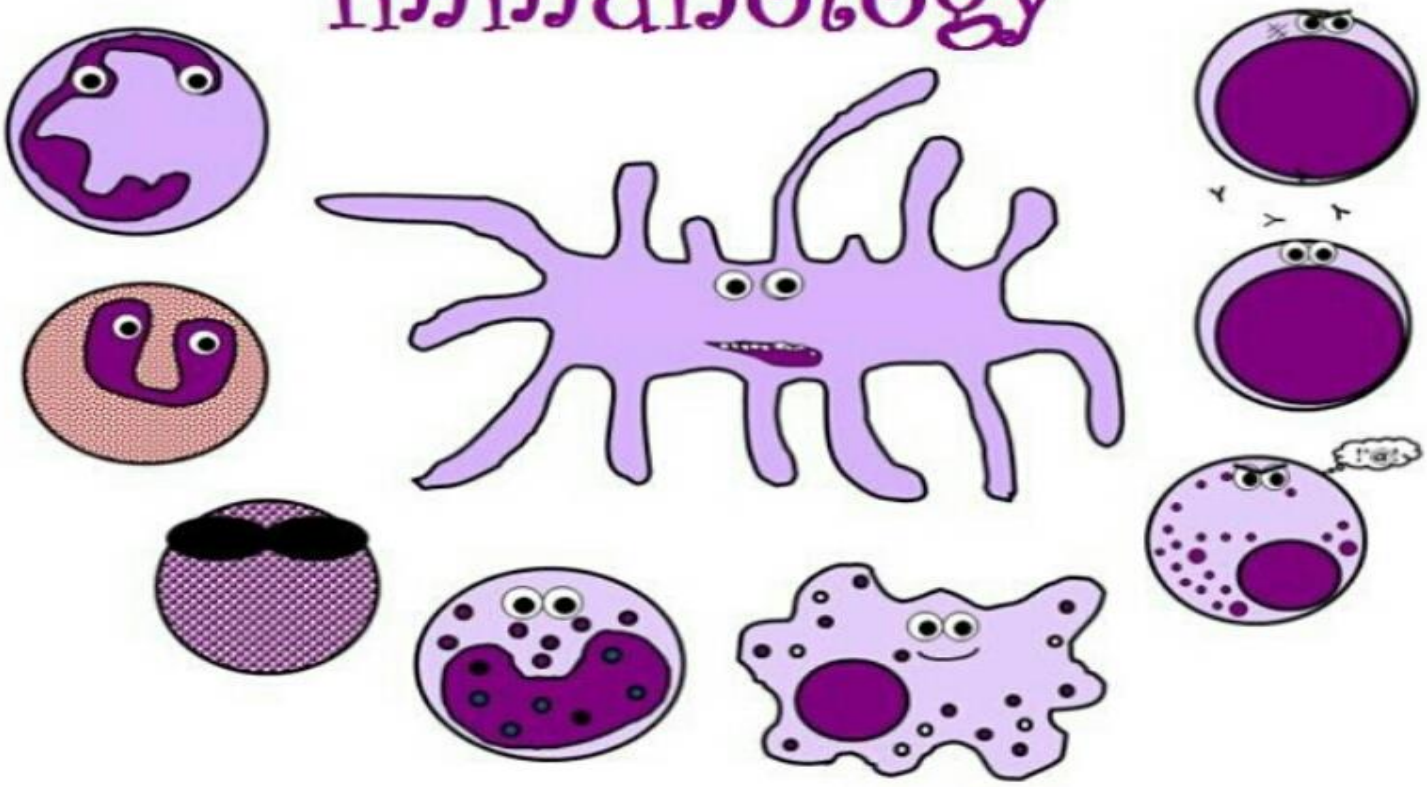
Immunology



Lecture: 5
Subject: antibodies
Edited by: Muhammad Dyaa Al-Shash

Antibodies (Immunoglobulin)

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General notes

- What is written in italic it is from doctor explanation during the lecture.
- Coloured statements what doctor emphasized.
- The symbol.  Means it is a new slide not the original

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

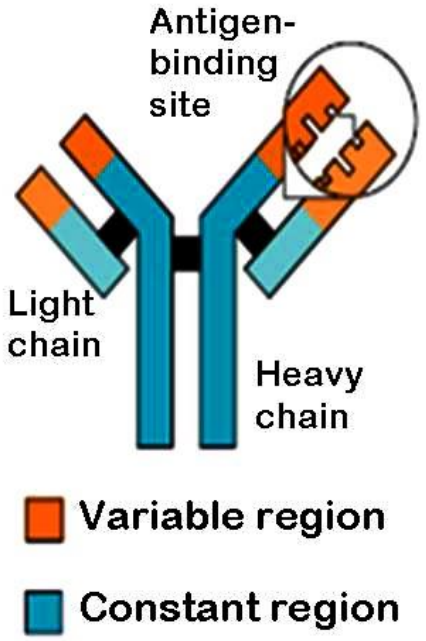
- Proteins that recognize and bind to a particular antigen with very high specificity.
- Belong to a group of serum proteins called immunoglobulins (Igs).
- Ab is produced by B cells in response to a stimulation of Ag.
- Ab possesses a high degree of specificity and affinity

Affinity means strength of binding

- Each antibody has at least two identical sites that bind antigen: Antigen binding sites.

At least 2 sites for all Abs classes , but can be more like pentamer IgM has 10 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

Each class of Abs has its own constant region

For ex. Fc of IgG differs from Fc of IgM and so on

The figure above represents an IgG with its 2 binding sites

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).**

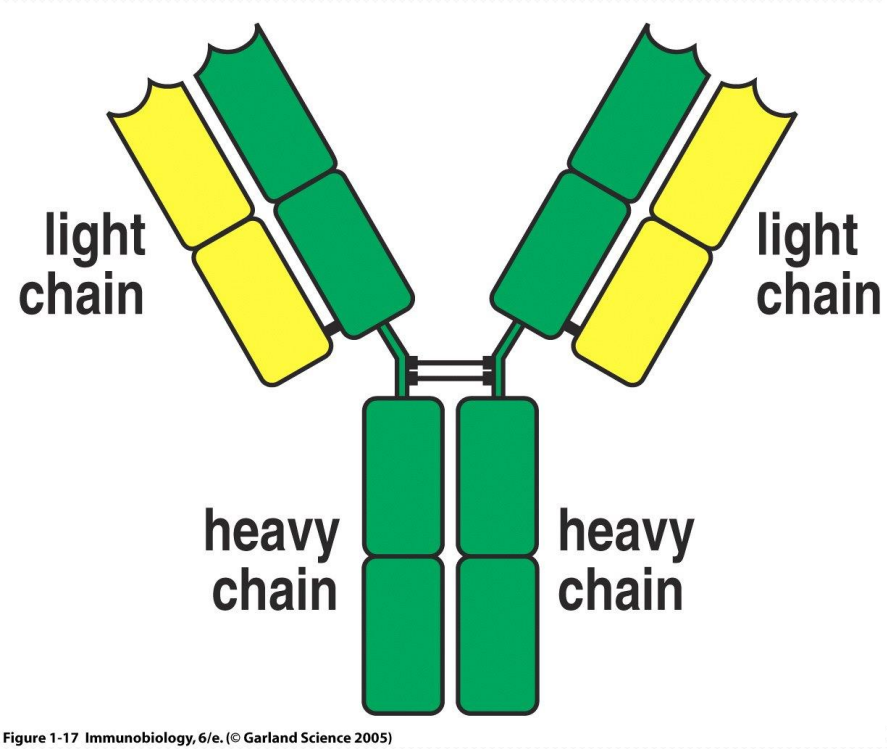
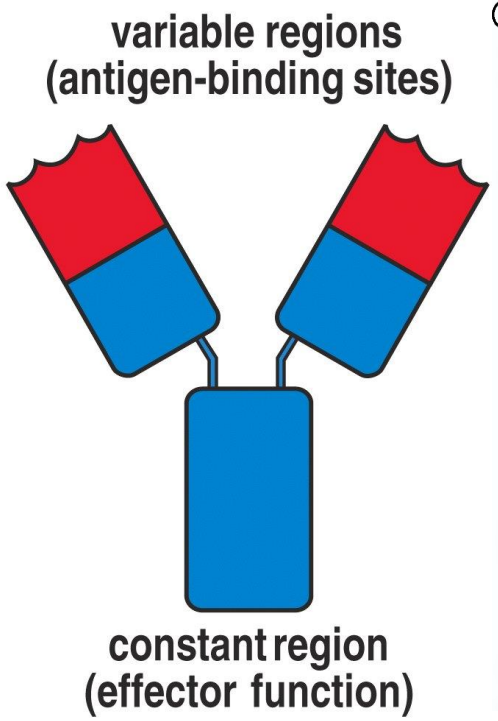


Figure 1-17 Immunobiology, 6/e. (© Garland Science 2005)



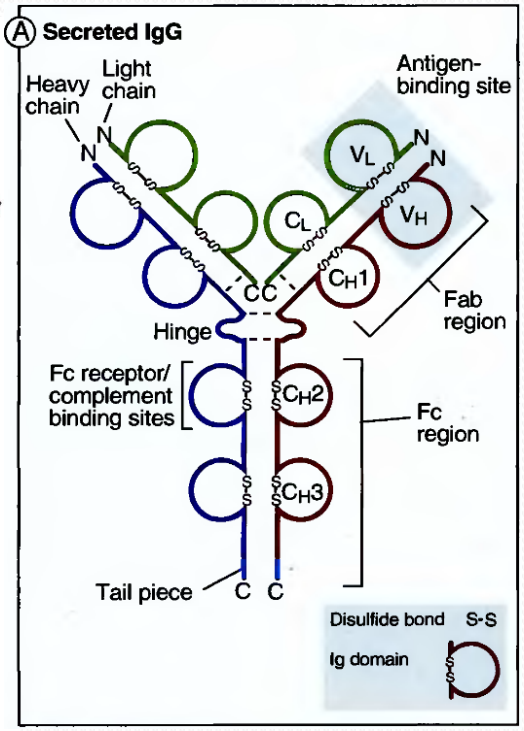


Figure 1-16 Immunobiology, 6/e. (© Garland Science 2005)

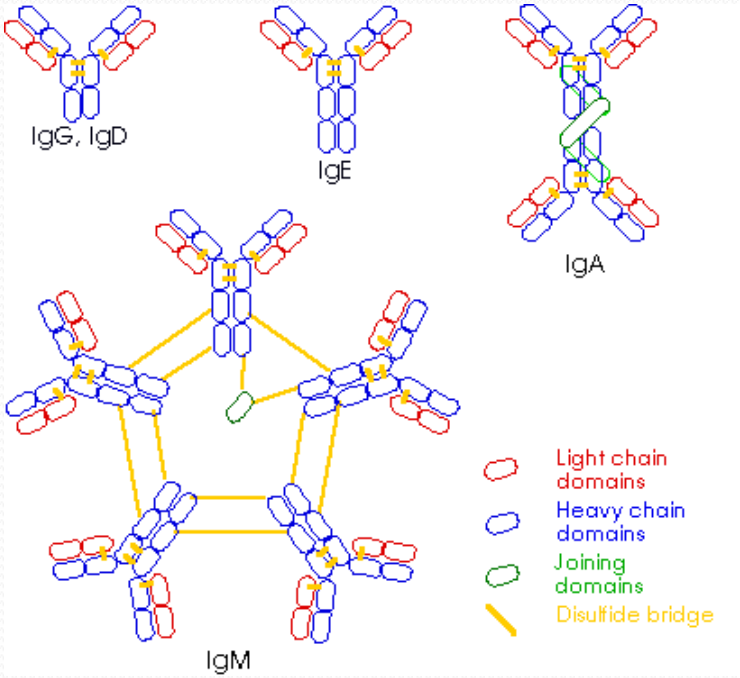
Noes

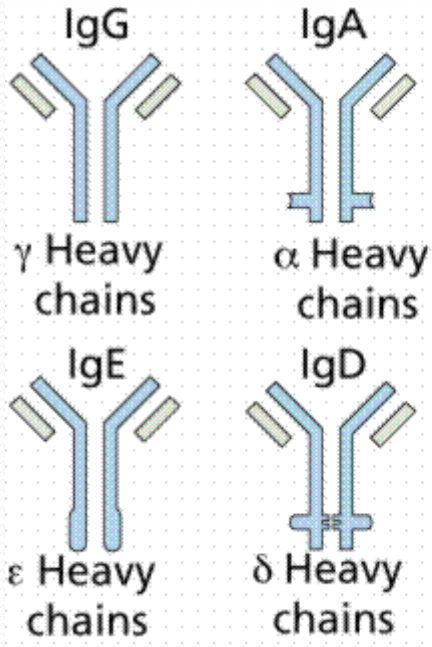
- *Th part of antigen which binds to the antibody is called epitope*

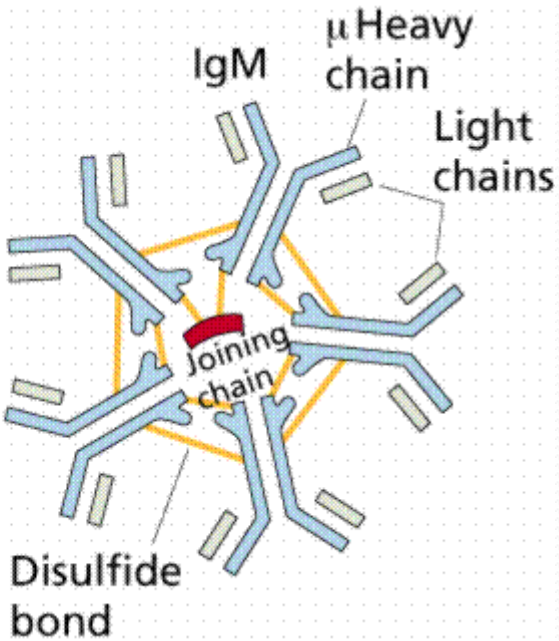
- *The part of antibody that binds to antigen is called antigen binding site , variable region , Fab portion or paratope*

Antibodies Classes

- Five classes of Antibodies:
  1. IgG *which is most common*
  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

Differences between Abs

- *Different structure.*
- *Different functions.*
- *Binding site , the Ab that has the highest number of binding sites is IgM then IgA and the least is IgG*
- *Fc portion.*

1. IgG

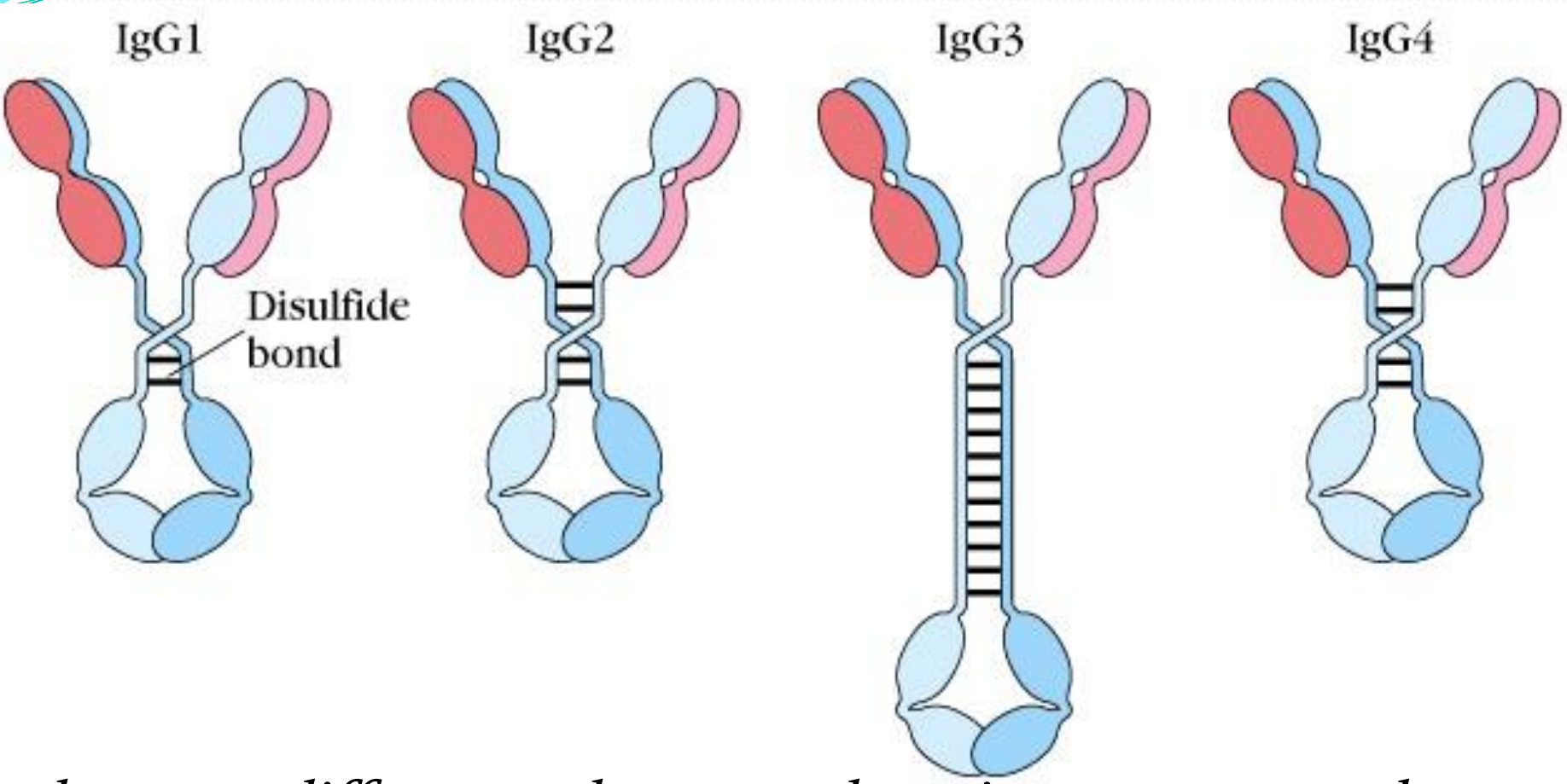
- Structure: Monomer
- Percentage serum antibodies: 80% *the highest one*
- Location: Blood, lymph, intestine
- Half-life in serum: 23 days
- Complement Fixation: Yes
- Placental Transfer: Yes
- Known Functions: Enhances phagocytosis, neutralizes toxins and viruses, protects fetus and newborn.

Notes

- *Rh disease occurs when IgGs of the pregnant crosses the placenta and bind rh+ rbcs of the fetus leading to hemolytic anemia.*

- *We don't fear about hemolysis caused by A or B antigens on rbcs because their Ab is IgM which cannot cross the placenta.*

Four subclasses: IgG1, IgG2, IgG3, IgG4



there are differences between them in structure and function but the main difference among them is the number and location of disulfide bonds

2. IgM

- Structure: Pentamer…*10 binding sites (2*5=10)*
- 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.

Complement fixation : bacterium(antigen) bound by Ab and then complement binds them leading to lysis

Notes

- *During infection the first Ab present is IgM then IgM begins to decrease meanwhile IgG increase.*
- *If we suspect a patient with hepatitis B how can we know if it is acute or chronic?*
- *If IgM is high and no IgG …acute*
- *If IgM is high and IgG starts to increase ….turns to be chronic*
- *If IgM is decreased and IgG is increased….chronic*
- *It is applied to all infectious diseases.*

Notes

- *If both IgM and IgG are negative ….no immunity, no previous infection.*
- *If only IgG positive …either the patient is infected or protected (by vaccination or immunity gained after a previos infection)*
- *Why IgM causes agglutination and IgG doesn't?*
- *Although IgG has 2 binding sites but the distance between these two sites is in nanometer and the size of rbcs is in micrometer and apply this fact on the pentamer IgM Although it has 10 binding sites but in fact it can binds two rbcs one at each side*

3. IgA

- Structure: Dimer…*4 binding sites*
- 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.
- *First Ab attacking antigens that eners via GI or Res. Sys*

4. IgD *resembles IgG in structure*

- 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. *And parasites*

The serum conc. is almost zero so any normal person should not have any IgE in his serum

Test yourself

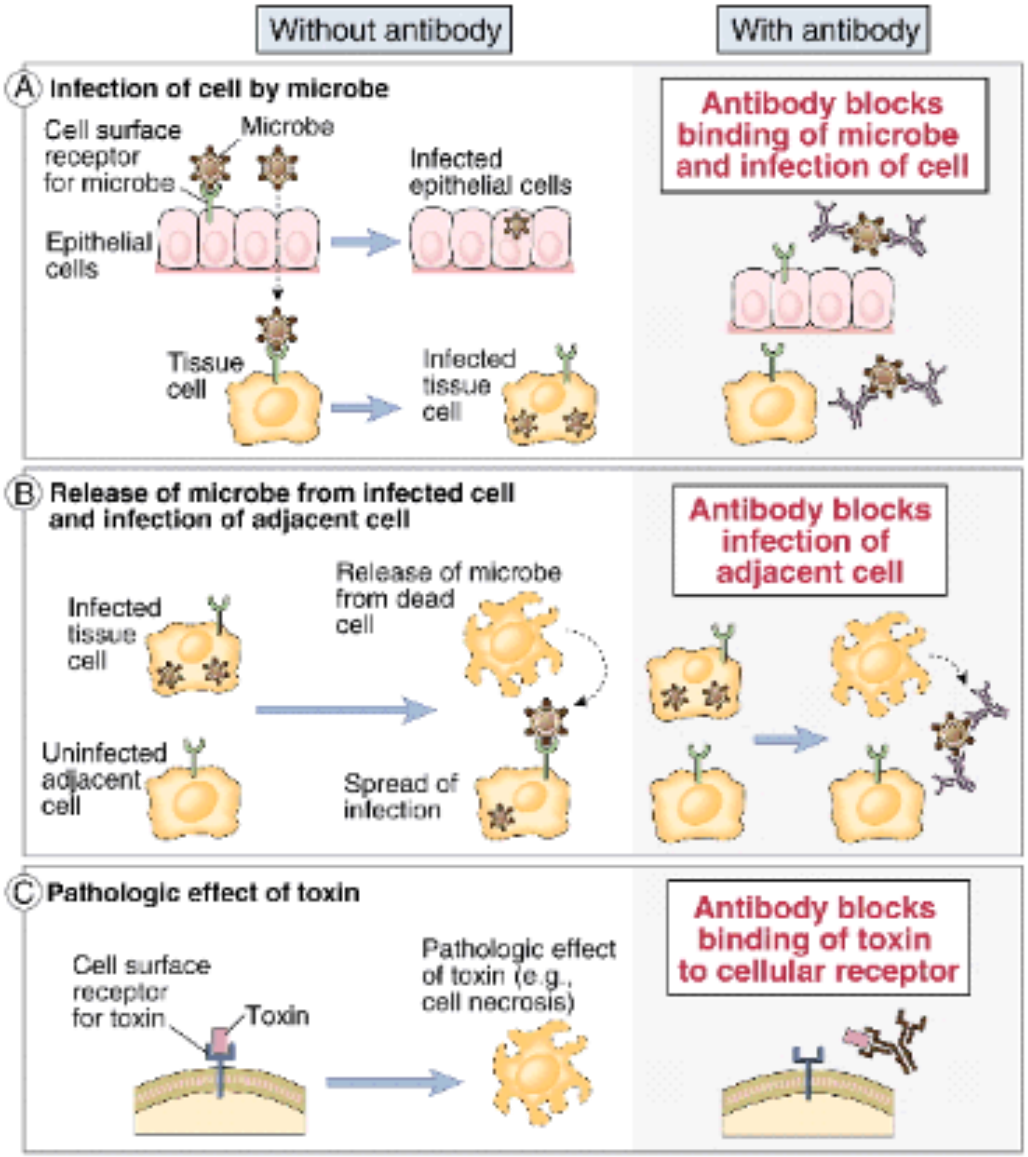
- *The lowest concentration of Abs is IgE*
- *The highest concentration of Abs is IgG*
- *The highest binding sites Ab is IgM*
- *The Ab that can cross placenta is IgG*
- *The Ab that has unknown function in serum is IgE*
- *The most neutralizing Ab is IgA (read the sildes next IgA is present in mucous)*
- *Abs that cause complement fixation are IgM and some IgG*

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

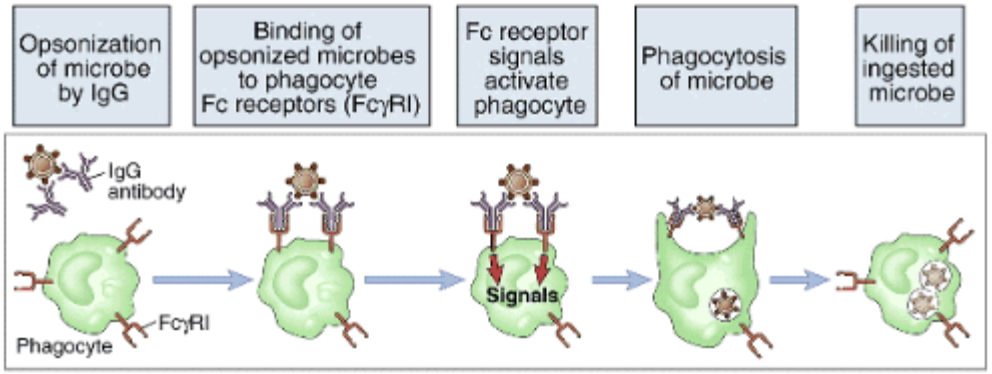
- The first step in a microbial infection involves attachment of the organism to the outside surface of the human body, either some part of the skin or the mucosal surfaces
- High-affinity antibodies that bind to the microbial ligand and prevent the microbe's attachment to human epithelium stop the infection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



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

*FC-**G**amma….Ig**G***



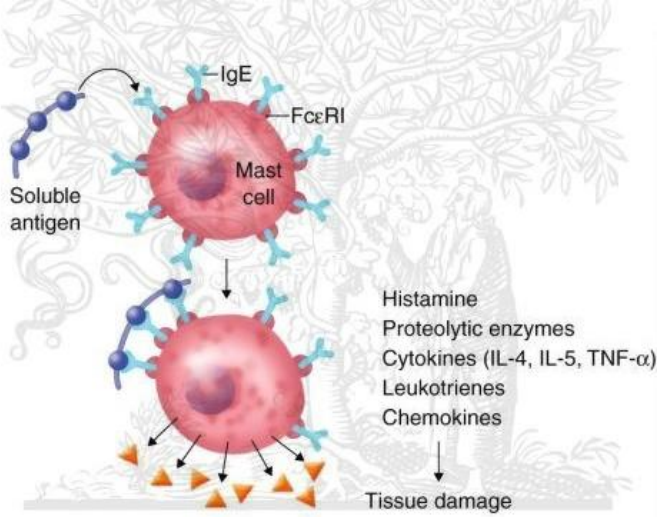
Notes

- *Some bacteria have capsules and the function of capsule is to protect the bacterium from phagocytosis by macrophages so IgG help macrophages to recognize microbes by labeling it..so the binding of macrophages to microbes in this case is not direct but via IgG bound to microbes*

3. Compliment Activation

- Classical: IgM or 2 adjacent IgG's binds to C1Q on bacterial surface results in cascade that can cause bacterial lysis …*Ab-mediated*
- Alternative: antibody binding attracts C3B → phagocytosis and opsonization..*without Abs*
- *Lectin pathway: here mannose has a role*

4. Mast Cell Activation

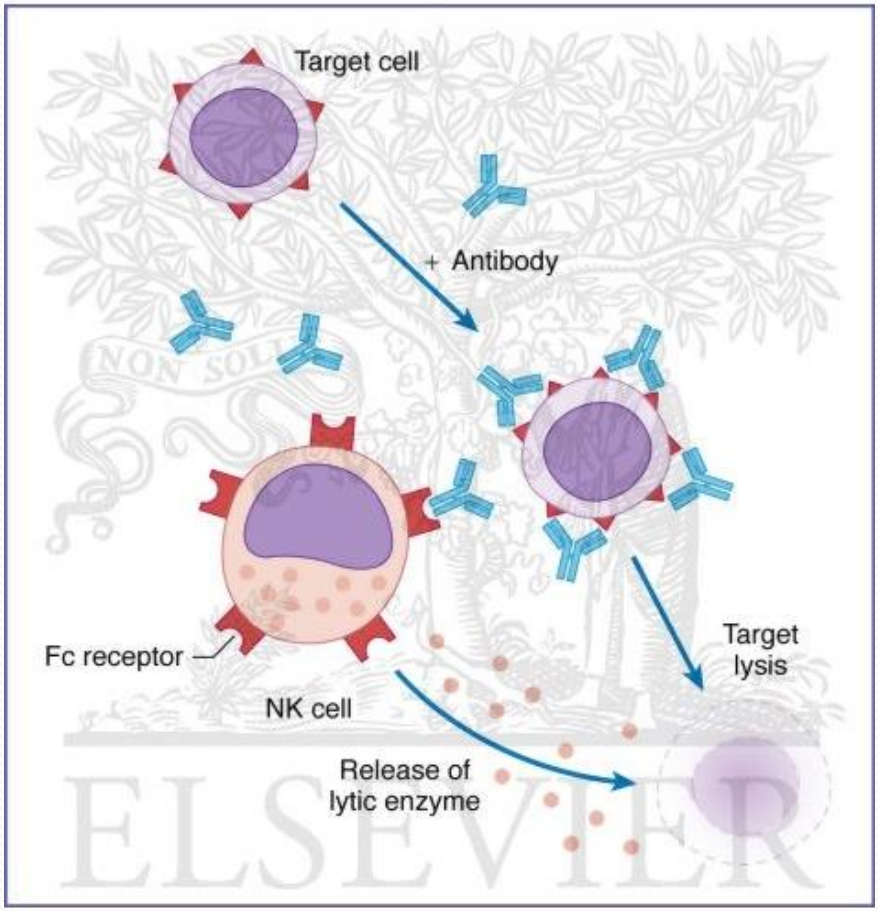


- IgE exists in serum at very low concentration (ng/ml)
- IgE binds to FC-ε recptors on Mast, Basophil, and Langerhan cells
- Antigen cross links bound antibodies → degranualtion and release of histamine, heparin, proteases, chemotaxins which attracts WBC's
- This induce Phospholipase activity → mucus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

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

Resembles opsonization but here the role is for NK



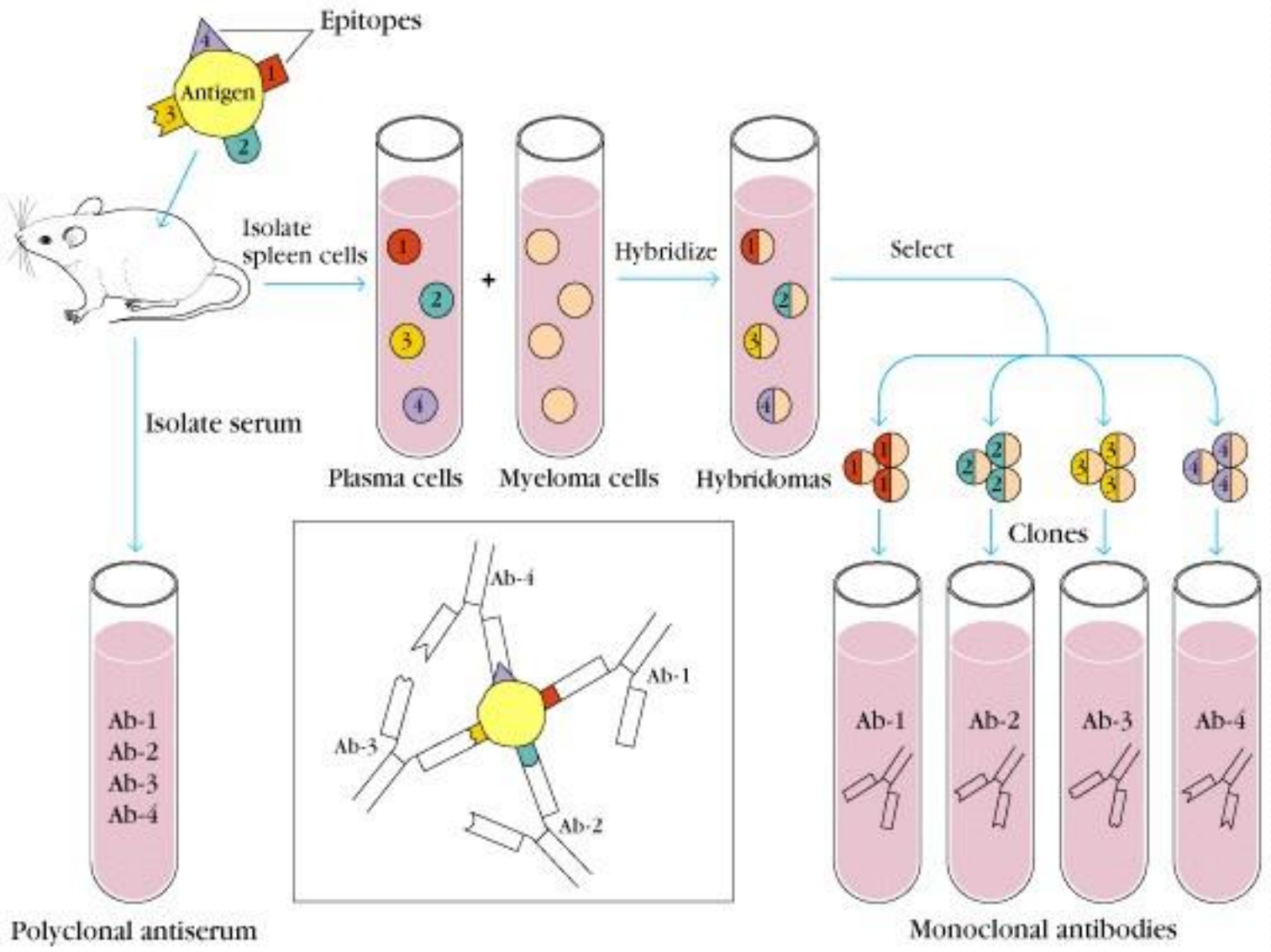
Artificial Antibodies

- 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.

Better for diagnosis because it's more specific

Higher affinity

Which is better to give passive Abs or to let the body deal with the infection and activate B and T cells and synthesize Abs ?

Surely , to let our immunity deal with infection in order to keep memory cells