Monoclonal Antibodies Production, Diagnostic and Therapeutic Applications

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Abstract

Introduction:Monoclonal antibody is an antibody that is specific for one antigen and is produced by a B-cell hybridoma. Hybridoma technology has been replaced by recombinant DNA technology and transgenic method (mice, plant and chickens). Once monoclonal antibodies for a given substance have been produced, they can be used to detect the presence and quantity of this substance and so monoclonal antibody has many diagnostic applications.

Aim of the work: To clarify the new methods for production of monoclonal antibodies and application of monoclonal antibodies in diagnostic and therapeutic approaches.

Conclusion: Hybridoma technology has been replaced by recombinant DNA technology and transgenic method (mice, plant and chickens). Once monoclonal antibodies for a given substance have been produced, they can be used to detect the presence and quantity of this substance and so monoclonal antibody has many diagnostic applications as in the analysis of cell surface and secreted molecules like CD molecules identification and identification of transcription factor FOXP3. Also, they have a role in the molecular genomics to identify the susceptible genes that predispose to development of autoimmune thyroid diseases.

Keywords:monoclonal antibodies, hybridoma technology, immune-diagnosis.

Introduction

Monoclonal antibody is an anti-body that is specific for one antigen and is produced by a B-cell hybridoma[1, 2].

By the introduction of hybridoma technology monoclonal antibodies have come to play an enormous role in biologic research and applications. They offer the advantages of relative ease of the production and purification of large quantities of antibodies[2, 3].

Hybridoma technology has been re-placed by recombinant DNA technology, transgenic mice and phage display[4, 5].

Monoclonal antibodies have had a profound impact on medicine by providing an almost limitless source of therapeutic and diagnostic reagents. Therapeutic use of mo-noclonal antibodies has become a major part of treatments in various diseases including transplantation, oncology, auto-immune, cardiovascular, and infectious diseases[6].

Antibodies are a key component of the adaptive immune response, playing a central role in the recognition of foreign antigens. The advent of monoclonal antibody tech-nology has made it possible to raise anti-bodies against specific antigens presented on the surfaces of tumors[7].

Monoclonal antibody therapy is the use of monoclonal antibodies to specifically target cells. The main objective is stimula-ting the patient's immune system to attack the malignant tumor cells and the prevention of tumor growth by blocking specific cell receptors[7].

Aim of the work:

To clarify the new methods for production of monoclonal antibodies and clarify application of monoclonal antibodies in diagnostic and therapeutic approaches.

Immunoglobulins:

Immunoglobulins **(**Antibodies)aregammaglobulinproteinst hat arefoundinbloodandareusedbytheimmu nesystem toidentifyand neutralizeforeignobjects, areproducedbyakindofwhite bloodcell

called a plasmacell[8]in response to an immunogen[9]. Antibodiesareheavy(~150 kDa)globularplasmaproteins.They areglycoproteins[10].

Thebasicfunctional unitofeachantibodyisanimmunoglobulin (Ig)mono-merwhichisa"Y"-shapedmoleculethatconsistsoffourpolypeptide chains;twoidentical heavychainsandtwoidenticallightchains connectedbydisulfidebond[11]. Eachchain iscomposedofstructuraldomains calledIgdomains.

Thesedomainscontainabout70-110aminoacids[12].

Most Igs mediate several effector functions which include fixation of complement that results to lyses of cells and release of biologically active molecules, binding of various cells to facilitate specific functions by bound cells e.g. phagocytic cells, lymphocytes, platelets etc. Most effector functions of Abs are carried out after the Ab binds to Ags[9].

Monoclonalantibodyisanantibodythat isspecificforoneantigen. It was firstly produced by hybridoma technologybut nowadays this hybridomatechnology has been replaced by recombinant DNA tech-nologyandtransgenicmethods[4].

I-Hybridoma Technology:

Anormal,activated,antibodypro-ducing Bcellisfusedwitha myelomacell(cancerous plasmacell)toproduceahydridecell,called hybridoma. Thehyb-ridoma cellhastheproperties ofboththenormal, activatedantibodyproducing cell(i.e.,thehybridoma cellsecretes antibodies) andthecancerousmyelomacell[(i.e.,thehybridomacell immortalized,(continuoustogrowindefinitely)[13].

II- Recombinant DNA technology:

Theproductionofrecombinantmonoclonal antibodiesinvolves techno-logies,referred to as repertoirecloning or phage display/yeast display.Recombinantantibodyengineering involves theuseofvirusesoryeasttocreateantibodies,ratherthanmice[14]. These techniquescan be used to enhance:the specificity with whichantibodies recognizeantigens, theirstabilityinvarious environmental conditio-ns, their therapeutic efficacy, and their detectability indiagnosticapplications[15].

III -Transgenic methods

Oneofthemostpromisingapproach-es totheproduction of therapeutic humanmonoclonalantibodiesis the creation of a mouse strainengineeredtoproducealargereperto ireofhuman antibodiesinthe absenceofmouseantibodies[16].Nowchick encanbegeneticallyengineered toproducecancer drugs intheireggwhites[17].

Thegenesformonoclonalantibodycanbeinsertedintoplants andexpressedbythem. Cornisthemostpopularplantforthesepurposes, buttobacco, tomatoes,potatoes,andricearealsobeingused[18].

Throughouttheprogressionofmono-clonaldrugdevelopmentthere havebeenfourmajorantibodytypesdeveloped: mu-rine,chimeric, humanized andhuman[19].

Humanized antibodies areproducedbygraftingmurine hypervariableaminoaciddomainsintohumanantibodies.Thisresultsin amoleculeofapproximately 95%humanorigin[20].

Humanmonoclonal antibodiesareproducedusingtransgenicmice or phagedisplaylibraries.Humanmonoclonalanti-bodiesareproducedby transferringhumanimmunoglobulingenesintothemurinegenome,after whichthetransgenic mouseisvaccinatedagainsttheimmuno-globulin, leading to the production of monoclonal antibodies. Once monoclonal antibodies for a given

substance have been produced, they can be used to detect the presence and quantity of this substance and so mono-clonal antibody has many diagnostic applications[4].

One of these most important applica-tions is analysis of cell surface and secreted molecules like CD molecules identification and identification of transcription factor FOXP3[7].

The diagnosis of many infectious diseases relies on the detection of particular antigens or antibodies in the circulation or in tissues by use of monoclonal antibodies in immunoassays, for example bacteria disea-ses (Chlamydiae pneumonia)[21, 22], sero-typing niesseria meningitides and diagnosis of H. pylori eradication 4 weeks after the end of treatment[23].Viral diseases (rubella virus, hepatitis E virus and SARS-CoVnucleocapsid protein)[24, 25]and airborne fungi[1].

In the field of molecular genomics the application of molecular biology with the help of monoclonal antibody has permitted identification of susceptibility genes that predispose to development of autoimmune diseases[26].

There is a very important application in the field of molecular imaging especially in Immune-positron emission tomography (ImmunoPET). Immuno-PET combines the high sensitivity and resolution of a PET camera with the specificity of a mAb. immuno-PET enables the confirmation of tumor targeting and the quantification of mAb accumulation (in fact, radioactivity uptake). Thus, we can select patients who have the best chance to benefit from expensive mAb-based therapy[27].

Monoclonal antibodies have had a profound impact on medicine by providing an almost limitless source of therapeutic reagents. Its therapeutic use has become a major part of treatments in various diseases including transplantation, cancer therapy, autoimmune, cardiovascular, and infectious diseases[28].

The first FDA-approved therapeutic monoclonal antibody was a murine IgG2a CD3 specific transplant rejection drug, Muromonab (OKT- 3), in 1986. This drug was found to be useful in solid organ transplant recipients who became steroid resistant. Currently, many FDA-approved therapies exist, and hundreds of therapies are undergoing clinical trials[28].

Trastuzumab (Herceptin)isa mono-clonal antibody that interferes with the human epidermal growth factor *(HER2/neu)* receptor[29].This increases the survival of people with cancer[30]. The original studies of trastuzumab showed that it improved survival in late-stage (metastatic) breast cancer[29].

Bevacizumab (trade name Avastin) is a humanized monoclonal antibody. It stops tumor growth by recognizing and blocking vascular endothelial growth factor (VEGF)[31].It is approved by the U.S. Food and Drug Administration (FDA) for metastaticcancers[32].

Rituximab (trade names Rituxan and MabThera) is a chimeric monoclonal antibody against the protein CD20, which is primarily found on the surface of B cells. It is used in the treatment of many lymphomas, leukemias, and some autoimmune disorders[33].

Also,it has been implicated as causing a Hepatitis E infection to become chronic (permanent) in a patient with a lymphoma. Hepatitis E infection is normally an acute (short-term) infection, suggesting the drug may have weakened the body's immune response to the virus[34].

Also, there are other anti-CD20 monoclonal antibodies as ocrelizumab, humanized (90%-95% human) B cell-depleting agent, ofAtumumab (HuMax-CD20) a fully human B cell-depleting agent and third-generation anti-CD20s have a glycoengineered Fe fragment

(Fe) with enhanced binding to Fe gamma receptors, which increase ADCC (antibody dependent cellular cytotoxicity)[35].

Radioimmunotherapy (RIT) involves the use of radioactively conjugated murine antibodies against cellular antigens. Most research currently involved their application to lymphomas, as these are highly radio-sensitive malignancies[36].

Antibody-directed enzyme prodrug therapy (ADEPT) involves the application of cancer associated monoclonal antibodies which are linked to a drug-activating enzyme. Subsequent systemic administration of a non-toxic agent results in its conversion to a toxic drug and resulting in a cytotoxic effect which can be targeted at malignant cells. The clinical success of ADEPT treatments has been limited now[37].

However, it does hold great promise and recent reports suggest that it will have a role in future oncological treatment. Monoclonal Antibodies may be used in autoimmune disease as in multiple sclerosis (MS)[38]and in rheumatoid arthritis and systemic lupus erythematosus[39].

Monoclonal antibody Anti-IgE monoclonal antibody also may be used for the treatment of asthma and other manifestations related to allergic diseases[40]. It has been demonstrated that systemic treatment with omalizumab reduced symptoms and caused improved quality of life in patients with moderate/severe allergic rhinitis[41].

Anti- T cell monoclonal antibodies derived from mouse B cells and directed against human T cells, particularly those reacting with the CD3 marker (Muromonab CD3, OKT3), have been extensively used in the management of transplanted patients. These antibodies are predominantly used for the treatment of acute rejection. In addition, some groups of doctors use the monoclonal antibody as "induction" treatment imme-diately before transplantation to prevent rejection[42].

The role of complement system in inflammation has been well established. Inflammation is a cornerstone of the post-myocardial infarction. Also, during a heart bypass procedure, the "Complement activation" causes an inflammatory response that can lead to side effects such as chest pain, heart attack, stroke, heart failure, or death[43].

One such agent which causes complement inhibition is Pexelizumab. Pexelizumab, a recombinant humanized single chain monoclonal antibody to C5, blocks the conversion of C5 to C5a and accordingly inhibits C5b-9. It is an engineered monoclonal antibody fragment designed to inhibit complement-mediated tissue damage associated with reperfusion injury and inflammation that occurs during open heart surgery.

Some trials in the last years have evaluated the role of Pexelizumab in patients undergoing Coronary Artery Bypass Graft surgery and also in the treatment of acute myocardial infarction[43].

New prophylactic and therapeutic strategies to combat human infections with highly pathogenic avian influenza (HPAI) H5NI viruses are needed. Neutralizing anti-H5NI human monoclonal antibodies ware generated and tested their efficacy for prophylaxis and therapy in a murine model of infection[44].

Some researchers are trying to use a new method of rapidly producing highly targeted monoclonal antibodies to develop a diagnostic test as well as a temporary therapy to stave off the HlNl (swine flu) virus[45].

Many diseases of the eye, such as age-related macular degeneration (AMD)

and diabetic retinopathy, damage the retina and cause blindness when blood vessels around the retina grow abnormally and leak fluid, causing the layers of the retina to separate. This abnormal growth is caused by VEGF, so bevacizumab has been successfully used to inhibit VEGF and slow this growth[(46)].

Drug abuse continues to be a major national and worldwide problem, and effective treatment strategies are badly needed. Antibodies are promising therapies for the treatment of medical problems caused by drug abuse (e.g. treatment of phencyclidine or methamphetamine overdose), with several candidates in preclinical and early clinical trials[(47)].

Conclusion

- In addition to its diagnostic applications monoclonal antibody have many therapeutic applications and one of the most important of these therapeutic applications is the use of monoclonal antibody in the treatment of cancer.
- Also Mab are used in the treatment of auto-immune diseases like multiple sclerosis (MS), rheumatoid arthritis and systemic lupus erythematosus.
- Mab are used also in the treatment of myocardial infarction and in coronary artery bypasses graft surgery.
- Mab are used in the treatment of some viral disease like West Nile virus and H5NI influenza infection.

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