MONOCLONAL ANTIBODIES PRODUCTION, DIAGNOSTIC AND THERAPEUTIC APPLICATIONS

Assay

Submitted for the partial fulfillment of Msc degree in Microbiology and Immunology

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2009

Abstract

Monoclonal antibody is an antibody that is specific for one antigen and is produced by a B-cell hybridoma**.** The process of producing monoclonal antibodies was invented by Georges Köhler, César Milstein, and Niels Kaj Jerne in 1975. 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of relative ease of the production and purification of large quantities of antibodies **.**Hybridoma technology has been replaced by recombinant DNA technology, transgenic mice and phage display **.**Monoclonal antibodies have had a profound impact on medicine by providing an almost limitless source of therapeutic and diagnostic reagents. Therapeutic use of monoclonal antibodies has become a major part of treatments in various diseases including transplantation, oncology, autoimmune, cardiovascular, and infectious diseases**.**Monoclonal antibody therapy is the use of monoclonal antibodies to specifically target cells. The main objective is stimulating the patient's immune system to attack the malignant tumor cells and the prevention of tumor growth by blocking specific cell receptors**.**

Key Words:

MONOCLONAL ANTIBODIES - PRODUCTION - DIAGNOSTIC AND THERAPEUTIC - APPLICATIONS

List of Abbreviation

^{111}In : indium-111

5-FU : 5-fluorouracil

^{99m}Tc : Technetium-99m

AChE: Acetylcholinesterase

ADCC: Antibody dependent cell-mediated cytotoxicity

ADEPT : Antibody directed enzyme prodrug therapy

ADEPT: Antibody-directed enzyme prodrug therapy

AIDS: Acquired immunodeficiency syndrome

AITD: The autoimmune thyroid diseases

AMD: Age-related macular degeneration

Anti-HAV: anti hepatitis A virus antibody

CABG: Coronary artery bypass graft

CD: Cluster of differentiation

CDC: Complement dependent cytotoxicity

CDC: Complement-dependent cytotoxicity

cDNAs: Chimeric DNAs

CDRs: Complementarity Determining Regions

CLT: Chronic lymphocytic thyroiditis

CML: Cell-mediated lympholysis

CNS: Central nervous system

CNV: Choroidal neovascular membrane

CRC: Colorectal cancer

EGFR: Epidermal growth factor receptor

ELISA: Enzyme-linked immunosorbent assay

Fab: Fragment antigen binding

Fc: Fragment crystallizable

FcγRIIs: low-affinity Fcγ receptors

FcγRIs: High-affinity receptors

FDA: Food and Drug Administration

FISH: Fluorescence in situ hybridisation

FPIA: Fluorescence polarization immunoassay

GBS: Group B streptococci

H pylori: Helicobacter pylori

HAT: Hypoxanthine, Aminopterin and Thymidine

HER2: Human epidermal growth factor receptor-2

HGPRT: Hypoxanthine-guanine phosphoribosyltransferase

HIV: Human immunodefeciency virus

HPAI: Highly pathogenic avian influenza

i.p.: Intraperitoneal

Ig: Immunoglobulin

IgSF: Immunoglobulin superfamily

IHC: Immunohistochemistry

Immuno-PET: Immuno-positron emission tomography

MAb: Monoclonal antibody

MHC II: Major histocompitability complex

M-mAb: Murine mAbs

MS: Multiple sclerosis

MTX: Methotrexate

MTX-PGs: MTX-polyglutamates

NHL: Non-Hodgkins lymphoma

PCa: Prostate cancer

PCR: Polymerase chain reaction

PEG: Polyethylene glycol

PML: Progressive multifocal leukoencephalopathy

RA: Rheumatoid arthritis

RBCs: Red blood cells

RCC: Renal cell carcinoma,

RIA: Riadioimmunoassay

RIT: Radioimmunotherapy

RSV: Respiratory syncytial virus

scFv: Single chain variable fragment

SLE: Systemic lupus erythematosus

SNCG: Synuclein-gamma

SPECT: Single photon emission computerized tomography

TK: Thymidine kinase

TKI: Tyrosine kinase inhibitors

VEGF: Vascular endothelial growth factor

WNV: West Nile virus

κ: kappa

λ: lambda

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Monoclonal antibody is an antibody that is specific for one antigen and is produced by a B-cell hybridoma **(Abbas and Litchman 2007)**.

The process of producing monoclonal antibodies was invented by Georges Köhler, César Milstein, and Niels Kaj Jerne in 1975.The key idea was to use a line of myeloma cells that had lost their ability to secrete antibodies, come up with a technique to fuse these cells with healthy antibody producing B-cells, and be able to select for the successfully fused cells **(Kohler and Milstein 2005).**

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 **(Williame 1998).**

Hybridoma technology has been replaced by recombinant DNA technology, transgenic mice and phage display **(Hudson and Souriau 2003).**

Monoclonal antibodies have had a profound impact on medicine by providing an almost limitless source of therapeutic and diagnostic reagents. Therapeutic use of monoclonal antibodies has become a major part of treatments in various diseases including transplantation, oncology, autoimmune, cardiovascular, and infectious diseases **(Nissim and Chernajovsky 2008).**

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 technology has made it possible to raise antibodies against specific antigens presented on the surfaces of tumors **(Janeway 2005)**.

Monoclonal antibody therapy is the use of monoclonal antibodies to specifically target cells. The main objective is stimulating the patient's immune system to attack the malignant tumor cells and the prevention of tumor growth by blocking specific cell receptors **(Waldmann and Thomas 2003).**

AIM OF THE WORK

This study was conducted to clearify

- The new methods for production of monoclonal antibodies
- Application of monoclonal antibodies in diagnostic and therapeutic approaches.

Immunoglobulins (Antibodies) are gamma globulin proteins that are found in blood and are used by the immune system to identify and neutralize foreign objects. Antibodies are produced by a kind of white blood cell called a plasma cell (**Eleonora and Papavasiliou 2003**).

Basic Unit Structure of Immunoglobulin

Antibodies are heavy (~150kDa) globular plasma proteins .They are glycoproteins. (**Mattu and Pleass 1998**).

The basic functional unit of each antibody is an immunoglobulin (Ig) monomer (containing only one Igunit). (**Roux 1999**), which is a "Y"-shaped molecule that consists of four polypeptide chains; two identical heavy chains and two identical light chains connected by disulfide bonds. **(Woof and Burton 2004).** Each chain is composed of structural domains called Ig domains. These domains contain about 70-110 amino acids. **(Barclay 2003).**

Heavy chain

There are five types of mammalian Ig heavy chain denoted by the Greek Letters: α, δ, ε, γ, and μ. **(Janeway and Charles 2001).**

The type of heavy chain present defines the class of antibody; these chains are found in IgA, IgD, IgE, IgG, and IgM antibodies, respectively. Each heavy chain has two regions, the constant region and the variable region. The constant region is identical in all antibodies of the same isotype, but differs in antibodies of different isotypes. Heavy chains γ, α and δ have a constant region composed of three tandem (in a line) Ig domains, and a hinge region for added flexibility **(Woof and Burton 2004).** Heavy chains μ and ε have a constant region composed of

four immunoglobulin domains. The variable region of the heavy chain differs in antibodies produced by different B cells, but is the same for all antibodies produced by a single B cell or B cell clone. The variable region of each heavy chain is approximately 110 amino acids long and is composed of a single Ig domain **(Janeway and Charles 2001).**

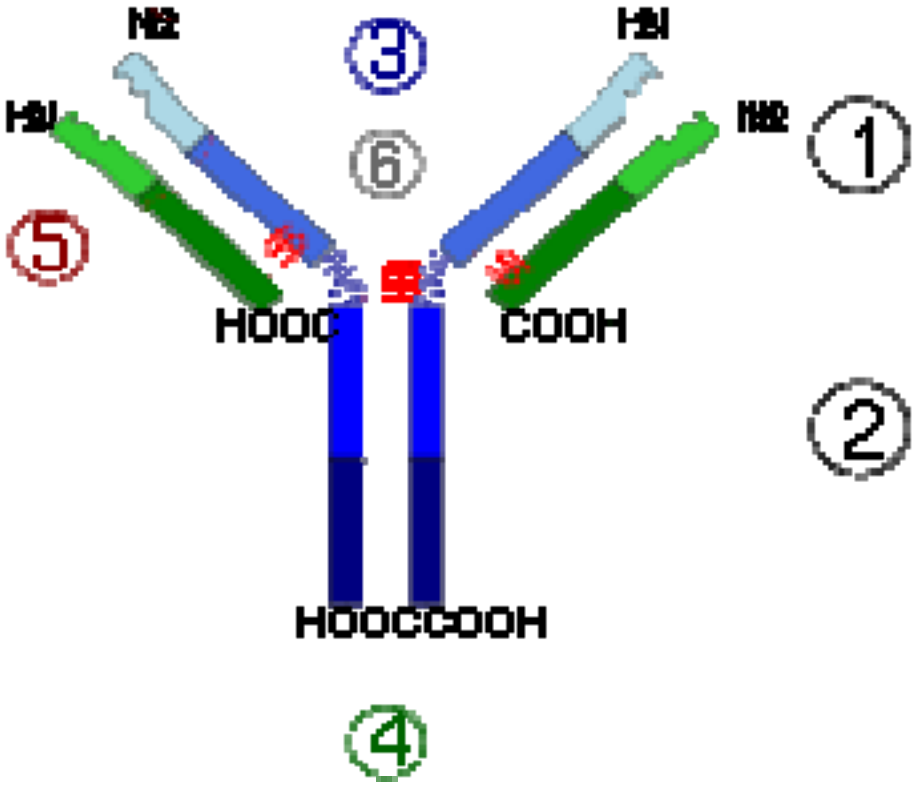


Figure 1: Antibody structure

1. Fab region
2. Fc region
3. Heavy chain with one variable (V_H) domain followed by a constant domain (C_H1), a hinge region, and two more constant (C_H2 and C_H3) domains.
4. Light chain with one variable (V_L) and one constant (C_L) domain
5. Antigen binding site (paratope)
6. Hinge regions **(Janeway and Charles 2001).**

Light chain

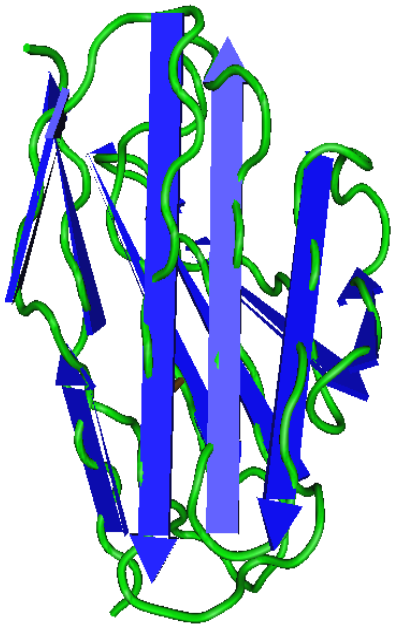
In mammals there are two types of Immunoglobulin light chain, which are called lambda (λ) and kappa (κ**).** A light chain has two successive domains: one constant domain and one variable domain. The approximate length of a light chain is 211 to 217 amino acids. Each antibody contains two light chains that are always identical; only one type of light chain, κ or λ, is present per antibody in mammals **(Janeway and Charles 2001).**

CDRs, Fv and Fab regions

Some parts of an antibody have unique functions which is called the Fab (fragment antigen binding) region. It is composed of one constant and one variable domain from each heavy and light chain of the antibody. The paratope is shaped at the amino terminal end of the antibody monomer by the variable domains from the heavy and light chains. The variable domain is also referred to as the FV region and is the most important region for binding to antigens. More specifically variable loops, three each on the light (VL) and heavy (VH) chains are responsible for binding to the antigen. These loops are referred to as the Complementarity Determining Regions (CDRs) **(Putnam and Liu 1979)**.

The Immunoglobulin Fold

A b barrel of 7 (C_L) or 8 (V_L) polypeptide strands connected by loops and arranged to enclose a hydrophobic interior. Hypervariable CDRs (are located on loops at the end of the Fv regions **(Leahy et al 2002).**



A barrel made of a sheet of staves arranged in a folded over sheet

Barrel under construction

Figure 2:

Single VL domain **A barrel made of a sheet of staves arranged in a folded over sheet**

Fc Region

The base of the Y plays a role in modulating immune cell activity. This region is called the Fc (Fragment, crystallizable) region, and is composed of two heavy chains that are formed of two or three constant domains depending on the class of the antibody **(Janeway and Charles 2001).** By binding to specific proteins the Fc region ensures that each antibody generates an appropriate immune response for a given antigen. The Fc region also binds to various cell receptors, such as Fc receptors, and other immune molecules, such as complement proteins **(Woof and Burton 2004).**

In an experimental setting, Fc and Fab fragments can be generated in the laboratory. The enzyme papain can be used to cleave an immunoglobulin monomer into two Fab fragments and an Fc fragment. The enzyme pepsin cleaves below hinge region, so a F(ab')2 fragment and