Use of monoclonal antibodies (mAbs) and their future prospects

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Abstract

The article discusses the areas of use of monoclonal antibodies (mAbs) and prospects for future use. The effectiveness of monoclonal antibodies in the modern stage, against diseases of different etiology, provides the basis for the assumption that in the future it will be further refined to obtain monoclonal antibodies, which will contribute to its widespread use not only in the treatment of diseases, but also in diagnostics.

Keywords

antibodies, monoclonal antibodies, mAbs, antigen, protein, diagnostics, COVID-19

Introduction

A monoclonal antibody (mAb) is obtained from a single cell clone and is a unique antigenic determinant. The technology for making monoclonal antibodies was first developed by Keller and Milstein in 1975. Several basic methods have been developed to generate human monoclonal antibodies, including: 1. Immunization of antigen-specific B cells (immortality) [1; 2]; 2. Extraction of antigen-specific B cells using the phage method [3; 4]; 3. Production of human monoclonal antibodies from transgenic mice [5-7]; 4. Method of cloning individual human B cells by direct cloning and expression of immunoglobulin genes in vitro [8-10].

Monoclonal antibodies are used in medical practice to treat various types of diseases, including in the therapy of infectious diseases. The use of monoclonal antibodies has also been shown to be effective in the treatment of COVID-19.

Antigen-specific B cell immortalization and hybridoma technology

In the early stages of human monoclonal antibody production, hybrid technology was used; Which is based on the fusion of antibody-producing human B-lymphocytes with mouse or human myeloma or lymphoblastic cells [11; 12]. Also used Ebstein-Barr virus (EBV), which is based on human antigen-specific B-lymphocyte immortalization techniques [13-15]. Each method has its pros and cons. Hybrids that produce antibodies, arise from a fusion of myeloma cells (which can multiply indefinitely) and from B-lymphoblasts; Which express antibody-specific genes. The best results were obtained with heteromyeloma technology (mouse X human myeloma). In addition, an alternative method is the human antibody Immortalization of cells as a result of EBV infection. However, EBV-transformed cells cannot grow indefinitely. The reason is that they are not malignant cells and do not produce clones. Therefore, small amounts of immunoglobulin are produced. EBV hybrid technology is based on a combination of 2 methods [2; 16]. Improving electroporation of B cell immortalization methods and refining the cell fusion process may reveal new trends in EBV hybrid technology [17].

Monoclonal antibodies obtained using transgenic mice

Using transgenic mice to obtain human monoclonal antibodies is another important technology. Genetically engineered mice were first created from human antibodies in 1994. Transchromosomal mice contained the genes encoding the heavy immunoglobulin chain and the light κ (kappa) chain [5-7; 18].

Significant results have been achieved in the last few decades by making additional changes in the genes of transgenic mice [19-21]. Currently, more than 50 human monoclonal antibodies have been obtained through transgenic mice. They are undergoing clinical trials and 6 of them have already been approved for medical use [22].

Areas of application of monoclonal antibodies (mAbs)

Monoclonal antibodies were found to be essential for fundamental immunological and molecular studies, Due to their high specificity ability. They are used in human therapy, in protein purification, In suppression of the immune response, In the diagnosis of diseases, In tumor therapy, In the diagnosis of allergies, In hormonal tests, In the purification of complex mixtures, in the identification of specialized cells, In vaccine production and in increasing the effectiveness of drugs [23-25].

The use of monoclonal antibodies in the diagnostic field is highly relevant. They can be used to detect antigen. Monoclonal antibodies have been used in biomedical applications, namely: Western blotting (method of detecting specific proteins in a sample,

Transfer of disintegrated/denatured protein fragments by gel electrophoresis to a paper filter for further identification, In which specific antibodies to search proteins are used); ELISA (immunoenzymatic analysis, method for determining the presence/concentration of antigen or antibody using enzyme-labeled antibodies and enzyme substrate); Radioimmunoassay (RIA-Radioimmuno assay); Transient cytometry; Immunohistochemistry; Fluorescence microscopy; Electron microscopy; Confocal microscopy and more.

mAbs are used to detect pregnancy during the first 1 or 2 weeks. They respond to chorionic gonadotropin secreted by the placenta.

With mAbs it is also possible to diagnose sexually transmitted diseases. E.g. Diagnosis of gonorrhea (caused by Neisseria gonorrhoeae) and chlamydial infection (caused by Chlamydia trachomatis) in 15-20 minutes. For comparison, the diagnosis of these infectious diseases by other methods takes 3-7 days. With mAbs it is possible to identify herpes virus 1 from herpes virus 2 [23-25].

Identification of cell types with monoclonal antibodies (mAbs)

mAbs help identify different types of cells involved in the immune response. E.g. MAbs are used in lymphocytes B lymphocytes, to detect T cell helpers and T suppressors. They differ from each other in surface antigens, which allows them to be identified [23; 26].

The biggest advantage of a monoclonal antibody is that it allows the analysis, labeling and purification of those antigens, which were unknown to the research process from the beginning. Different antibodies can be used to purify molecules or to detect them in cells [25].

With monoclonal antibody technology, it is possible to separate and analyze cell populations from tissues using cell surface antigens. With antibodies that detect only 1 antigenic determinant, made it possible to identify individual surface components of the cell, which had not been previously identified. In this way the surface composition of the cells can be analyzed and functionally differentiated.

Antibodies can be used to identify antigenic determinants, which allow us to identify other types of cells, e.g. Brain, kidney, liver, etc. Sh. [25].

Tumor diagnosis and therapy

mAbs can be used against tumor cell-specific antigens. The availability of mAbs that recognize immune cell antigens, Improved diagnosis of certain types of leukemia and lymphoma. mAbs, also, use lung, breast, in the diagnosis of colorectal cancer. In particular, examination of sputum or biopsy specimens to identify tumor cells or substances produced

by tumor cells. Currently, special mAbs are available for colorectal cancer, ovarian and lung tumors. mAbs-mediated immunotherapy recruits (attracts) cells that have cytotoxicity, Such as monocytes and macrophages, via antibody-dependent cellular cytotoxicity (ADCC). During tumor immunotherapy, mAbs bind to complement proteins that directly induce cellular toxicity. This is complement-dependent cytotoxicity [27; 28].

mAbs can block the growth factors secreted by tumor cells, by inhibiting growth factor receptor, thus effectively stopping the proliferation of tumor cells. Rituximab (IDEC-C2B8) is an IgG mAb chimeric antibody that is directly directed against the CD20 molecule and it is effective in treating malignant tumor cells of B cells. IbritumomabmAb is a CD20 antigen against lymphoma. It is conjugated to the radioactive isotope indium-111 (111In) or yttrium-90 (90Y), For the treatment of patients with lymphoma. Initially, patients are treated with the indium-111 version, followed by the yttrium-90 version. Ibritumomab is added to Rituximab.Tositumomab, which is a mAb against CD20, is used to treat lymphoma. 131I-Tositumomab is a single reliable drug for the treatment of B-cell lymphoma [27-30].

These mAbs can be modified for radioisotope (radioimmunotherapy), toxin, cytokine or other active conjugates. Bispecific mAbs can be structured in such a way that their antigen-binding fragment can bind to the target antigen and the effector cell. mAbs play a crucial role in the diagnosis of breast cancer. They block HER-2 (human epidermal growth factor receptor 2) using Trastuzumab. HER-2 is hyperexpressed in almost 20% of breast cancer cases. Cetuximab, a drug used to treat some forms of breast cancer and lymphomas, blocks HER-1 in some cancer cells. mAbs can be used not only to detect tumor cells, but also to destroy them. In addition, clinical studies show that mAbs cause partial remission of pathologies.

Recent studies show that mAbs conjugates, drugs, and toxins can kill leukemia cells [23-25; 27-31]. Anticancer therapeutic mAbs such as Gemtuzumab and Alemtuzumab are used against leukemia, Rituximab for non-Hodgkin lymphoma, Trastuzumab for breast cancer, Nimotuzumab and Cetuximab for carcinomas. Alemtuzumab provides complete remission in chronic lymphocytic leukemia by binding to the CD52 molecule on lymphocytes. It also prevents organ donor incompatibility during kidney transplantation [27-31].

Gemtuzumabozogamicin (Mylotarg) antibody-based therapy for acute myeloid leukemia (AML-Acute myeloid leukemia). It is injected intravenously. It has been shown to be effective as a specific anti-cancer agent in preclinical and clinical trials. An antibody fragment of this drug detects tumor-associated CD33 antigen, which is present on the surface of malignant (leukemic) blast cells and on normal cells of the myelomonocyte line, But not on normal hematopoietic pluripotent stem cells. This antibody is a monoclonal

anti-CD33 immunoglobulin G (IgG) that binds to a gamma derivative of calicemycin (N-acetyl-calicemycindimethylhydrazide-CalichDMH).

One of the side effects of gemtuzumabozogamicin is venous occlusive disease. Out of 62 patients with acute myelogenous leukemia or myelodysplastic syndrome, who underwent allogeneic stem cell transplantation and underwent retrospective examination, 14 of them received Gemtuzumab before stem cell transplantation. 13 of them developed veno-occlusive disease. 9 out of 10 patients underwent stem cell transplantation within 3.5 months of receiving Gemtuzumab. All of them developed veno-occlusive disease. And 4 patients who underwent stem cell transplantation 3.5 months after receiving Gemtuzumab did not develop any of these diseases [32].

Herceptin (chemical name Trastuzumab) can be used to treat early or metastatic stages of HER-2 positive breast cancer, both in parallel with chemotherapy and after separate chemotherapy, which includes anthracyclines to reduce the risk of breast cancer recurrence. Herceptin is currently approved by the US Food and Drug Administration (FDA).

As for the side effects of Trastuzumab. Like almost all medicines, Herceptin can cause side effects, although not everybody gets them, some of which are quite serious. The most common side effects of Herceptin are: Headache, diarrhea, nausea, chills, fever, heart problems, infection, insomnia, cough, rash. Trastuzumab can cause serious side effects such as heart and lung problems.

Problems can go asymptomatic, E.g. Decreased cardiac function and may manifest symptoms, E.g. Congenital heart failure. The following symptoms should definitely be considered: Swelling of the feet, shortness of breath, cough or weight gain of more than 5 kg in 24 hours. The risk of heart problems is higher when Herceptin is taken in combination with anthracycline chemotherapy. An echocardiogram should be taken before starting therapy with Herceptin to check how the patient's heart is working. Herceptin can cause pneumonia, which can be fatal if complicated [33].

Nimotuzumab is a human monoclonal antibody used to treat squamous cell carcinoma of the head and neck. He underwent several clinical trials. Like Cetuximab, Nimotuzumab binds to EGFR (epidermal growth factor receptor). It is a signaling protein that controls the normal division of cells. In some forms of cancer, this receptor changes, resulting in cells dividing uncontrollably. These monoclonal antibodies block EGFR and inhibit uncontrolled cell proliferation [34; 35].

The toxicity and safety of Nimotuzumab have been evaluated in several preclinical and clinical trials. It should be noted that the side effects that typically characterize EGFR

inhibitors are, Rash and other toxic effects on the skin, In the case of Nimotuzumab, these symptoms were mild. The reason for this is that Nimotuzumab only binds to cells that are characterized by hyperexpression of EGFR [36].

Clinical studies have shown that Nimotuzumab is quite tolerable. The most common adverse reactions in patients treated with Nimotuzumab are: Colds, fever, nausea and vomiting, dry mouth, asthenia, hypertension / hypotension.

Autoimmune disease

Immunotherapy is an important field for the treatment of autoimmune diseases. At this time, it is possible to block the target connecting the antibodies, As a result, the immune system acts on the host cells. The main target in the treatment of Alzheimer's disease is amyloid-β peptide (Aβ peptide). Passive therapy with mAbs is an important area of research. However, the results of the study are mixed. E.g. The Gantenerubam Phase II / III study, which involved 799 people, no significant improvement in either brain activity or Aβ peptide levels was observed. A common explanation for these conflicting results was the participation of people in the study who had late-stage Alzheimer's disease. This problem is quite difficult to solve, because it is difficult to find people who have this disease in the early stages of progression. In addition, a more in-depth study of Alzheimer's disease is needed [37].

The specific role of Aβ peptide is not yet fully understood. In particular, how closely its role is related to the symptom or cause of Alzheimer's disease [37].

mAbs and coronaviruses

Recent advances in the development of mAbs against coronavirus, in particular SARS-CoV and MERS-CoV, could be a potential way to prevent or effectively treat a new coronavirus, SARS-CoV-2. Passive immunization with mAbs is unlikely to lead to a complete cure, but may help to stop the aggravation of the disease and limit the replication of the virus. This method of treatment will be especially effective for patients with moderate to severe medical conditions and help these people save lives.

Tocilizumab was used by Chinese doctors in the treatment of COVID-19 in May 2020 in patients with severe clinical picture, and the result was successful [38].

Tocilizumab-recombinant monoclonal antibody to the human interleukin-6 (IL-6, immunoglobulin class IgG1) receptor. Selectively binds to and inhibits both soluble and membrane IL-6 receptors. IL-6 is a multifunctional cytokine produced by different types of cells and participates in the paracrine regulation of systemic, physiological and pathological processes, Such as immunoglobulin secretion, T cell activation, and more.

The drug tocilizumab is used under the trade name Actemra as an immunosuppressant. The purpose of its use in the treatment of COVID-19 is to inhibit cytokine storm by inhibiting IL-6 signaling pathways. Clinical studies have shown a positive effect [38-41]. Inclusion of tocilizumab in the treatment of COVID-19 has reduced the incidence of acute respiratory distress syndrome [42]. However, the existence of severe side effects remains a topical issue.

Conclusion

Over the past decade, mAbs has been one of the fastest growing classes of pharmaceuticals. Whereas more potential targets have been identified based on in-depth study of the molecular mechanisms of disease, this creates opportunities for the development of new mAbs-based drugs. The use of mAbs as a diagnostic tool is promising. The use of monoclonal antibodies is very effective in treating a wide range of diseases.

To date, approximately 80 monoclonal antibodies have been approved by the FDA for the detection, diagnosis and treatment of various diseases.

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