The Application and Mechanism of Monoclonal Antibodies in Diseases

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Abstract. Monoclonal antibodies (mAbs), as a kind of highly specific biological drugs, play an increasingly important role in the treatment and prevention of diseases. Monoclonal antibodies were invented in the 1970s and went through four stages of development to become fully humanized antibodies. The principle is to use cell fusion technology to fuse myeloma cells and B lymphocytes, and then use HAT culture medium to screen successfully fused hybridoma cells. The preparation process of mAbs is varied, and the required biotechnology (protein purification, ELISA, immunofluorescence) and equipment are complex. With the continuous progress of biotechnology, mAb has been widely used in many fields such as tumor, autoimmune disease and infectious disease. At present, there are many mAb drugs on the market for the treatment of various intractable diseases. In tumor therapy, mAbs can inhibit the growth and spread of tumor cells by targeting tumor cell surface antigens, and improve the therapeutic effect. In autoimmune diseases, mAb can target autoantigens, regulate the immune response, reduce symptoms, and improve the condition. In addition, mAbs also play an important role in the treatment of infectious diseases, such as neutralizing viruses, bacteria and other pathogens, preventing their infection of cells. The mechanism of mAbs, including binding to antigens, triggering signal transduction, endocytosis and degradation, and inducing immune response, is also introduced. There are also many binding sites where mAbs act on different diseases, such as PD-1, PD-L1, CD20, F and G surface proteins of RSV. MAb has excellent specificity and effectiveness, but it is expensive and has its own risks. With the continuous development and optimization of monoclonal antibody technology, its application prospect in the future disease treatment will be broader.

Keywords: mAb; tumors; autoimmune diseases; infectious diseases.

1. Introduction

Antibodies are produced by B lymphocytes, which can specifically bind to pathogen antigens and block pathogen infection. Antibodies were discovered from antitoxin sera. But their effectiveness is limited due to the unstable titer and polyclonal nature of serum antibodies. Additionally, these methods are often associated with allergic reactions and an increased risk of blood-borne pathogens.

The development of hybridoma technology has endowed antibody with epoch-making significance in clinical application. Compared with polyclonal antisera, the high specificity of monoclonal antibodies(mAbs) to recognize antigens greatly reduces non-specific cross-reactivity [1]. Monoclonal antibody(mAb) is the use of genetic engineering technology to extract mAb from hybridoma in specific mice and make mAb drugs. Hybridoma cells are formed by the fusion of an antigen-activated B cell with a myeloma cell. Due to the good tolerance and outstanding efficacy of mAb drugs, it is mainly used in the adjuvant treatment of some malignant tumors.

In 1975, the first mAb was produced. British scientist Milstein and French scientist Kohler fused antibody-producing B lymphocytes with tumor cells to form hybridoma cells. Hybridoma cells have the characteristics of parent cells, which can produce antibodies, but also have the characteristics of infinite proliferation of tumor cells, so as to continuously secrete mAb. This breakthrough discovery not only provided experimental evidence for the clonal selection theory of lymphocyte development, but also made it possible to produce a large number of antibodies with highly defined antigen specificity, which laid the foundation for future clinical applications and led to the great success of mAb therapies over the past three decades [1]. In 1986, Orthoclone OKT3 was the first murine mAb approved by the U.S. FDA to prevent host rejection after kidney transplantation. Since the first murine

mAb was introduced in 1986, there are now nearly 100 mAbs on the market worldwide. Hybridoma technology for the production of mAbs was one of the most important medical revolutions of the 20th century. It offers hope for the research, diagnosis, prevention and treatment of different types of diseases. However, a newer technique, phage display, is considered an alternative to the traditional hybridoma technique for the rapid production of mAbs. Many mAbs have been introduced into biomedical research as fairly quick diagnostic tools, but their progress as therapeutic drugs has been quite slow. Currently, four main types of mAbs have been developed, including mouse mAbs, chimeric mAbs, humanized mAbs, and fully humanized mAbs. Due to the production of human anti-mouse antibodies (HAMA), the mouse antibodies cause high immunogenicity in the human host, which may limit its efficacy.

With the advent of genetic engineering methods, the immunogenicity of mAbs has gradually decreased, respectively, from mouse to chimeric humanized to fully human mAbs. MAb is now recognized as an effective treatment for immune diseases, including autoimmune diseases, allergies, autoinflammatory diseases, and cancer. The main advantage of mAb over most small molecule drugs is that it has a larger surface area for binding and therefore has excellent specificity, which reduces "off-target" effects. However, mAbs are expensive compared to abiotic therapies and, like all drugs, have their own risks [2].

At present, the mAb can be used in a variety of fields, including tumors (such as non-small-cell lung carcinoma, breast and stomach cancers), autoimmune diseases (such as Rheumatoid Arthritis, systemic lupus erythematosus), and infectious diseases (such as hepatitis, AIDS, and syphilis). In cancer therapy, it can induce the death of tumor cells by functioning on the related antigens or epidermal growth factor receptor (EGFR) that differentiate the membrane of malignant tumor cells. At the same time, mAb can also inhibit the proliferation of tumor cells by inhibiting tumor angiogenesis or blocking the mediation between tumor cells, and ultimately control the development of malignant tumors to some extent. In addition, mAb is widely used in the treatment of autoimmune diseases. It can alleviate the symptoms of autoimmune diseases and control the disease progression by modulating the immune response or inhibiting the activity of immune cells. For example, mAb to B or T cells can block the activation or proliferation of immune cells, thus reducing attacks on their own tissues. To treat the infectious diseases, the mAb can bind to pathogens or disease-related molecules, such as viral surface proteins or bacterial surface receptors. By binding to these molecules, mAb can block the entry of pathogens into cells in order to prevent infection. This paper will illustrate the structures, preparation procedures and functions of different kinds of mAbs from these three fields.

2. Structure and preparation of mAbs

Antibody is a kind of protein secreted by plasma cells produced by immune system in response to antigen stimulation, which has the characteristics of high specificity for antigen. Human antibodies are mainly composed of five different subtypes: IgG, IgA, IgM, IgD and IgE. Among them, human IgG1 and IgG4 subtype antibodies are often used in clinical therapy[3].

2.1. Structure and principle

2.1.1 Structural characteristic

Structurally, an antibody consists of two identical heavy (H) chains and two identical light (L) chains (κ or λ), each containing a variable area (V) and a constant area (C). The C region is held together by disulfide bonds, and both the L and H chains consist of variable nitrogen ends and constant carbon ends. In every region of the V are the three hypervariable regions [i.e., immunoglobulin complementarity determining region (CDR)], they are the molecular basis of antibody specificity, three complement area (CDR1, CDR2 and CDR3) with variable area (VH and VL), It is located at the nitrogen end of the two L chains and the two H chains [3].

2.1.2 Cell fusion technique

The basis of hybridoma technology is cell fusion technology. In 1958, Japanese scholar Okada found that Sendai virus has the effect of triggering cell fusion. In 1974, Canadian scholar Gao Guoan created the polyethylene glycol (PEG) chemical fusion method. Kohler and Milstein developed hybridoma technology based on these techniques. This method overcomes the obstacle of continuous cultivation of primary B lymphocytes in vitro, and enables them to obtain the ability of continuous proliferation [1].

2.1.3 Screening of hybridoma cells

HAT medium (H-hypoxanthine, A-aminopterin, T-thymine) was used for screening hybridoma cells. There are two ways of DNA synthesis in cells: endogenous and exogenous. Aminopterin in HAT medium is an inhibitor of dihydrofolate reductase, which can effectively block the endogenous pathway of DNA synthesis. Hybridoma cells can survive in HAT medium for a long time because they inherit the dual characteristics of B lymphocytes and myeloma cells.

2.2. Preparation of mAbs

2.2.1 Preparation of mAbs for the treatment of tumors

Take the preparation process of SOAT1 mAb for liver cancer as an example. The SOAT1 gene was obtained by reverse transcription from hepatocellular carcinoma cells Huh-7, and after prokaryotic expression and protein purification, the purified recombinant SOAT1 protein was used as antigen to immunize mice, and a total of 5 SOAT1 mAb cell lines were obtained by hybridoma and other techniques. They were named 1F3, 1G3, 1D6, 2F8 and 4D11, among which 4D11 could recognize the SOAT1 protein in liver cancer cells Huh-7 and clinical liver cancer tissue samples. Finally, 4D11 was sent to a biological company for sequencing to obtain the amino acid sequence of the variable region of the antibody [4].

2.2.2 Preparation of mAbs for the treatment of autoimmune diseases

Because the abnormal expression of NLRP and the overactivation of inflammatory bodies drive, affect the occurrence and development of immune diseases (including IBD, RA, SLE, psoriasis, MS, and diabetes), infectious diseases. Therefore, NLRP3 has become a popular research target/gene at present. The recombinant plasmid MS-N3-ThroHFC was constructed by inserting the gene fragment encoding mouse NLRP3 gene exon 3(Ms-N3) into the vector P3-G3-ThrohFC, and then transfected into HEK293F cells for eukaryotic protein expression. Ms-N3 protein was purified by protein an affinity chromatography column and immunized NLRP3 gene knockout (NLRP3-/-) mice. Spleen cells of immunized mice were fused with SP2/0 myeloma cells to form hybridoma cells. Hybridoma cells that secrete mAb that specifically recognizes mouse NLRP3 were screened by ELISA and immunofluorescence [5].

2.2.3 Preparation process of mAbs for the treatment of infectious diseases

The researchers used the insect baculovirus expression system to express E2 protein of CSFV gene 2.1b subtype JL23, and immunized BALB/c mice with purified protein. Spleen cells of immune mice were fused with myeloma cells by electrofusion, and hybridoma cells were screened by semi-solid selective medium. The hybridoma cell supernatant (mAb) reacting with JL23 was identified by indirect immunofluorescence assay (IFA), and a stable hybridoma cell line reacting with JL23 was obtained by subcloning. IFA and western blot were used to determine the reaction spectra of mAb with swine fever vaccine strains and epidemic strains of different genotypes. Amino acid truncation and point mutation were used to identify the recognized epitopes. The neutralization level of mAb was determined by fixed virus dilution antibody method [6].

3. Mechanism of mAbs in disease treatment

The mechanism of mAbs in disease treatment is to act by binding to specific antigens, which can be antigens on the surface of tumor cells, cytokines free in the blood circulation, pro-angiogenic factors, biotoxins, etc. To achieve precise treatment of diseases. With the continuous development and improvement of mAb technology, its application in disease treatment will be more and more extensive.

3.1. Mechanism of mAbs in tumor therapy

Tumors are a general term for a group of diseases that can affect any part of the body. It begins in cells of the body that proliferate abnormally and form new organisms that do not function as normal cells. Cancer cells have the ability to grow and multiply uncontrollably, and can invade adjacent normal tissues, and even spread to distant tissues and organs. MAbs can inhibit cell signal transduction pathways through a variety of mechanisms, including neutralizing signal transduction factors [e.g., vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF)], bind to and block cell surface receptor expression, and reduce the expression level of cell surface receptors.

Ligand blocking is the primary mechanism of action of Bevacizumab (Avastin), which prevents VEGF from binding to its homologous receptors (VEGFR1 and VEGFR2), thereby inhibiting angiogenesis. In most cases, the blocking of cell signal transduction does not require the involvement of the crystallizable fragments (Fc) domain of mAbs, but is achieved through the antigen-binding fragments (Fab) domain. For example, Bevacizumab and Cetuximab work with epidermal growth factor receptor, EGFR and the human epidermal growth factor receptor (hEGFR).

Another mechanism is cytotoxic effects, Direct cytotoxicity of target cells is the primary mechanism of action of CD20-targeting type II mAbs in B-cell malignancies, such as Obinutuzumab, which does not mediate the aggregation of CD20 molecules on the surface of target cells, but induces programmed cell death of malignant B cells through caspase activation. Antibody-dependent cellular cytotoxicity (ADCC) is mediated by the interaction between the Fc domain of the antibody and FC-γriiia on the surface of immune cells. MAbs can bind to cell surface targets via their Fab domain and then bind to white blood cells expressing FC-γriiia via the Fc domain, causing cell killing [3].

3.2. Mechanism of mAbs in the treatment of autoimmune diseases

Autoimmune diseases are diseases in which the body reacts to its own antigens and causes damage to its own tissues. MAbs act specifically on specific inflammatory targets and corresponding pathways of action (mainly tumor necrosis factor and interleukin), which can reduce inflammatory damage, reduce adverse drug reactions, and have no obvious damage to normal immune function [7]. MAbs act through various mechanisms, including binding to soluble cytokines and growth factors, blocking receptors, regulating receptors and receptor signaling, inducing apoptosis through antibody-dependent cell-mediated cytotoxicity, antibody-dependent cytophagocytosis (ADCP), complement-dependent cytotoxicity(CDC), and mediating cell signaling. Antibody therapy can replace hormone therapy, and when combined with glucocorticoids, the dosage of hormones can be reduced, so that the incidence of adverse drug reactions is reduced.

For example, TNF-α inhibitors are currently the most successful biologic agents for the treatment of autoimmune diseases, and their mechanism of action is to bind to TNF-α, thereby preventing TNF-α from binding to the target receptor, inhibiting the transmission of downstream signaling pathways, thereby preventing the development of the disease. For another example, BAFF and APRIL are important B cell stimulating molecules in B cell maturation, plasma cell survival and class conversion, respectively, which can stimulate the proliferation and differentiation of B cells. Inhibiting the biological activity of BAFF can reduce the level of antibodies in the disease, thereby controlling the development of the disease. Belimumab is a humanized mAb against BAFF, and Atacicept is constructed from the extracellular region of TACI and the Fc fragment of human immunoglobular egg white G(IgG). TACI-Ig fusion protein, in animal experiments, can significantly inhibit joint

inflammation, and can inhibit the destruction of bone and cartilage and the development of disease. So both are being developed for the treatment of systemic lupus erythematosus [8]. Rituximab is a chimeric mAb targeting CD20+B cells. CD20 is stably present and highly expressed on B cells, but not on stem cells and plasma cells. Therefore, B-cell depletion becomes a target for the treatment of rheumatoid arthritis. Rituximab consumes B cells through cell-mediated and complement-dependent cytotoxicity by binding to CD20 molecules located on the surface of B cells for the treatment of refractory rheumatoid arthritis [9].

3.3. The mechanism of mAbs in the treatment of infectious diseases

Infectious disease is a kind of disease caused by various pathogens that can be transmitted between people, animals and animals or between people and animals. MAbs play an important role in the treatment of infectious diseases caused by a variety of deadly viruses. Antibodies can bind to the virus specifically and efficiently, and neutralize the activity of the virus, and then play the role of eliminating invading pathogens. Antibodies play an important role in bodily fluids' immunity to infection. They bind to soluble toxins, blocking their function, and bind to antigens on the surface of pathogens, thereby neutralizing their ability to infect human cells or mark them for destruction [10]. For example, the viral glycoprotein of the enveloped virus or the protein shell of the non-enveloped virus prevents binding to the target host cell. These viral proteins have two main functions during the viral life cycle: binding to cell receptors and mediating the fusion of the virus and cell membrane (in the case of enveloped viruses) or penetrating into the cytosol (in the case of non-enveloped viruses). For example, the entry of SARS-CoV-2 into host cells is mediated by the interaction between the viral spike (S) glycoprotein and the angiotensin-converting enzyme 2 (ACE2) receptor on the surface of the host cell. ACE2 is expressed in respiratory, gastrointestinal and endothelial cells. Spike-ACE2 interactions can be blocked by antibodies that target the spike-receptor binding domain (RBD), inhibiting viral infection. Or elimination of labeled pathogens by complement activation of immune cells, ADCC, or ADCP. Antibody effect function mediated by binding to the complement protein C1q or FC-γRS on white blood cells may also be involved in fighting viral infection. Complement activation of antibodies results in direct lysis of the virus and infected host cells, and antibodies can also promote or induce phagocytosis, or trigger the release of toxic chemicals such as cytokines or reactive oxygen species. For example, recent studies have shown that the optimal protection of monoclonal antibody therapy against SARS-CoV-2 requires Fc effector function; When administered after infection, intact mAbs were more effective than FC-variant mAbs with loss of function in reducing SARS-CoV-2 burden and lung disease in animals by reducing inflammation and improving respiratory mechanics [11].

All antibodies are composed of Fab that give their target specificity and Fc that drive biological functions. Both Fab and Fc region changes affect the specificity, persistence, and outcome of antibody-dependent reactions. The simple biological design of antibodies, combined with their broad versatility and a range of effective effector functions, make them of major interest as biologics for the treatment of disease.

MAbs are well-defined and highly specific biologics because they target key antigens of pathogens with minimal off-target effects. They can have effector function, regulating different biological effects depending on the pathogen. In addition, they can be mass-produced by culture in vitro and could potentially be engineered to further refine their properties. Avoiding sources of human or animal serum/plasma can also reduce the risk of contamination by undetected pathogens or other factors. Finally, mAbs that target different epitopes can be used in combination to achieve synergistic or additive effects, or in combination with other types of therapies. Current FDA-approved mAbs for the treatment of infectious diseases include Trogarzo, which is humanized IgG4 for the treatment of HIV-1; mAb114, human IgG1, is used to treat Ebola virus infection; REGN10933 and REGN10987 combination therapy, also human IgG1, for the treatment of novel coronavirus pneumonia (COVID-19) [10].

4. Binding site of mAbs in diseases

The binding site of a mAb in a cell mainly refers to the location where it binds to a specific antigen. These antigens can be receptors, enzymes, structural proteins, or other molecules on the cell surface. When a mAb binds to these antigens, it can affect cell function and activity in a variety of ways, such as neutralizing activity, signal transduction, endocytosis and degradation, and inducing an immune response

4.1. Binding site of mAbs in tumor therapy

Programmed cell death protein (PD-1) and its ligand Programmed death ligand 1 (PD-L1) or programmed death ligand 2 (PD-L2) are important immune checkpoints, and their upregulated expression in T cells and tumor cells can induce immunosuppression. The up-regulation of expression in tumor cells is the mechanism of immune escape of cancer cells. MAb immunotherapies based on blocking PD-1/PD-L1 have been shown to restore anti-tumor immune responses in patients. Two PD-1-targeting mAbs (Pembrolizumab and Nivolumab) have been approved by the FDA for melanoma, non-small cell lung cancer, and neck squamous cell carcinoma [12].

4.2. Binding site of mAbs in the treatment of autoimmune diseases

There is growing evidence for the role of B cells in an autoimmune pathology called multiple sclerosis (MS). The cluster of differentiate 20 (CD20) receptor is a transmembrane phosphoprotein expressed on the surface of B cells and is involved in T cell independent antibody response. As a result, B-cell depletion mAbs targeting CD20+ B-cell specific Fab domains were developed and have recently been more widely used, including rituximab, ocrelizumab, ofatumumab, and ublituximab. These drugs treat disease through NK cell-mediated ADCC, CDC, and antibody-triggered apoptosis that deplete B cells [13].

4.3. Binding site of mAbs in the treatment of infectious diseases

Respiratory syncytial virus (RSV) is a pulmonary virus in the paramyxoviridae family that causes severe lower respiratory disease in infants and young children. Studies have shown that RSV encodes 11 proteins, among which F and G surface proteins induce protective immunity. MK-1654 (clesrovimab) is an investigational fully human IgG1 mAb derived from parental RB1 and binds to the conserved site IV of RSV F glycoprotein [14]. The antibody effectively neutralizes a variety of strains containing RSV F in vitro. G protein binds to the CX3C chemokine receptor CX3CR1 and plays an important role in inducing and regulating the host immune response to infection. MAb 131-2G binds to the central conserved region of G protein, blocking G protein binding to CX3CR1 and reducing RSV disease presentation [15].

5. Conclusion

The development and application of mAb in diseases is a major breakthrough in the medical field. MAb is a single antibody against a specific antigen, which can accurately recognize and attack disease-related antigens, thus playing a therapeutic role. In the treatment of diseases, the application of mAb is increasingly widespread. They are used in cancer therapies to attack tumors by blocking their growth signals or activating the immune system; In autoimmune diseases, mAbs can regulate the function of the immune system and reduce inflammation. In infectious diseases, mAbs neutralize pathogens and prevent them from invading human cells.

The mechanisms of action of mAbs are varied. Some mAbs can directly bind and neutralize pathogens, preventing them from infecting cells; Some can activate or suppress immune cells and regulate immune responses; Others can block the growth signal of tumor cells and inhibit their proliferation. These mechanisms make mAbs highly specific and effective in the treatment of various types of diseases. At the same time, mAbs also have many different binding sites which make the therapies more accurate and efficient. With the continuous development of biotechnology, the

preparation technology of mAb is also improving. Today, scientists have been able to produce high-purity, high-affinity, low-immunogenicity mAbs, making their application in disease treatment more extensive and effective. The application and mechanism research of mAb in the treatment of diseases have brought revolutionary progress to the medical field. With the deepening of research and technological progress, it is believed that mAbs will have significant impacts on the treatment of more diseases in the future.

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