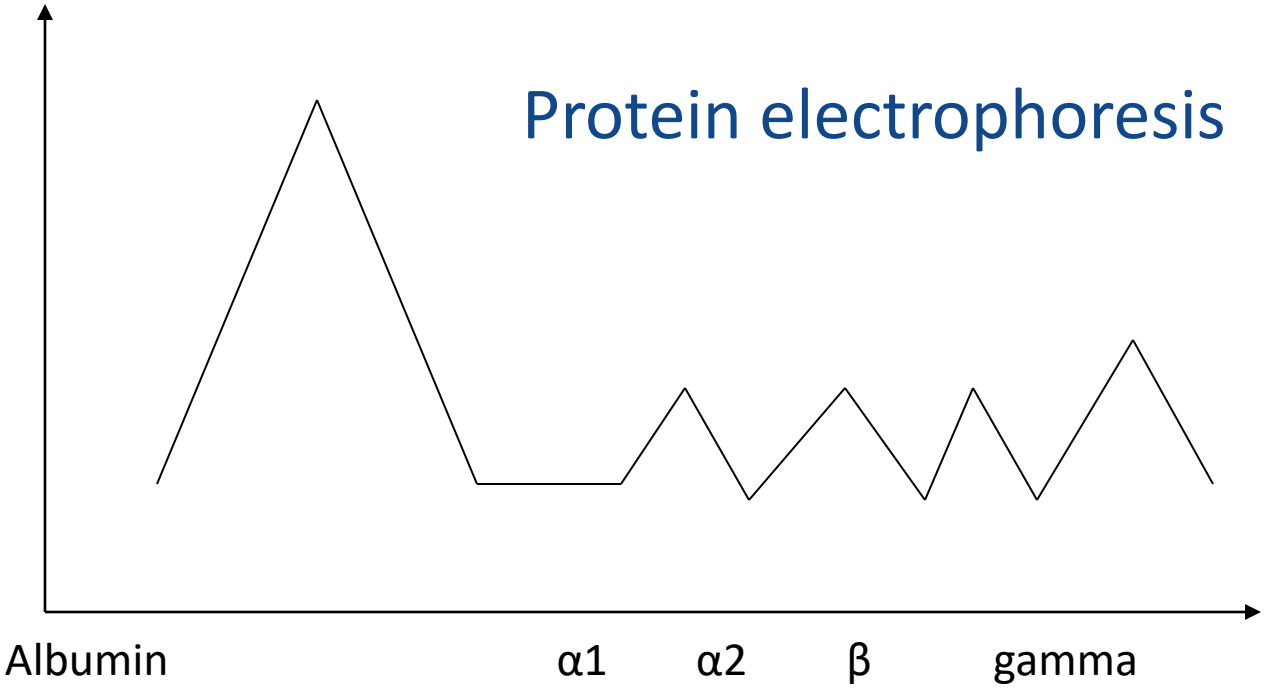
بسم الله الرحمن الرحيم

Objectives
IMMUNOGLOBULIN
ANTIBODY (Ab)
Structure
Functions
Immune response
complement

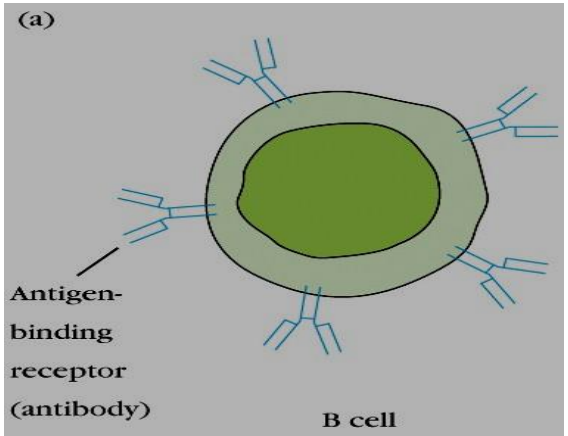
Blood from an individual and put it in a plain tube without anticoagulant and left it for half an a hour.
Blood will coagulate and you will get serum.

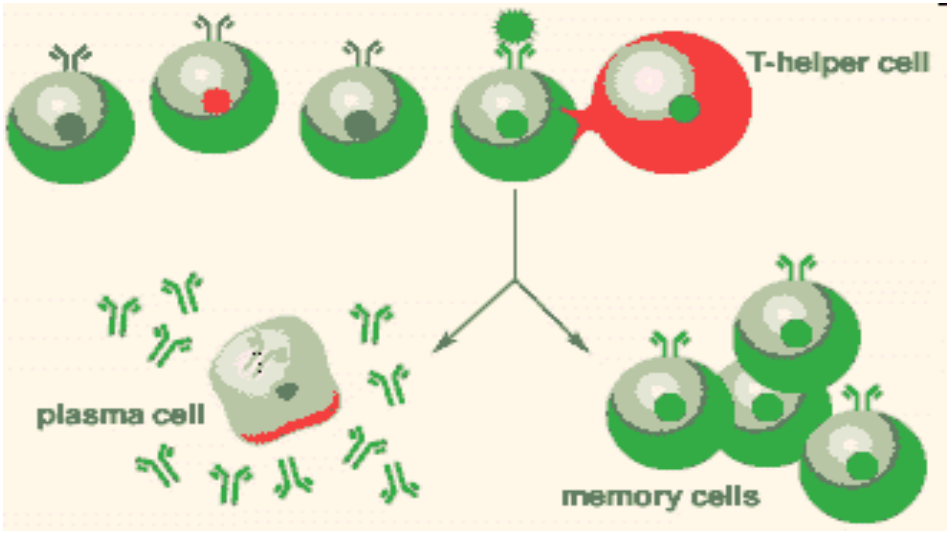


Gamma globulin fraction of protein has antibody activity

Antibodies are made by B-Cells armed on its surface and act as a surface molecule bind an antigen

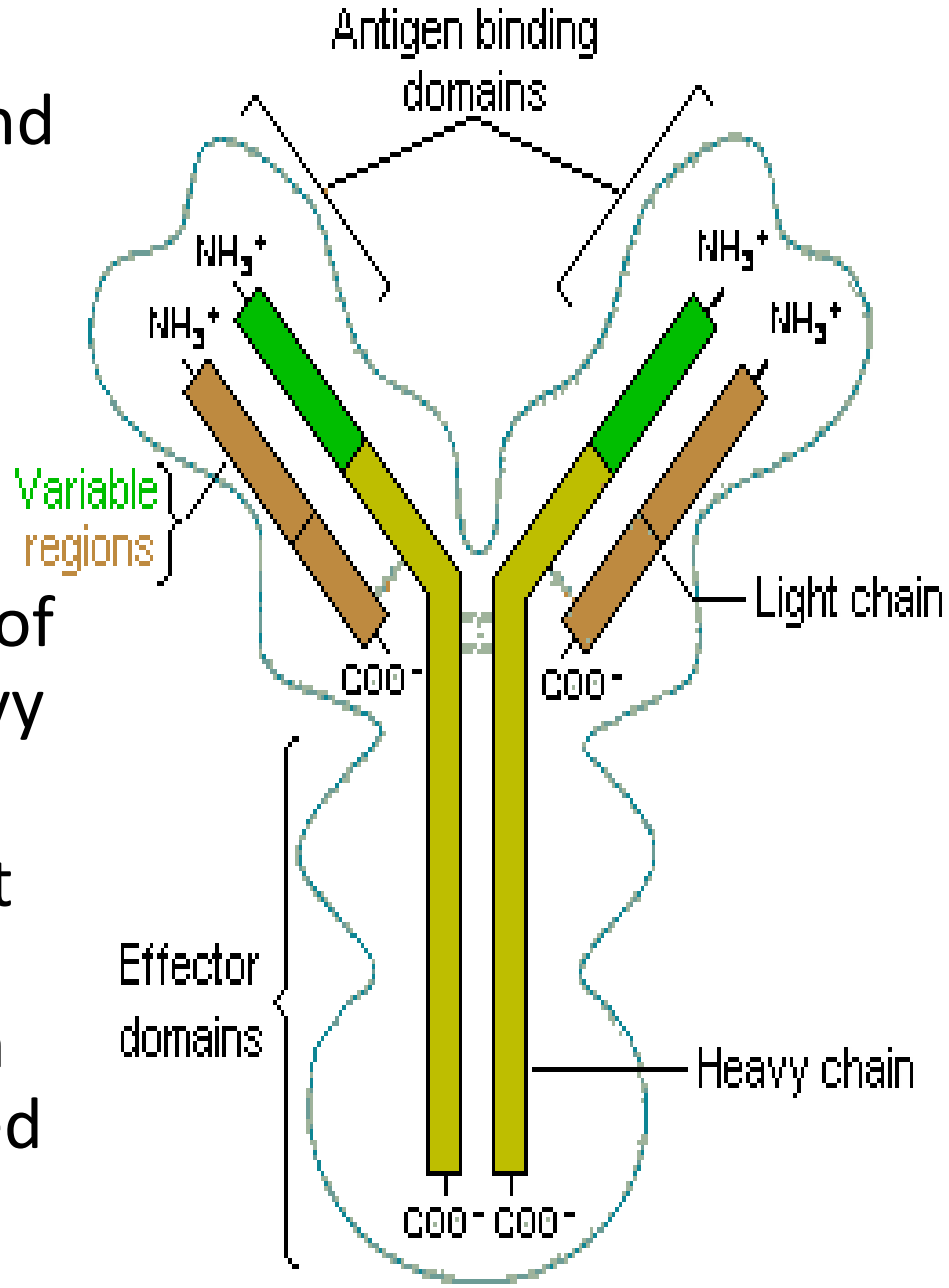
B cell when it encountered a specific Ag will differentiate into plasma cell that secrets Abs and memory cell





Structure of Ab

- Each antibody is made up of two identical heavy chains and two identical light chains, shaped to form a Y shape. Linked covalently by a disulfide bounds.
- Heavy chain (H) has a molecular weight twice that of light chain (L) , so called heavy and light.
- Each polypeptide chain is not linear but folded to form domes or loops by intrachain disulfide bonds (-s-s) an called domains.



- Light chain had one VL and CL domain
- Heavy chain had one VH and three CH called CH1, CH2 and CH3
- Hinge region : area of heavy chain between CH1 and CH2 domains where the disulfide bond is present. It is a flexible area permits the movement of Ab binding fragment (Fab) from 30-180^{o} .

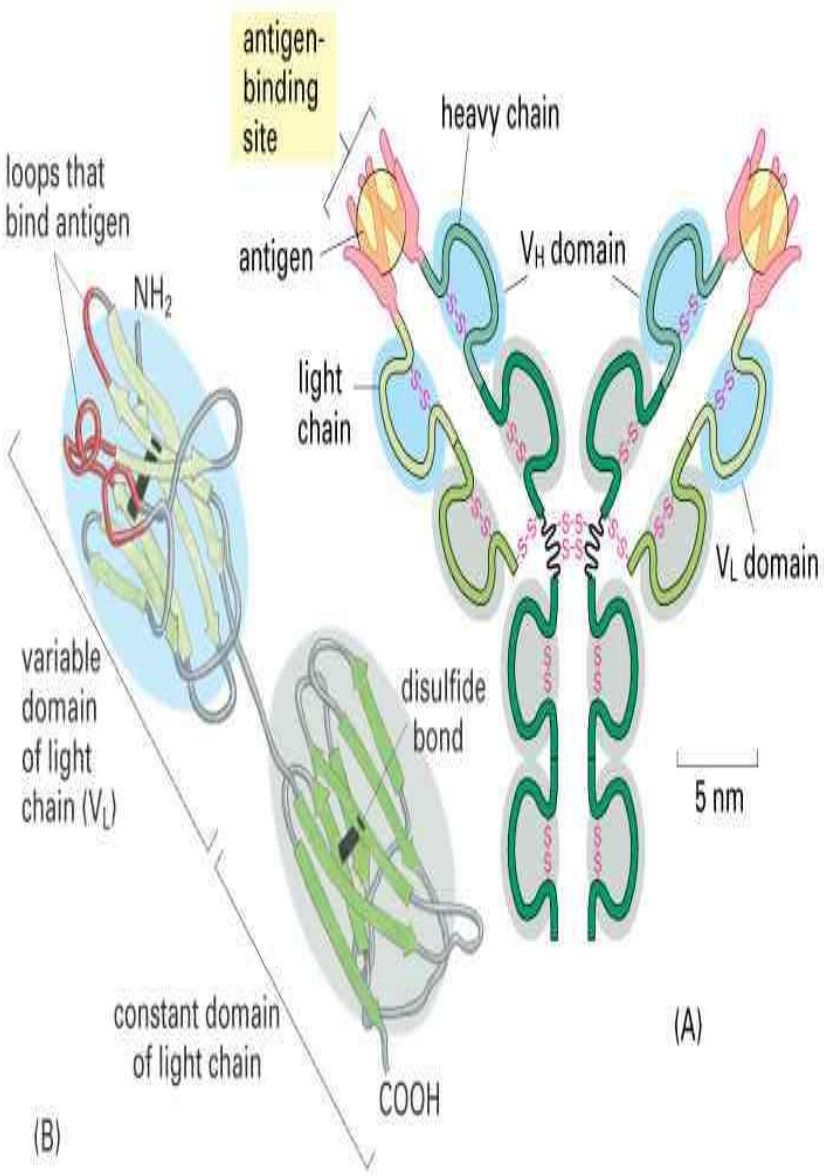
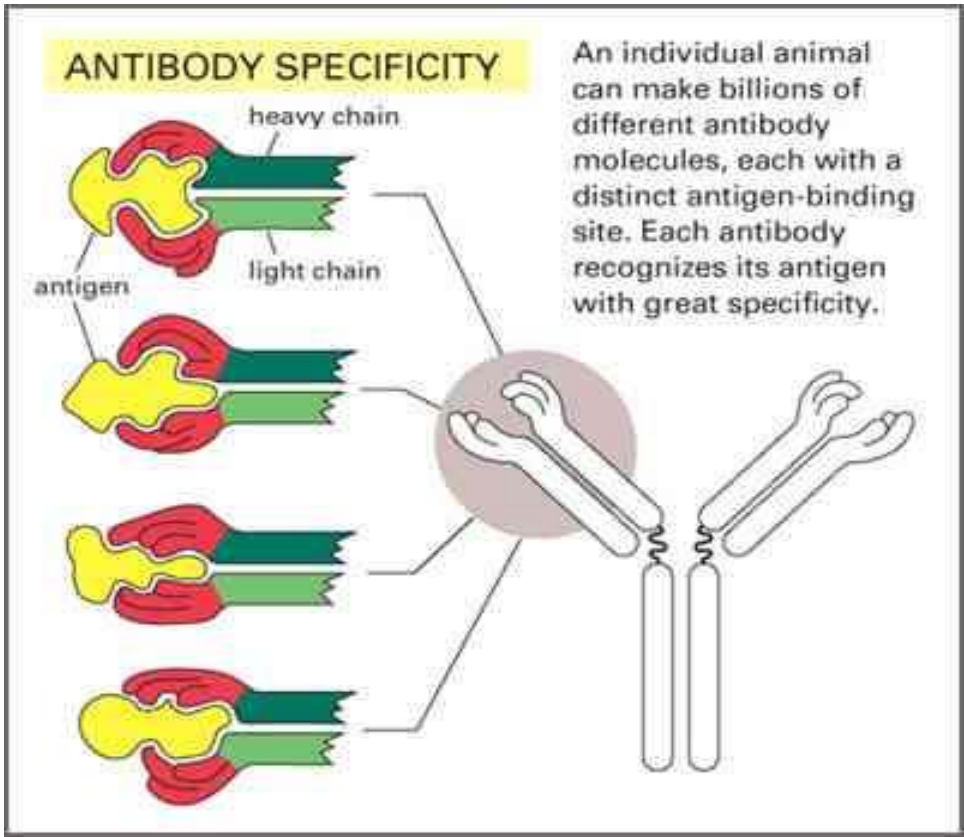


Figure 4-32 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Each chain has two regions:

1- The Variable Region: This sections that makes up the tips of the Y's arms, represent the **amino terminal** of poly peptide chain, varies greatly in contour from one antibody to another. This variation is due to a **change in aa sequence**. for this reason called the variable region .

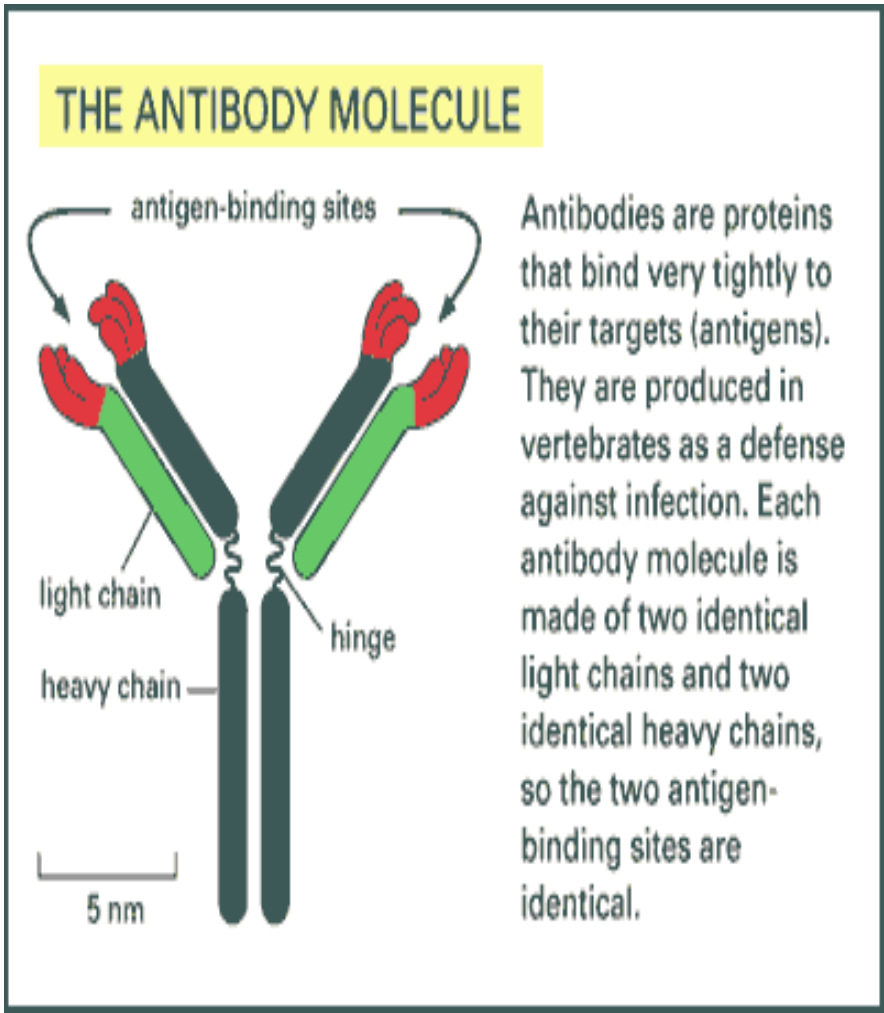
it has unique contours that "match" antigen to antibody., such as a **lock matches a key**



- At the outer end of each arm of the antibody molecule, a specific amino acid sequence exists. This is where the antibody molecule reacts with the antigenic determinant (epitope) that provoked its production.

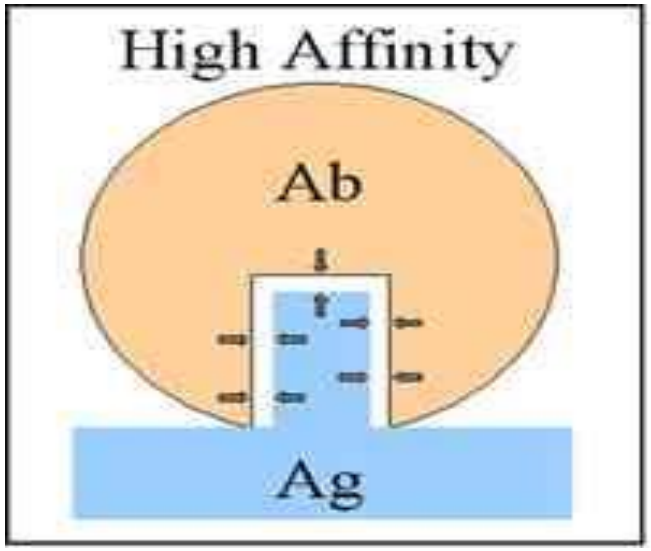
The combining site is known as the **Fab region.**

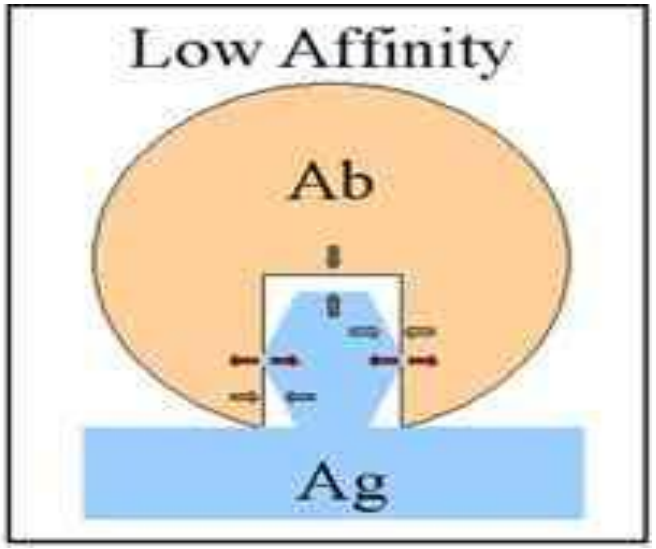
The most common antibody molecules have two Fab regions and are said to be **bivalent** (having two combining sites).



Paratope: It is a small region (of 15–22 amino acids) of the antibody's Fv region and contains parts of the antibody's heavy and light chains

- Affinity: Strength of interaction between single epitope and single paratope.





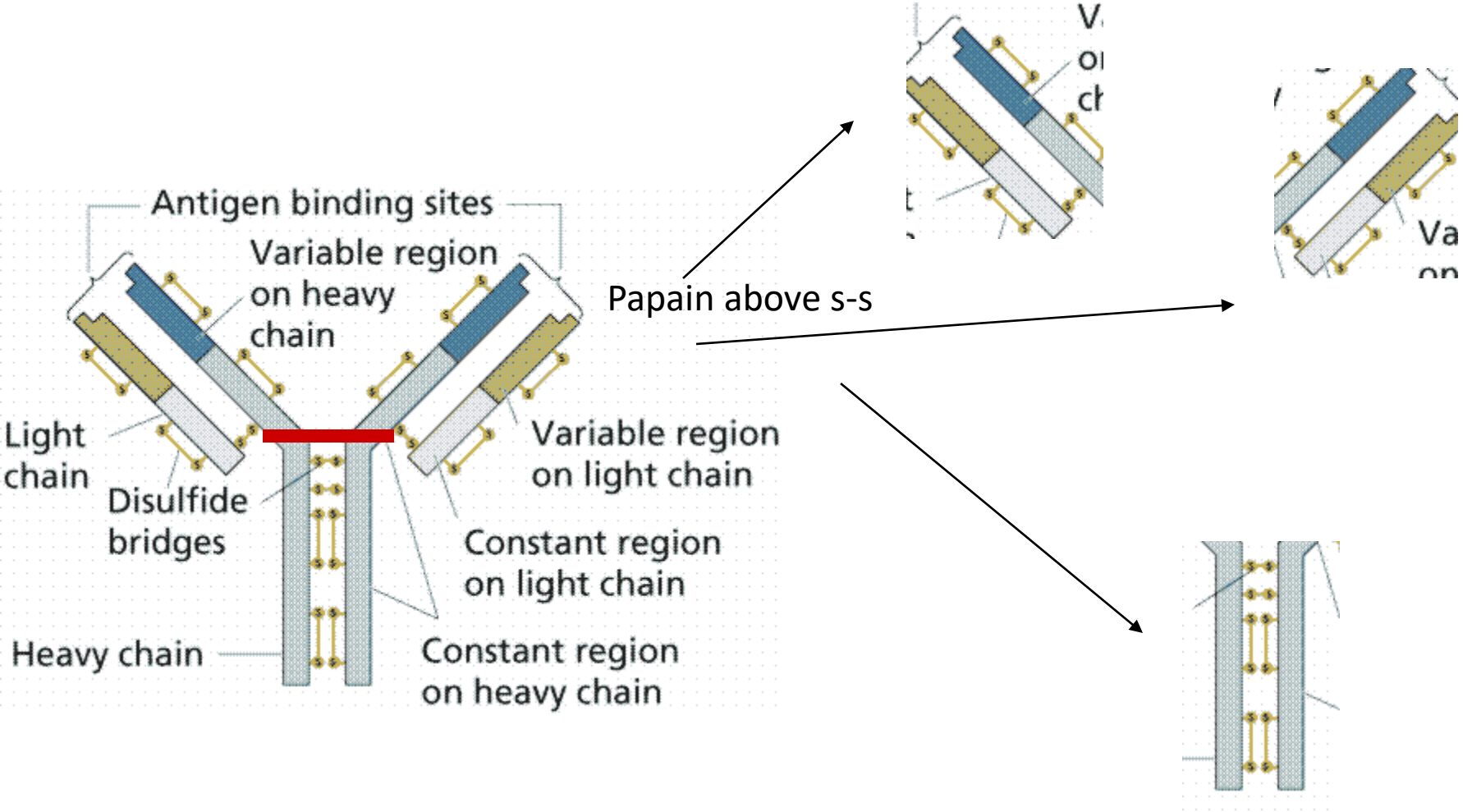
2- **The Constant Region:** The stem of the Y links the antibody to other participants in the immune defenses. This area is identical in all antibodies of the **same class** and it's called the constant region.It represent the carboxy terminal of polypeptide chain.

This region of the antibody molecule is called the **Fc region** because it can be crystallized. Its amino acid content and sequence is relatively constant and characteristic for its class. This portion of the molecule activates the complement system and encourages phagocytosis.

- According to constant region of heavy chain, we have five classes of Abs (isotypes) (IgG,IgM,IgA,IgE and IgD)
- The constant region of light chain either kappa (κ) or lambda (λ)

Proteolysis enzymes degrade Ab into different fragments:

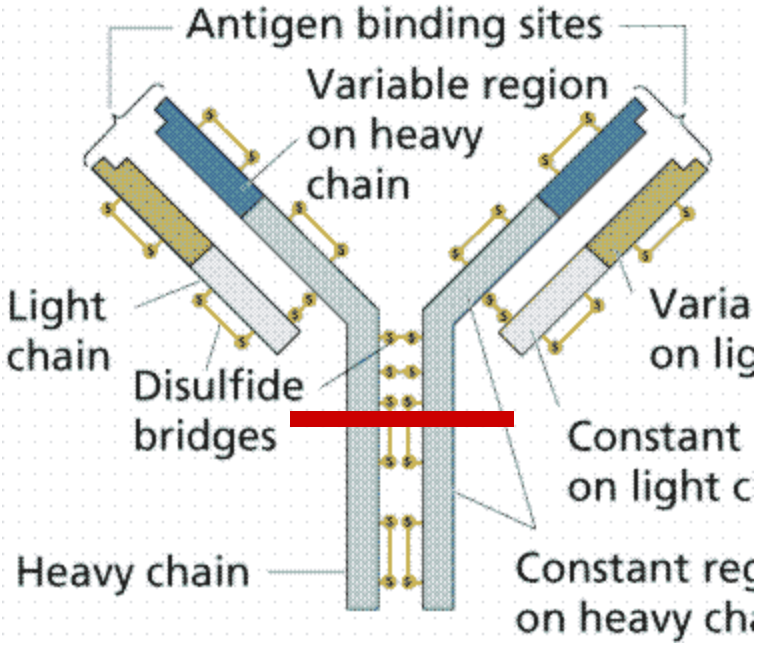
1- Papain: split Ab at hinge region above interchain disulfide bonds into 3 fragments (Two Fab and one Fc)



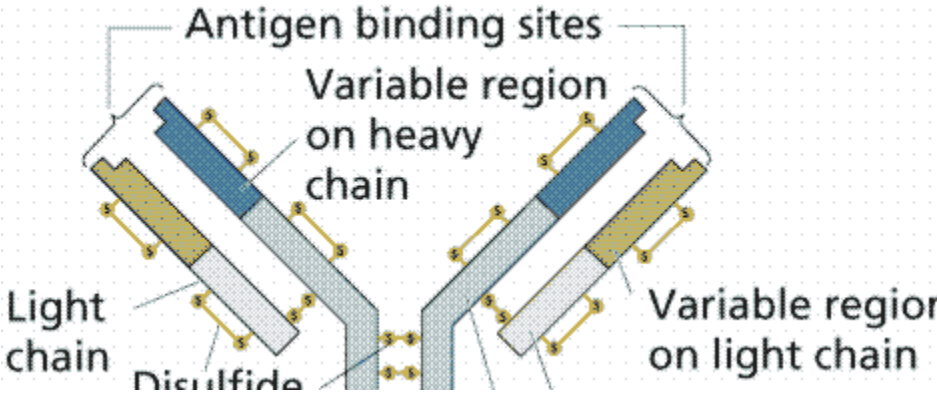
Pepsin: digest Ig below the interchain disulfide bonds at hinge region into two fragments: F(ab)2

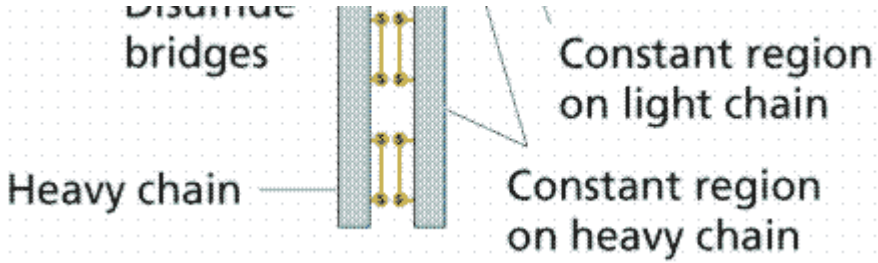
a- large one fragment (Fab2) which consist of two Fab fragments joine by disulfide bonds and has two Ag binding sites.

b-one Fc fragment



Pepsin below disulfide bonds





Functions of Igs

1. Activation of complement
2. Opsonization
3. Ab dependent cell mediated cytotoxicity

   ADCC by NK cell

4- Neutralization of toxins

5- Agglutination of RBC

Immunoglobulin classes(Isotypes)

- **Immunoglobulin classes**
  The immunoglobulins can be divided into five different classes, based on differences in the amino acid sequences in the constant region of the heavy chains.
- 1. IgG - Gamma heavy chains
- 2. IgM - Mu heavy chains
- 3. IgA - Alpha heavy chains
- 4. IgD - Delta heavy chains
- 5. IgE - Epsilon heavy chains

Immunoglobulin Subclasses

- The classes of immunoglobulins can de divided into subclasses based on small differences in the amino acid sequences in the constant region of the heavy chains. All immunoglobulins within a subclass will have very similar heavy chain constant region amino acid sequences.
- **1. IgG Subclasses**
- a) IgG1 - Gamma 1 heavy chains
- b) IgG2 - Gamma 2 heavy chains
- c) IgG3 - Gamma 3 heavy chains
- d) IgG4 - Gamma 4 heavy chains
- **2. IgA Subclasses**
- a) IgA1 - Alpha 1 heavy chains
- b) IgA2 - Alpha 2 heavy chains

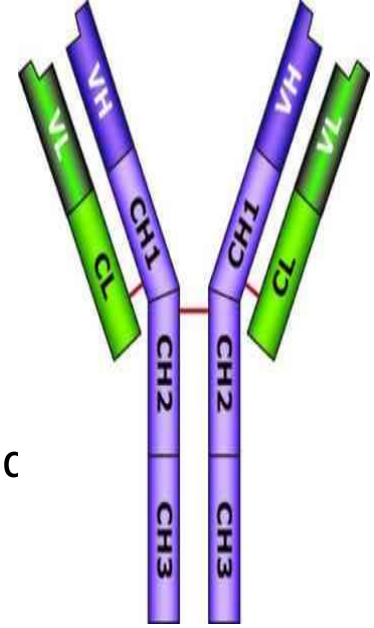
Immunoglobulin Types

- Immunoglobulins can also be classified by the type of light chain that they have. Light chain types are based on differences in the amino acid sequence in the constant region of the light chain.

1-Kappa light chains

2- Lambda light chains

IgG



Structure

The structures of the IgG are made up of two identical heavy chains and two identical light chains .

All IgG's are monomer .

MW=150 000 d.

called so because of its gamma heavy chain

The subclasses (IgG1,IgG2,IgG3, IgG4)differ in the number of disulfic and length of the hinge region.

.Properties:

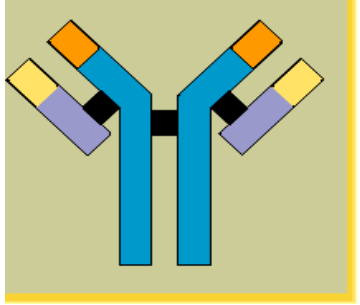
1- **IgG is the major Ig in serum - 75% of serum Ig**

2- **IgG is the only class of Ig that crosses the placenta. IgG2 does not cross well.**

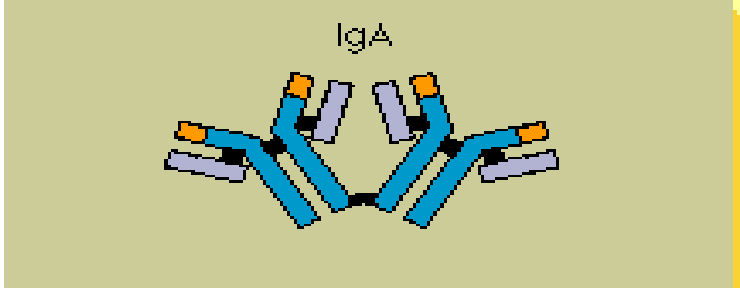
3- **Fixes complement and mediate ADCC by NK cell . IgG4 does not fix complement**

4- **Binding to cells like Macrophages ,PMN by Fc region of IgG . The antibody has prepared the antigen for eating by the phagocytic cells called opsonin**

5- **) main Ig in the secondary immune response**



IgA



Structure

Serum IgA is a monomer but IgA found in secretions is a dimer - MW=150 000-600 000 d

When IgA exits as a dimer, a J chain is associated with it.

- **When IgA is found in secretions is also has another protein associated with it called the secretory piece , the secretory piece is made in epithelial cells and is added to the IgA as it passes into the secretions**
- **J chain:** linked to the carboxy terminal portions of heavy chains.
- **Properties**
- **a) IgA is the 2nd most common serum Ig. constitutes 10-15 % of serum Ig**
- **b) IgA is the major class of Ig in secretions - tears, saliva, colostrum, mucus. Since it is found in secretions secretory IgA is important in local (mucosal) immunity.**
- **c) IgA does not fix complement**
- **d) IgA can binding to some cells - PMN's and NKT and mediate ADCC**
- J chain: small glycoprotein that are covalently linked to the carboxy terminal portions of heavy chains.
- Secretary component: is a polypeptide chain synthesized by exocrine epithelial cells that enables IgA to pass through mucosal tissues into secretions and protect IgA from protease enzymes.

IgM

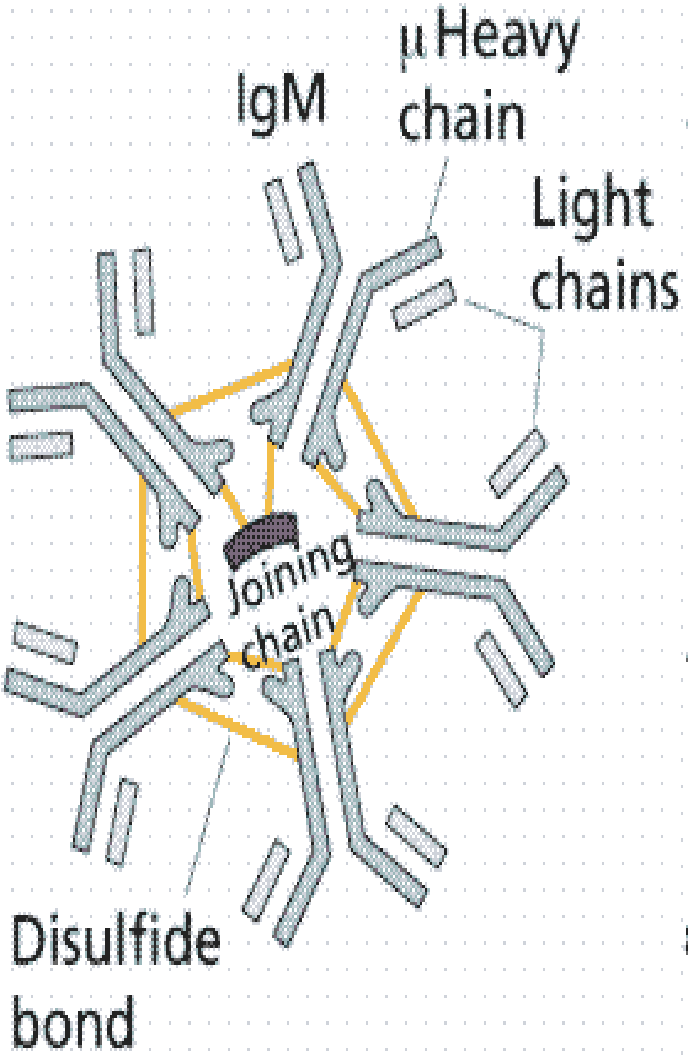
Structure

IgM normally exists as a pentamer but it can also exist as a monomer. MW=900 000 d

In the pentameric form all heavy chains are identical and all light chains are identical. Thus, the valence is theoretically 10 times.

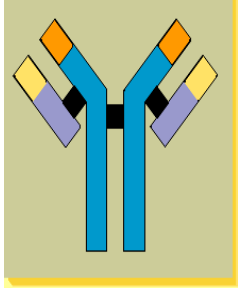
IgM did not has a hing region and replaced by an extra domain on the mu chain (CH4) , so it has 4 constant heavy domains and it has another protein covalently bound via a S-S bond called the J chain. This chain functions in polymerization of the molecule into a pentamer.

Present as monomer on the membrane of mature B cells.



- **Properties**
- **a) IgM is the third most common serum Ig.Constitute 5-10% of total serum Ig .**
- **b) IgM is the first Ig to be made by the fetus and the first Ig to be made by primary immune response**
- **c) As a consequence of its pentameric structure, IgM is a good complement fixing Ig.**
- **d) IgM is also a good hemagglutinating Ig .**
- **e) IgM binds to some cells via Fc receptors.**

IgE



Structure

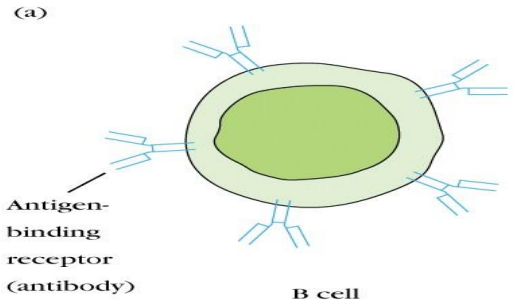
-IgE exists as a monomer and has an extra domain in the constant region , had four CH domains

-MW=190 000 d.

Properties

- **IgE is the least common serum Ig - about 0.002% of total serum Ig**
- **since it binds very tightly to Fc receptors on basophils and mast cells even before interacting with antigen.**
- **Involved in allergic reactions - As a consequence of its binding to basophils an mast cells, Binding of the allergen to the IgE on the cells results in the release of various pharmacological mediators that result in allergic symptoms.**
- **It is called homocytotropic (bind cell) and called reagenic Ab**
- **IgE also plays a role in parasitic helminth and protozoal diseases.**
- **Eosinophils have Fc receptors for IgE and binding of eosinophils to IgE-coated helminths results in killing of the parasite.**
- **IgE does not fix complement.**

IgD



Structure

IgD exists only as a monomer. MW=150 000 D

Properties

- **IgD is found in low levels in serum; constitutes about 0.2% of total serum Ig**
- **its role in serum uncertain.**
- **IgD is primarily found on B cell surfaces where it functions as a receptor for antigen.**
- **c) IgD does not bind complement**

Variation of Igs

- Isotypes: All classes and subclasses of Ig that are present in normal individuals (IgG,IgM,IgA,IgE,IgD)
- Allotype: there is a single aa difference in the peptide chain in CH and CL chain.
- Idiotype :The unique aa diference in the sequence of VH and VL chain

polyclonal antibody

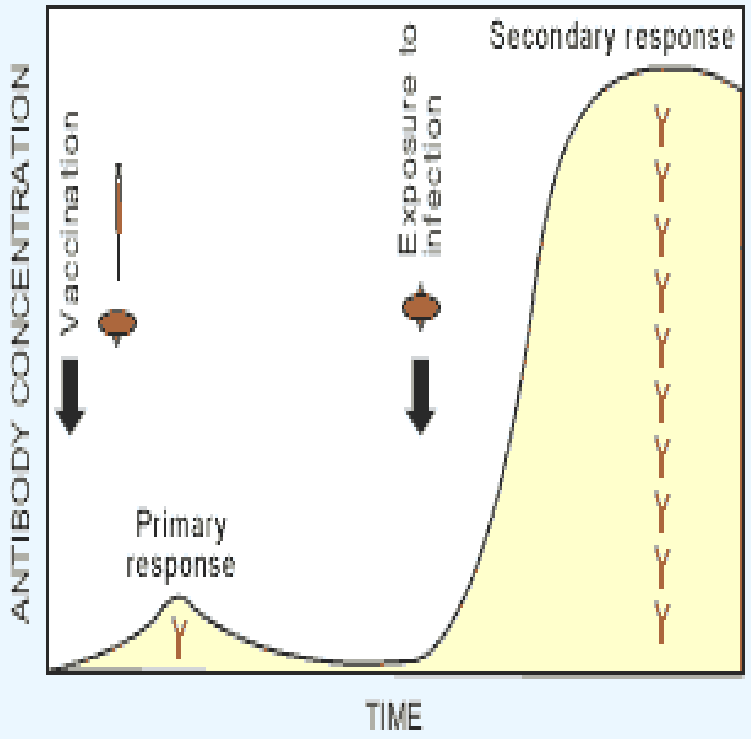
- Most Ags possess multiple epitopes and each one of them induce different B cells to proliferate into a clone of cells that recognize different epitopes, these B cells secret Abs, resulting into a mixture of Abs called polyclonal Abs
- Monoclonal antibody A clone of single B-cells that recognize a single epitope that secret Abs spesific to a single epitope so its called monoclonal Abs. Its used for diagnostic and theraputic purposes .

Immune response

- The first contact of an exogenous Ag with an individual leads to generation a primary humoral immune response.
- Characteristics:

1- longer lag phase: during this period , the naive B cells undergo clonal selection, clonal expansion and differentiation into memory and plasma cells

2- Log phase (logarithmic): increase in IgM concentration.



Primary antibody response: the antibody concentration rises gradually and peaks about 2 weeks after vaccination.

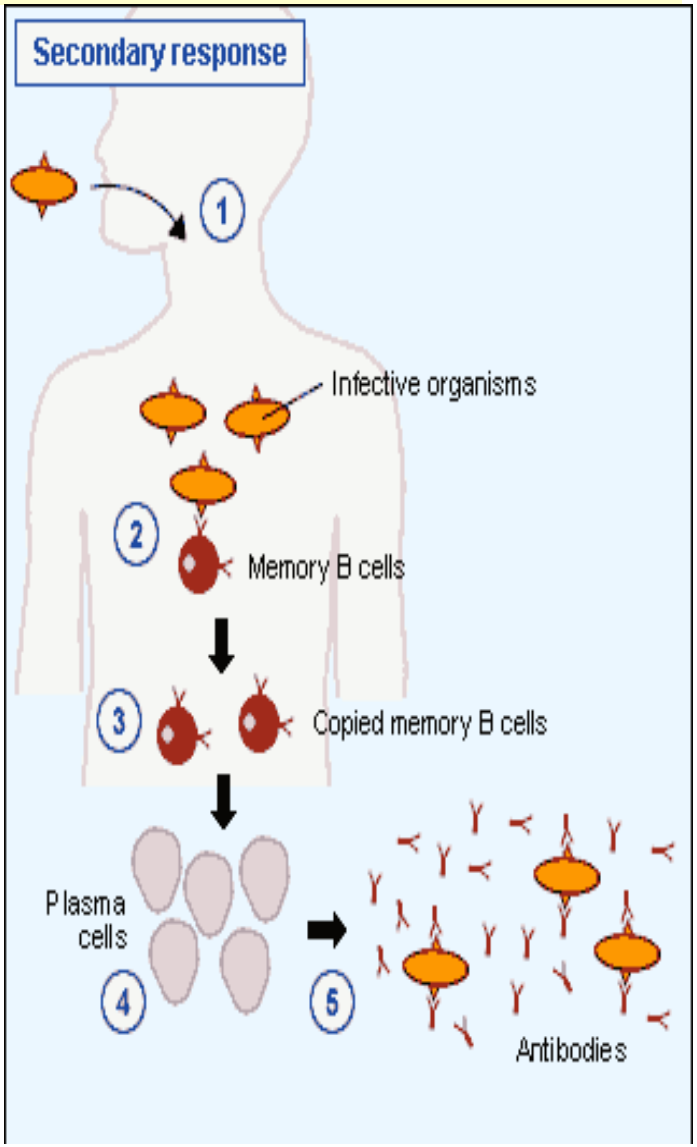
Secondary antibody response: the antibody concentration rises quickly, and the response is more intense. The antibody concentration remains higher for longer.

Secondary immune response

- Second contact with same exogenous antigen, generates secondary humoral immune response.
- Characterization:

1-shorter lag phase

2-Rapid reaches a greater magnitude of IgG and last for longer time. This is because of memory B-cells specific for this Ag are existed. The processes of affinity maturation and class switching are responsible for higher affinity to Ag and different isotype



Vaccination (immunization)

- Used to provoke a positive immune response by an individual to various pathogenic microorganisms to confer protection.
- 1- natural(passive like maternal Abs and active like natural infection)
- 2-artificial (passive like Abs against hepatitis B virus and active like vaccination with tetanus vaccine)

Comparism between active and passive immunization

- Active immuization

1-Delay before protection

2-long lived

3-stimulate immune system

- Passive

1- acts immediately

2-short-lived

3-no stimulation to immune system

Complement (C)

- Complement components are proteins or glycoprotein that are synthesized mainly in the liver hepatocytes monocytes, macrophages, epithelial cells.
- **Its function is to complete the action of antibodies to eliminate the pathogen.**
- Its present in the blood serum.
- Its designated by the numerals (C1-C2 …..C9) **Numbered in order of discovered, not sequence of action**

  and letter symbols like D, B, …..
- ~ 15% of globulin fraction
- The complement destroyed by heating the serum at $56C^0$ for 30 mins. circulate in **inactive state**
- once it is activated a peptide fragment will be formed and denoted by a small letters:

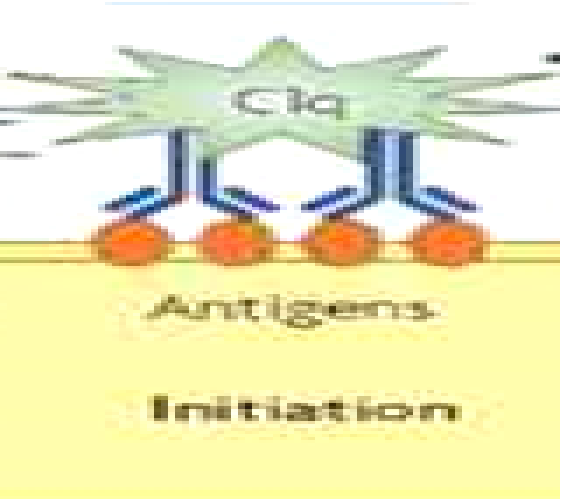
- **small fragment designated ((a))**
- **large fragment designated ((b))**

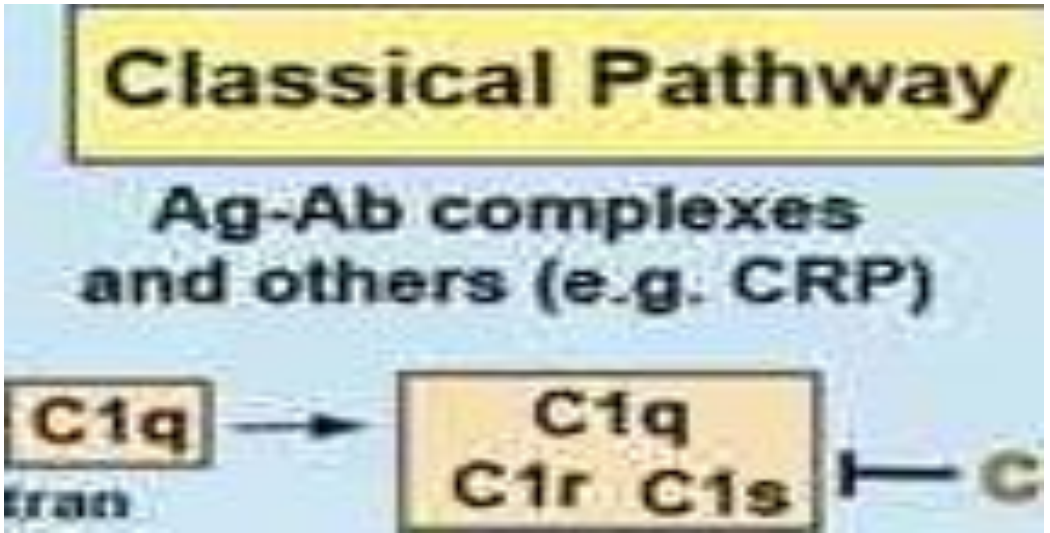
Complement activation

- Classical pathway.
- Alternative pathway.
- Lectin pathway.

Classical Pathway

- Adaptive immunity
- Ab(IgM, IgG3, IgG1, IgG2)+Ag called immune complex
- Fc portion of Ig recognized by C1
- C1q binds to Ag-Ab complex then C1r thenC1s (C1qrs)





C1

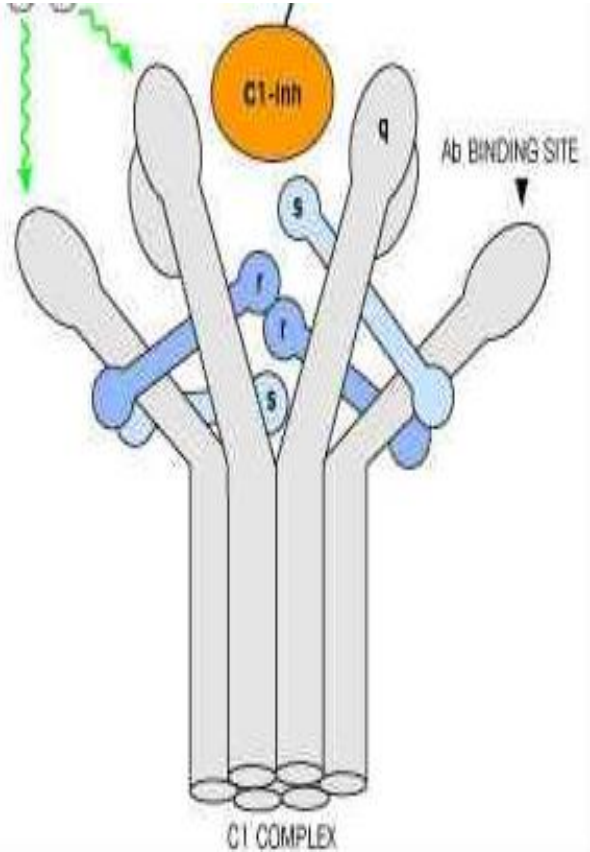
- is a macromolecule complex present in the serum . Consisting of 3 subunits

-C1q

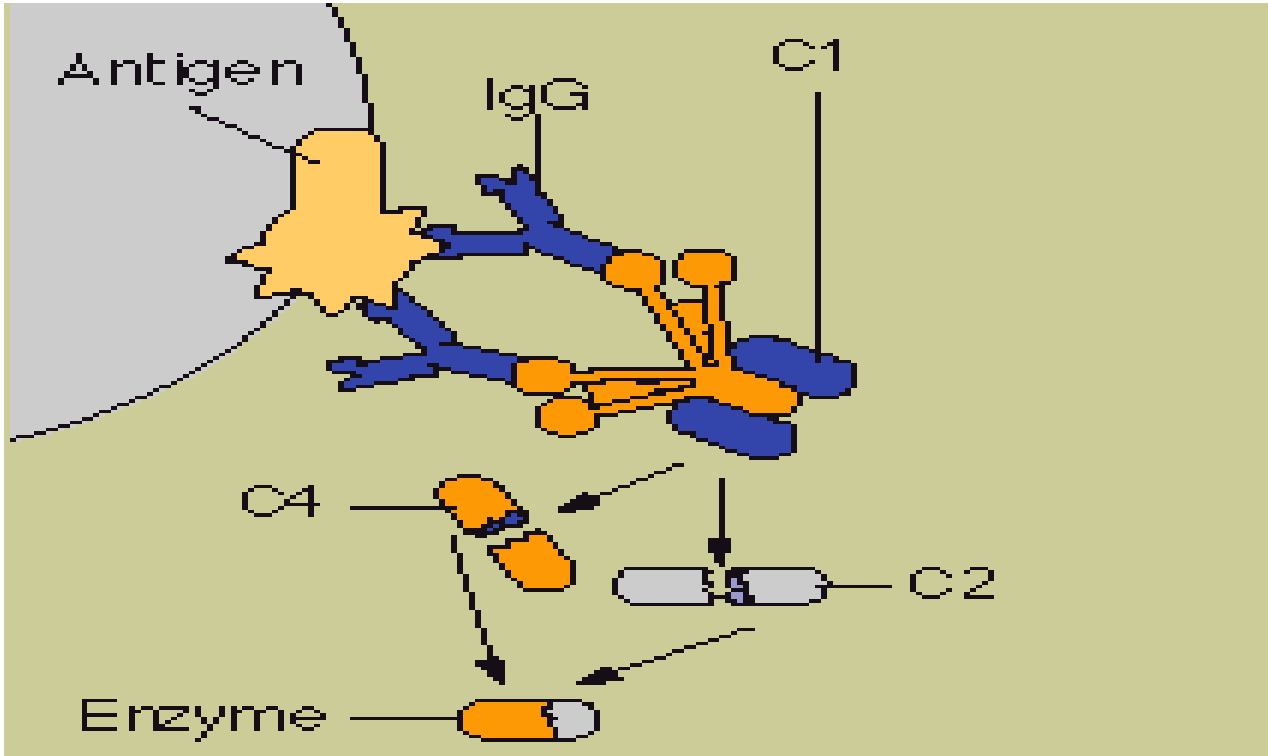
-C1r

-C1s

Connected to each other by Ca++ dependents bounds.



- *AgAb Activate C1q then C1r then C1s
- *C1s had enzymetic activity (estrase activity) cleaves C4 into two fragments
- –C4a
- -C4b



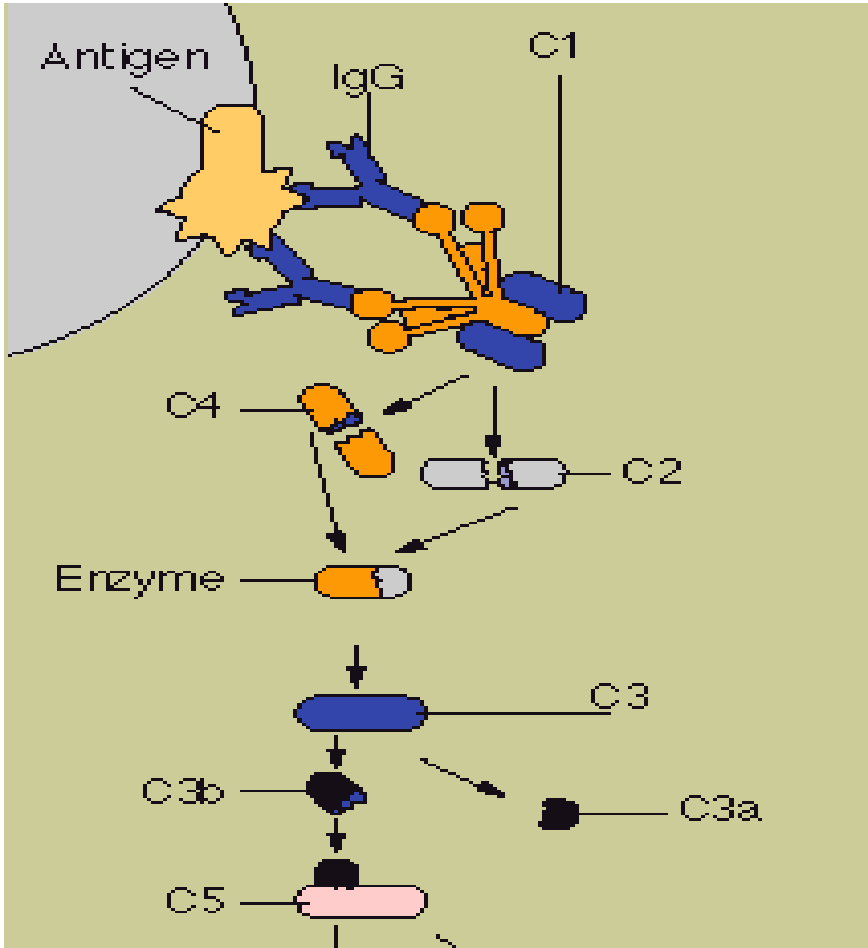
C1s in the presence of C4b will split C2 into two fragments (C2a and C2b) and C2

Formation of

C3 convertase(C4b2a)

that Cleaved C3 into C3a and C3b forming a complex called

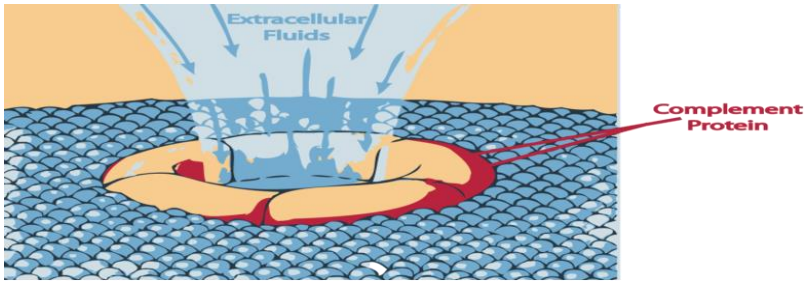
C5 convertase (C4b2a3b)

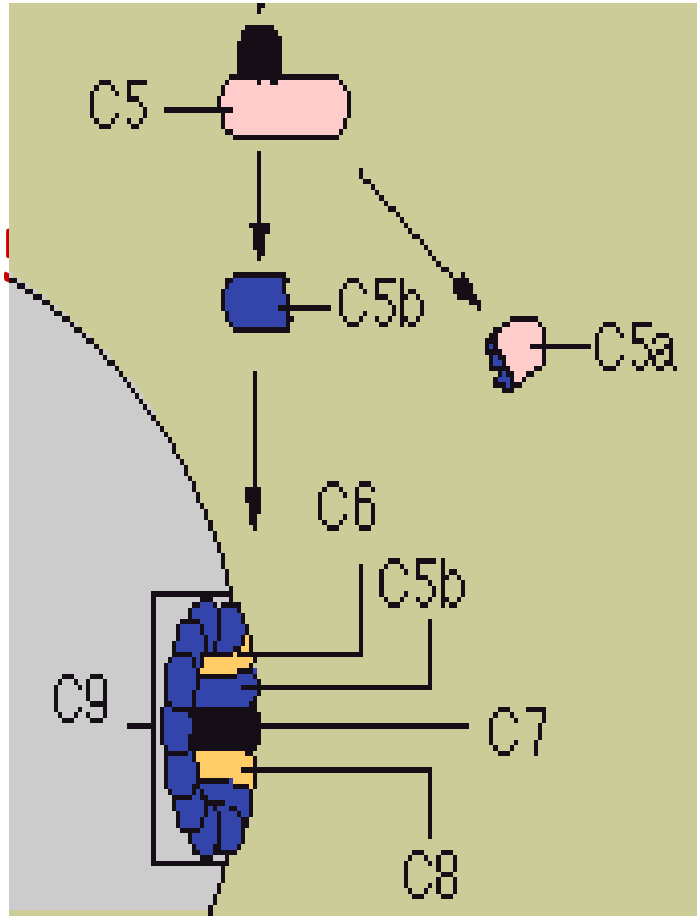


C5 is cleaved by C5 convertase which had enzymatic activity that cleave C5 into:

C5a and C5b

-C5b attached to cell membrane to form C4b2a3b5b act as a **receptor to C6 then C7 then C8 and C9** to form a complex called membrane attachcomplex**(MAC)(C** causing a pore on the cell surface →influx of Na+ and water→lysis of the cell.

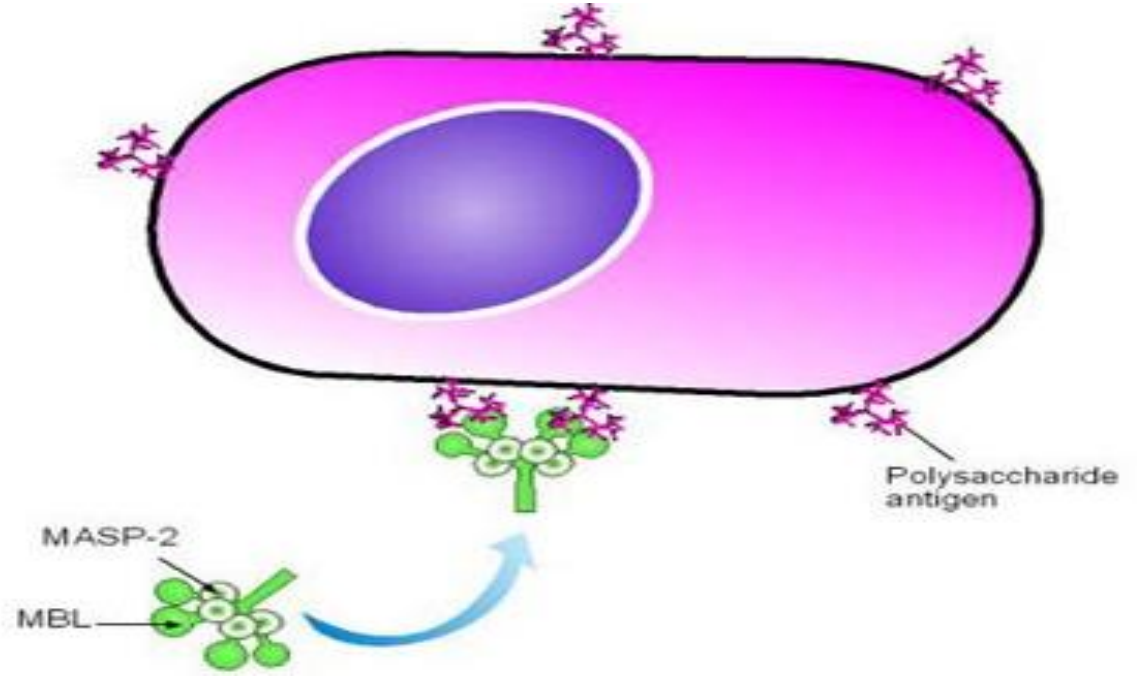




- C3a, C4a = act as a mediator of anaphylaxis and chemotactic substance.
- C3b = immune adherence in opsonization

Mannose Binding Lectin (MBL)pathway

- MBL: is a serum protein that binds to mannose in microbial cell wall
- Is part of Innate immunity
- This complex had a function as **C1qrs**
- This complex cleaves C4 and C2 ……C9



Alternative Pathway

- **Can be initiated by three distinct ways:**

1-Tickover pathway: by spontaneous hydrolysis of C3 yielding C3(H_2O)that bind to factor B in the presence of factor D will be cleaved into :

C3(H_2O)Bb called C3 convertase(fluid phase)and Ba

- **this initiate more breaking down of C3 into C3a and C3b**
- **This C3b bound to microbial surface and bind to factor B that cleaves into Ba and Bb in the presence of factor D lead to formation C3bBb called C3 convertase (Membrane bound) and Ba.**

- **Formation of C3 convertase (C3bBb)**

requires properdin (Factor P) that Breaks more C3 . This is called positive feedback loop.

- This leads to formation **C5 convertase (C3bBbC3b)** that act on C5 cleaves it into C5a and C5b and continue the same reaction as classsical pathway (C6789)→cell lysis.

2- Alternative Properdine –activated pathway:

-Properdine may also serve to initiate complement

-Proberdine binds to components of microbial membranes and stabilizes the binding of C3b to Bb complex resulting C3bPBb complex that act as C3 convertase

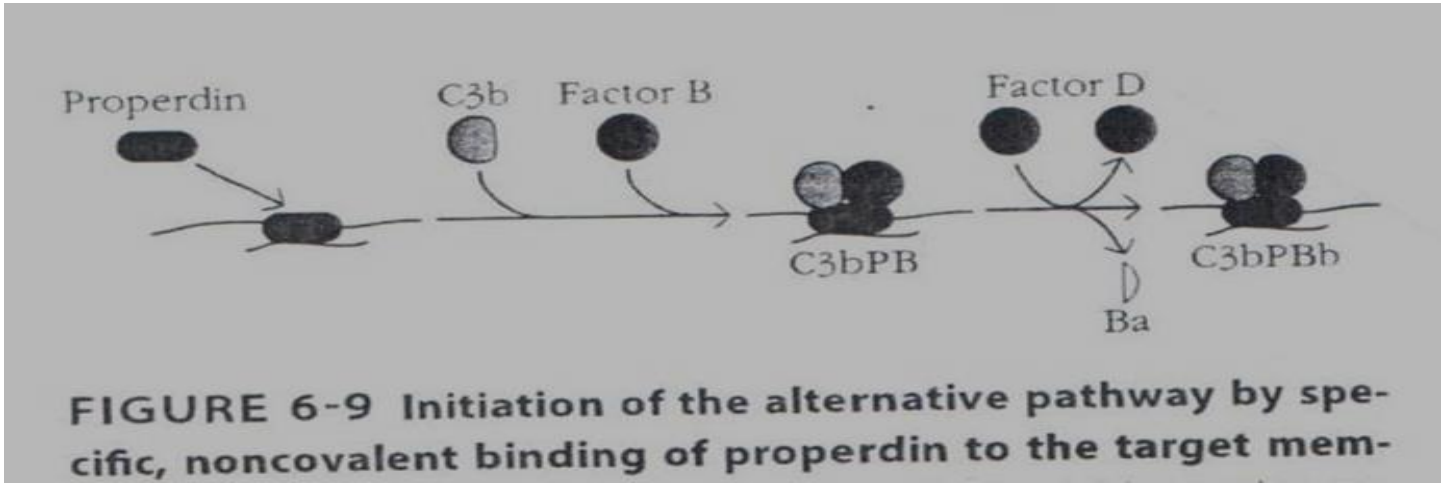
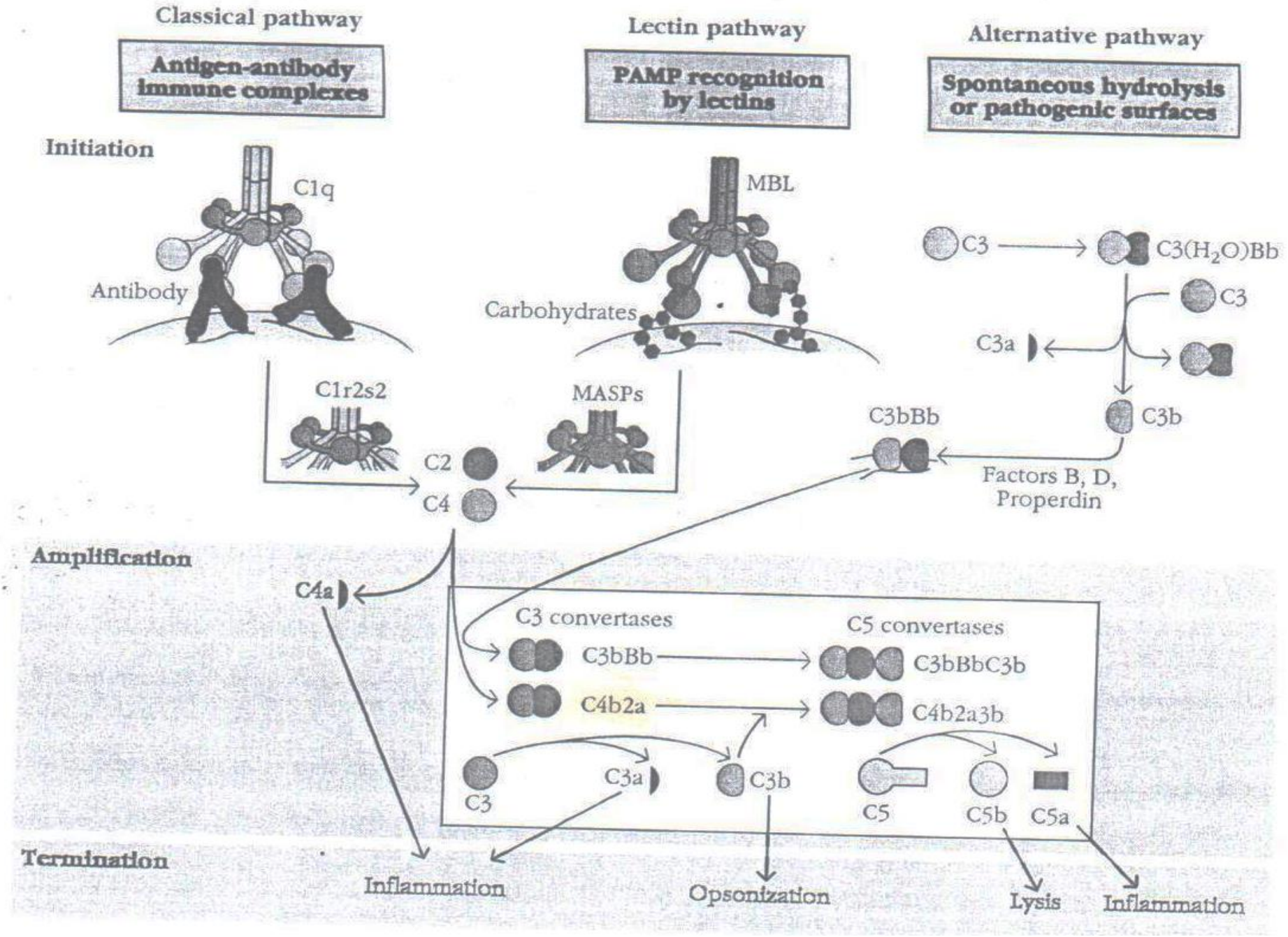


FIGURE 6-9 Initiation of the alternative pathway by specific, noncovalent binding of properdin to the target mem-

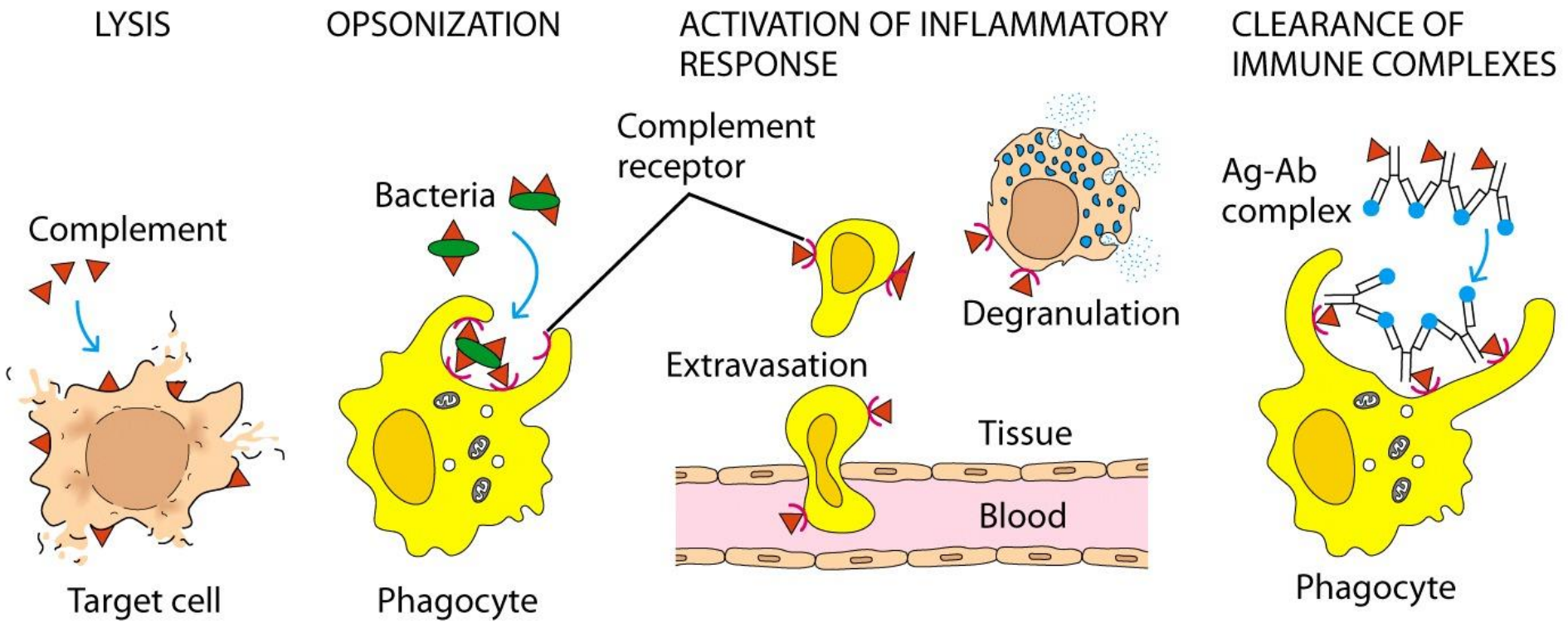
3- Alternative Protease –activated pathway

-Proteins factors involved in blood clotting such as thrombin and plasmin are capable of generating both C3a and C5a

-C5b binds to microbial cell wall and stabilized by C6


Classical pathway
Antigen-antibody immune complexes
Lectin pathway
PAMP recognition by lectins
Alternative pathway
Spontaneous hydrolysis or pathogenic surfaces
Initiation
C1q
Antibody
MBL
Carbohydrates
C3
C3(H2O)Bb
C3
C3a
C1r2s2
MASPs
C3bBb
C3b
C2
C4
Factors B, D, Properdin
Amplification
C4a
C3 convertases
C5 convertases
C3bBb
C3bBbC3b
C4b2a
C4b2a3b
C3
C3a
C3b
C5
C5b
C5a
Termination
Inflammation
Opsonization
Lysis
Inflammation

Biological Effects of Complement Activation



Cell Receptors for Complement

- CR1(complement receptor 1)(CD35)
  - Present on Macrophages, neutrophils
  - Binds C3b
  - clearance of immune complexes and enhancement of phagocytosis
- CR3(complement receptor 3)
  - present on Macrophages, neutrophils
  - Binding to adhesion molecules facilitates extravasation

Regulation of Complement activity

1. C1 inhibitor promotes dissociation of C1 components.
2. Decay accelerating factor promotes decay of C3 convertase
3. Protein S prevents insertion of C3b67 into host cell membrane

Complement Deficiencies

- C3 deficiency leads to recurrent bacterial infection
- C9 Deficiency leads to increase Neisseria infection (Gonococcal and Meningococcal)
- C1 inhibitor Deficiency leads to Hereditary angioneurotic edema.

Thank you