Complement

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Learning objectives

At the end of the session, you will be able to understand:

- Definitions
- Complement activation pathway : Classical, alternate, lectin
- Role of complement
- Complement deficiencies

COMPLEMENT

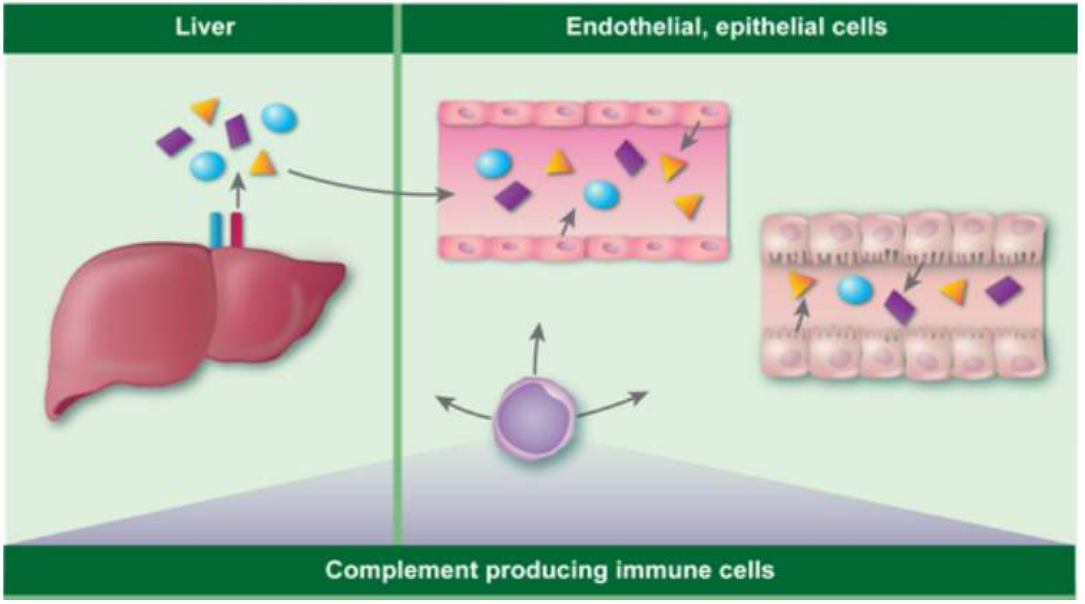
- A group of proteins, normally found in serum in inactive form. In activated form, augment the immune responses.
- Constitute about 5% of normal serum proteins.
- Level **does not** increase following infection /vaccination.
- Bind to Fc region of antibody
- Not activated by only antigen or only antibody
- Species nonspecific
- Heat labile

Complement Components

- Complement system- about 30 serum proteins
- **Complement components**, the **properdin system** and the **regulatory proteins.**
- Components-named by numerals.
- Nine components- C1 to C9.
- C1 has 3 subunits- C1q, C1r,C1s.
- Properdin system and the regulatory proteins are named by letter symbols, e.g.factor-B

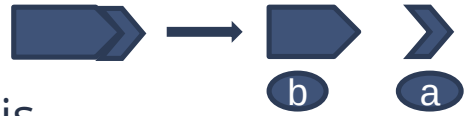
Synthesis

- **Liver**: major site of synthesis of complement proteins.
- Minor sites: blood monocytes, tissue macrophages, epithelial cells of GIT and genitourinary tract.



Complement Activation

- Complement proteins -synthesized in inactive form



- Activated by proteolysis.
- Components have 2 unequal fragments (large & small).
- Larger fragments designated as ‘b’ (e.g. C3b)
- Smaller fragments designated as ‘a’ (e.g. C3a).
- Exception -C2a is larger fragment.

Complement Activation (Cont..)

- During proteolysis, smaller fragment is removed, exposing the active site of the larger fragment.
- Larger fragment participates in the cascade reaction of complement pathway
- Smaller fragment diffuses away to mediate other functions.

Complement Activation (Cont..)

- **Cascade reaction-** Fragments of complements interact in a definite sequential manner with a cascade like effect, which leads to formation of complex.
- Complex having enzymatic activity is designated by putting a bar over the number or symbol (e.g. C $\overline{3bBb}$).

COMPLEMENT PATHWAYS

1. **Classical pathway:** Antibody dependent pathway, triggered by the Ag-Ab complex formation.
2. **Alternative pathway:** Antibody independent pathway, triggered by the antigen directly.
3. **Lectin pathway:** Recently described pathway. resembles classical pathway but is antibody independent.

Stages of complement activation

- **Four main stages** in the activation of any of the complement pathways.
  - Initiation of the pathway
  - Formation of C3 convertase
  - Formation of C5 convertase
  - Formation of membrane attack complex (MAC)

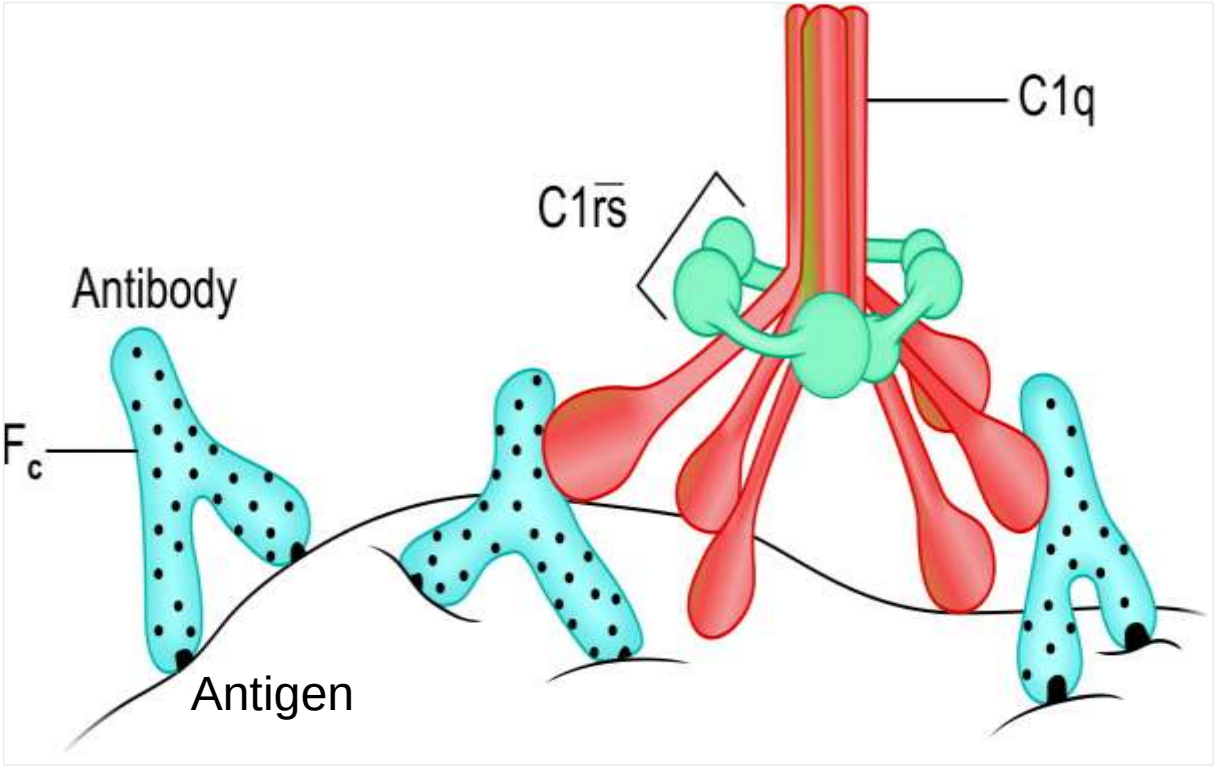
- Three pathways differ from each other only in their **initiation till formation of C3 convertase.**

1.Classical Pathway

- Antibody dependent
- All Abs can not bind to complement of classical pathway.
- Ability of Abs to fix complement is-

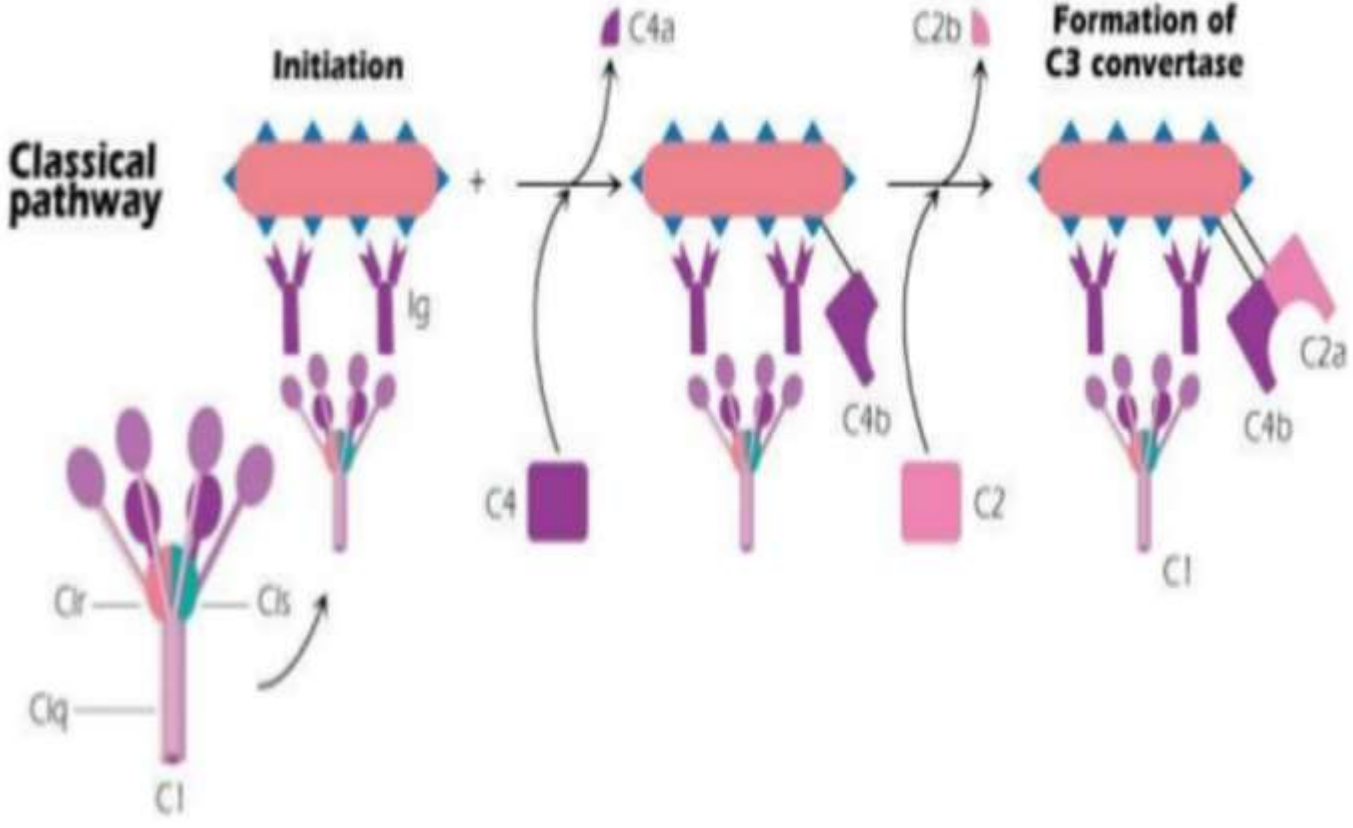
  IgM (most potent) > IgG3> IgG1> IgG2.
- Other classes of Abs do not fix complements.
- C_H2 domain on IgG, C_H4 on IgM participate in complement binding.
- The classical pathway begins with activation of C1

- **First step -** binding of C1 to Ag-Ab complex.
- C1q binds first with Fc portion of IgM /IgG bound to Ag.
- C1q -hexamer(6 globular heads)has 6 combining site.
- Effective activation of classical pathway begins only when C1q attaches by **at least two** of its 6 globular binding sites.
- C1q binding (in the presence of calcium ions)activates sequentially C1r followed by C1s.


C1q
C1r̄s̄
Antibody
Fc
Antigen

- Activated C1s acts as an esterase (C1s esterase)
- It cleaves C4 to produce **C4a** (an anaphylatoxin) & **C4b** which binds to C1
- $\overline{C14b}$ cleaves C2 into **C2a** (remains linked to C complex), and **C2b** (has kinin like activity), is released outside.

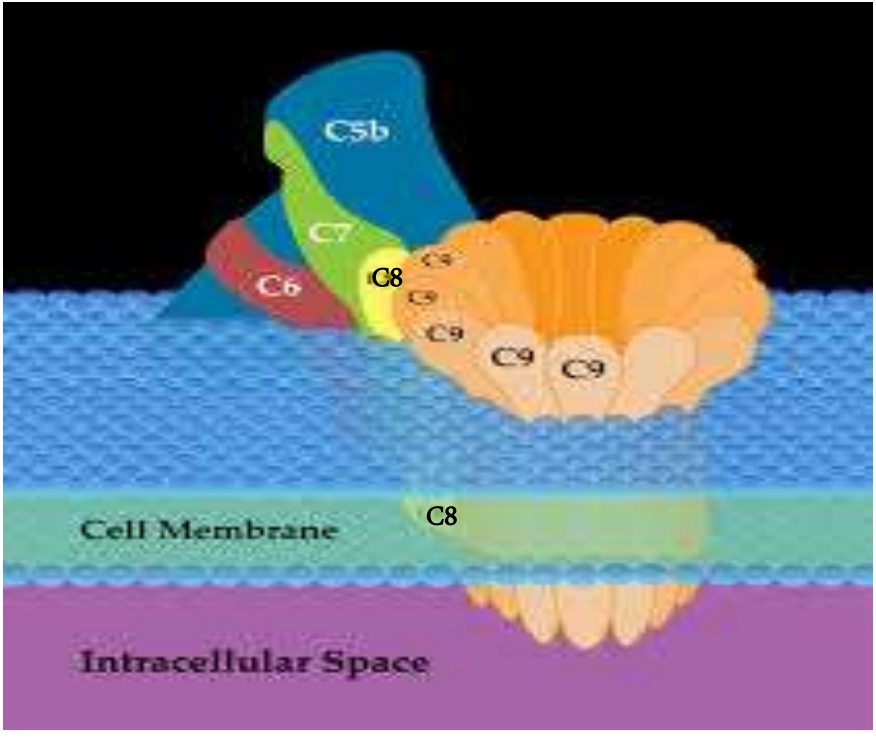
➢ **$\overline{C14b2a}$** is referred to as **C3 convertase** of the classical pathway.


Classical pathway
Initiation
Ig
C1r
C1s
C1q
C1
C4a
C4
C4b
C2b
C2
Formation of C3 convertase
C2a
C4b
C1

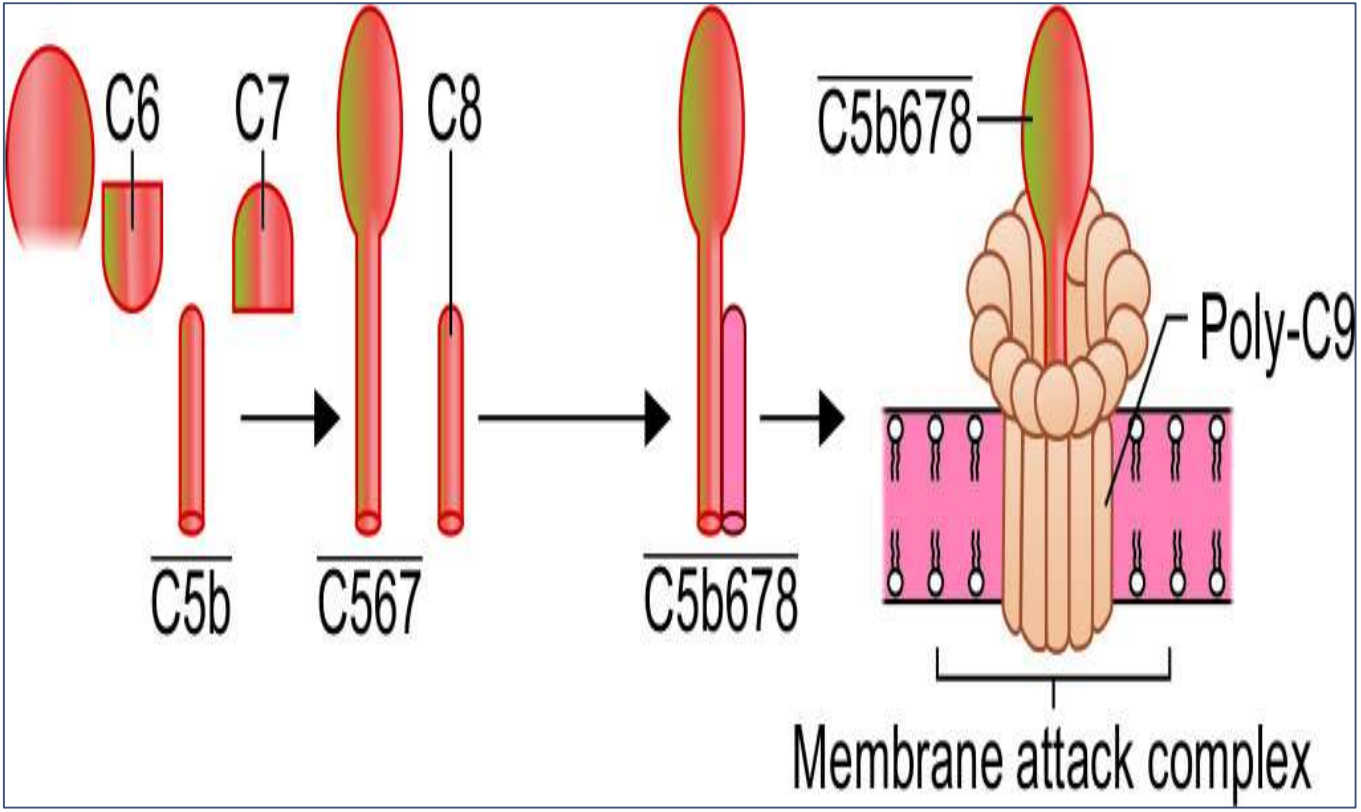
- C3 convertase hydrolyses many C3 molecules into-
  - **C3a** (an anaphylatoxin)
  - **C3b** remains attached to $\overline{C14b2a}$ to form $\overline{C14b2a3b}$ complex which acts as **C5 convertase** of classical pathway.

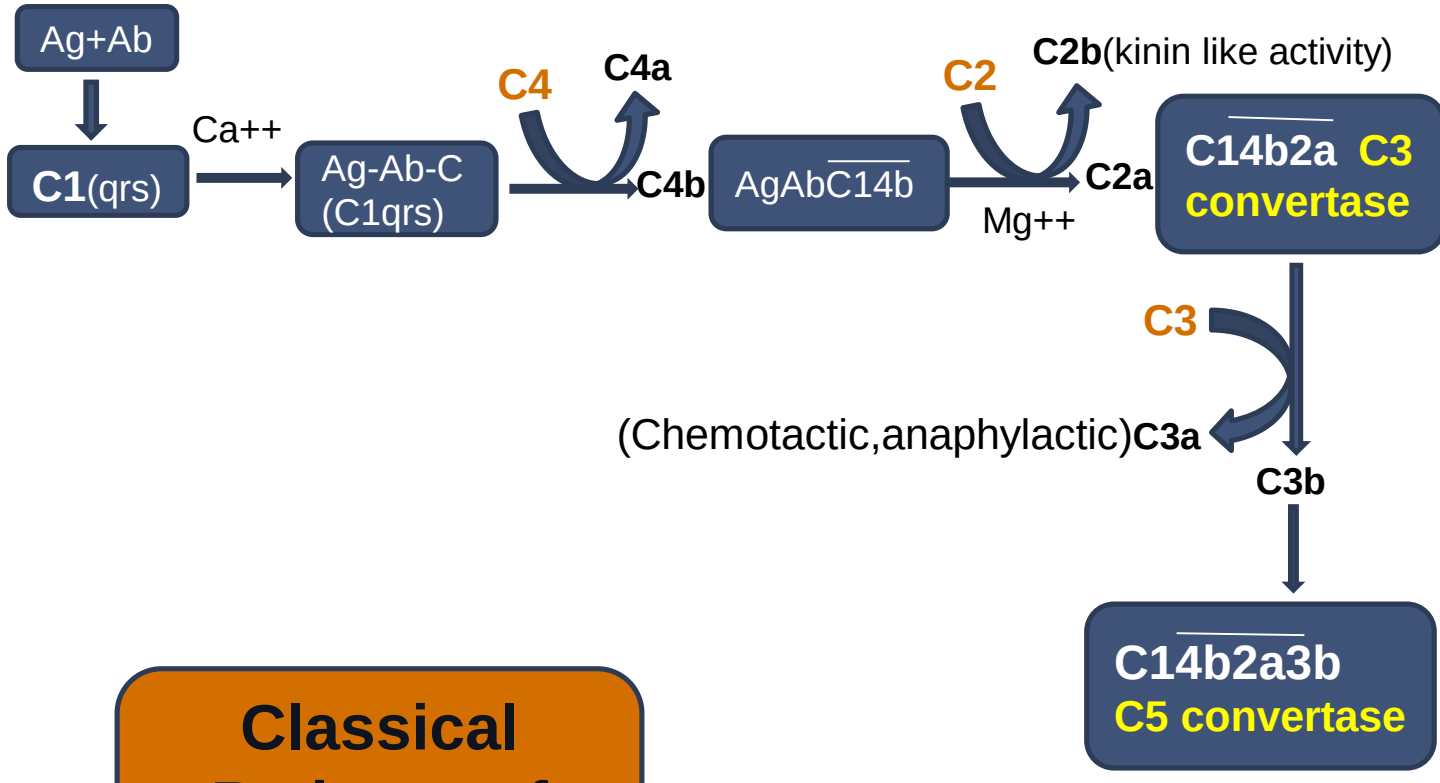
- C5 convertase cleaves C5 into **C5a** (an anaphylatoxin) and **C5b**, continues with the cascade.
  - C5b is extremely labile, gets stabilized by binding with C6 and C7 followed by addition of C8.
  - Hydrophobic regions on C7 and C8 help in penetration into the target cell membrane.
  - Inserted membrane complex ($\overline{C5b678}$) binds to C9 molecule

- Penetration of C9 ⟶ pores (10 nm) on target cell membrane
- Each tubular channel-hydrophobic outside, hydrophilic inside
- Free passage of ions & water ⟶ cellular swelling or lysis.

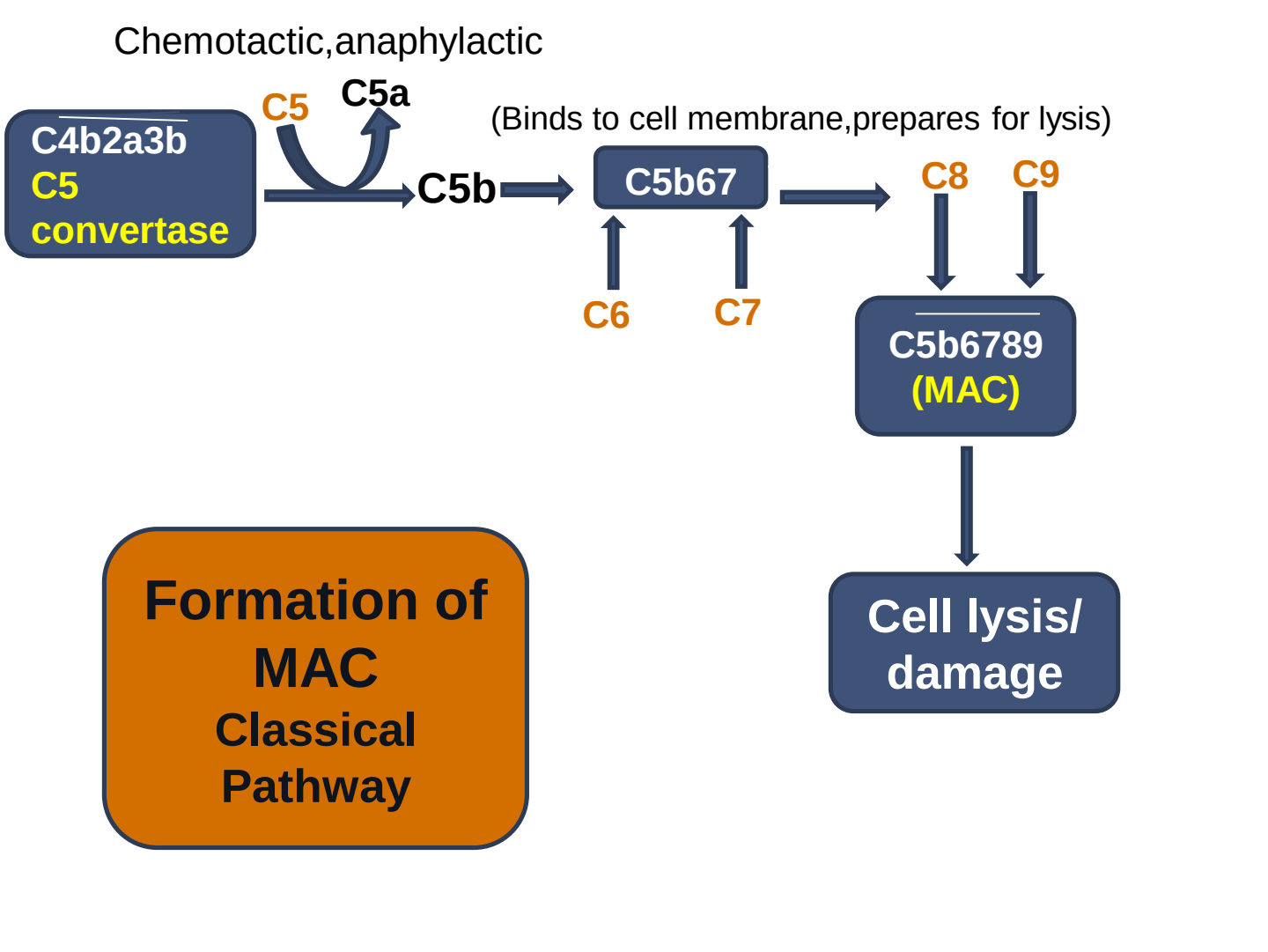


- $\overline{C5b6789}$ destroys the target cell by **MAC.**
- Process of cytolysis is ‘complement-mediated cytotoxicity’





Classical Pathway of Complement


Chemotactic,anaphylactic
C5
C5a
C4b2a3b
C5
convertase
C5b
(Binds to cell membrane,prepares for lysis)
C5b67
C6
C7
C8
C9
C5b6789
(MAC)
Cell lysis/
damage
Formation of
MAC
Classical
Pathway

2. Alternative Pathway

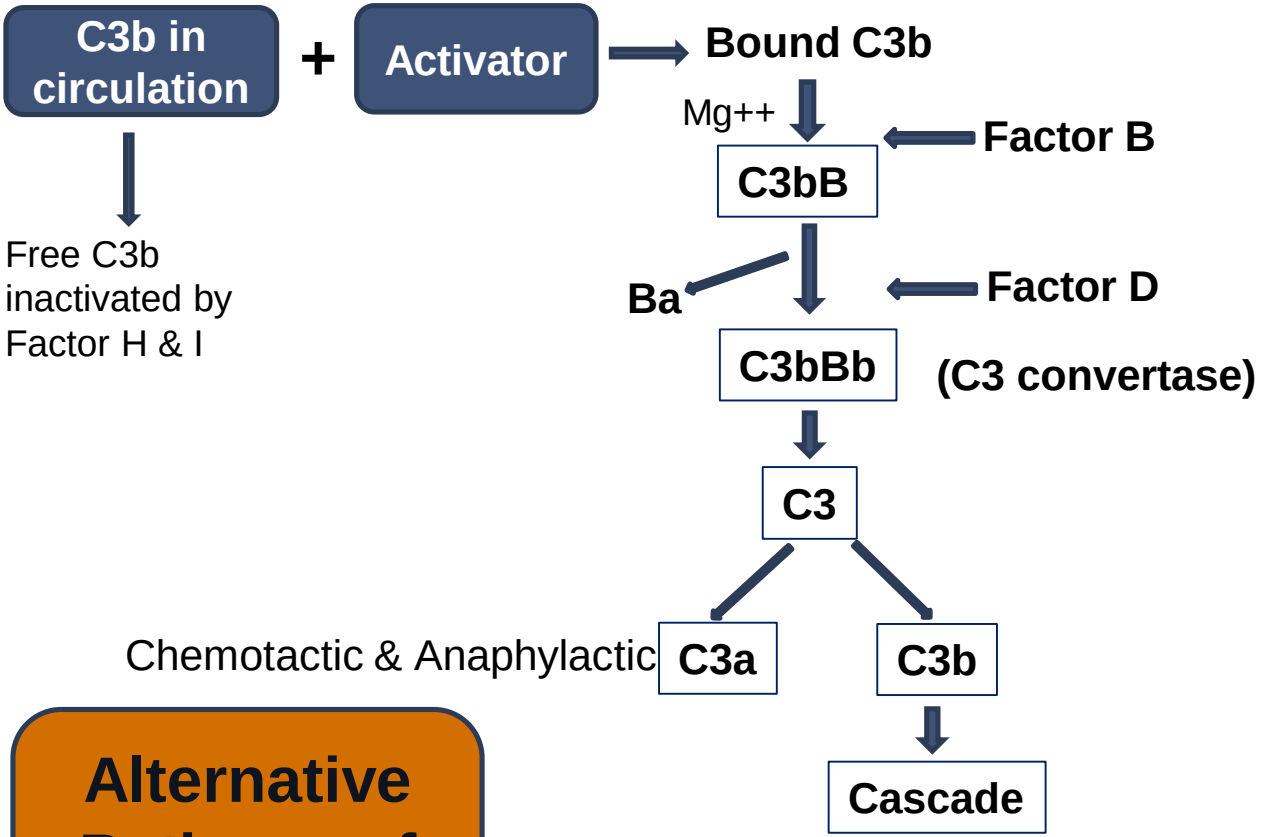
- Independent of antibody; hence a part of innate immunity.
- Four stages.
- Differs from the classical pathway in first two stages.
- Complement components C1, C4 and C2 are not involved.
- Requires three other complement proteins present in serum named **factor B, factor D and properdin**.

Alternative pathway-Initiation (Cont..)

- First component to be involved is **free C3** in the serum.
- C3 hydrolyzes spontaneously, to generate **C3a** (diffuses out) and **C3b** (attaches to foreign cell surface antigen).

Formation of C3 Convertase

- **Factor B** binds to C3b coated foreign cells.
- **Factor D** acts on factor B, cleaves it into **Ba** (diffuses out) and **Bb** (remains attached).
- $\overline{C3bBb}$ - C3 convertase.
- $\overline{C3bBb}$ has a very short half-life of 5 minutes.
- Stabilized by **properdin** (half-life is increased to 30 min.)
- C5 convertase & MAC formation-identical to the classical pathway.



Alternative Pathway of Complement

Initiators of Alternative pathway

Antigens from pathogen	Non microbial initiators
Endotoxin or LPS (lipopolysaccharide) from Gram negative bacteria	Human antibodies in complexes- IgA, IgD
Teichoic acid from Gram positive bacteria	Tumor cells
Fungal cells- Yeast cells	Cobra venom factor
	Heterologous RBCs from mouse, rabbit and chicken
Parasites like Trypanosomes	Anion polymer like dextran sulphate
Virus infected cells	Pure carbohydrates like agar, inulin

3. Lectin Pathway

- Works independent of antibody.
- Mediated through **lectin proteins** of the host that interact with **mannose** residues present on microbial surface.
- Lectin pathway involves all complement components used for classical pathways except C1.
- Instead of C1, host lectin protein called **mannose binding lectins** mediate the first 'initiation' stage.

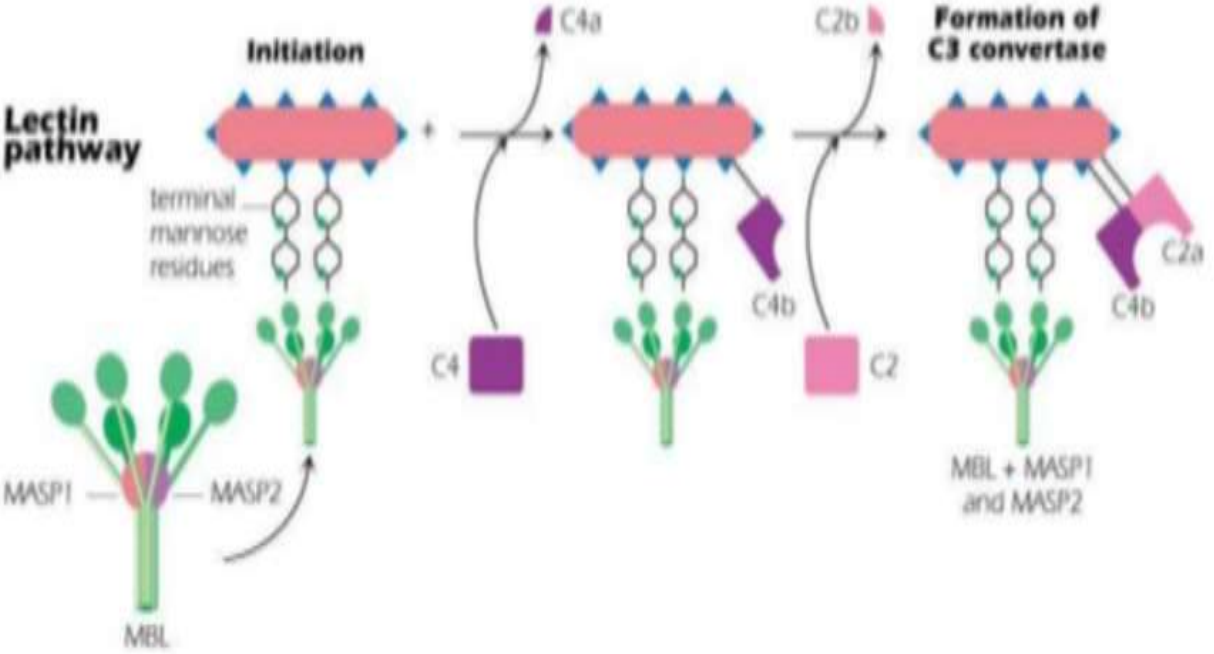
Initiation

- **Activation-**Mannose carbohydrate residues of glycoproteins on microbial surfaces.
- Mannsoe binding lectins (MBL) bind to mannose residues on microbial surface.
- **MBL:** an acute phase reactant protein, structurally similar to C1q

Initiation (Cont..)

- After binding of MBL to microbial surface, another host protein called MASP (**M**BL **A**ssociated **S**erine **P**rotease) gets complexed with MBL.
- MASP is similar or C1r and C1s and mimics their functions.
- MBL-MASP complex cleaves C4 which in turn splits C2.
- $\overline{\text{MBL/MASP-C4b2a}}$ acts as C3 convertase.

Mannose-binding Lectin Pathway



Differences between complement pathways

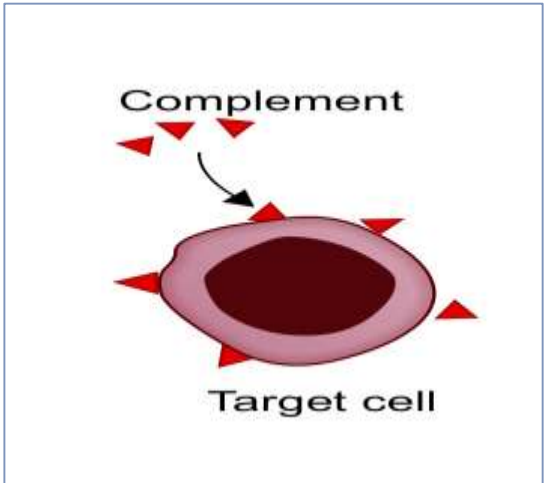
Features	Classical pathway	Alternative pathway	Lectin pathway
Activator (initiator)	Antigen + antibody complex	Endotoxin IgA, IgD, Cobra venom, Nephritic factor	Carbohydrate residue of bacterial cell wall (mannose binding protein) that binds to host lectin antigen.
1st complement activated	C1	C3b	C4
C3 convertase	$C1\overline{4b2a}$	$\overline{C3bBb}$	$\overline{MBL/MASP-C4b2a}$
C5 convertase (C3convertase+ 3b)	$C1\overline{4b2a3b}$	$C3b\overline{Bb3b}$	$\overline{MBL/MASP-C4b2a3b}$
Complement level in the serum	All C1-C9: Low	C1,C4,C2- Normal Others- Low	C1- Normal Others- Low
Immunity	Acquired	Innate	Innate

EFFECTOR FUNCTIONS OF COMPLEMENT

1. Target cell lysis by MAC

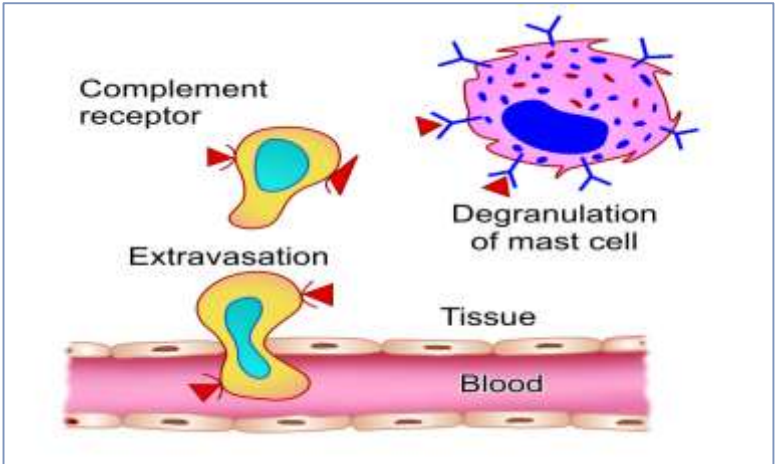
- MAC makes pores or channels in target cell membrane.
- Allows free passage of various ions and water into the cell leading to cell swelling, lysis and death.

e.g. Bacteria, enveloped viruses, damaged cells, tumour cells etc



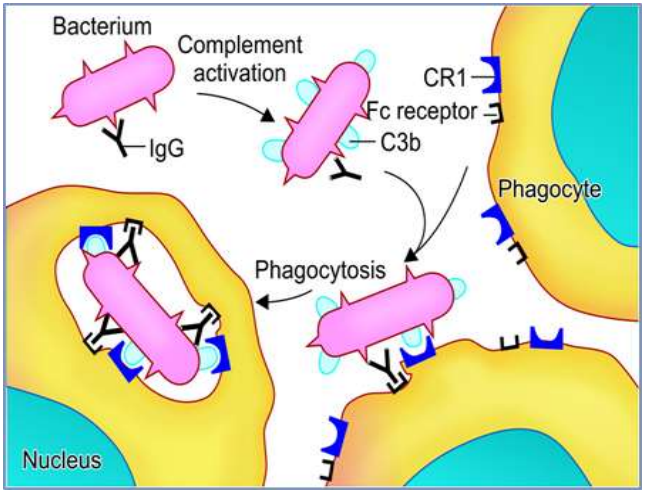
2. Inflammatory response

- C3a, C4a and C5a - Anaphylatoxins.
- Bind to surface receptors of mast cells, induce their degranulation ➡ release of histamine and other inflammatory mediators.
- Causes vasoconstriction, and increased vascular permeability.



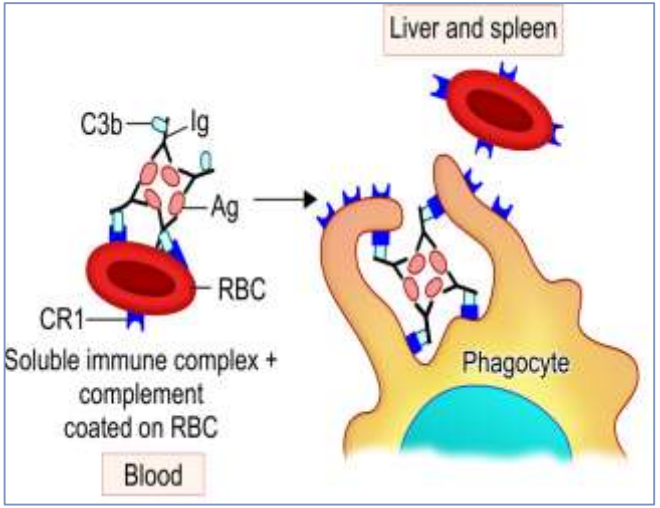
3.Opsonization

- C3b & C4b -major opsonins - coat the immune complexes and particulate antigens.
- Phagocytic cells express complement receptors for complement components (C3b, C4b).
- Bind to complement coated antigens & enhance phagocytosis.
- C5a - ↑CR1 expression on phagocytes by 10 folds.



4.Removing the immune complexes from blood

- C3b - important role.
- C3b bound immune complexes - Recognized by complement receptor CR1 present on RBCs.
- Immune complexes bound to RBCs are taken to liver ,spleen where they are phagocytosed after being separated from the RBCs.



5. Viral neutralization

- Complements coated on virus surfaces neutralize the viral infectivity by blocking their attachment sites.
- C3b mediated opsonization of viral particles
- Lysis of the enveloped viruses by:
  - Activation of classical pathway (most viruses)
  - Alternative or lectin pathways (viruses like Epstein Barr virus, rubella etc)

COMPLEMENT RECEPTORS

- Receptors play an important role in mediating the activities of complement and in their regulation.
- Many complement receptors **(CR1 to CR5)** - distributed on various cell types and bind to specific ligands to mediate specific function.
- e.g. **CR2** (present on B cells) is involved in humoral immune response - also acts as receptor for Epstein-Barr virus.

Evasion of complement system by microorganisms

Mechanisms	Examples
Shown by Gram negative bacteria	
Long polysaccharide side chain of bacteria can prevent MAC insertion	*Escherichia coli* *Salmonella*
Non covalent interactions between bacterial cell wall components can prevent MAC insertion	*Neisseria gonorrhoeae*
Elastases destroy C3a & C5a	*Pseudomonas*
Shown by Gram positive bacteria	
Thick peptidoglycan cell wall prevents MAC insertion	*Staphylococcus* *Streptococcus*
Bacterial capsule forms a physical barrier between C3b and CR1 interaction	*Streptococcus pneumoniae*

Evasion of complement system by microorganisms.....

Mechanisms	Examples
Shown by other microbes	
Proteins mimicking complement regulatory proteins	Vaccinia virus, Herpes simplex virus, Epstein-Barr virus, *Trypanosoma cruzi*, *Candida albicans*

REGULATION OF COMPLEMENT PATHWAYS

- Antigen non-specific.
- Capable of attacking microorganisms as well as host cells.
- Regulatory mechanisms: to restrict complement activity only to the designated target cells.
- Series of regulatory proteins, which inactivate various complement components at different stages.

REGULATION OF COMPLEMENT PATHWAYS (Cont..)

Examples:

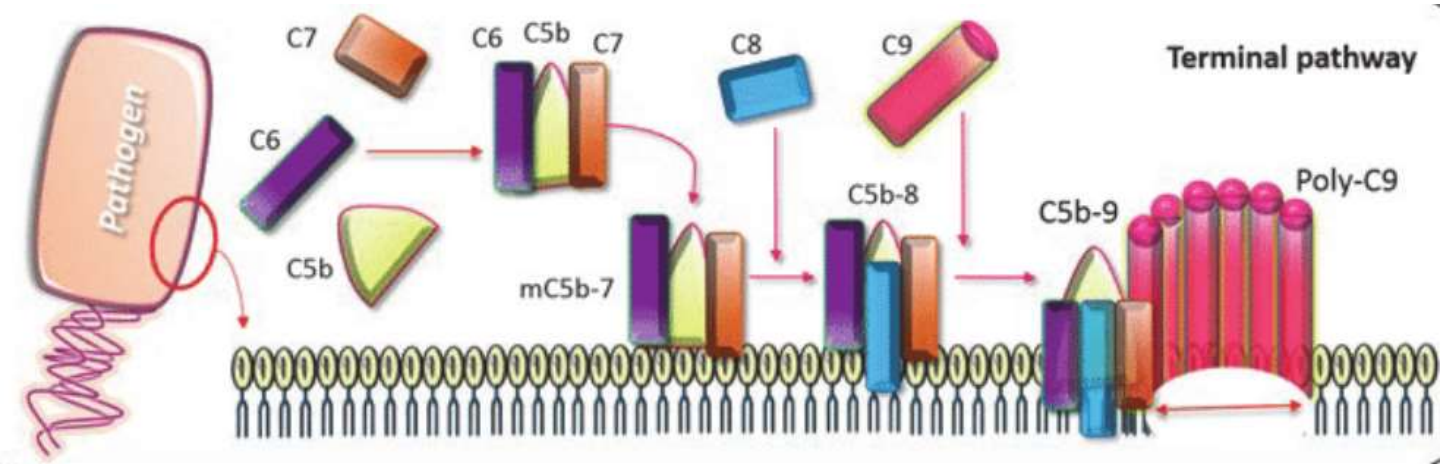
- **C1 inhibitor (or C1 esterase inhibitor):** soluble glycoprotein, inhibits the action of C1q by splitting C1qrs into C1rs and C1q - whole classical pathway is inhibited.
- **DAF (Decay accelerating factor):**CD55 molecule present on cell membrane, accelerates dissociation of C3 convertase - inhibiting all three pathways.

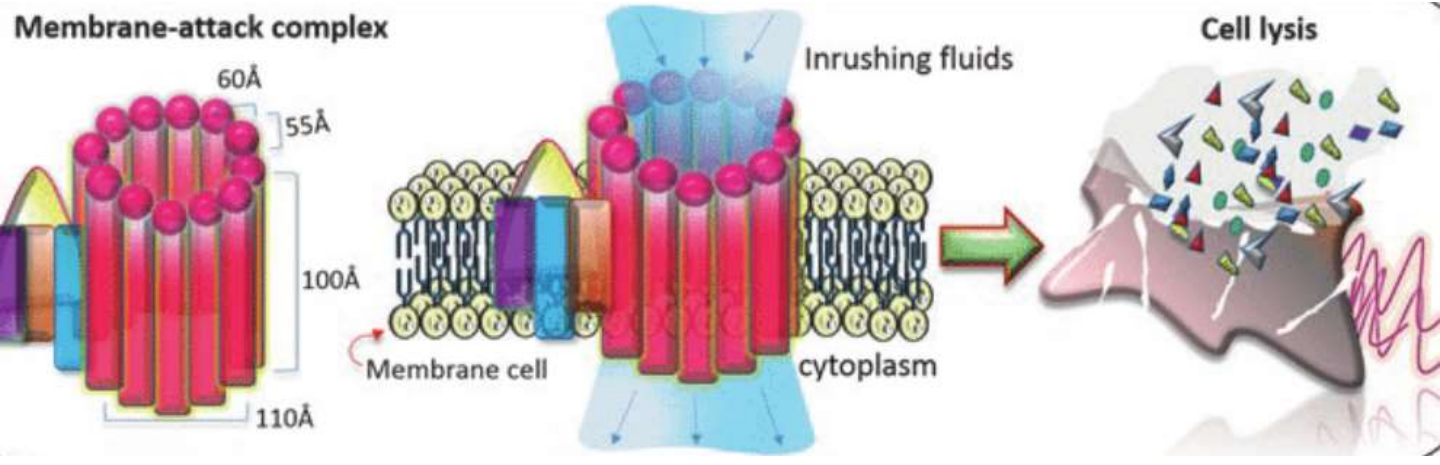
COMPLEMENT DEFICIENCIES

Complement protein deficiencies	Pathway(s) involved	Disease/pathology
C1, C2, C3, C4	C1, C2,C4-Classical pathway C3- Common deficiency	SLE, glomerulonephritis & pyogenic infections
Properdin, Factor D	Alternative pathway	*Neisseria* and pyogenic infection
Membrane attack complex (C5-C9)	Common deficiency	Disseminated *Neisseria* infection

COMPLEMENT DEFICIENCIES (Cont..)

Complement regulatory protein deficiencies	Pathway(s) involved	Disease/pathology
C1 esterase inhibitor	Overactive classical pathway	Hereditary angioneurotic edema
DAF (Decay accelerating factor) & CD59	De-regulated C3 convertase Increased RBC lysis	PNH (Paroxysmal nocturnal hemoglobinurea)


Terminal pathway
Pathogen
C7
C6
C5b
C6
C5b
C7
C8
C9
mC5b-7
C5b-8
C5b-9
Poly-C9


Membrane-attack complex
60Å
55Å
100Å
110Å
Inrushing fluids
Membrane cell
cytoplasm
Cell lysis