



Antibodies (Immunoglobulin)

Objectives



- Immhugbuline structure and binding site/s
- Immunoglobiuline classes and their characteristics
- the role of Immuoglobulines in neutralization, opsonization, antibody-dependent cellular cytotoxicity (ADCC), complement and mucosal immunity
- Introduction to artificial antibodies including monoclonal and polyclonal antibodies

Introduction

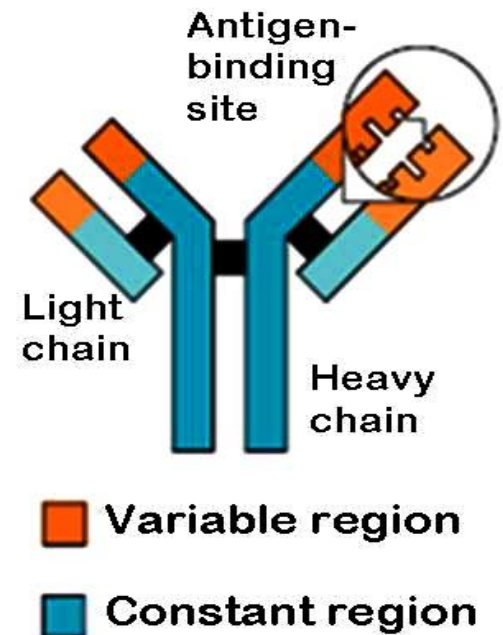


- Proteins that recognize and bind to a particular antigen with very high specificity.
- Belong to a group of serum proteins called immunoglobulins (Igs).
- Ab is produced by B cells in response to a stimulation of Ag.
- Ab possesses a high degree of specificity and affinity
- Each antibody has at least two identical sites that bind antigen: Antigen binding sites.

Antibodies Structure



- Immunoglobulins are glycoproteins made up of Four polypeptide chains (IgG):
 - Two light (L) polypeptide chains
 - Two heavy (H) polypeptide chains
- The four chains are linked by disulfide bonds





Variable (V) and Constant (C) Regions

- Each H-chain and each L-chain has V-region and C-region
 1. **V region:** Terminal portion of L-chain and terminal portion of H-chain compose antigen binding site and located within the “Fab” fragment of antibody. It shows wide variation in amino acid sequences
 2. **C-region:** lies in carboxyl or terminal portion of molecule. C-region shows an unvarying amino acid sequence and forms Fc fragment. It is responsible for biologic functions. H-chains are distinct for each of the five Immunoglobulins



• An antibody molecule is composed of two identical **Ig heavy chains (H)** and two identical **light chains (L)**, each with a **variable region (V)** & **constant region (C)**.

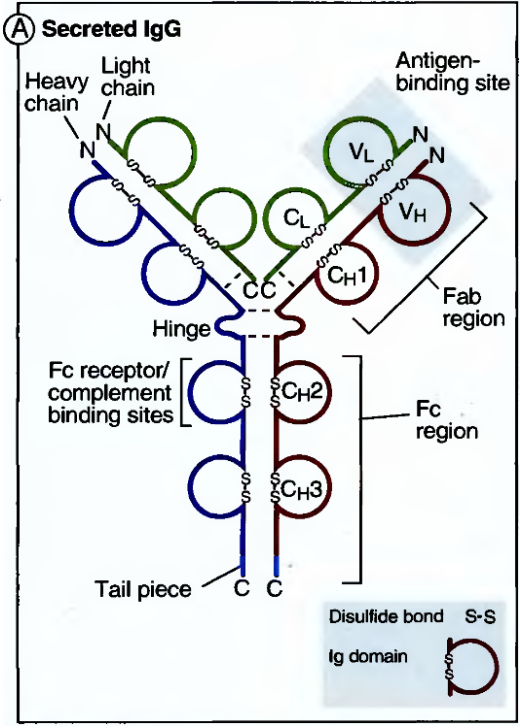
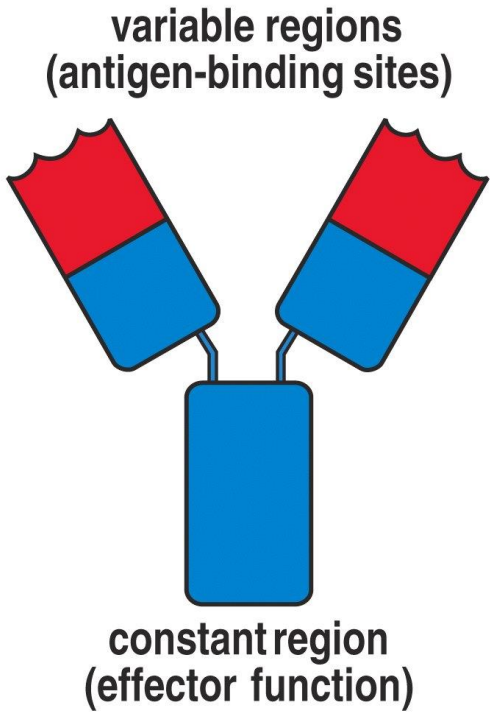
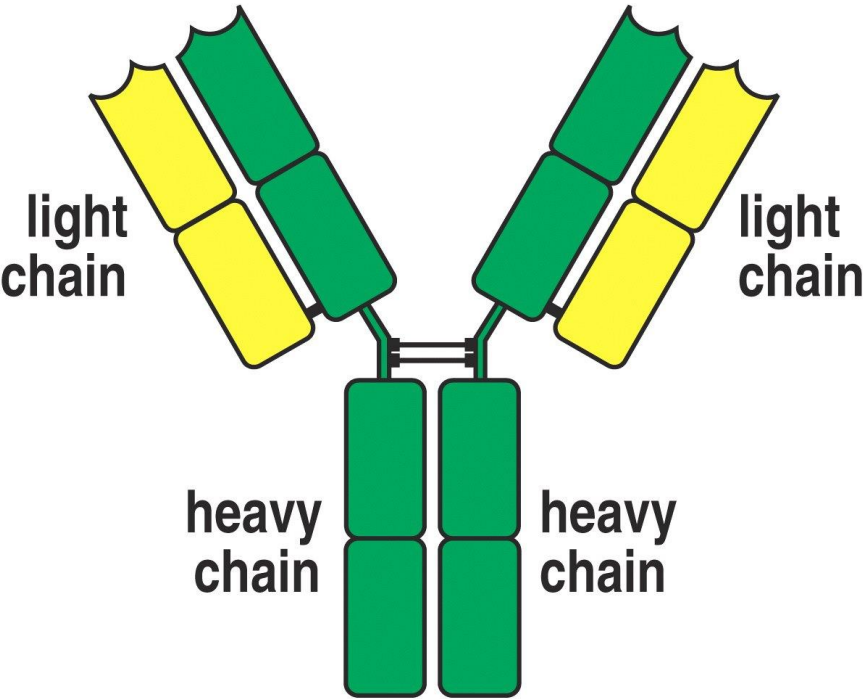


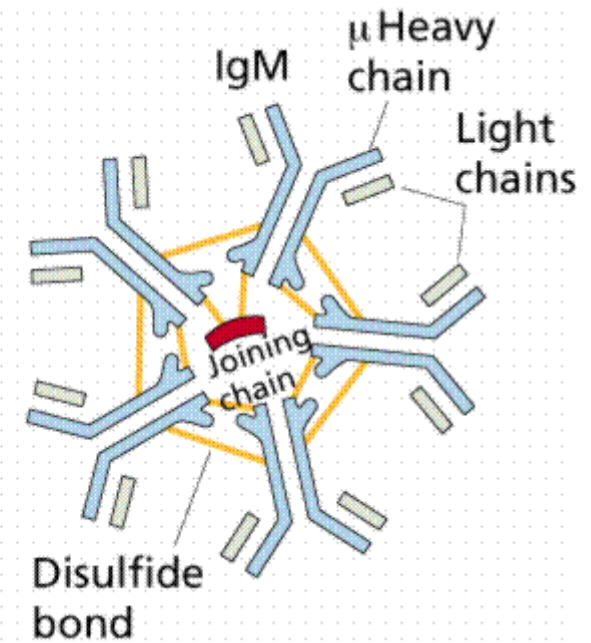
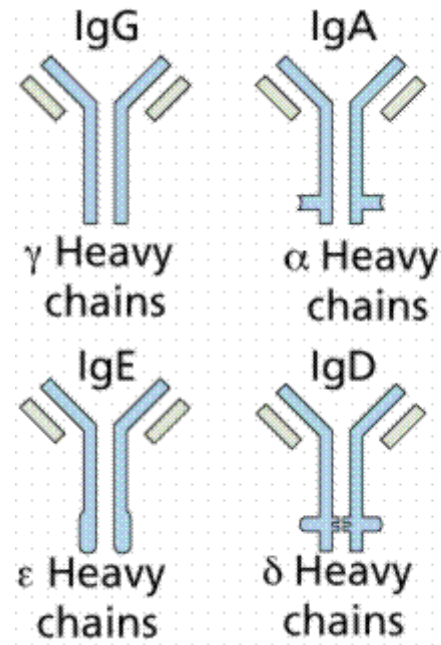
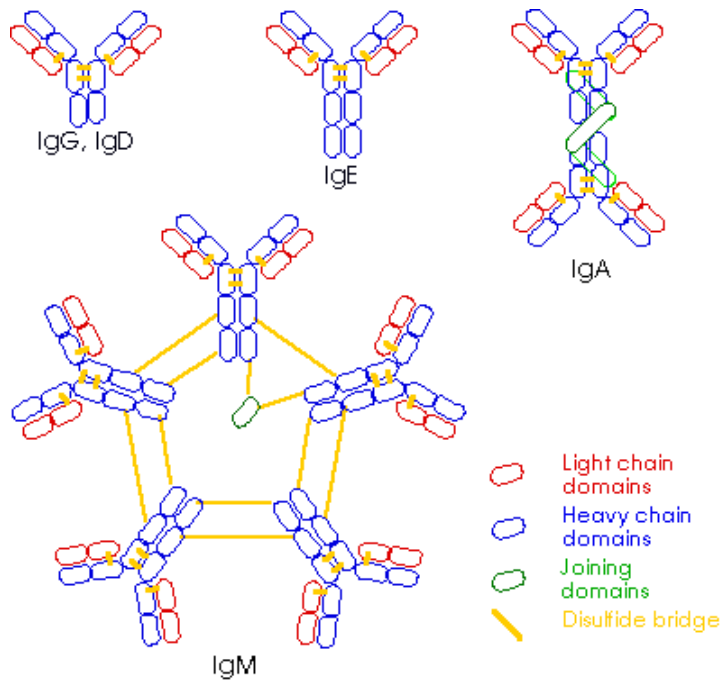
Figure 1-17 Immunobiology, 6/e. (© Garland Science 2005)

Figure 1-16 Immunobiology, 6/e. (© Garland Science 2005)

Antibodies Classes



- Five classes of Antibodies:
 1. IgG
 2. IgM
 3. IgA
 4. IgD
 5. IgE





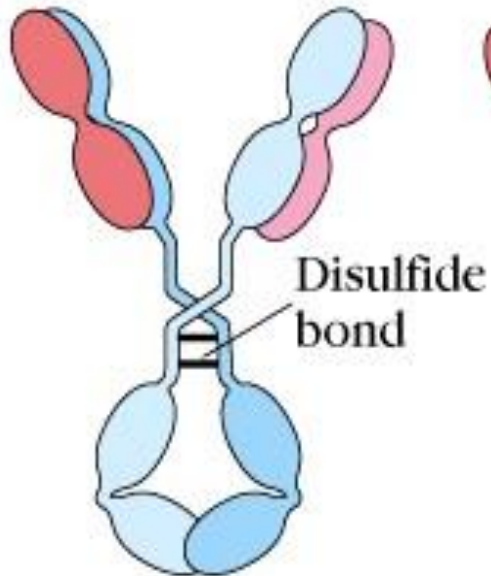
1. IgG

- Structure: Monomer
- Percentage serum antibodies: 80%
- Location: Blood, lymph, intestine
- Half-life in serum: 23 days
- Complement Fixation: Yes
- Placental Transfer: Yes
- Known Functions: Enhances phagocytosis, neutralizes toxins and viruses, protects fetus and newborn.

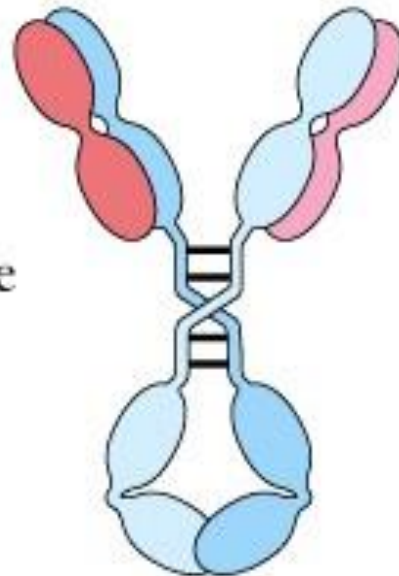
Four subclasses: IgG1, IgG2, IgG3, IgG4



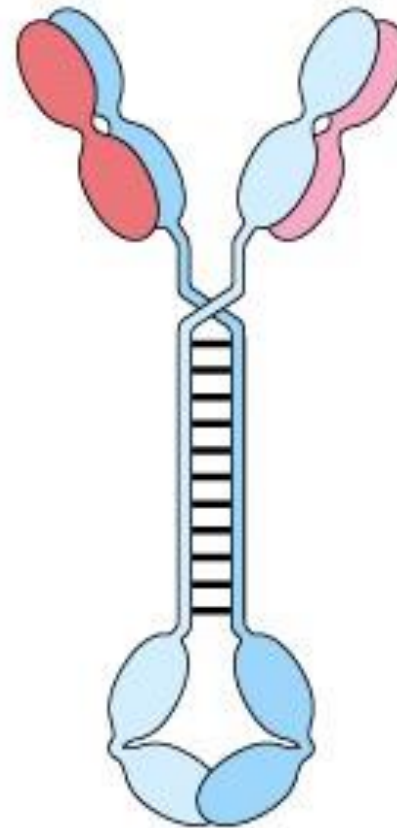
IgG1



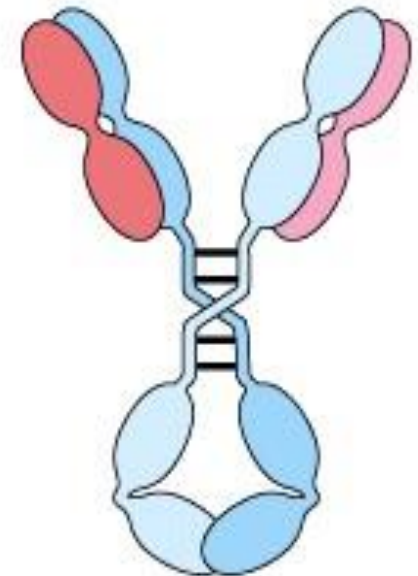
IgG2



IgG3



IgG4



2. IgM



- Structure: Pentamer
- Percentage serum antibodies: 5-10%
- Location: Blood, lymph, B cell surface (monomer)
- Half-life in serum: 5 days
- Complement Fixation: Yes
- Placental Transfer: No
- Known Functions: First antibodies produced during an infection. Effective against microbes and agglutinating antigens.



3. IgA

- Structure: Dimer
- Percentage serum antibodies: 10-15%
- Location: Secretions (tears, saliva, intestine, milk), blood and lymph.
- Half-life in serum: 6 days
- Complement Fixation: No
- Placental Transfer: No
- Two subclasses : IgA1, IgA2
- Known Functions: Localized protection of mucosal surfaces. Provides immunity to infant digestive tract.

4. IgD



- Structure: Monomer
- Percentage serum antibodies: 0.2%
- Location: B-cell surface, blood, and lymph
- Half-life in serum: 3 days
- Complement Fixation: No
- Placental Transfer: No
- Known Functions: In serum function is unknown.
On B cell surface, initiate immune response.

5. IgE



- Structure: Monomer
- Percentage serum antibodies: 0.002%
- Location: Bound to mast cells and basophils throughout body. Blood.
- Half-life in serum: 2 days
- Complement Fixation: No
- Placental Transfer: No
- Known Functions: Allergic reactions. Possibly lysis of worms.

Antibodies Functions

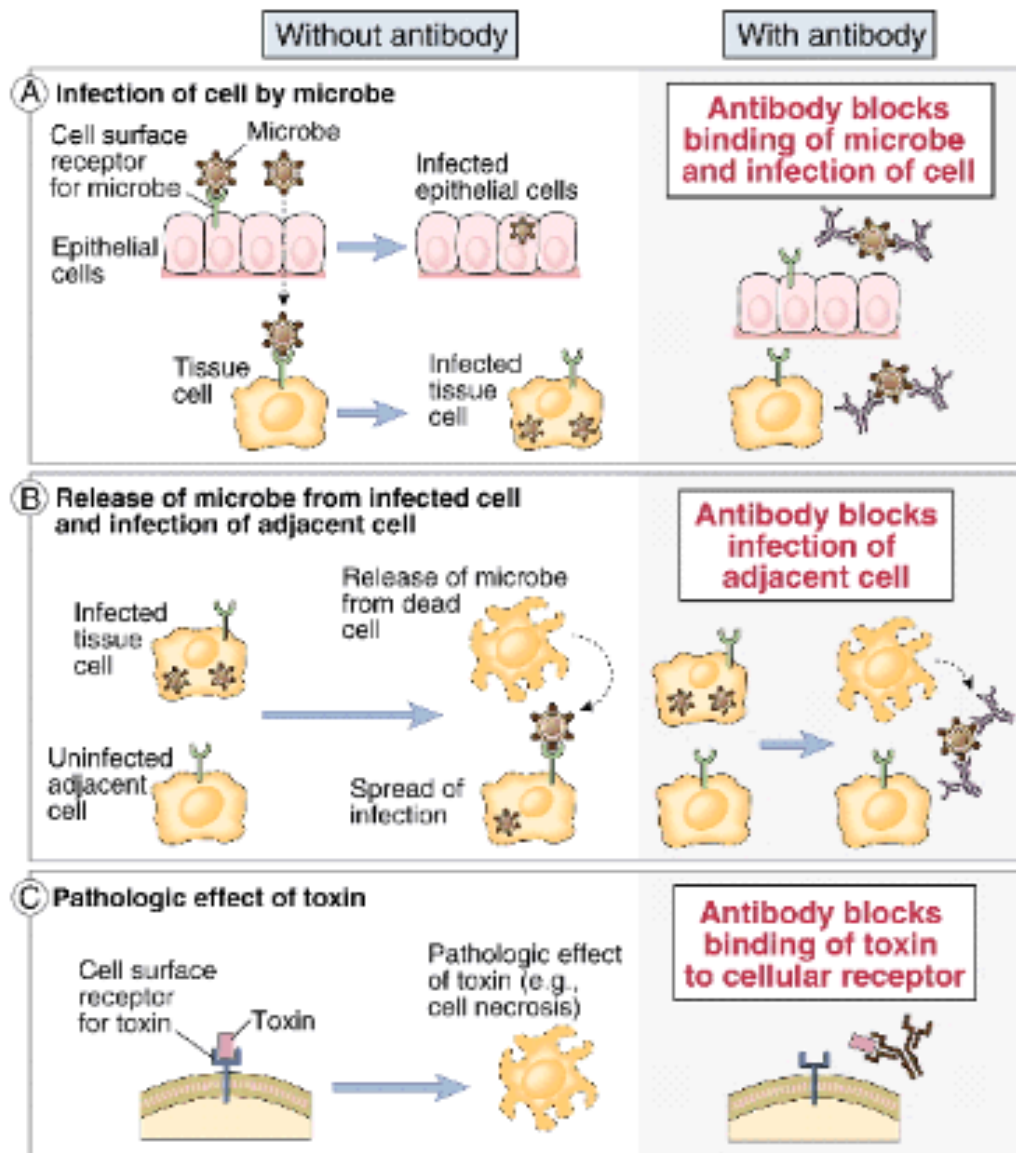


- Neutralization: Bind antigen- neutralize toxins, virus particles
- Opsonization
- Complement activation- IgG,M
- ADCC
- Mast cells activation
- Transcytosis- movement across epithelial cells



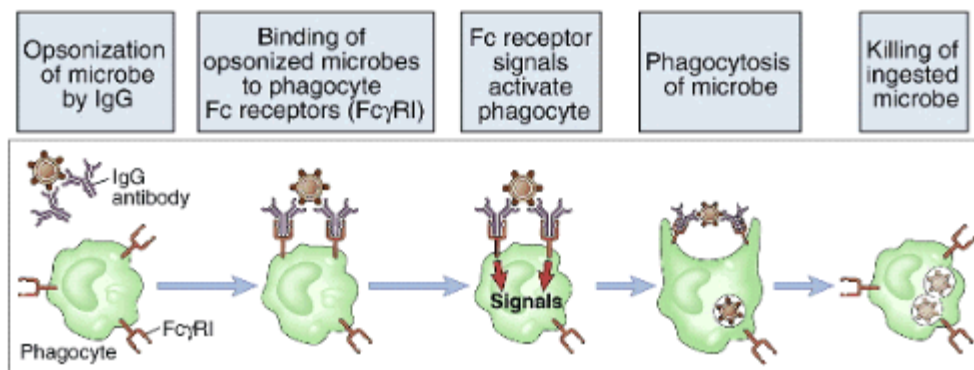
1. Neutralization

- The first step in a microbial infection involves attachment of the organism to the outside surface of the human body, either some part of the skin or the mucosal surfaces
- High-affinity antibodies that bind to the microbial ligand and prevent the microbe's attachment to human epithelium stop the infection before it starts
- Antibodies thus bind and inactivate foreign antigenic entities directly.



2. Opsonization

- Many bacteria are coated with polysaccharide
→ slippery and hard to endocytose
- But IgG can bind polysaccharide
- Macrophage can specifically bind IgG via Fc- γ receptors

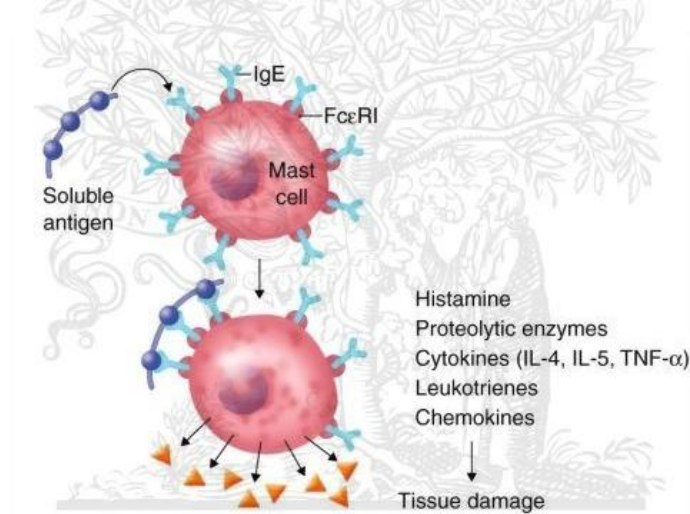




3. Compliment Activation

- Classical: IgM or 2 adjacent IgG's binds to C1Q on bacterial surface results in cascade that can cause bacterial lysis
- Alternative: antibody binding attracts C3B → phagocytosis and opsonization

4. Mast Cell Activation

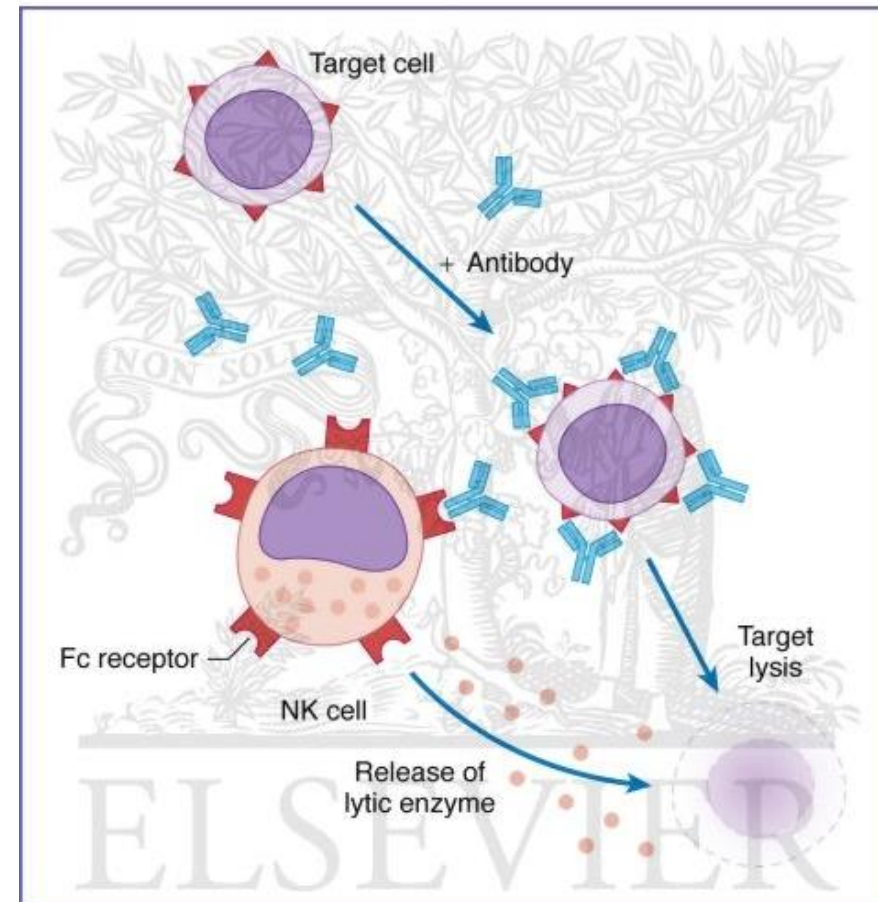


- IgE exists in serum at very low concentration (ng/ml)
- IgE binds to FC- ϵ receptors on Mast, Basophil, and Langerhan cells
- Antigen cross links bound antibodies \rightarrow degranualtion and release of histamine, heparin, proteases, chemotaxins which attracts WBC's
- This induce Phospholipase activity \rightarrow mucus production, sneezing and other allergic symptoms

5. ADCC: Antibody-Dependent Cell Mediated Cytotoxicity



- IgG binds target cell (virally infected or tumorigenic)
- FC- γ R on NK (non B, non T, natural killers) bind IgG
- Crosslinking of receptors \rightarrow perforin/protease release by NK



6. Transport/trancytosis



- Submucosal lymphoid follicles secretes IgA (trachea, for example)
- Epithelial M cells phagocytose/pinocytose foreign particles in lumen transport digested antigens into follicle and stimulate antibody production
- Placental transport: active transport of IgG across placenta give protection for baby up to six months

Artificial Antibodies

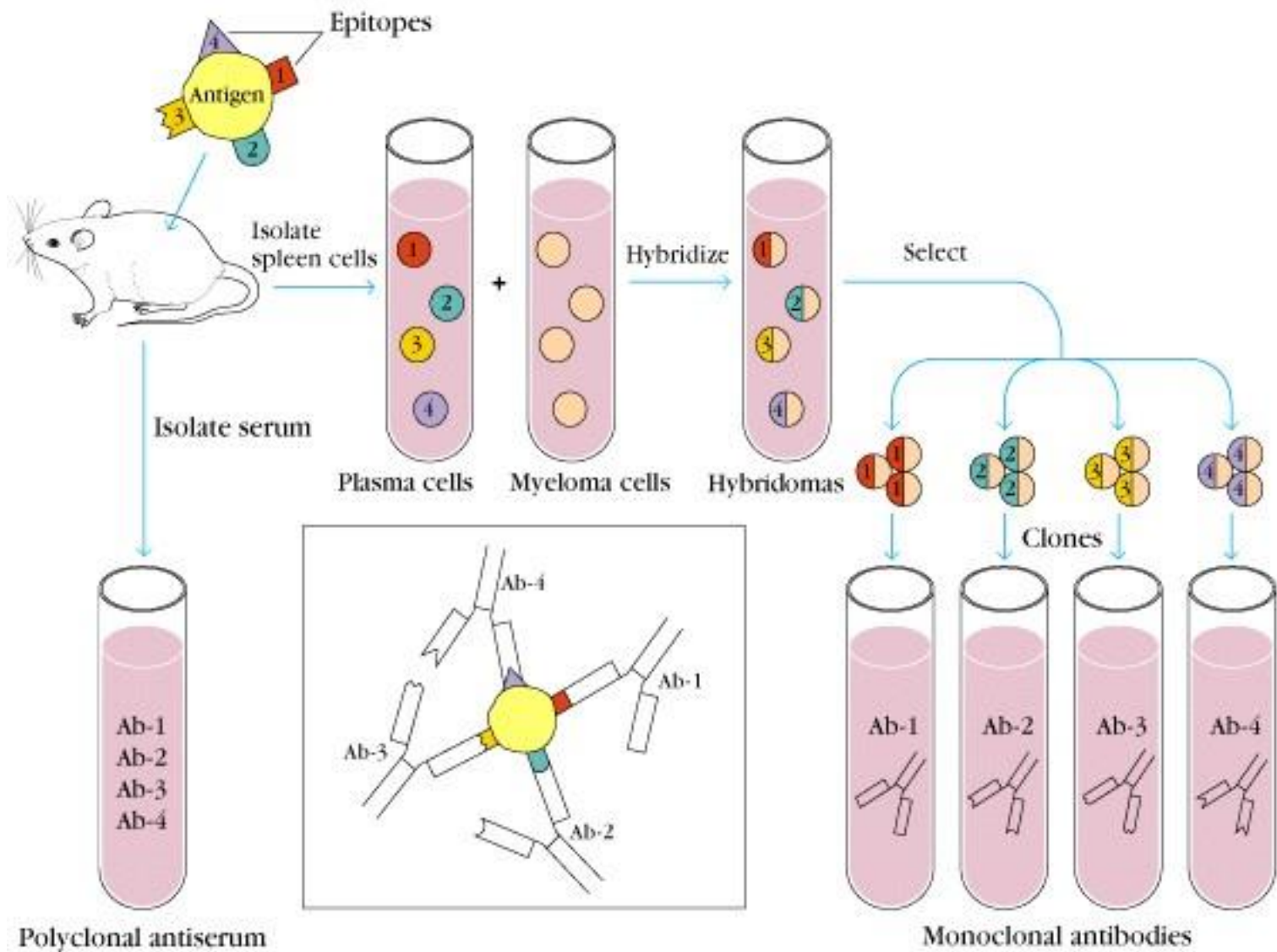


- Antibodies made artificially
- Two types:
 1. Polyclonal Ab:
 - A mixture Ab with different specificities and affinities
 - Generate in a natural response or artificial immunization
 2. Monoclonal Ab:
 - Ab produced by single clone (or one hybridomas clone) and having a single specificity



Monoclonal Ab Applications

- Diagnostic Tests
 - mAbs 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
- Immunotoxins
 - mAbs conjugated with toxins
- mAbs To Clear Pathogens
- mAbs for treatment (thrombotic diseases, cancer..)



Artificial antibodies

POLYCLONAL.

Derived from different B Lymphocytes cell lines

Batch to Batch variation affecting Ab reactivity & titre

NOT Powerful tools for clinical diagnostic tests

MONOCLONAL.

Derived from a single B cell clone

mAb offer Reproducible, Predictable & Potentially inexhaustible supply of Ab with exquisite specificity

Enable the development of secure immunoassay systems.