Antibodies (Immunoglobulin)

Mohammad Altamimi, MD, PhD
Jordan University
Faculty of Medicine

Objectives

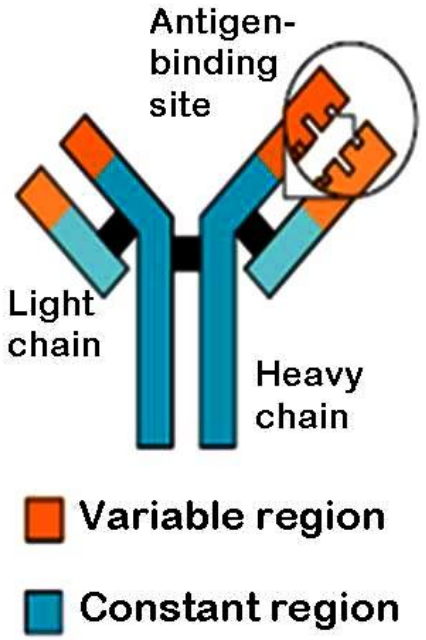
- Immhuglobuline structure and binding site/s
- Immunoglobiuline classes and their characteristics
- the role of Immuoglobulines in neutralization, opsonization, antibody-dependent cellular cytotoxicity (ADCC), complement and mucosal immunity
- Introduction to artificial antibodies including monoclonal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

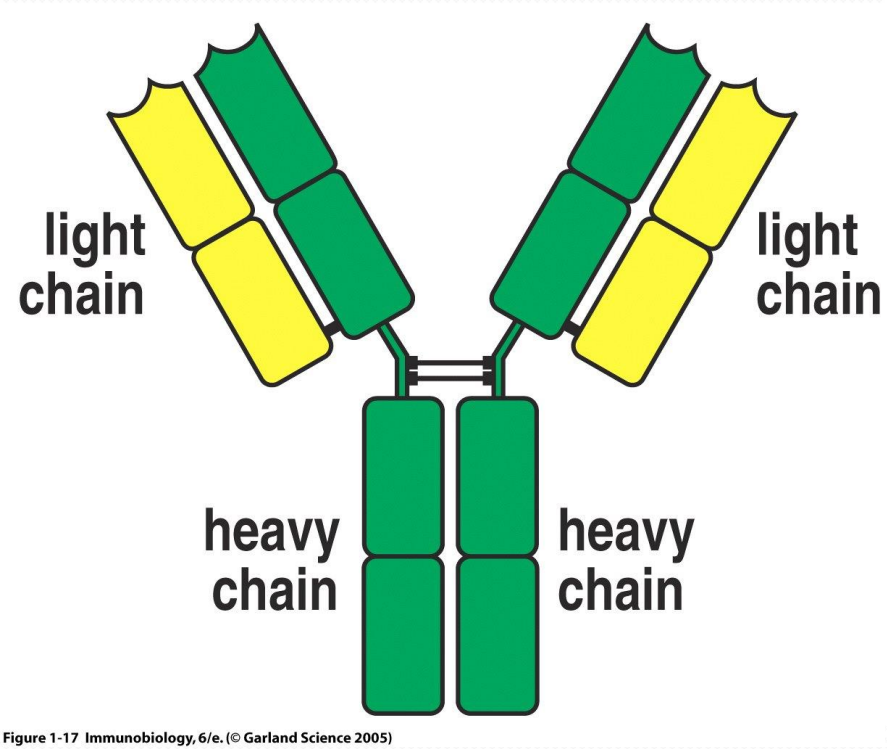
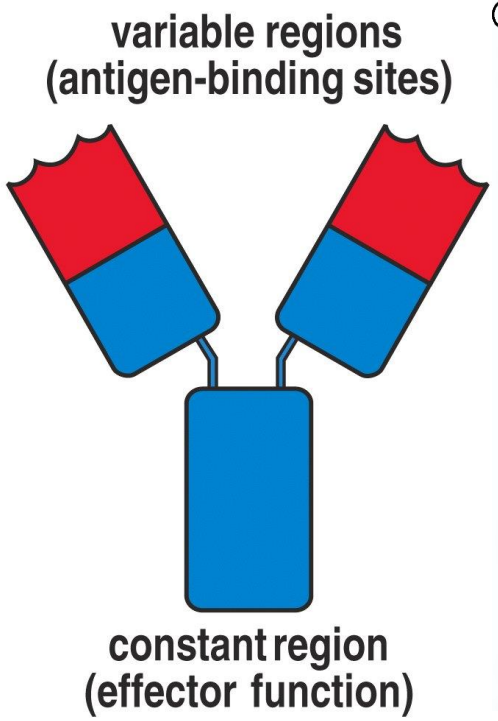


Figure 1-17 Immunobiology, 6/e. (© Garland Science 2005)



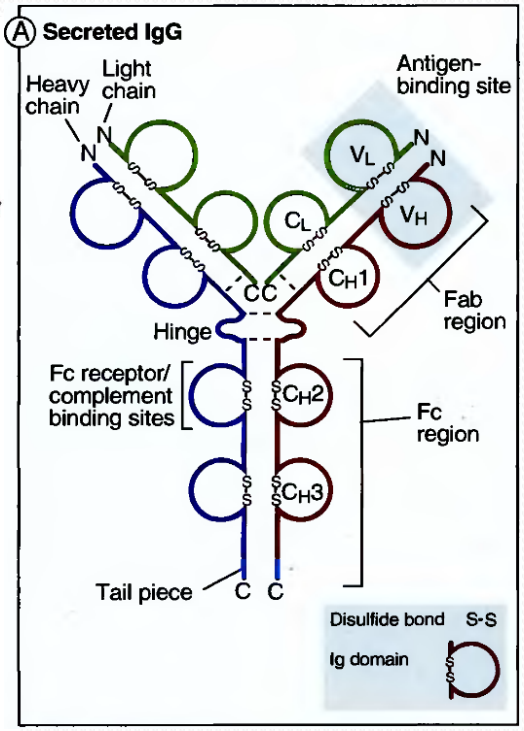
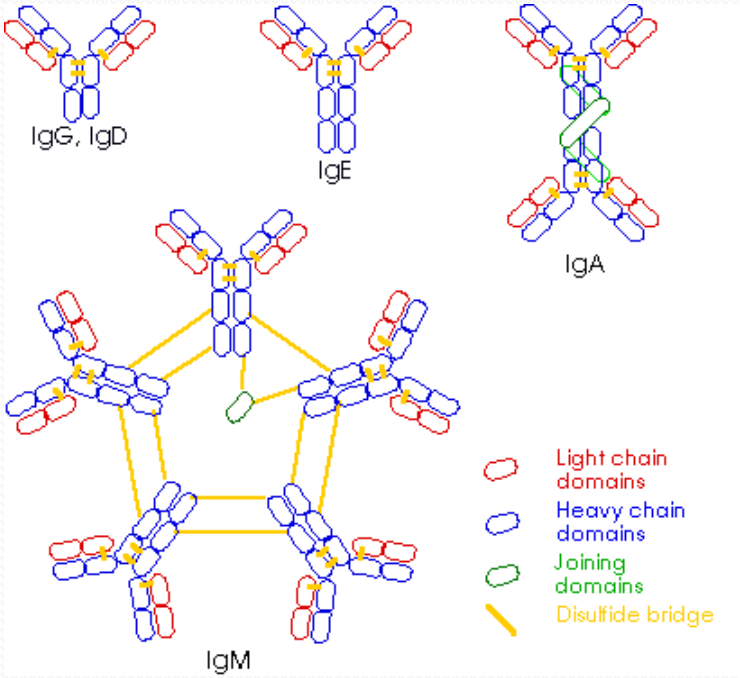
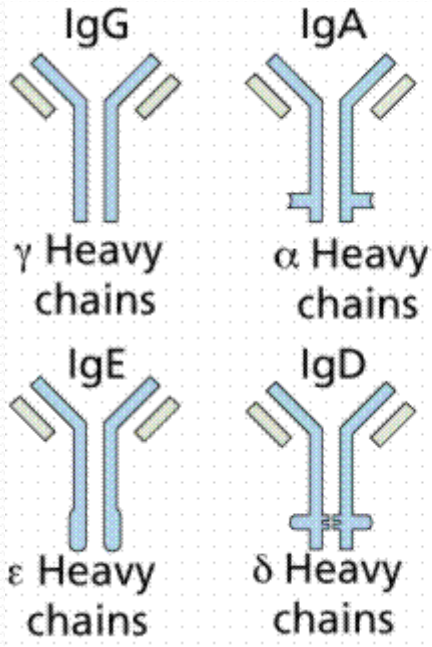


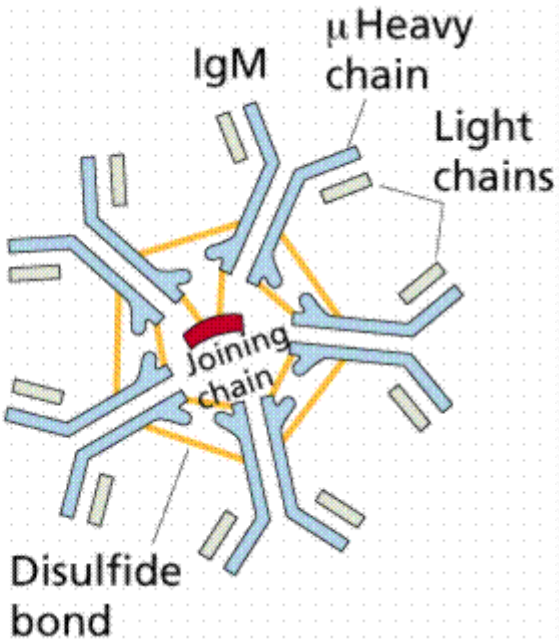
Figure 1-16 Immunobiology, 6/e. (© Garland Science 2005)

Antibodies Classes

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


IgG, IgD
IgE
IgA
IgM
Light chain domains
Heavy chain domains
Joining domains
Disulfide bridge

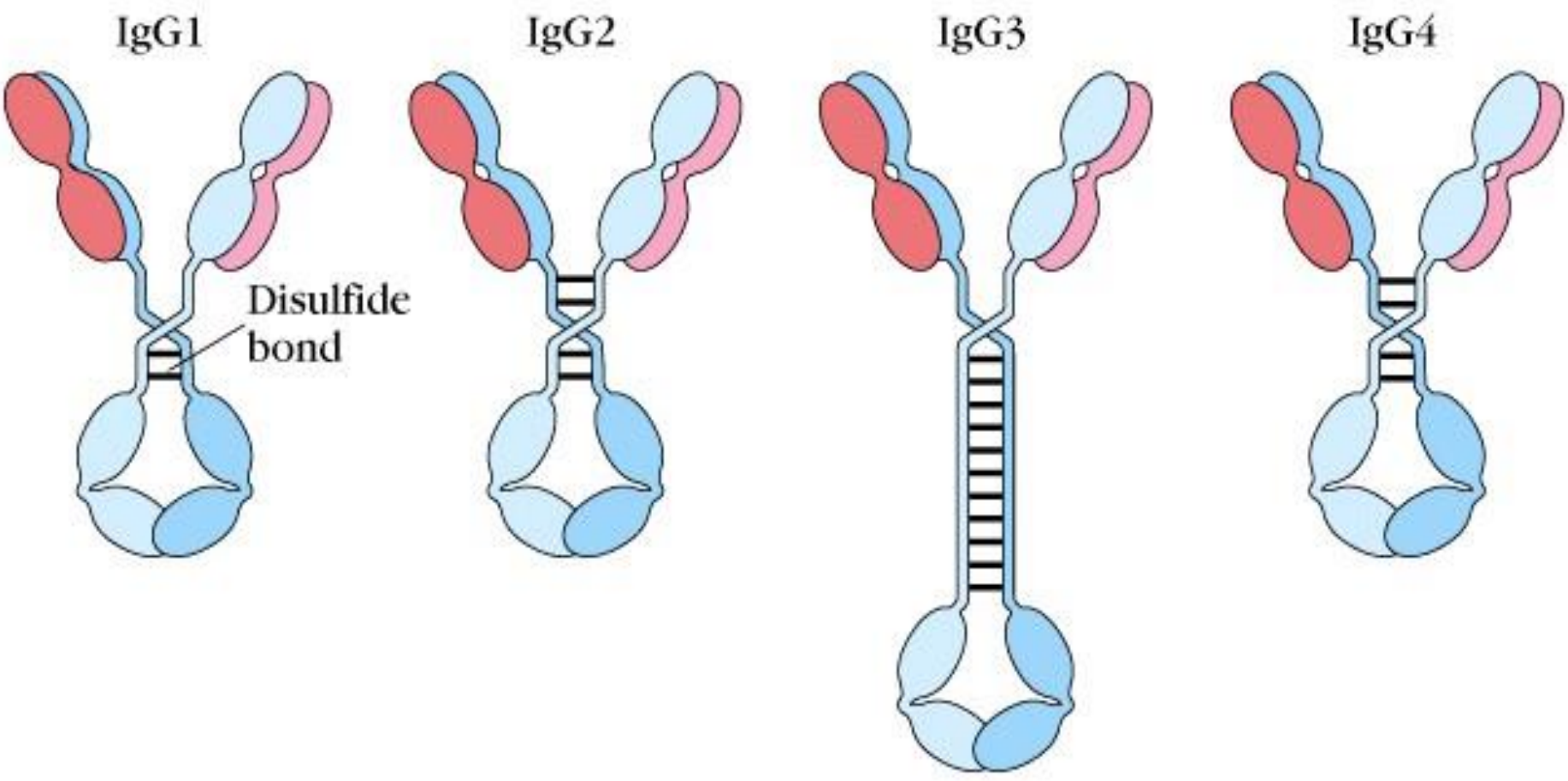

IgG
γ Heavy chains
IgA
α Heavy chains
IgE
ε Heavy chains
IgD
δ Heavy chains


IgM
μ Heavy chain
Light chains
Joining chain
Disulfide bond

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



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

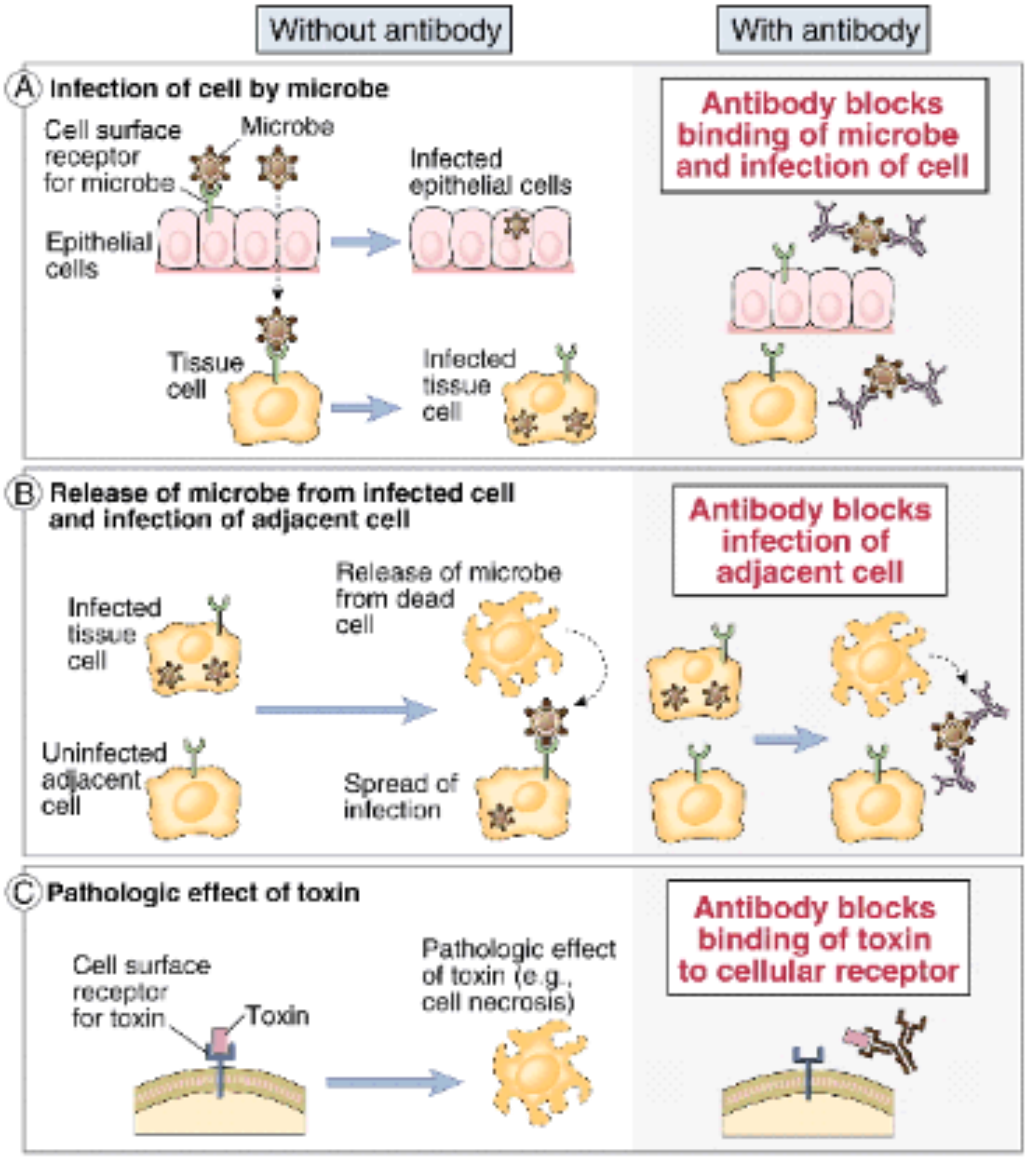
- Structure: Monomer
- Percentage serum antibodies: 0.002%
- Location: Bound to mast cells and basophils throughout body. Blood.
- Half-life in serum: 2 days
- Complement Fixation: No
- Placental Transfer: No
- Known Functions: Allergic reactions. Possibly lysis of worms.

Antibodies Functions

1. Neutralization: Bind antigen- neutralize toxins, virus particles
2. Opsonization
3. Complement activation- IgG,M
4. Antibody-Dependent Cell Mediated Cytotoxicity (ADCC)
5. Mast cells activation

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.



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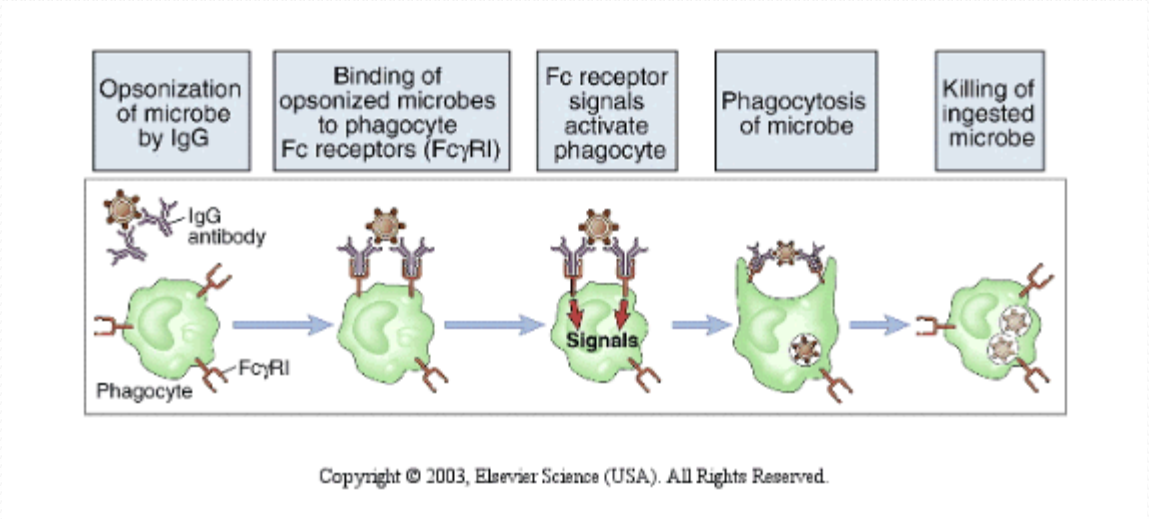
2. Opsonization

- 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



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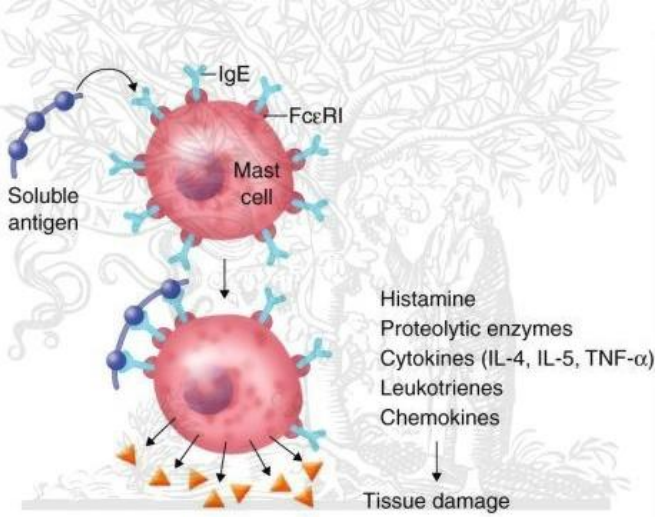
3. Compliment Activation

- 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

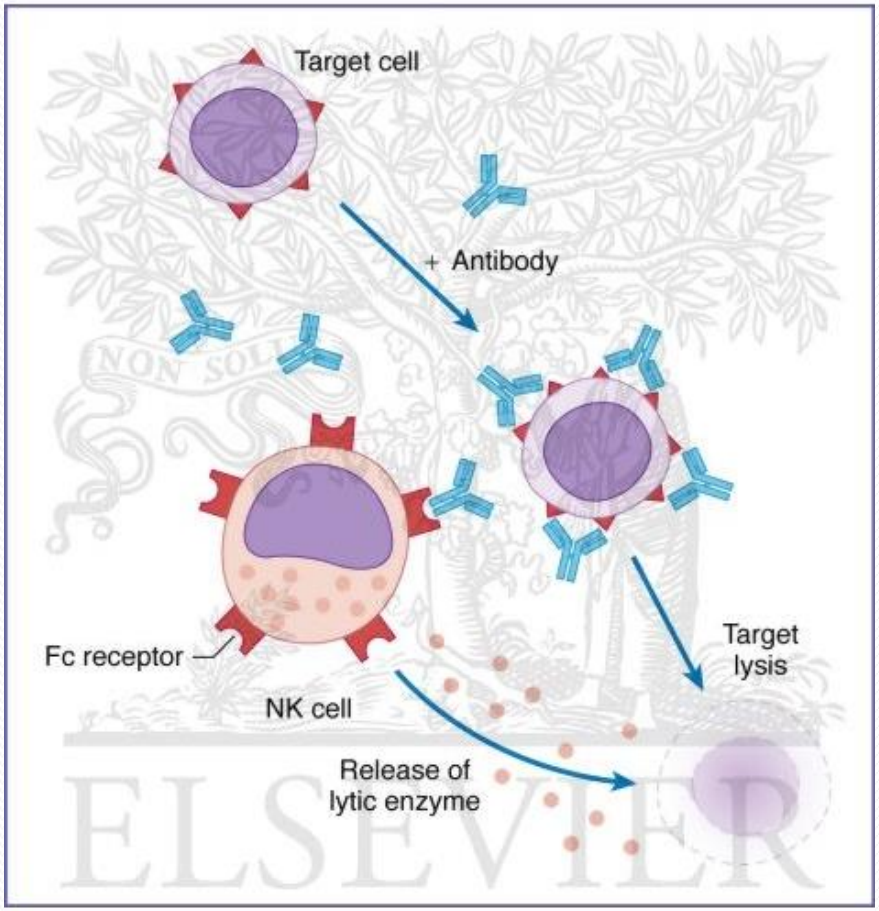
4. Mast Cell Activation



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

5. ADCC: Antibody-Dependent Cell Mediated Cytotoxicity

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



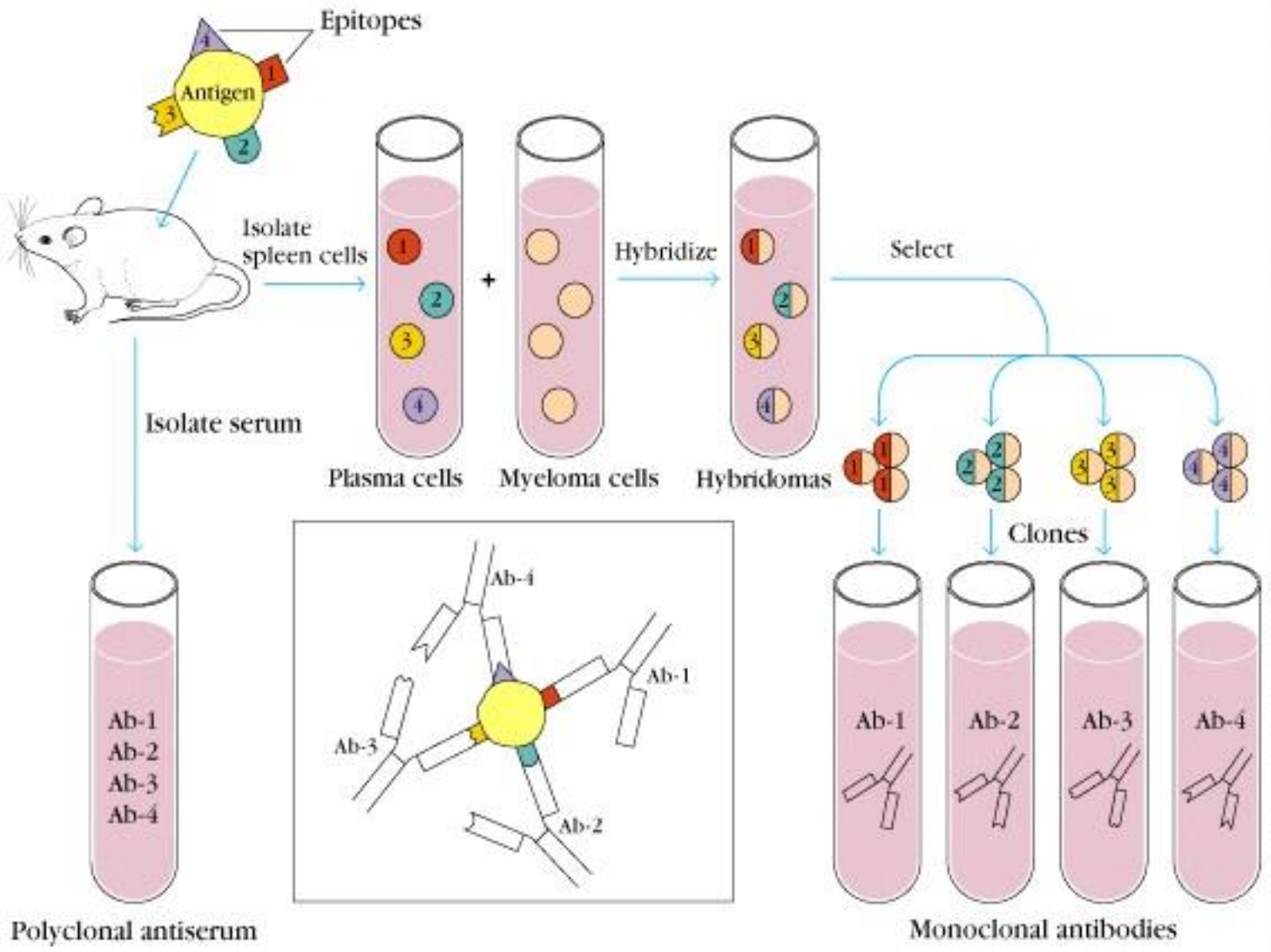
Artificial Antibodies

- Antibodies made artificially
- Tow 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 amouns (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..)


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

Artificial antibodies

POLYCLONAL.	MONOCLONAL.
Derived from different B Lymphocytes cell lines	Derived from a single B cell clone
Batch to Batch variation affecting Ab reactivity & titre	mAb offer Reproducible, Predictable & Potentially inexhaustible supply of Ab with exquisite specificity
NOT Powerful tools for clinical diagnostic tests	Enable the development of secure immunoassay systems.