Complement

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Learning objectives

At the end of this session students should be able to

- Define complement
- Understand classical, alternate and lectin pathway
- Role of complement

Complement

- Represents a group of proteins normally found in serum in inactive form, but when activated they augment the immune responses.
- Complements constitute about 5% of normal serum proteins.
- Their level does not increase following either infection or vaccination.

GENERAL PROPERTIES

- Bind to Fc region of antibody
- Role of antigen
- Species nonspecific
- Heat labile

Complement Components

- Complement system comprises of about 30 serum proteins grouped into complement components, the properdin system and the regulatory proteins.
- Complement components are named by numerals. There are nine components; C1 to C9. C1 has three subunits- C1q, C1r and C1s.
- Properdin system and the regulatory proteins are named by letter symbols, e.g., factor-B

Synthesis

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

Complement activation

- All the complement proteins are synthesized in inactive form (e.g. zymogens) and are activated by proteolysis.
- Complements have two unequal fragments (large and small fragment).
- The larger fragments are usually designated as ‘b’ (e.g. C3b) and the smaller fragments are designated as ‘a’ (e.g. C3a). An exception is C2a which is larger fragment.
- During proteolysis, the smaller fragment is removed exposing the active site of the larger fragment.

Complement activation

- The larger fragment participates in the cascade reaction of complement pathway and the smaller fragment diffuses away to mediate other functions.
- **Cascade reaction-** Fragments of complements interact in a definite sequential manner with a cascade like effect, which leads to formation of complex. Such complex having enzymatic activity is designated by putting a bar over the number or symbol

  (e.g. $\overline{C3bBb}$).

COMPLEMENT PATHWAYS

- *Classical pathway*- Antibody dependent pathway. Pathway is triggered by the antigen antibody complex formation.
- *Alternative pathway*- Antibody independent pathway, triggered by the antigen directly.
- *Lectin pathway* is a recently described pathway. It resembles classical pathway but it is antibody independent.

Stages of complement activation

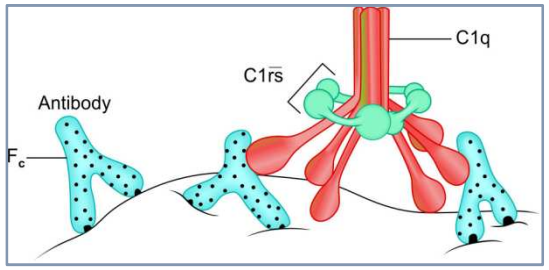
- There are four main stages in the activation of any of the complement pathways.
  - Initiation of the pathway
  - Formation of C3 convertase
  - Formation of C5 convertase
  - Formation of membrane attack complex (MAC)
- All the three pathways differ from each other in their initiation till formation of C3 convertase. Then, the remaining stages are identical in all the pathways.

CLASSICAL PATHWAY

- Antibody dependent
- Not all antibodies can bind to complements of classical pathway.
- Decreasing order of ability of antibodies to fix complement is- IgM (most potent) > IgG3> IgG 1> IgG2.
- The other classes of antibodies 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 and binding to antigen-antibody complex.

CLASSICAL PATHWAY - Initiation

- The first step is the binding of C1 to the antigen- antibody complex.
- The first binding portion of C1 is C1q, which reacts with the Fc portion of IgM or IgG bound to antigen.
- C1q is a hexamer having six globular heads each acting as a combining site.
- Effective activation of classical pathway begins only when C1q is attached to the Fc portion of antibody by at least two of its globular binding sites.



CLASSICAL PATHWAY - Initiation

- IgM being pentameric, has five Fc regions, hence one molecule of IgM can initiate the pathway.
- Whereas IgG is monomeric, therefore two IgG molecules are needed to initiate the process. Hence IgM is much efficient stimulator of classical pathway.
- C1q binding in the presence of calcium ions, in turn activates sequentially C1r followed by C1s.

CLASSICAL PATHWAY - Formation of C3 convertase

- Activated C1s acts as an esterase (C1s esterase), which can cleave C4 to produce C4a (an anaphylatoxin), and C4b which binds to C1 and participates further in complement cascade.
  - oC14b in the presence of magnesium ions cleaves C2 into C2a, which remains linked to complement complex, and C2b (has kinin like activity), which is released outside.
  - oC14b2a is referred to as C3 convertase of the classical pathway.

CLASSICAL PATHWAY - Formation of C5 convertase

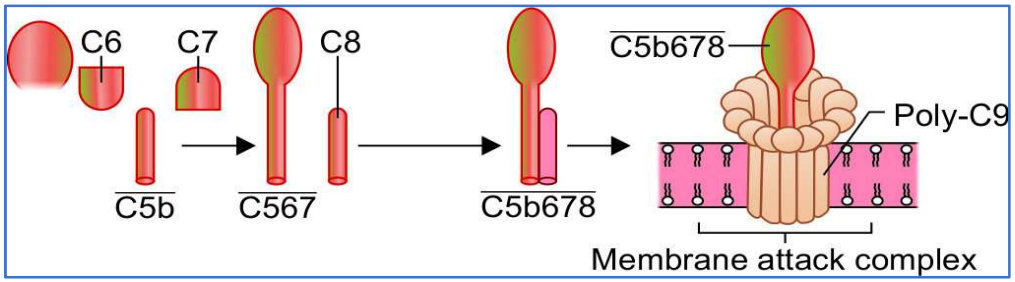
- C3 convertase hydrolyses many C3 molecules into two fragments:
  - oC3a (an anaphylatoxin)
  - oC3b which remains attached to C14b2a to form C14b2a3b complex which acts as C5 convertase of classical pathway.

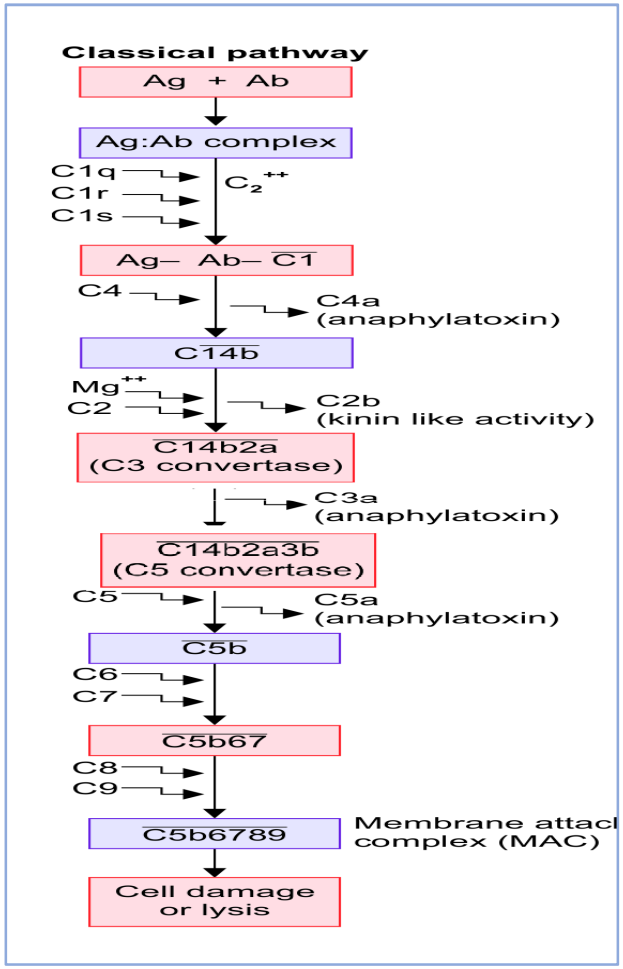
CLASSICAL PATHWAY – Formation of Membrane attack complex

- Begins with C5 convertase cleaving C5 into C5a (an anaphylatoxin, released into the medium) and C5b, which continues with the cascade.
  - oC5b is extremely labile, gets stabilized by binding soon with C6 and C7 to form C5b67 followed by addition of C8.
  - oHydrophobic regions on C7 and C8 help in penetration into the target cell membrane.
  - oThis inserted membrane complex (C5b678) has a catalytic property to bind to C9 molecule and then it polymerizes the C9 into a tubular channel of 10 nm diameter

CLASSICAL PATHWAY –
Formation of Membrane attack complex

- Penetration of C9 - channels or pores on the target cell membrane
- Each tubular channel - hydrophobic outside, hydrophilic inside - free passage of ions and water into the cell - cellular swelling - lysis.
- $\overline{C5b6789}$ destroys the target cell by attacking the cell membrane – MAC,
- Process of cytolysis is referred to as complement-mediated cytotoxicity.




Classical pathway
Ag + Ab
Ag:Ab complex
C1q
C1r
C1s
C_2^{++}
Ag– Ab– $\overline{C1}$
C4
C4a
(anaphylatoxin)
$\overline{C14b}$
Mg^{++}
C2
C2b
(kinin like activity)
$\overline{C14b2a}$
(C3 convertase)
C3a
(anaphylatoxin)
$\overline{C14b2a3b}$
(C5 convertase)
C5
C5a
(anaphylatoxin)
$\overline{C5b}$
C6
C7
$\overline{C5b67}$
C8
C9
$\overline{C5b6789}$
Membrane attacl
complex (MAC)
Cell damage
or lysis

ALTERNATIVE PATHWAY

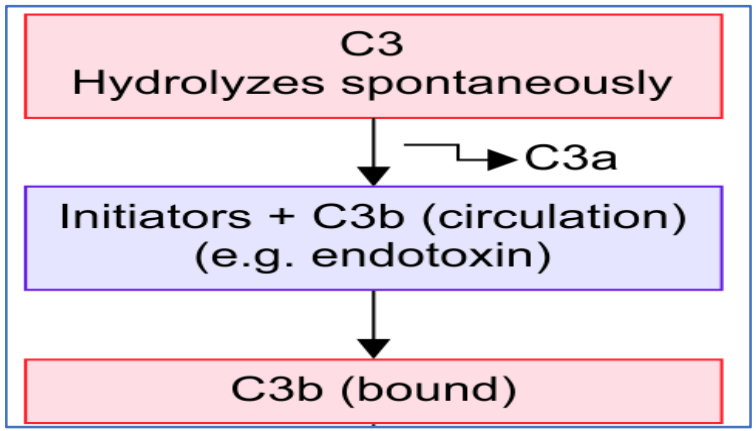
- Independent of antibody; hence is considered as a part of innate immunity.
- Four stages.
- Differs from the classical pathway in first two stages.
- Three complement components C1, C4 and C2 are not involved. Requires three other complement proteins present in serum named factor B, factor D and properdin.

ALTERNATIVE PATHWAY- Initiation

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	Cobra venom factor
Yeast cells	Heterologous RBCs from mouse, rabbit and chicken
Parasites like Trypanosomes	Anion polymer like dextran sulphate
Virus infected cells	Pure carbohydrates like agar, inulin

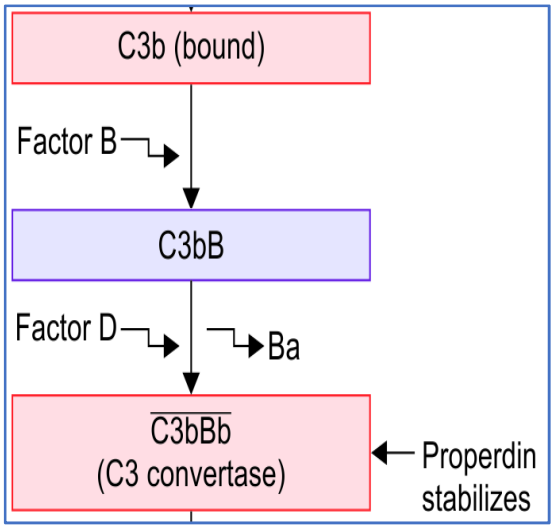
ALTERNATIVE PATHWAY

- First complement component to be involved in alternative pathway is free C3 in the serum.
- C3 hydrolyzes spontaneously, to generate C3a which diffuses out and C3b fragment which attaches to foreign cell surface antigen.



ALTERNATIVE PATHWAY-
Formation of C3 convertase

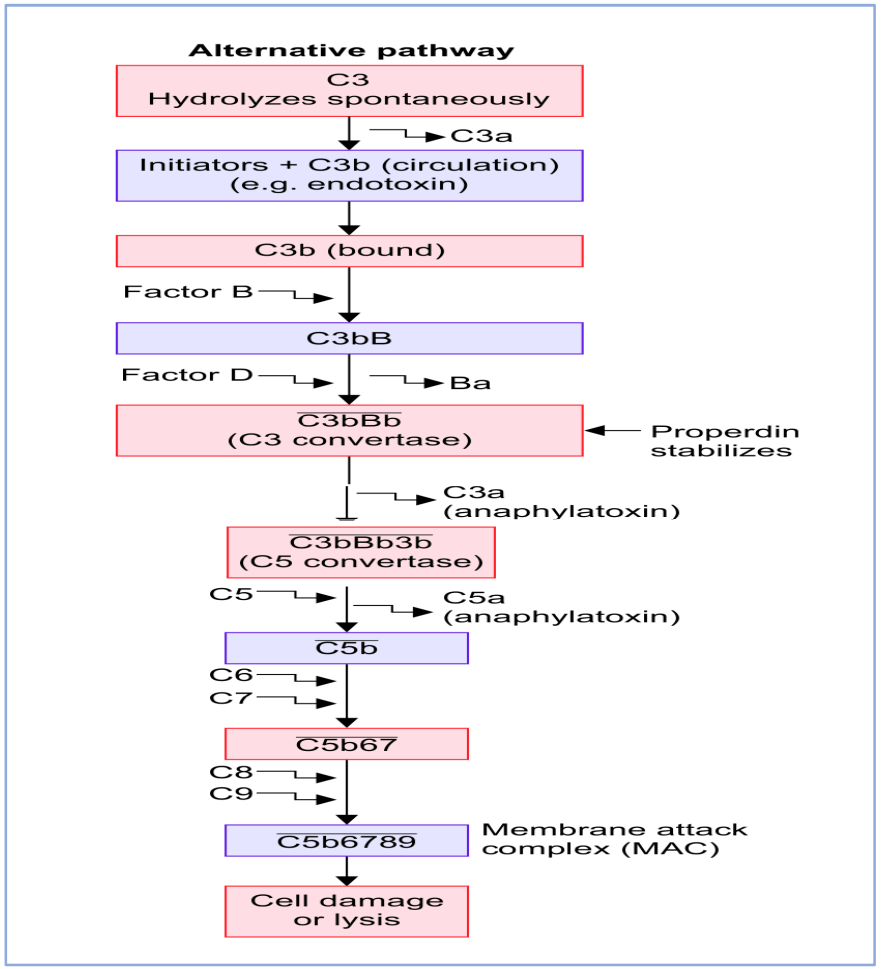
- **Factor B** binds to C3b coated foreign cells.
- **Factor D** - acts on factor B, and cleaves it into Ba (diffuses out) and Bb (remains attached).
- C3bBb - C3 convertase.
- C3bBb has a very short half-life of 5 minutes.
- Stabilized by properdin (half-life is increased to 30 minutes).



ALTERNATIVE PATHWAY

- Formation of C5 convertase.
- Formation of MAC

} Identical to that of classical pathway.

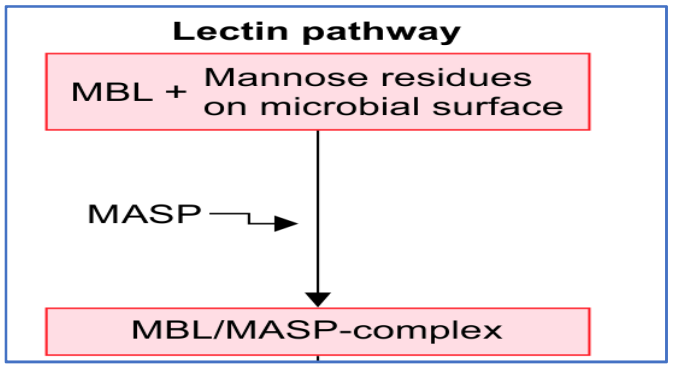

Alternative pathway
C3
Hydrolyzes spontaneously
C3a
Initiators + C3b (circulation)
(e.g. endotoxin)
C3b (bound)
Factor B
C3bB
Factor D
Ba
C3bBb
(C3 convertase)
Properdin
stabilizes
C3a
(anaphylatoxin)
C3bBb3b
(C5 convertase)
C5
C5a
(anaphylatoxin)
C5b
C6
C7
C5b67
C8
C9
C5b6789
Membrane attack
complex (MAC)
Cell damage
or lysis

LECTIN PATHWAY

- Complement pathway of innate immunity -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.

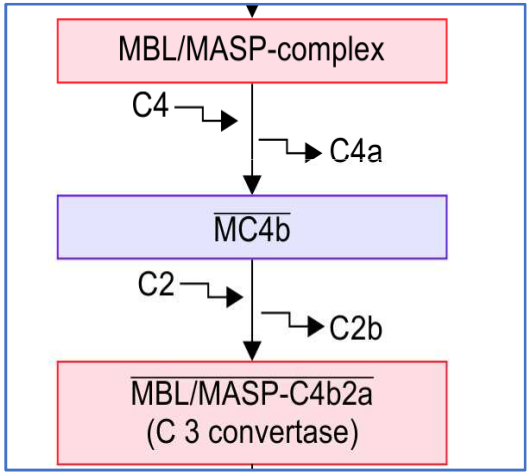
LECTIN PATHWAY-
Initiation

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



LECTIN PATHWAY-
Initiation

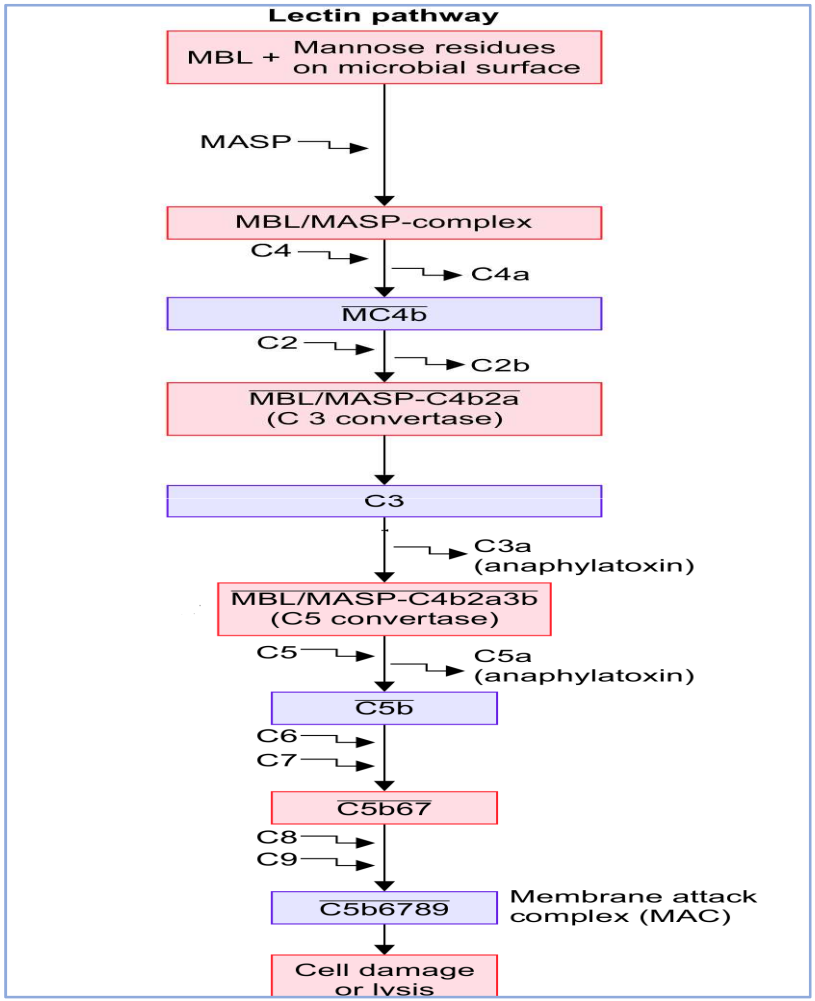
- After binding of MBL to microbial surface, another host protein called MASP (MBL associated serine protease) gets complexed with MBL.
- MASP is similar or C1r and C1s and mimics their functions.
- MBL-MASP complex cleaves C4 which in turn splits C2 and the MBL/MASP-C4b2a acts as C3 convertase.



ALTERNATIVE PATHWAY

- Formation of C5 convertase.
- Formation of MAC

} Identical to that of classical pathway.


Lectin pathway
MBL + Mannose residues on microbial surface
MASP
MBL/MASP-complex
C4
C4a
MC4b
C2
C2b
MBL/MASP-C4b2a (C 3 convertase)
C3
C3a (anaphylatoxin)
MBL/MASP-C4b2a3b (C5 convertase)
C5
C5a (anaphylatoxin)
C5b
C6
C7
C5b67
C8
C9
C5b6789
Membrane attack complex (MAC)
Cell damage or lysis

Differences between the three complement pathways

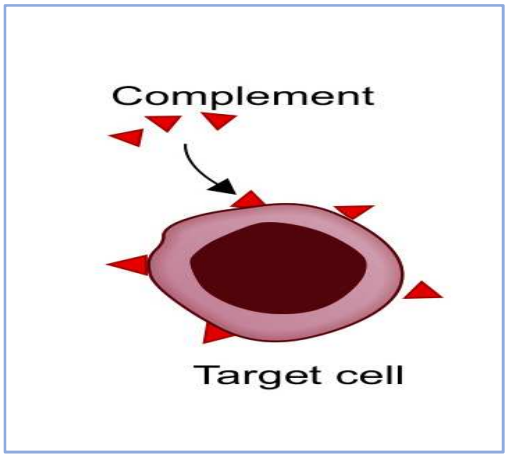
	Classical pathway	**Alternative pathway**	**Lectin pathway**
Activator (initiator)	Antigen antibody complex	Endotoxin IgA, IgD, Cobra venom, Nephritic factor	Carbohydrate residue of bacterial cell wall (mannose binding protein) that binds to host lectin antigen.
1st complement activated	C1	C3b	C4
C3 convertase	C14b2a	C3bBb	MBL/MASP-C4b2a
C5 convertase (C3 convertase + 3b)	C14b2a3b	C3bBb3b	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

- MAC and other complement by-products produced during the activation augment the immune response in many ways.
  - oTarget cell lysis by MAC
  - oInflammatory response
  - oOpsonization
  - oRemoving the immune complexes from blood-
  - oViral neutralization

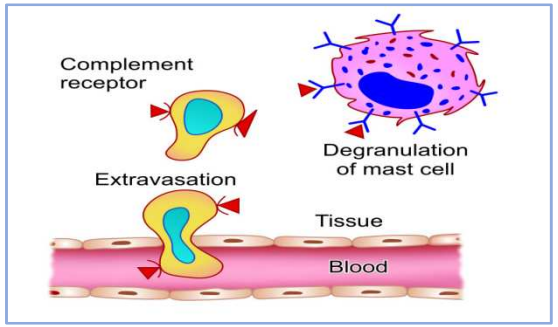
Target cell lysis by MAC-
complement mediated cell lysis

- MAC makes pores or channels in the target cell membrane.
- Allows the free passage of various ions and water into the cell leading to cell swelling, lysis and death.
- E.g. Bacteria, enveloped viruses, damaged cells, tumor cells, etc



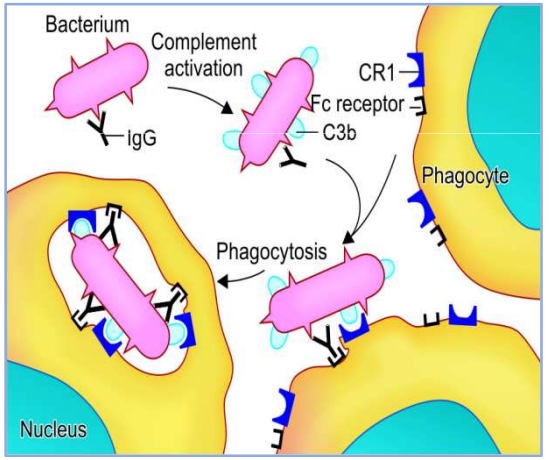
Inflammatory response

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



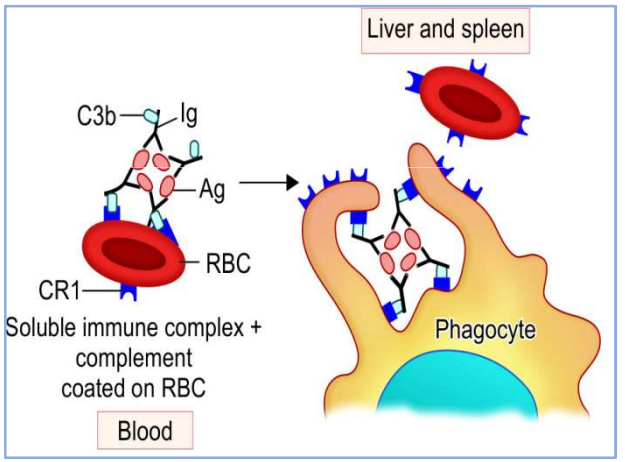
Opsonization

- C3b and C4b - major opsonins - coat the immune complexes and particulate antigens.
- Phagocytic cells express complement receptors (CR1, CR3 and CR4) for complement components (C3b, C4b).
- Bind to complement coated antigens and enhance phagocytosis.
- C5a - enhances the CR1 expression on phagocytes by 10 folds.



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 and spleen where they are phagocytosed after being separated from the RBCs.



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

CR	Ligands	Distribution	Function
CR1 (CD35)	C3b, C4b	RBCs, phagocytes All blood cells	1. Regulates complement pathway by inhibiting C3 convertase 2.Helps in removal of immune complexes
CR2 (CD21)	C3d, C3dg	B cells, T cells, Follicular dendritic cells	1. Forms a part of B cell co-receptor-involved in humoral responses 2. Acts as EBV receptor
CR3, CR4	iC3b	Phagocytes	1. Opsonization 2. Binding and extravasation of neutrophils
CR3a, CR4a, CR5a	C3a, C4a, C5a	Mast cells, Basophils	Degranulation of mast cells and basophils

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*

EVASION OF COMPLEMENT SYSTEM BY MICROORGANISMS

Mechanisms	**Examples**
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*
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

Regulatory proteins	Pathway affected	Type of protein	Regulatory function
C1 regulator			
C1 inhibitor (C1 Inh, or C1 esterase inhibitor)	Classical only	Soluble	It is a glycoprotein, inhibits the action of C1q by splitting C1qrs into C1rs and C1q. Thus the whole classical pathway is inhibited
C3 convertase regulators			
C4b-binding protein (C4bBP)	Classical and lectin	Soluble	It blocks formation of C3 convertase by binding C4b; It acts as cofactor for cleavage of C4b by factor I
CR-1(Complement-receptor-1)	All three pathways	Membrane bound	Blocks formation of C3 convertase by binding C3b or C4b
MCP(Membrane-cofactor protein)			
Factor H	Alternative only		

REGULATION OF COMPLEMENT PATHWAYS

Regulatory proteins	Pathway affected	Type of protein	Regulatory function
C3 convertase regulators..			
DAF (Decay accelerating factor) or CD55	All three pathways	Membrane bound	Accelerates dissociation of C3 convertase
Factor-I	All three pathways	Soluble	Cleaves C4b or C3b by using C4b-binding protein
MAC formation regulators			
S protein	All three pathways	Soluble	Binds soluble C5b67 and prevents its insertion into cell membrane
Membrane inhibitor of reactive lysis (MIRL or CD59)		Membrane bound	Inhibit MAC formation by blocking C9 binding
Homologous restriction factor		Membrane bound	Inhibit MAC formation by blocking C9 binding

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

Complement regulatory protein deficiencies		**Diseases**
C1 esterase inhibitor	Overactive classical pathway	Hereditary angioneurotic edema
DAF (Decay accelerating factor) & CD59	Deregulated C3 convertase Increased RBC lysis	PNH (Paroxysmal nocturnal hemoglobinurea)
Factor I	Deregulated classical pathway with over consumption of C3	Immune complex disease; recurrent pyogenic infections
Factor H	Deregulated alternative pathway with increased C3 convertase activity	Immune complex disease; pyogenic infection