Antibodies: generation, interactions with antigen and their functions

Learning objectives

- By the end of this lecture you should be able to:
  - Outline the structure of antibodies
  - Outline the classes and biological activities of antibodies
  - Discuss the interactions of antibodies with antigens
  - Describe the functions of antibodies
  - Describe monoclonal antibodies
  - Outline the functions of monoclonal antibodies

Definition

- Also known as immunoglobulins
- Glycoproteins produced by plasma cells in response to an immunogen

Definition

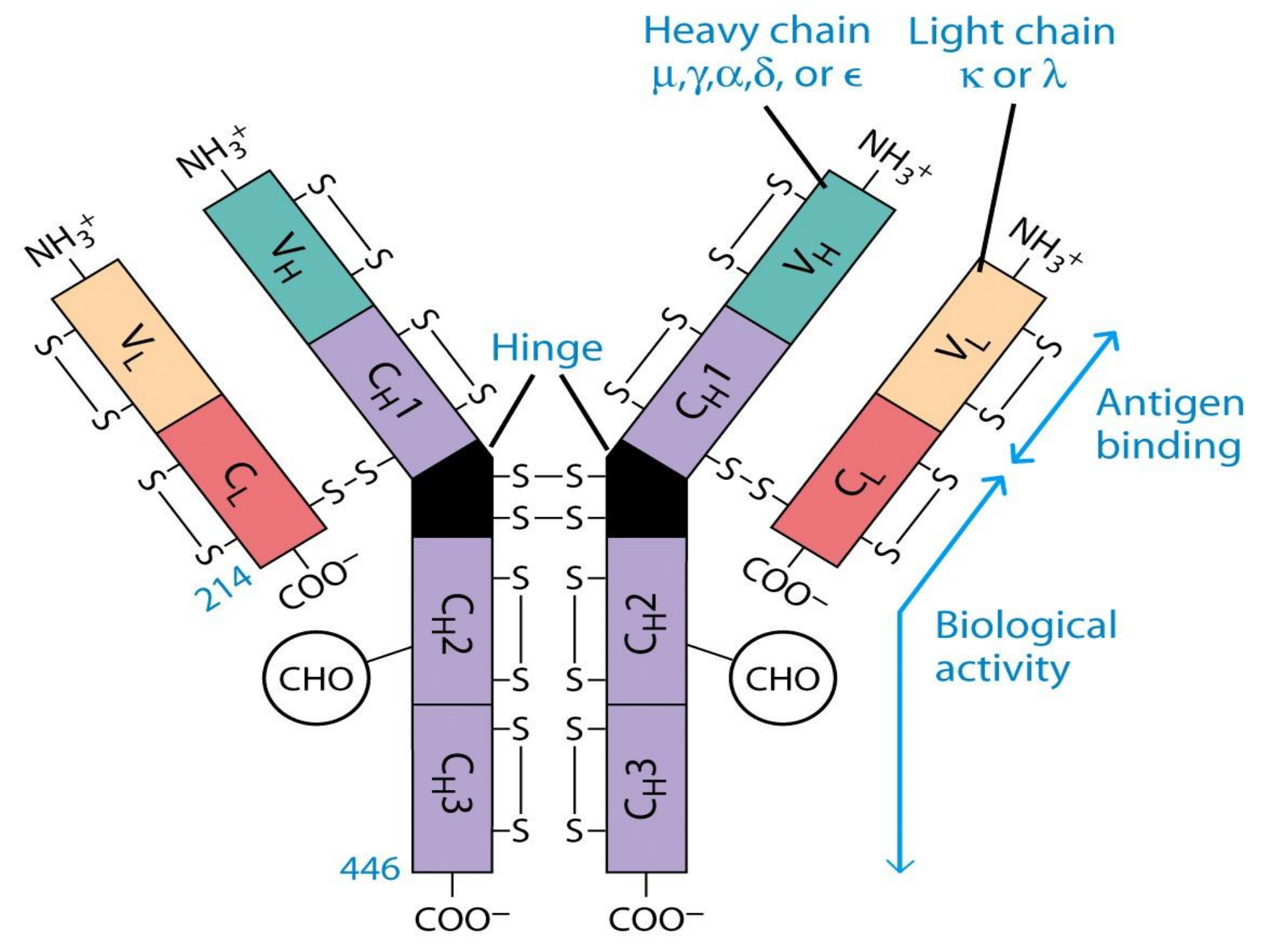
- Expressed as:
  - Membrane-bound receptors on surface of B cells; or
  - Soluble molecules (secreted from plasma cells); present in serum and tissue fluids

Structure of antibodies

- Antibodies are made up of:
  - 2 light polypeptide chains
  - 2 heavy polypeptide chains
- Each light chain bound to heavy chain by disulfide bonds (H-L)
- Heavy chain bound to heavy chain (H-H)

Structure of antibodies

- First 100 amino acids of amino terminal of both H and L chains are variable
- Referred to as V_L , V_H, C_H and C_L


Heavy chain
μ,γ,α,δ, or ε
Light chain
κ or λ
NH3+
VH
CH1
VL
CL
Hinge
S—S
CHO
CH2
CH3
214
446
COO−
Antigen
binding
Biological
activity

Structure of antibodies

- Fab and Fc portions
- Within the variable regions of both heavy and light chains, some polypeptide segments show exceptional variability – **hypervariable regions**
- Hinge region – allows for mobility (Y and T-shaped structures visualized on electron microscopy)

Sequencing of heavy chains

- The light and heavy chains are folded into discrete domains, and the type of heavy chain determines the class and subclass of the antibody

Fc receptors: functions

- Transport Ab across membranes
  - Secretion of immunoglobulin A across epithelium into lumen
  - Transport of maternal antibody across placenta (immunoglobulin G)
- Many cell types use FcR
  - Mast Cells, macrophages, neutrophils, B, T, NK cells

Fc receptors: Functions

- Opsonization, antibody-dependent cellular cytotoxicity

Antibody classes and subclasses

- Based on amino acid sequence in the constant region of the heavy chain

Class	Heavy chain	Subclass
IgG	γ	IgG1, IgG2, IgG3, IgG4
IgA	α	IgA1, IgA2
IgM	μ	
IgD	δ	
IgE	ε	

ANTIBODY CLASSES AND BIOLOGICAL ACTIVITIES

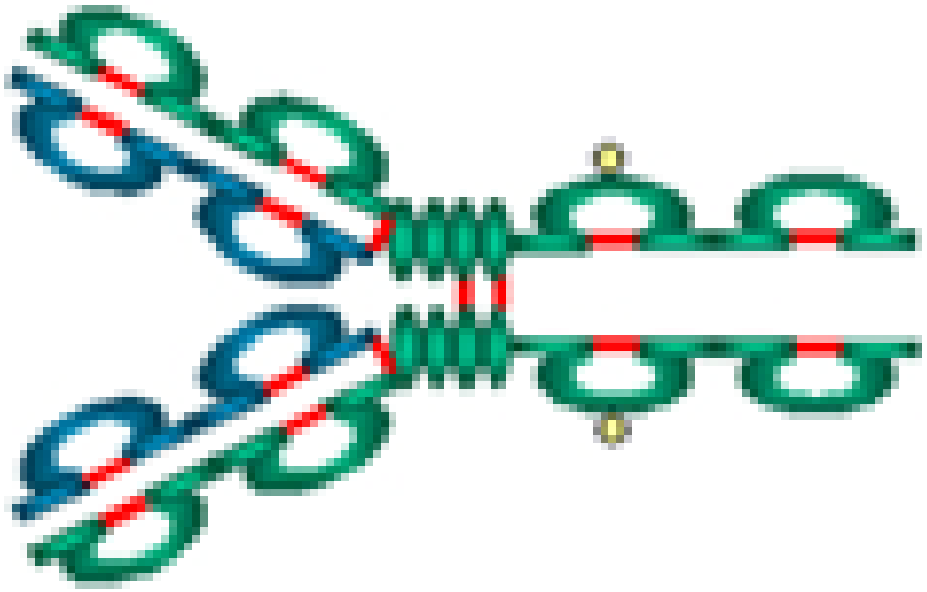
IgG

- Exists as a monomer
- Immunoglobulin G accounts for 70-75% of the total serum immunoglobulin pool; 146-170kDa
- Subclasses differ in number of disulfide bonds and length of the hinge region
- Major immunoglobulins in serum 80%

IgG

- Placental transfer-mediated by a receptor on placental cells for the Fc region of IgG
- Functions:
  - Fixes complement (except IgG4)
  - Binds phagocytic FcyR
  - Opsonin

IgG

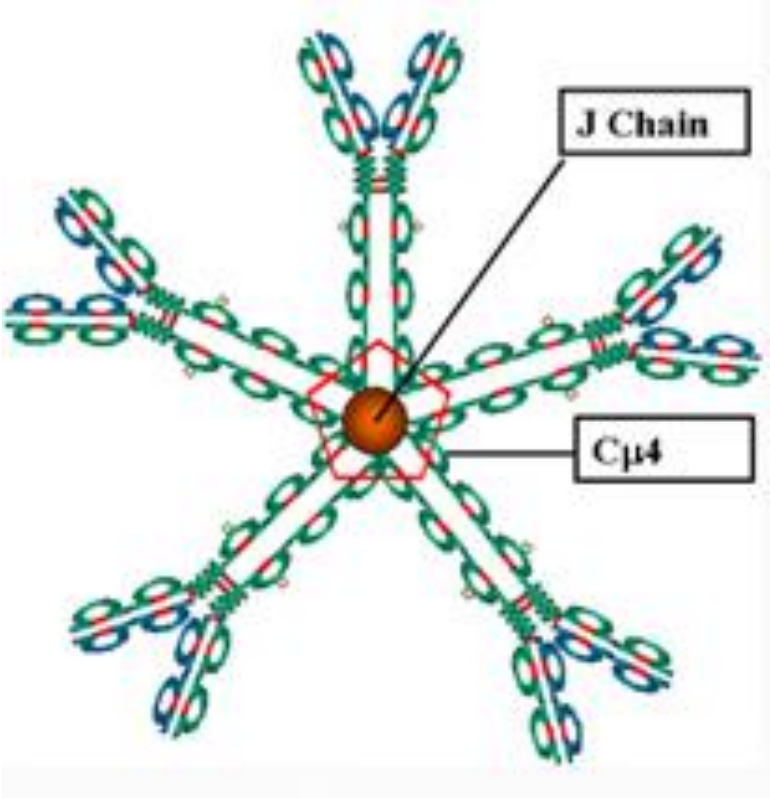


IgM

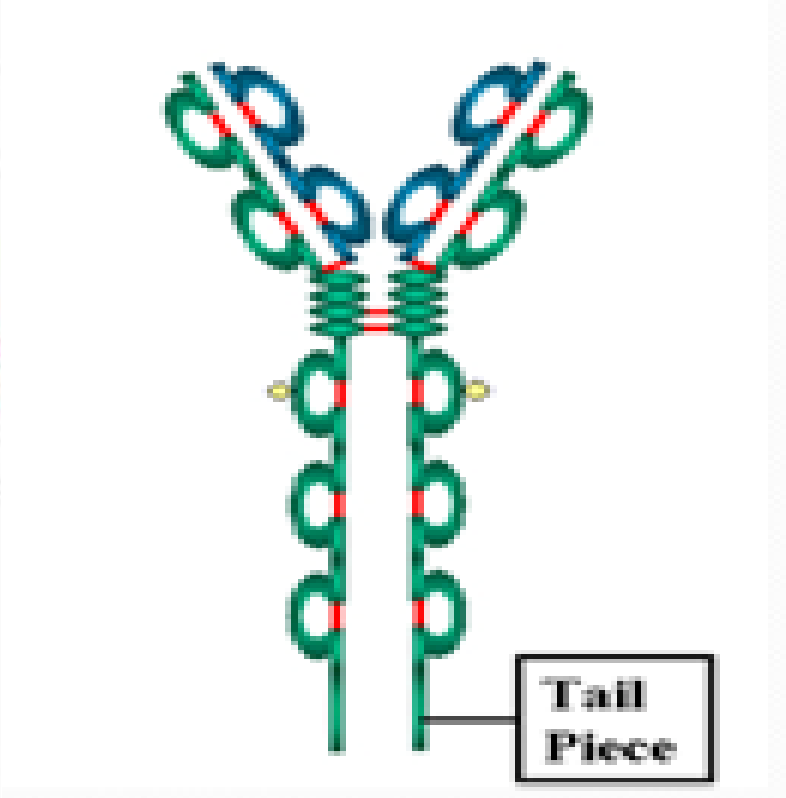
- Exists in 2 forms - pentamer, monomer
- Accounts for 10% of the total serum immunoglobulin pool; 970kDa
- Has an extra domain μ CH4
- Pentamer has 5 subunits linked by a J chain.
- J chain- polymerization of the molecule into a pentamer

IgM

Pentamer



Monomer



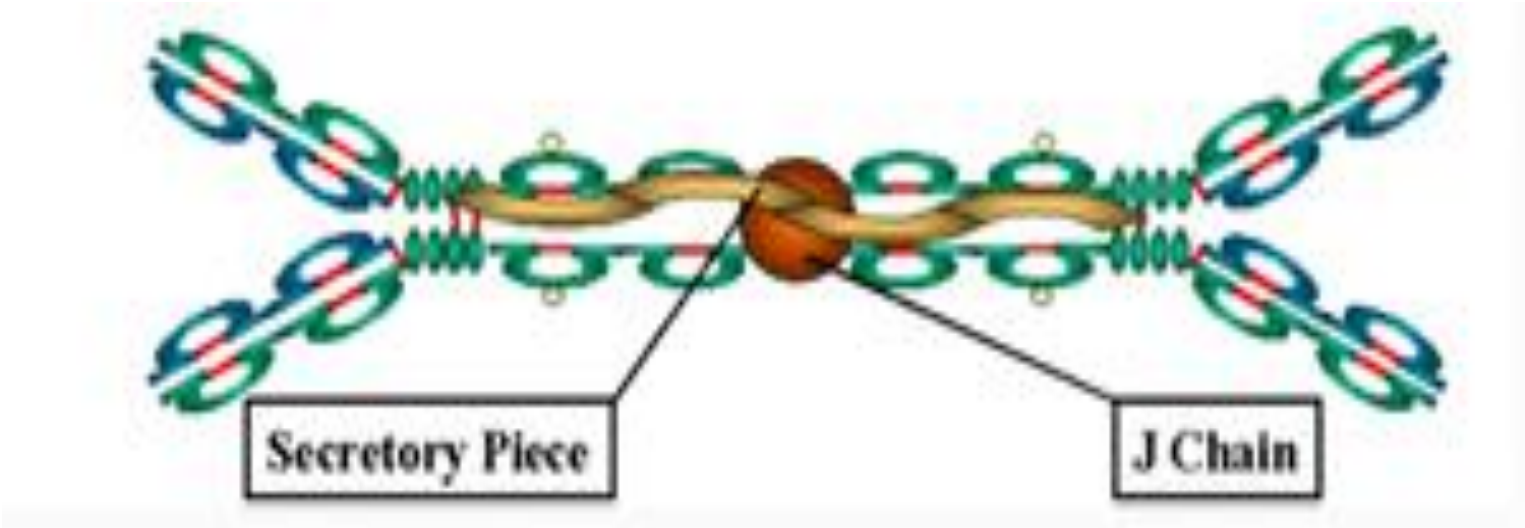
IgM

- First immunoglobulin to be made by the fetus & virgin B cells
- Functions:
  - Complement fixation
  - Agglutination
  - Immunoglobulin of primary immune response
  - A transmembrane form (monomer) is present as an antigen-specific receptor on mature B cells

IgA

- Exists as a monomer, but can interact with J chain to form a polymer.
- Accounts for 15-20% of serum immunoglobulin pool
- Predominant Ig in seromucous secretions – saliva, colostrum, milk, tracheobronchial and genitourinary secretions

IgA



IgD

- Exists a monomer
- Found in low levels in serum (accounts for <1% of serum Ig pool)
- A transmembrane form of immunoglobulin D is present as an antigen-specific receptor on mature B cells

IgE

- Exists as a monomer
- Serum levels are very low relative to the other immunoglobulin isotypes
- Basophils and mast cells express an immunoglobulin E-specific receptor of very high affinity, and so are continuously saturated with immunoglobulin E

IgE

- Functions:
  - Involved in allergic reactions
  - Plays a role in parasitic helminthic infections

Selected properties of immunoglobulin subclasses

	IgG	IgA	IgM	IgD	IgE
Mwt (Da)	160, 000	170, 000	900, 000	160, 000	180, 000
Half-life (days)	23	6	5	3	2.5
Special property	Placental passage	Secretory immunoglobulin	Primary response	Lymphocyte surface	Reagin

Antibody interaction with antigens

- Hypervariable regions are clustered at the end of Fab arms to form a unique surface topography that is complementary to structures on the antigen

Antibody interaction with antigens

- This complementary surface is called a **paratope**
- Corresponds to antigenic determinant (**epitope**)

Interaction

- Antigen-antibody interaction results from formation of multiple non-covalent bonds
- These attractive forces consist of
  - Hydrogen bonds
  - Electrostatic bonds
  - Van der Waals forces
  - Hydrophobic forces

Interaction

- Each bond is relatively weak in comparison with covalent bonds, but together they generate a high-affinity interaction

Interaction

- For a paratope to combine with its epitope, the interacting sites must be complementary in shape, charge distribution and hydrophobicity, and in terms of donor and acceptor groups capable of forming hydrogen bonds

Interaction

- The strength on interaction between an antigen and an antibody is referred to as **affinity**
- Is the sum of the attractive and repulsive forces resulting from binding between the binding site of a monovalent Fab fragment and its epitope

Interaction

- Each antibody unit of 4 polypeptide chains has 2 antigen-binding sites
- Therefore, antibodies are potentially multivalent in their reaction with antigen
- Antigen can be monovalent (e.g. small chemical groups, haptens), or multivalent (microorganisms)

Interaction

- The strength with which a multivalent antibody binds a multivalent antigen is termed **avidity**
- (affinity is determined for a univalent antibody fragment binding to a single antigenic determinant)

Interaction

- Avidity of an antibody for its antigen is dependent on the affinities of the individual antigen-combining sites for the epitopes on the antigen

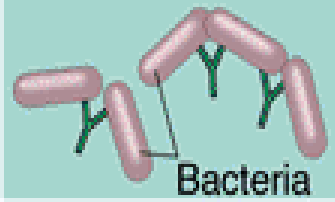
Functions of antibodies

- Functions mediated by antibody alone
- Functions mediated by antibody and additional molecules or cells
- Functional properties of engineered antibody molecules

PROTECTIVE MECHANISM OF BINDING ANTIBODIES TO ANTIGENS

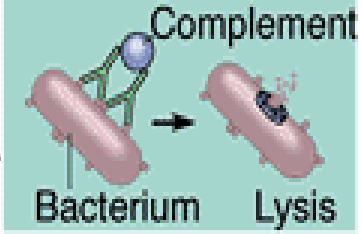
Agglutination

Enhances phagocytosis and reduces number of infectious units to be dealt with



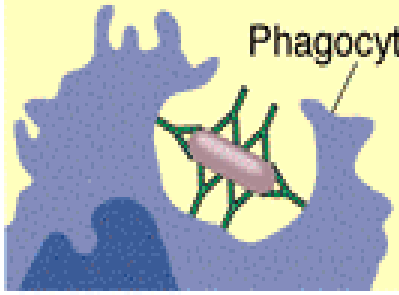
Activation of complement

Cell lysis

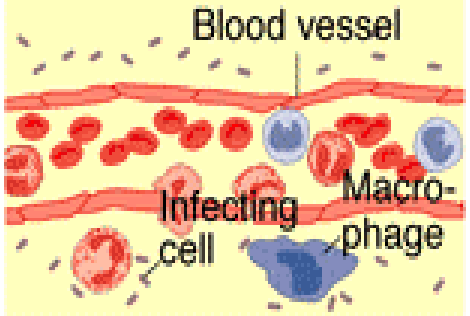


Opsonization

Coating antigen with antibody enhances phagocytosis



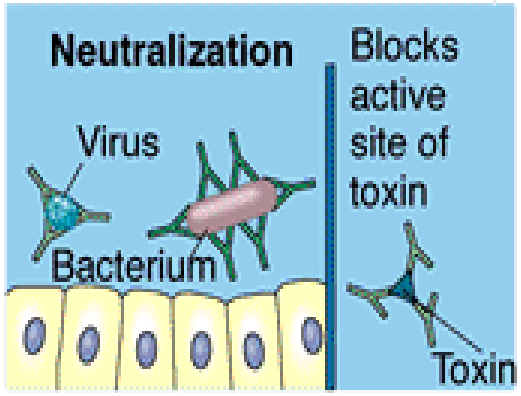
Inflammation



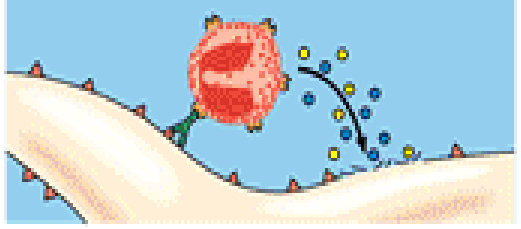
Disruption of cell by complement/ reactive protein attracts phagocytic and other defensive immune system cells

Neutralization

Blocks adhesion of bacteria and viruses to mucosa



Antibody-dependent cell-mediated cytotoxicity



Antibodies attached to target cell cause destruction by non-specific immune system cells

Functions

- Each immunoglobulin is bifunctional (except serum immunoglobulin D):
  - Recognize and bind antigen; then
  - Promote the killing and/or removal of the immune complex formed through activation of effector mechanisms (complement activation, phagocytosis)

Functions mediated by antibody alone

- Antibodies inactivate pathogens, parasites, toxins and enzymes.
  1. Pathogen neutralization
     - Blocking attachment to host cell surface
     - Prevent fusion between virion and cell membranes e.g. influenza virus

Functions

2. Neutralization of toxins and enzymes
   - Results from direct competition between Ab and the target molecule or substrate of toxin/enzyme
   - Induction of conformation incompatible with normal function of toxin/enzyme.
3. Immunity against helminth parasites (immunoglobulin E)

Functions mediated by antibody and additional molecules or cells

1. Complement activation
   - Classical pathway activators - IgM, IgG1,IgG3
   - Alternative pathway activators - IgA, IgG2
   - Functions of complement:
     - Opsonization, chemotaxis, lysis, vascular permeability etc

Functions

2. Receptors for Fc regions
   - Fc receptors are the specific molecules with which cells recognize antibodies

Functions

2. Receptors for Fc regions
   - Results that follow ligation of FcR by Ab-Ag complexes include:
     - Phagocytosis
     - Antibody-dependent cellular cytotoxicity
     - Release of inflammatory mediators
     - IgA- Ab facilitated antigen secretion

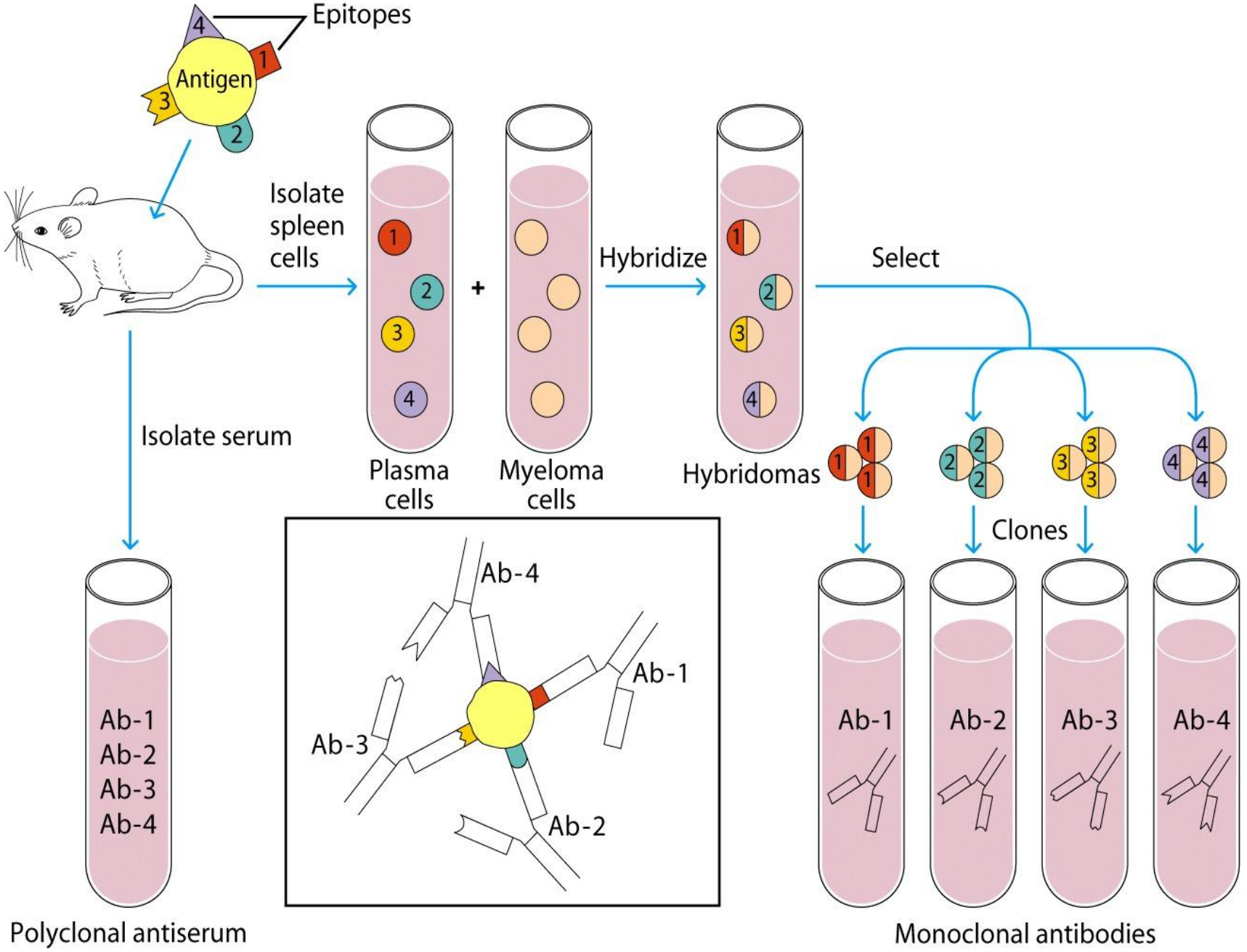
Functional properties of engineered antibody molecules

- **Monoclonal antibodies**

  Derived from a clonal population of B cells thus all the molecules express identical V domains and antigen specificities.
- Hybridomas are created by fusing normal lymphocytes from animals immunized with an antigen of choice, and transformed cells of B cell lineage.

Functional properties of engineered antibody molecules

- Multiple clones are generated
- Produces an antibody recognizing one epitope


Epitopes
Antigen
4
1
3
2
Isolate spleen cells
Plasma cells
Myeloma cells
Hybridize
Hybridomas
Select
Clones
Isolate serum
Ab-1
Ab-2
Ab-3
Ab-4
Polyclonal antiserum
Monoclonal antibodies

Applications of monoclonal antibodies

- Diagnostic tests
  - Abs are capable to detect tiny amounts (pg/mL) of molecules
  - Ex. Pregnancy hormones
- Diagnostic imaging
  - mAbs that recognize tumor antigens are radiolabeled with iodine I-131

Applications of monoclonal antibodies

- Therapy
  - Autoimmune diseases, lymphoma, breast cancer