene of one or several hematopoietic stem cells, resulting in abnormal glycosylphosphatidylinositol (GPI) synthesis, resulting in the loss of a group of membrane proteins anchored to the cell membrane by GPI, including CD16, CD55, and CD59 [12]. Typical PNH is characterized by chronic intravascular hemolysis, hemoglobinuria, and hemosiderosuria, but most patients are often atypical, with insidious onset, prolonged course of disease, and varying severity of disease. The peak age of incidence is between 20~40 years old, and it occurs in children or the elderly, and there are significantly more males than females. Among the 203 patients with PNH in China, 56.7% of the first symptoms were anemia, 12.8% had hemoglobinuria, and 5.9% had jaundice and anemia. PNH is caused by a mutation in the PIG-A gene that prevents the synthesis of N-acetylgalactosaminylphosphatidylinositol, an important component of glycosylphosphatidylinositol anchor protein (GPI-AP) [12]. Chronic uncontrolled complement activation on the surface of clonal cells is due to the lack of complement inhibitory proteins anchored by GPI, particularly CD55 (decay-accelerating factor) and CD59 (membrane attack complex inhibitory factor) [12,13]. However, mutations in the PIG-A gene alone are not sufficient to cause the pathogenesis of PNH, and clonal proliferation of PNH relies on two mechanisms: selective immune attack and anti-apoptotic [13]. The mechanism of selective immune attack is that T lymphocytes mediate an autoimmune response in patients with PNH, and the immune system selectively attacks normal hematopoietic stem cells, but due to the lack of GPI-anchored complement inhibitory proteins, PNH clonal proliferation escapes immune attack [13]. As for the anti-apoptotic mechanism, the PIG-A gene itself has the advantage of apoptosis resistance, coupled with the selective immune attack mechanism, so that the clonal proliferation of PNH can fight apoptosis [13]. At present, eculizumab is commonly used for the treatment of PNH. Eculizumab is a humanized monoclonal antibody prepared from murine myeloma cell line NS0 cells and expressed using recombinant DNA technology. Eculizumab is based on strong binding to complement protein C5 and inhibiting its cleavage to C5a and C5b, thereby preventing the production of the terminal complement complex C5b-C9 [15]. Eculizumab has greatly improved vasolysis, transfusion dependence, and quality of life in patients due to its strong binding to complement protein C5, and has significantly improved the survival rate of this rare disease [14]. At the same time, eculizumab has limitations. Because eculizumab improves hemolysis by blocking the downstream channels of complement activation, it does not resolve the GPI anchor defect caused by the PIG-A mutation [14]. After discontinuation of the drug, there is a large accumulation of red blood cells with GPI defects, which can lead to an outbreak of hemolysis. Due to the influence of currency exchange rates and the global economy, the price of eculizumab has remained high in the global market, so once clinical patients choose to use eculizumab, they need to face the risk of hemolytic outbreak after discontinuation due to economic problems.

3. Conclusion

Currently, monoclonal antibodies that can be used to treat hepatitis B virus are still in the experimental stage and have not yet entered clinical practice. It still requires in-depth research by scholars. In the treatment of HER-2-positive breast cancer, trastuzumab has been utilized extensively and has significantly improved the state of breast cancer care worldwide. Presently, eculizumab is extensively utilized in the management of patients suffering from paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), neuromyelitis-optic nerve disease positive for varicella-4 (AQP4), and generalized myasthenia gravis. It also plays a crucial role in treating rare diseases. With the development of high-throughput sequencing, bioinformatics and other technologies, it is increasingly convenient to obtain antibodies with high safety, high effectiveness, and high stability [1]. Safe, efficient, and highly specific monoclonal antibodies have established an indispensable position in the diagnosis and treatment of pathogenic infections, cancer, and neurological diseases. Due to limits of current medical technology, many diseases remain difficult
to treat with traditional medications. Monoclonal antibody treatment may be the mainstream research direction of scholars in the future, and it will also be one of the main means for clinical doctors to treat unconventional diseases in the future. However, there are still safety issues with monoclonal antibodies. Relevant reports indicate that some monoclonal antibodies may increase the risk of worsening lymphoma or other malignant tumors during treatment [1]. In addition, in terms of administration method, because monoclonal antibodies are proteins and are easily decomposed by gastric acid, intravenous, intramuscular injection, and subcutaneous injection must be used. Compared with the administration method of small molecule drugs, it is more convenient [16]. Therefore, in-depth study on selecting optimal targets for monoclonal antibodies in clinical applications and research on revolutionary improvements in drug delivery techniques will become one of the research hotspots in the global biomedicine area in the future. Additionally, as monoclonal antibody patents become available, academics, businesses, and other organizations in the international biomedical field will devote a great deal of their research resources to the study of biosimilars, which is expected to become a future research hotspot in the field.

References


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