ASIAN JOURNAL OF BIOMEDICAL & PHARMACEUTICAL SCIENCES

e-ISSN: 2249-622X

REVIEW ARTICLE

Monoclonal Antibodies in Therapeutics

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Received:
9th April 2013
Received in revised form:
25th April 2013
Accepted:
4th May 2013
Available online:
12th May 2013

OPEN ACCESS

Online ISSN 2249–622X
http://www.jbiopharm.com

ABSTRACT

Monoclonal antibodies (MAb) are antibodies that are identical because they are produced by one type of immune cell; all are clones of a single parent cell. Given (almost) any substance, it is possible to create monoclonal antibodies that specifically bind to that substance; they can then serve to detect or purify that substance. Monoclonal antibodies (MAbs) are an integral part of targeted therapy approach for various diseases which result in decrease in adverse effects and increase in efficacy. They target various receptors or various growth factors on the cell surface and modulate their vital functions and cause cell death by various mechanisms. They are produced by conventional hybridoma technique. They have been modified to humanized forms to decrease adverse effects. Since then many MAbs have been produced which have changed the clinical course of many diseases. In this article, briefly the technique of production of these monoclonal antibodies, their mechanism of action in therapy and clinically important monoclonal antibodies will be discussed.

Keywords: newer monoclonal antibodies, targeted cancer therapy, radiolabelled MAbs, mepolizumab, MAbs

1. INTRODUCTION

In the year 1975, Kohler and Milsten provided the proof of clonal selection theory by the fusion of normal cells and the constantly dividing myeloma cells. This discovery was so spectacular that they were awarded with the prestigious Nobel Prize in the year 1984.There were few immunological reactions due to the murine nature of the older antibodies. In the year 1988 Greg Winter pioneered and mastered the technique of humanizing these antibodies.

The first FDA-approved therapeutic monoclonal antibody was a murine IgG2a CD3 specific transplant rejection drug, OKT3 (also called muromonab), in 1986. This drug found use in solid organ transplant recipients who became steroid resistant. Hundreds of therapies are undergoing clinical trials. Most are concerned with immunological and oncological targets.

Monoclonal antibodies (MAbs) are an integral part of targeted therapy approach for various diseases which result in decrease in adverse effects and increase in efficacy.

In this article, briefly the technique of production of these monoclonal antibodies, their mechanism of action in therapy and clinically important monoclonal antibodies will be discussed.

MONOCLONAL ANTIBODIES (MAbs) are antibodies that are identical because they are produced by one type of immune cell; all are clones of a single parent cell. Given (almost) any substance, it is possible to create monoclonal antibodies that specifically bind to that substance; they can then serve to detect or purify that substance.[1] This has become an important tool in biochemistry, molecular biology and medicine.

Types of monoclonal antibodies[1]:

Murine source MAbs: rodent MAbs with excellent affinities and specificities generated using conventional hybridoma technology.

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2. Chimeric MAbs: chimers combine the human constant regions with the intact rodent variable regions. Affinity and specificity unchanged.
3. Humanized MAbs: contained only the CDRs of the rodent variable region grafted onto human variable region framework
4. Recombinant DNA engineered MAbs

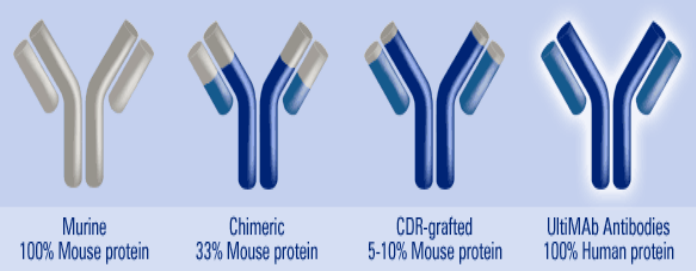

Figure 1: Evolution of therapeutic antibodies[2]

Production of monoclonal antibodies[1,3]:

Step 1: Immunization of mice

Mice are immunized with an antigen (attached to adjuvant). The antigen can be whole cells, membrane fragment, or complex molecules. Mice serum's are screened using various techniques such as ELISA. When sufficient titre is reached the mice are euthanized and spleen is removed as a source of cells for cell fusion.

Step 2: Preparation of Myeloma Cells

Myeloma cells are immortalized cells that are capable of dividing indefinitely. These cells are treated with 8-azaguanine to ensure sensitivity to HAT

Step 3: Fusion of myeloma cells with Spleen cells: spleen cells harvested from mice are fused with myeloma cells. Fusion is done through co-centrifuging in polyethylene glycol. Cells are plated in selection medium Hypoxanthine-aminipterin-thymidine (HAT) selection medium – inhibitor aminoterin which blocks nucleotide synthesis.Only fused cells with grow on HAT. Cells are distributed on feeder cells (murine bone-marrow) to promote growth of the hybridomal cells.

Step 4: Cloning of Hybridoma cells .A mouse is inoculated with the cell and thereby becomes a factory for producing the MAbs. Ascites fluid collected from the mouse.

Step 5: Antibody is screened and purified: Antibodies are screened using specific Ag binding. Advantage of in vivo

Step 6: Desired antibodies are cloned: This is done in vitro on culture bottle[1,3]

Problems with use of murine and chimeric antibodies[3]:

The therapeutic use of rodent monoclonal antibodies in humans is limited by their immunogenicity-human patients mount a immune response against them, producing HAMA. (Human anti mouse antibodies). These not only cause the therapeutic antibodies to be quickly eliminated from the host, but also form immune complexes that cause damage to the kidneys. This is due to differences between the mouse and humans.

HUMANIZED ANTIBODIES: [3,4]

To address this problem, the complementary regions (CDRs), which are the responsible for antigen binding within the variable regions, have been transferred to human frameworks creating ''CDR-grafted'' or ''humanized'' antibodies. This is, in essence a human antibody with small segments containing mouse antibody genes.

RECOMBINANT DNA ENGINEERED MAbs[3]

Transgenic plants: transgenic tobacco plants to produce IgA antibodies.

Transgenic animals: transgenic mouse to make humanized IgG antibodies, using embryonic stem cell method.

Uses of MAbS[1, 4]

Diagnostic: [4]

Once monoclonal antibodies are formed against any substance, they can be used to detect the presence of that substance anywhere in the body. Few of the examples are:

Western blot test -to detect a protein on a membrane.

Immunofluorescence test -to detect a substance in a cell.

Immunohistochemistry - to detect antigen in fixed tissue sections.

Immunoprecipitation and affinity chromatography - to purify a substance.

Therapeutic applications of MAbs[4]

Inhibition of allo-immune reactivity

Inhibition of auto-immune reactivity

Cancer therapy

Mechanism of action of monoclonal antibodies in cancer therapy[3,4]

Monoclonal antibodies induce an immunological response in the body by binding with extra-cellular domain of receptor involved in cell growth & proliferation.

Types of MAbs (clinical) [1,4]

Naked MAbs

MAbs coated with toxins, radionucleotides

'Naked' monoclonal antibodies[1, 3,4]

'Naked' means these antibodies are not fused to a toxin. Naked Monoclonal antibodies can kill cells via a variety of mechanisms, including:

Antibody-Dependent Cellular Cytotoxicity (ADCC),

Complement-Dependent Cytotoxicity (CDC) and

Direct induction of apoptosis.

Apoptosis induction in cancer cells[1]

MAbs target growth factor receptors on the cell surface to exert an inhibitory effect on the growth and survival of the cancer cells by antagonizing ligand-receptor signaling. MAbs can target the cell surface antigens and directly elicit apoptotic signaling.

Role of monoclonal anti bodies in cancer therapy:

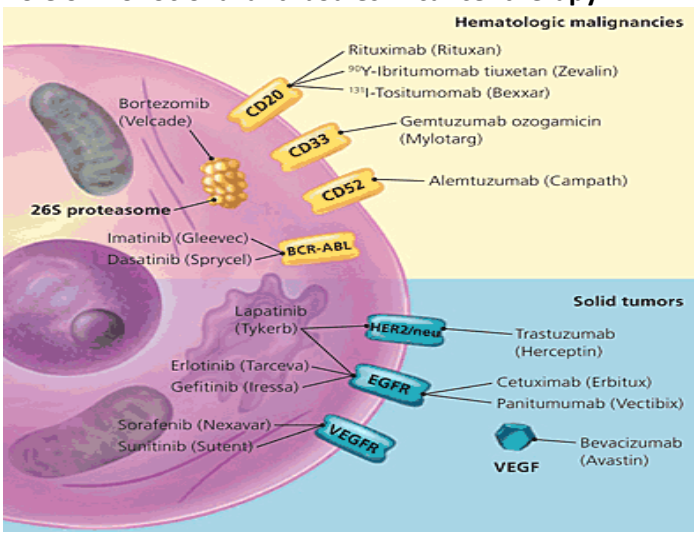

Figure 2: Targeted therapy: monoclonal antibodies and small molecules in chemotherapy of various cancers. [1, 5]

Monoclonal antibodies in therapeutic use:

ANTI CD3:[1, 3]

CD 3 is a co receptor which plays an important role in T cell receptor signaling.

MUROMUMAB (OKT3) is a drug which blocks this receptor. It blocks killing by cytotoxic T cells and many other T cell functions. It kills cells by blocking vital functions of CD3, approved for treatment of renal allograft rejection crisis.

TEPLIZUMAB[7] is a newer anti CD3 drug. It is used for protecting remaining beta cells in newly diagnosed type I DM. It is currently in phase III clinical trials.

ANTI CD20:

RITUXIMAB[1, 2]: Its mechanisms of action in killing cancer cells are- ADCC ,CDC and direct induction of apoptosis with proven efficacy against wide range of NHL B-cell malignancies. Dose: 375mg/m2 IV infusion weekly for 4 weeks. Side effects are fever with rash, dyspnoea and late onset neutropenia.

OCRELIZUMAB[1] is a newer CD20 drug .It targets mature B lymphocytes and thus is an immunosuppressive drug. It is currently in Phase III. It is used in RA, SLE, MS and lymphomas.

OFATUMUMAB[1] is also a newer CD20 drug. It inhibits early B lymphocyte activation. It targets different epitope of that by rituximab. It was approved in February 2010 for refractory CLL. [1, 5]

Radiolabelled Anti CD20:[1, 3]

Radiolabelling the monoclonal antibodies increases their efficacy. They can also be used for various imaging purposes. Yttrium 90-ibritumomab (Y^{90}), Indium 111-ibritumomab (In^{111}), Iodine 131- tositumomab (I^{131} sub) are the 3 commonly radiolabelled anti CD20 MAbs. They show increase in efficacy than their naked counterparts. Effective in relapsed /refractory/advanced cases of follicular B-cell lymphoma and NHL in the doses of 0.4 ci/kg IV. Its side effects are myelodysplasia and hematological toxicities.[6]

ANTI CD 22:

CD22 is a co receptor important for B cell receptor signaling.[7]

EPRATUZUMAB is a drug which blocks CD22 signaling. It is currently in phase III clinical trials[8]. It is active against malignant B cells and used in SLE. It produces cell death by ADCC. [1, 5]

ANTI CD52:

CD52 is present on thymocytes, macrophages, lymphocytes and monocytes.

ALEMTUZUMAB[1, 2] is a drug which blocks the CD52 signaling. It kills tumor cells by ADCC, CDC and apoptosis. It is Administered IV 30mg/day thrice weekly. Premedication with diphenhydramine and acetaminophen should precede this drug since hypersensitivity reactions are common. It used in B cell and T cell lymphomas and MS. Its side effects are T cell depletion and immunosuppression.[1,3]

ANTI CD33:

CD33 is a co receptor found on myeloid cell surface.

GEMTUZUMAB OZOGAMICIN (MAb linked to a toxin). Humanized MAb covalently linked to a semisynthetic derivative of calicheamicin .It causes DS DNA breaks and cell death. Its dose is 2 doses of 9mg/m2 IV separated by 14 days. It is used in AML. Its side effects are hematopoietic suppression and vaso occlusive disorders. [1, 2]

LINTUZUMAB is a newer anti CD33 MAb. It is currently in phase III for AML. [9]

ANTI CD 11a:

CD 11a is a co receptor found on B cells and important in cell to cell adhesion and co-stimulation.

EFALIZUMAB[1.6] is a drug that blocks CD11a signaling. It is approved for the treatment of adult patients with severe psoriasis. It administered by SC injections.

ANTI HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2/ NEU: [1,2,10]

Human epidermal growth factor receptor is a factor which plays an important role in growth of many breast cancers.

TRASTUZUMAB[1, 2] is a drug which blocks this receptor. it causes cell death by inhibition of HER2 signaling with G1 arrest and also by ADCC and apoptosis .Its dose is loading dose of 4mg/kg IV followed by 2 mg/kg weekly. It is used in HER2-positive metastatic breast cancers. Its side effects are cardiomyapathy and flu like syndrome.

PERTUZUMAB is a newer HER2 blocking MAb. It is currently in clinical trials. [10]

ANTI EPIDERMAL GROUTH FACTOR RECEPTOR (EGFR)[1,2]

Epidermal growth factor receptor plays a vital role in growth of many cancers. CETUXIMAB is a drug which blocks EGFR signaling and causes cell death by ADCC.

Its dose is loading dose of 400mg/kg infusion followed by 250mg/kg weekly. It is used in metastatic colorectal and head and neck cancers. ITS SIDE effects are infusion related toxicity and skin rash. [1, 2]

PANITUMUMAB - It is an another EGFR receptor blocking MAb..Produces cell death by similar mechanism as cetuximab. It was approved in September 2006. It is used in metastatic colorectal and head and neck cancers. Its adverse effects are skin rash and fatigue. First MAb approved by FDA developed from transgenic mice. [1, 2]

MATUZUMAB and NIMOTUZUMAB are the other EGFR blocking MAbs which are in phases III of clinical trials. [11]

ANTI VASCULAR ENDOTHELIAL GROUTH FACTOR (VEGF)[1, 2]

Vascular endothelial growth factor plays an important role in angiogenesis and neo vascularization of various tumors.

BEVACIZUMABis a drug which blocks VEGF. It inhibits angiogenesis and neovascularization in the dose of 5mg/kg IV every 14 days until disease progression stops. It is used in colorectal and head and neck cancers. Its side effects are hypertension, pulmonary hemorrhages, GI perorations and CCF. [1, 2]

RANIBIZUMAB is a newer VEGF blocking drug. It is used in neovascular macular degeneration. It is injected directly into vitreous cavity. [1, 2,4]

These are few other new important monoclonal antibodies which are either in the pipeline of development or recently approved:

ADALIMUMAB, GOLIMUMAB AND CERTOLIMUMAB: These are the monoclonal antibodies against the TNF alpha. Certolimumab and Golimumab are the newer drugs recently approved. Golimumab is administered as SC injections once monthly, in Rheumatoid Arthritis. [12]

OMALIZUMAB is a drug against IgE. It prevents histamine release from the mast cells. It is used in bronchial asthma. [1,2]

ECULIZUMAB is a drug against complement protein C5. It prevents the formation of terminal components of the complement chain C5b-C9. It is used in Paroxysmal Nocturnal Hemoglobinuria and Hemolytic Uremic Syndrome. [13]

PEXELIZUMAB is a newer anti C5 drug which is currently in phase III trials for patients of coronary artery bypass grafting surgeries. [5, 13]

PALIVIZUMAB is a MAb against fusion protein of respiratory syncitial virus. [14]

TANEZUMAB is a MAb against nerve growth factor, which plays an important role in mediation of pain in patients of Rheumatoid arthritis. It is used for pain relief in RA patients. [15]

BAPINEUZUMAB is a MAb against A beta amyloid protein. It removes the A beta amyloid which accumulates in patients of Alzheimer's disease, currently in phase III trials. [16]

MEPOLIZUMAB is a MAb against IL5. It stops eosinophil activation and is used in hypereosinophilic syndrome. It has been given an orphan drug status. [17]

DENOSUMAB is a MAb against Receptor activator of nuclear factor kappa-B ligand (RANKL). It is used in osteoporosis. [1,2]

ATLIZUMAB is a MAb against IL6. It is used in Crohn's disease, RA and Castleman's disease. [18]

FONTOLIZUMAB is a MAb against interferon 3 γ, under trial for crohn's disease. [19]

LABETUZUMAB is a MAb against carcinoembryonic antigen. It is under trial for medullay thyroid carcinoma. [4]

NATALIZUMAB is a MAb against cellular adhesion molecule α 4 integrin protein. It is under trial for multiple sclerosis. It is approved for the use in patients of crohn's disease. Its rare side effect is progressive multifocal leukoencephalopathy. [16]

SIBROTUZUMAB is a MAb against fibroblast activation protein. It is under trial for many cancers. [20]

SIPLIZUMAB is a MAb against CD2 receptor. It is under trail for NK cell NHL. [9]

BAVITUXIMAB (TARVACIN) it targets the protein phosphatidyl serine in the outer envelope coat of the enveloped viruses. It is under trial for diseases with enveloped viruses. [21]

ALIROCUMAB and LODELCIZUMAB are the MAbs under trial for hypercholesterolemia.[23]

BEZLOTOXUMAB is a MAb under phase III clinical trials for clostridium difficile infections.[23]

DALOTUZUMAB is a MAb is in phase III clinical trials for various cancers. It is a MAb against insulin-like growth factor I receptor.[23]

FELVIZUMAB is a MAb useful against respiratory syncitial virus infection.[23]

EXBIVIRUMAB is a MAb under trial for hepatitis B infection.[23]

SECUKINUMAB is a MAb targeting IL-17A protein and is under trial for uveitis and rheumatoid arthritis.[23]

TALIZUMAB is a MAb targeting Ig E antibody used in treatment of allergic reactions.[23]

TEFIBAZUMAB is a MAb against clumping factor A, useful against life threatening staphylococcal infections.[24]

IBALIZUMAB is a non-immunosuppressive monoclonal antibody that binds CD4, the primary receptor for HIV, and inhibits the viral entry process. [25]

GALIXIMAB is a MAb against CD80 protein and prevents co stimulation and is used in relapsed hodgkin lymphoma.[26]

MAbs against deaddiction are also under trial.

MAbs WITH RADIOISOTOPES[2]

Monoclonal antibodies can be labeled with radioisotopes and can be used for imaging and diagnostic purposes in many cancers.

ARCITUMOMAB- a murine fragment from an anti CEA ab used for imaging in ca colon

CAPROMAB PENDETIDE- murine MAb specific for prostatic specific antigen Coupled with iridium 111 and used in immunoscintigraphy of prostate.

NOFETUMOMAB- used in small cell lung ca imaging.

SATUMOMAB- murine monoclonal IgG that binds to TAG-72 found in ovarian ca and used for imaging purpose in ovarian cancers.

OBSTACLES TO USE OF MAbs IN CANCER THERAPY[1,3,4]

Antigen distribution of malignant cells is highly heterogeneous, so some cells may express tumor antigens, while others do not. Tumor blood flow is not always optimal, thus MAbs may not reach the tumor tissues if blood flow is not optimal. High interstitial pressure within the tumor can prevent the passive monoclonal antibody from binding. More importantly high cost of these antibodies can be a major obstacle for the use of these drugs in developing countries like India.

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Conflict of Interest: None Declared

Cite this article as:
Somesh Rayasam, Sujata Dudhgaonkar, Prashant Deshmukh, Swati Sarda, Parag Kinge, Rohan Hire. Monoclonal Antibodies in Therapeutics. Asian Journal of Biomedical and Pharmaceutical Sciences, 2013, 3: (19), 1-13.

