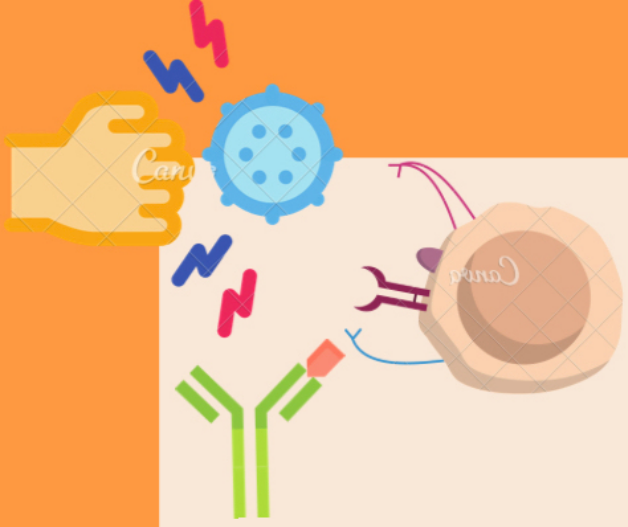
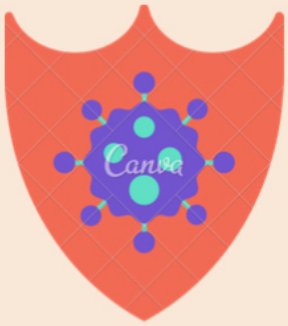


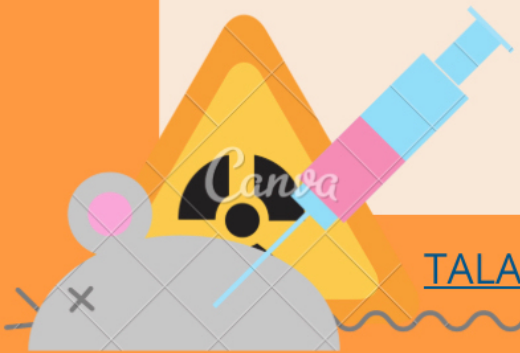
2nd year

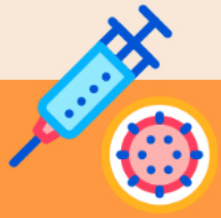


Immunology notes

Molecules of the immune system:

- complement
- cytokines
- MHC Molecules





TALA IYAD

1) Complements

C1 C3 C4 C4

complement system : group of proteins that are normally present in the blood produced by

1) liver (most of complement molecules)

2) C1 produced by the mucous of git

3) many of them produced by macrophages

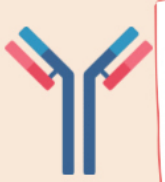
After production they released to bloodstream

1) no immune response thus inactive (pro enzymes)

2) infection lead to activation

- Mechanism of action :

3 pathways:

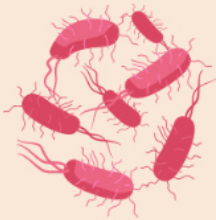


1) classical pathway : antibody involved humeral immunity

2) alternative pathway : no antibodies

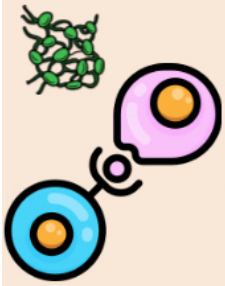
3) mannone binding pathway: innate immunity (lactic pathway)

1) classical pathway: 1)humeral immunity activation

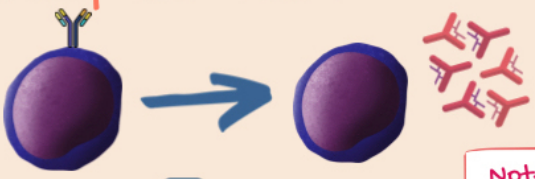


1) invasion of the body by bacteria lead to infection

2) Phagocytosis by macrophage ,, then migrate to closet lymph node



3) macrophage present antigen to t helper which then activate B cells



4) B cell ➡ Plasma cells (

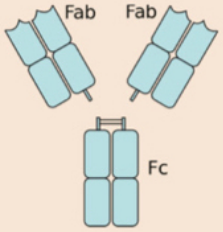
Antibody on the surface

antibody secreting cells

Note :antibodies involved in complement activation are IgM & IgG

- Revision: antibody structure:

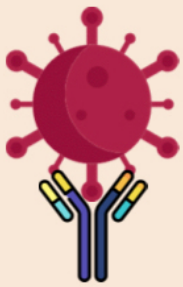
Immunoglobulins are classified as IgA ,IgD ,IgE,IgG,IgM. epitopes binds immunoglobulin on paratopes (antigen binding site)

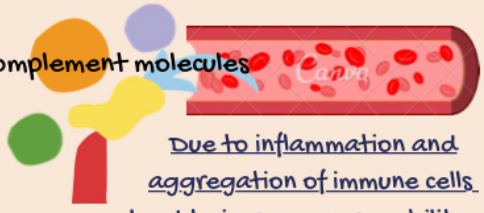


Tail portion of the antibody is called Fc prtion where it binds to the Receptor and the receptor is named according to this portion

TALA IYAD

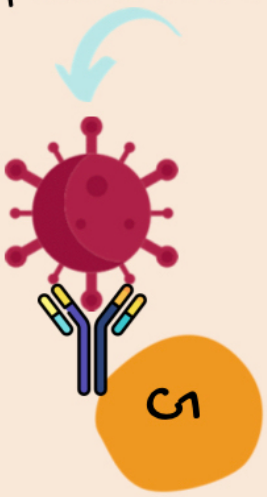
1) classical pathway: 1)humeral immunity activation





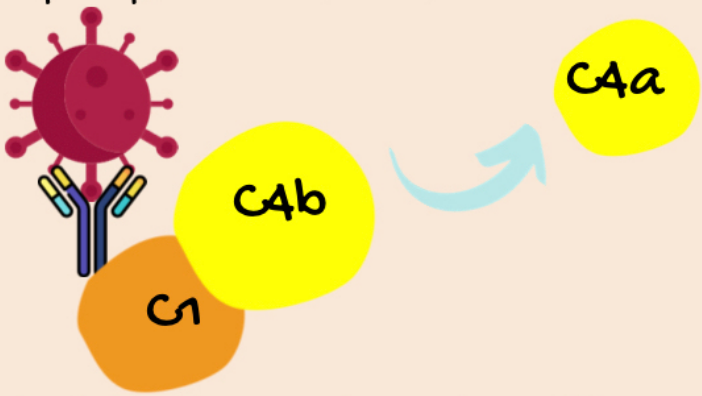
Due to inflammation and aggregation of immune cells lead to increase permeability of vessel allowing complements aggregation

5) Epitope binds the antibody and triggers a conformational change in the tail (fc portion) that allow bindingto complements

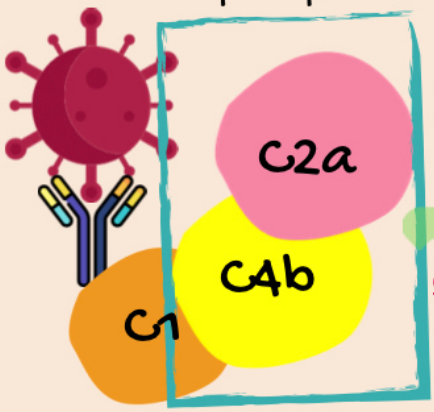


note: if Ig not binding to antigen and no change of FC so the loop That bind to complement won't be exposed and that explains why Abs and complement aren't always activated when they circulate

6) C1q activated by the Ab which then activateother complement molecule C4 and hydrolyse it into C4a & C4b

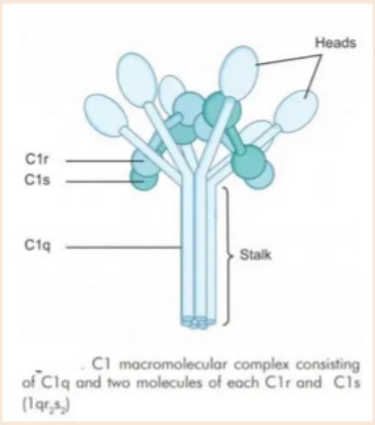


7) C4b bind the complex and C4a diffuse away , then C4b attract and activate C2 which also hydrolyse into C2a and C2b .



C4b2a complex are called c3 convertase that activate C3

- structure of C1:



Has 3 protein domain (QRS), Q interact with the antibody, and only domain that activated

- structure of other complements :

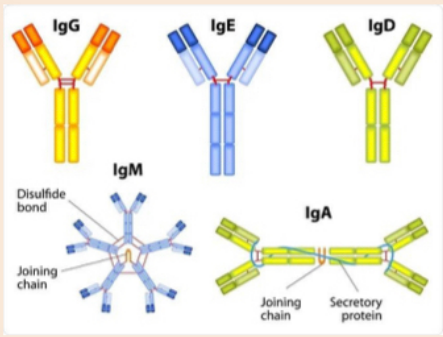
most of complements consist of 2 domain that are associated togatherincase of inactive and dissociate when activated

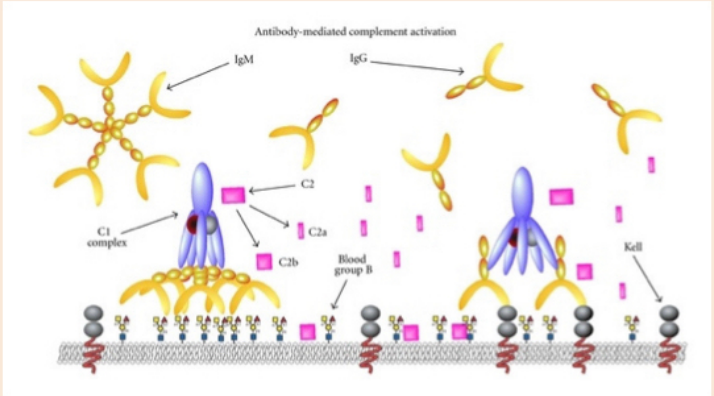
- Ig complement notes:

Immunoglobulin that activates complement system:

1) one molecule of IgM

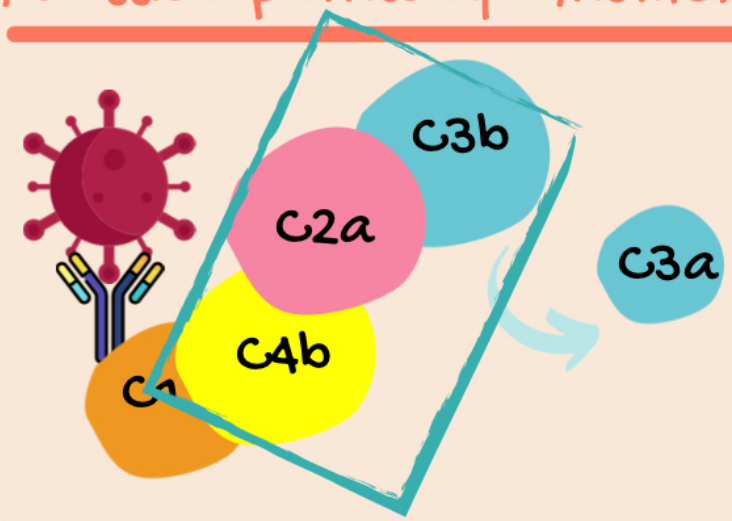
2) 2 molecules of IgG (4 type only 1-3 can activate)



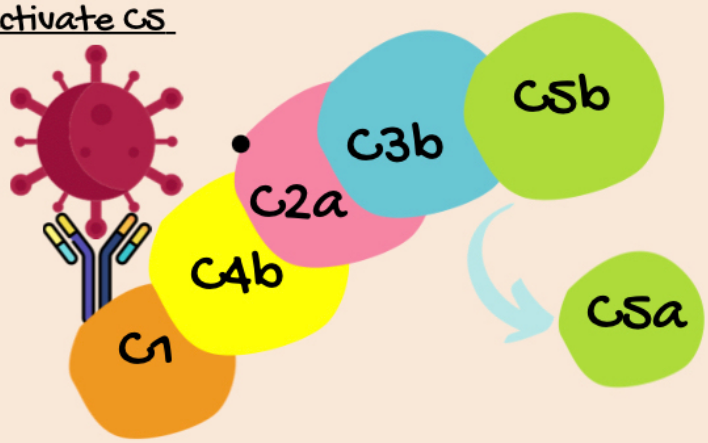


TALA IYAD

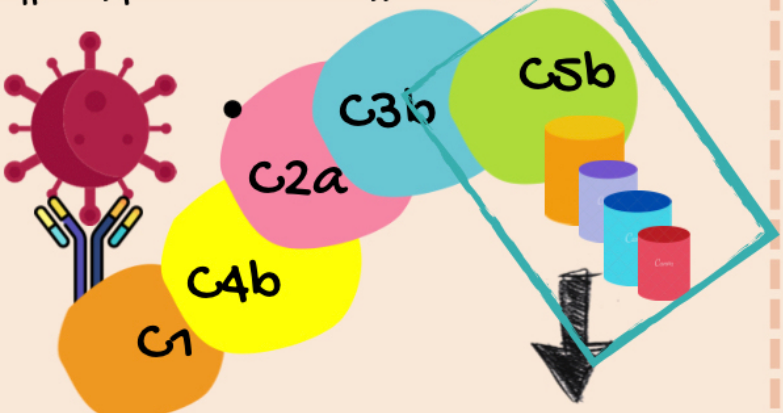
1) classical pathway: 1)humeral immunity activation



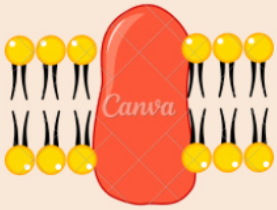
8) C4b2a3b complex (C5 convertase) that activate C5



9)C5b will activate C6-C9 , which are special type of protein with different structure

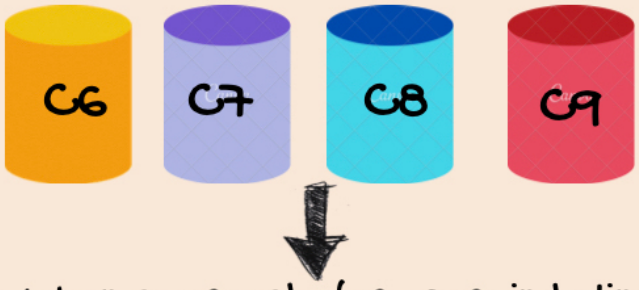


10)C5b - C9 are called membrane attack complex , disturb the physical integrity of cell membrane by dissolving into lipid bilayer and allow leak out of substances and entrance ofunwanted thing into the cell lead to kill the bacteria



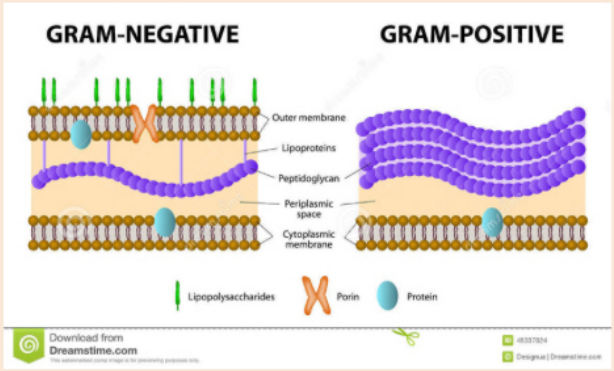
this mechanism is more effective on gram negative bacteria

- Structure of C6-C9 :



- when aggregate form pore including C5b

- Gram positive vs gram negative bacteria:



People who lack the C6-C9 or any of them more susceptible to gram negative bacteria infection

- Complement receptor and gram positive bacteria:

C3b has receptor on macrophage and neutrophils that bind it and activate phagocytosis

Aggregation of complement complex C1 +C4b +C2a + C3b then bind to C3bR on macrophage

Note :

Mainly RBCs are coated by IgG and thus it may be opsonisation by C3b and lead to phagocytosis by macrophage to RBCs which may lead to haemolytic anemia

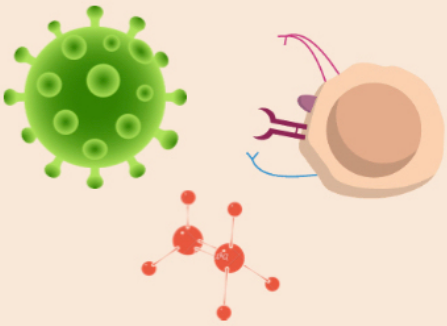
TALA IYAD

2) Alternate pathway that Abs aren't involved:

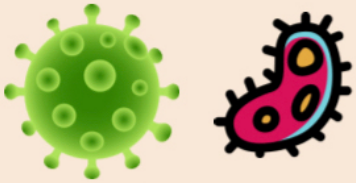
classical pathway requires production of Abs that need needs time to be produced thus there's more quick pathway until classical pathway activation that need for Abs

• Alternative pathway :

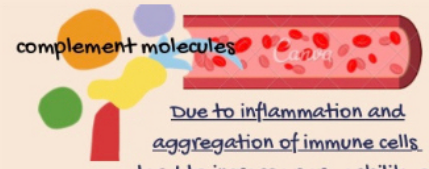
- induced by endotoxins
- induced by IgA

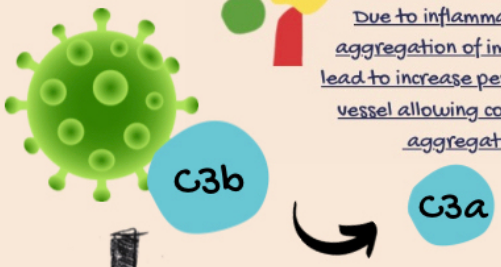


• Alternative pathway : induced by endotoxins



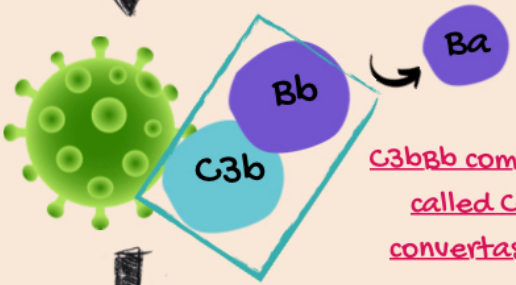
1) PAMPS and surface molecules that present on pathogens can directly activate C3 to give more quick response than classical pathway.





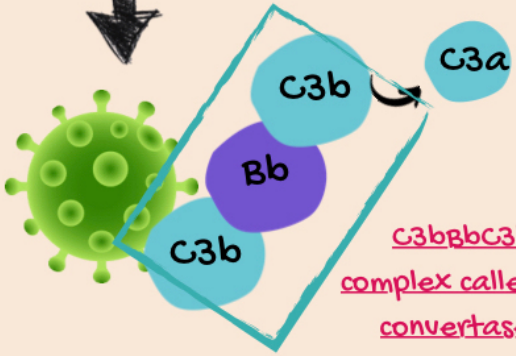
2) LPs are examples of PAMPS that can activate C3 present on gram- bacteria, that hydrolyse C3 into 2 domains C3b diffuse away and C3b bind to the LPS and C3b activate another complement protein called B ,which also split into Bb , and Ba diffuse away by D factor .

3) C3 convertase activate another C3 molecule in the same way to form C5 convertase

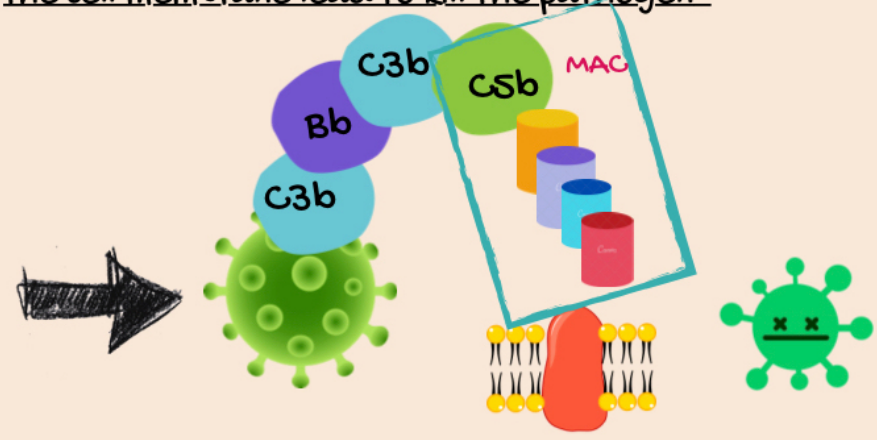


C3bBb complex called C3 convertase

4) C5 convertase activates C5 and C5b activate membrane attack complex which then forms pores in the cell membrane lead to kill the pathogen



C3bBbC3b complex called C5 convertase

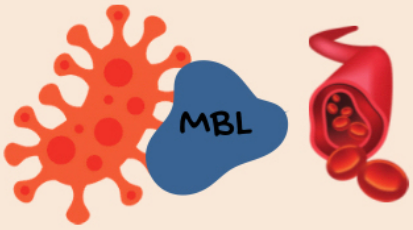


TALA IYAD

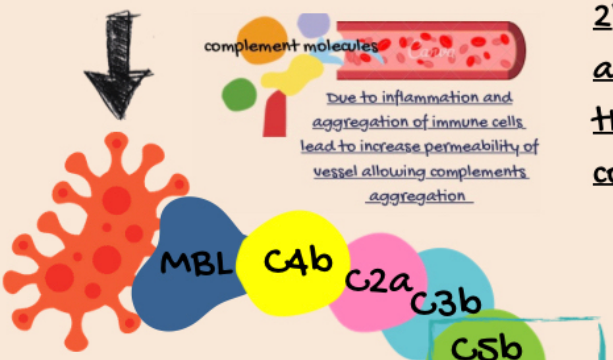
3) mannose binding pathway: an innate immune

Mannose molecule present on surfaces of many bacteria,,, mannose binding lectin a protein which is normally present in the blood

• mannose binding pathway: an innate immune

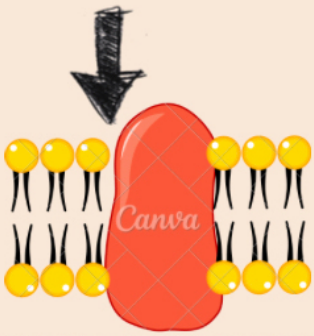


1) mannose binding lectin bind Mannose on bacterial surface and activate proteolytic enzymes which are actually C4 and C2



2) C4b and C2a are C3 convertase that activate C3b and C4b2a3b are C5 convertase that activate C5 which then activate and Form membrane complex (MAC)

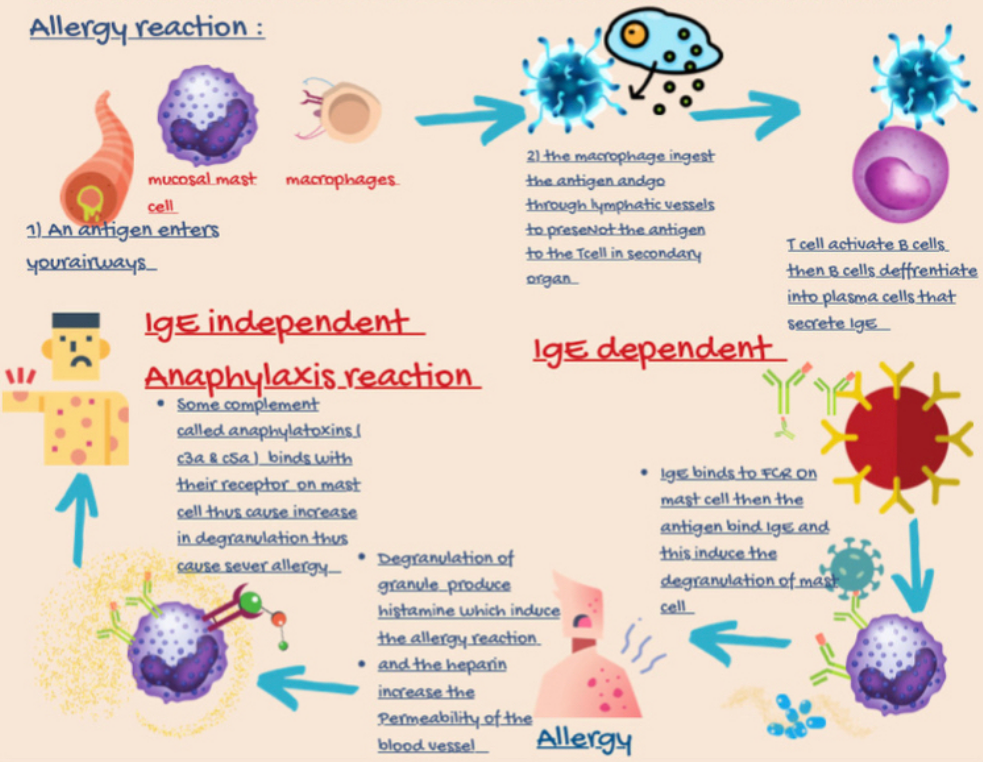
3) membrane attack complex form pores that disturb the bacterial membrane integrity and kill bacteria



• function of a domains of complement system :

Remember :

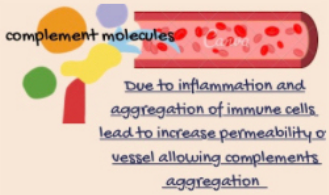
so C5b and C3a act as anaphylactic molecules on granulocytes specifically mast cells



TALA IYAD

• function of a domains of complement system :

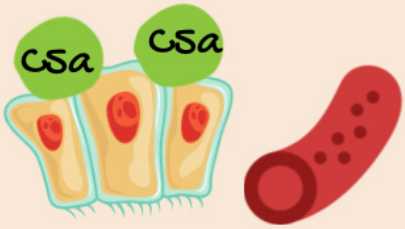
2) function of a domains of complement S :



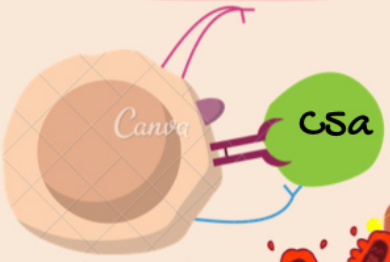
1) Act as anaphylaxis

2) bind to endothelial cells and infancy expressions of adhesion molecules

3) act as chemotaxis for neutrophils

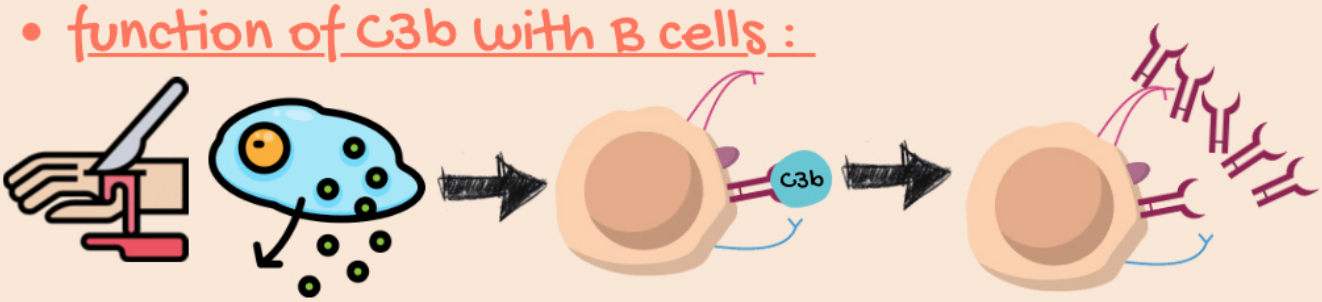


CSa bind to its receptor on endothelium cells to increase expression of adhesions to allow migration of immune cells to site of infection



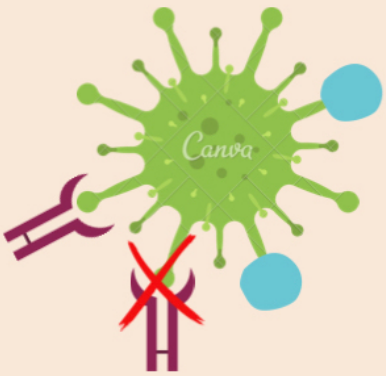
CSa bind's it's receptor on neutrophils and attract it to site of infection

• function of C3b with B cells :



- phago By APCSdue to infection ➡ Migrate to closet lymph node ➡ Activate T helper which then activate B cells ➡ Differentiate to plasma cells. That secret immunoglobulins
- here C3b bind to underdiffrentiation B cell to its receptor and act as antibody producing amplifier
- thus patiants that lack C3 produce Abs in low level

• Virus neutralising proteins:

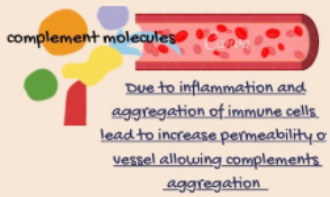


- each virus has surface molecules that can bind to specific receptors in our body and enter the cells and cause infection
- some complement act to neutralise these pathogenic molecules by coating them and prevent their binding to specific receptors in our body
- mainly are complement: C1 &C2 & C3

TALA IYAD

• Regulations of complement function:

1) classical pathway regulations:



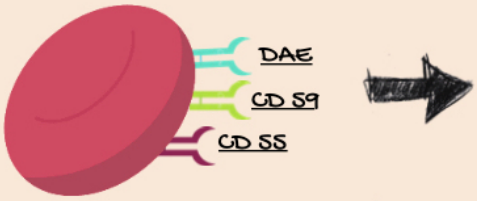
1) Prescence of Ab For C1 activation

2) Prescence of antigen for Ab activation

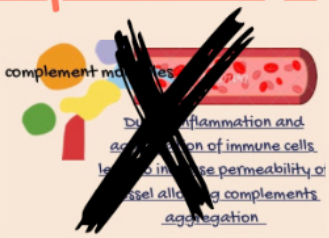
3) C1 esterase inhibitor (Ifactor)andIt factor that negative regulators

- C1INH that inhibit function of C1 important, in case of deficiency of this protein to inhibition for C1 thus activate
- more complements molecules and producing more C5a and C4a and lead to enhance the inflammation and produce edema
- and anaphylactic reaction
- this called heredity angioedema

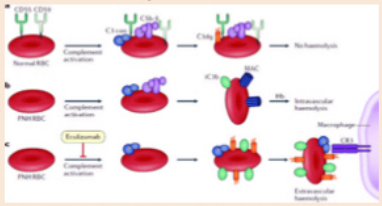
• Body cell protection against activated complements :



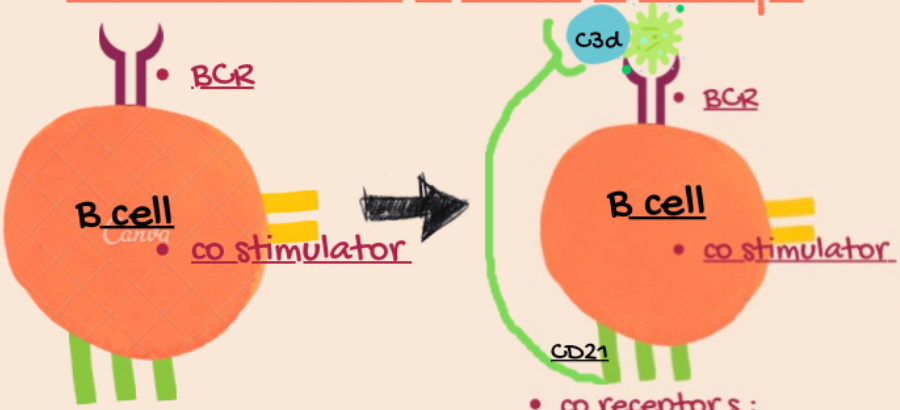
- Complement inactivators



- RBCS express on their surfaces molecules that inactivate complement
- when these molecules are deficient ▶ Complement opsonise RBCs and cause heamolysis
- increase globulin secretion in urine due to heamolysis
- paroxysmal nocturnal heamoglobinuria



• Cd3 and humeral immunity:



- co receptor s : CD21 ,CD19 ,CD81

- co receptor s : CD21 ,CD19 ,CD81

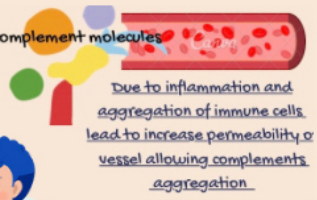
- C3b molecule that produced from MBL and classical pathway can be further cleavage into C3d and other molecul
- C3d can bind to CD21 a complement receptor type 2 present on B cell surface and in the same time bind to antigen on B cell receptor
- thus Ag binding send ICs that accelerate B cell activation

- CD 21 :
  - receptor for C3d
  - receptor and entrance for Epstein bar virus

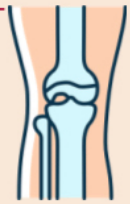
TALA IYAD

• Immune complex and complements :

1) immune complexs that circulate in blood are too heavy and insoluble
2) immune complexes start to deposit in blood vessels and tissues
3) the body attack these immune complexes
4) autoimmune disease develop
(SLE -RA-DM)

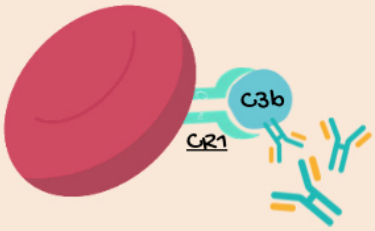


Due to inflammation and aggregation of immune cells lead to increase permeability o vessel allowing complements aggregation

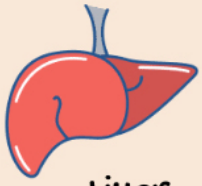


• Clearance mechanism by complements:

1) inside the blood vessel:



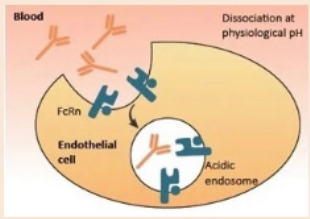


spleen

Liver

- Immune complex that bind C(most common)3b or C4b are insoluble
- C3b or 4 bind to CR1 on RBCS surfaces making immune complex soluble
- carry it to liver or spleen to be distracted
- also C3b can bind to Receptor on macrophage in blood then phagocytosis

2) inside the tissue:

- Binding CR1 on macrophage and neutrophils by C3b with immune complex and induce phagocytosis
- Abs bind Fc receptor and induce phagocytosis



• Autoimmune disease can occur due to :

1) deficiency of CR1 :
- in tissue : no phagocytosis for immune complex
- in blood: insoluble complex that deposits in body tissue

2)deficiency in molecules that bind to RC1:
- c3 : meeting point for all pathway that are effective to gram negative so when lack ▶️More risk of infection of gram negative
- c2 : lack lead to inability to activate C3 , but still c4 present but less effective
- C1 lack : no activation for C3 and C4
- C4
- C9 : not very significant because C5-C8 are able to form MAC but less efficient

TALA IYAD

• MCQ :

32) A complement component which is strongly chemotactic for neutrophils is Select one

a C9
b. C5a
c C3
d. C3b
e. C5

5) The classical and alternative pathways meet at complement component Select one

a .C4
b. C4b
C. FactorD .
d. C5
e C3

4)The initial complement component that is bound by complement-foing antibodies is Select one

A.C1q
B.C1s
C. C3b
D. C5a
e. C9

93)Which of the following key components of the complement pathway can be directly activated by the lectin, pathway?

a) C1
b) C2
c) C5
d) C7
e) C9

94)Complement component C3 in alternative pathway is cleaved by

Select one:

a) C3b
b) C3bBb
c) Factor B
d) Simultaneously by antigen
e) Simultaneously by antigen and antibody

Complements

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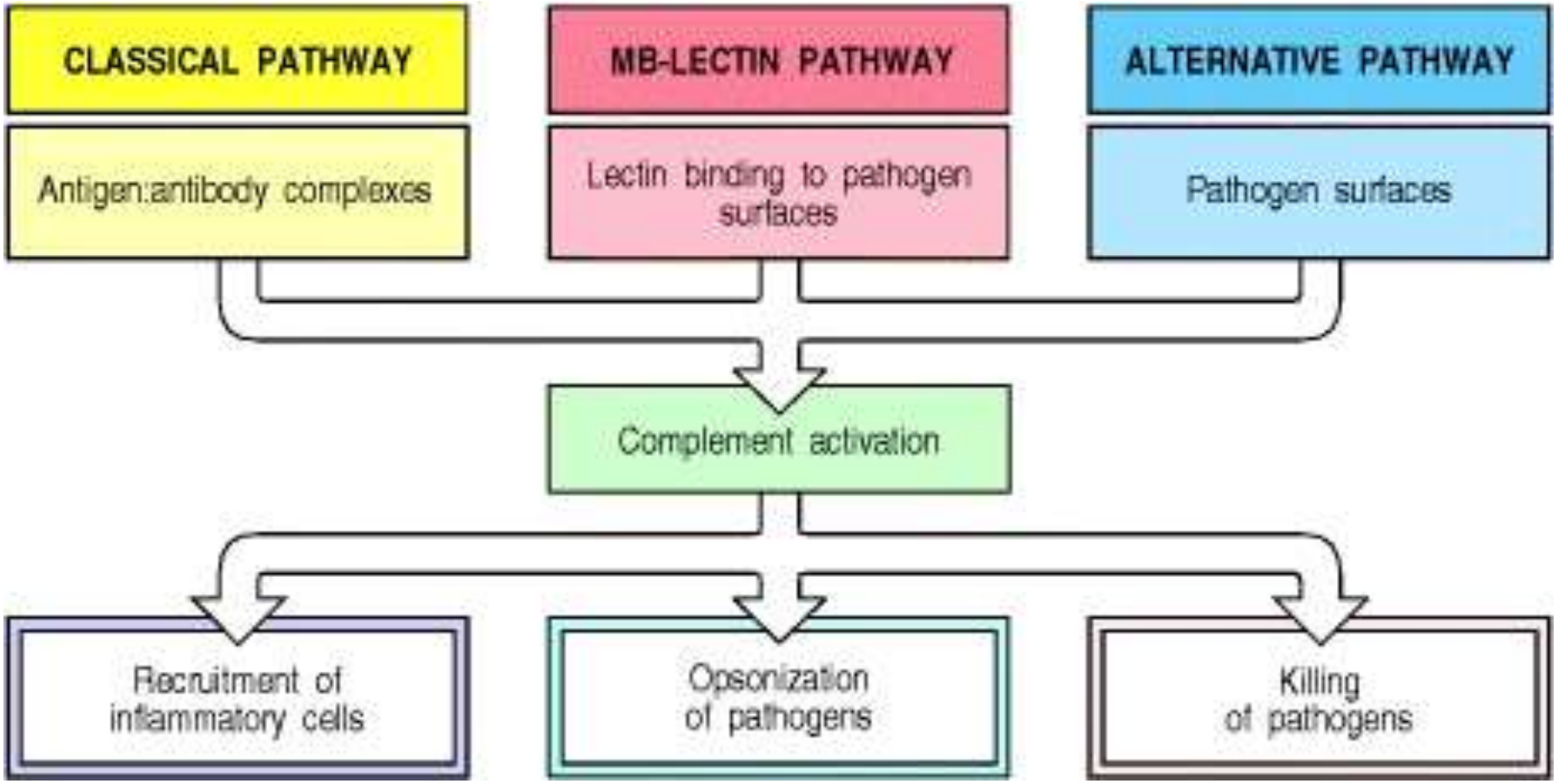
Immunology, 2nd year students

- The complement system consists of a number of small proteins found in the blood, generally synthesized by the liver, and normally circulating as inactive precursors (pro-proteins).
- When stimulated by one of several triggers, activation cascade is started and lead to functional effects.
- Over 25 proteins and protein fragments make up the complement system. They account for about 5% of the globulin fraction of blood serum.

- Complement was discovered many years ago as to ‘complement’ the antibacterial activity of antibody, hence the name.
- Although first discovered as an effector arm of the antibody response (Adaptive), complement can also be activated early in infection in the absence of antibodies (innate) .

- In the case of the complement system, the precursor are widely distributed throughout body fluids and tissues without adverse effect. At sites of infection, however, they are activated locally and trigger a series of potent inflammatory events
- There are three distinct pathways through which complement can be activated on pathogen surfaces. These pathways depend on different molecules for their initiation, but they converge to generate the same set of effector molecules


CLASSICAL PATHWAY
MB-LECTIN PATHWAY
ALTERNATIVE PATHWAY
Antigen:antibody complexes
Lectin binding to pathogen surfaces
Pathogen surfaces
Complement activation
Recruitment of inflammatory cells
Opsonization of pathogens
Killing of pathogens

Complement pathways activation

- Classical pathway; recognize antibody binding microbe as viruses or bacteria (IGG1, IGG3, IGA and IGM) it is arm of humoral immunity
- Alternative; recognize LPS or endotoxins of microbe (part of innate response)
- Lectin pathway. The lectin is a protein bind carbohydrates on microbe (mannose)

Complements

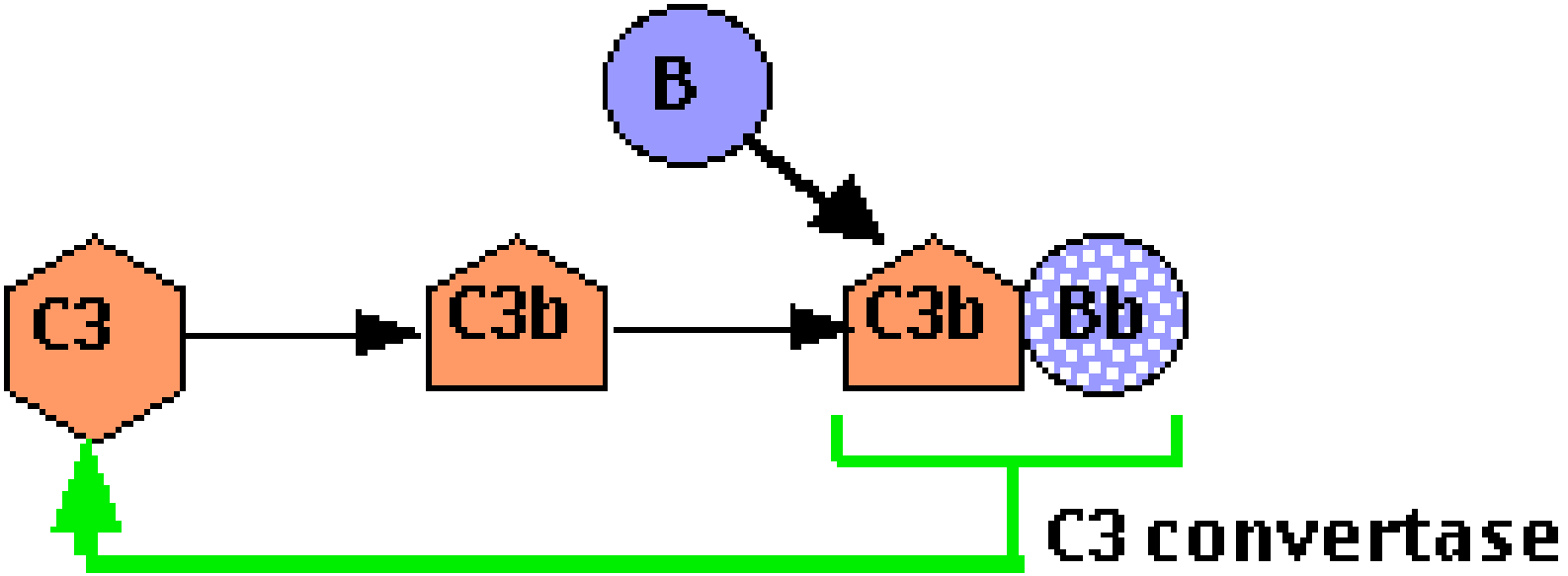
- The **classical pathway**, so called because it was discovered first, uses a plasma protein called C1q to detect antibodies bound to the surface of a microbe or other structure. Once C1q binds to the Fc portion of the antibodies, two associated serine proteases, called C1r and C1s, become active and initiate a proteolytic cascade involving other complement proteins (C2, C4) to make C3 convertase. The classical pathway is one of the major effector mechanisms of the humoral arm of adaptive immune responses.

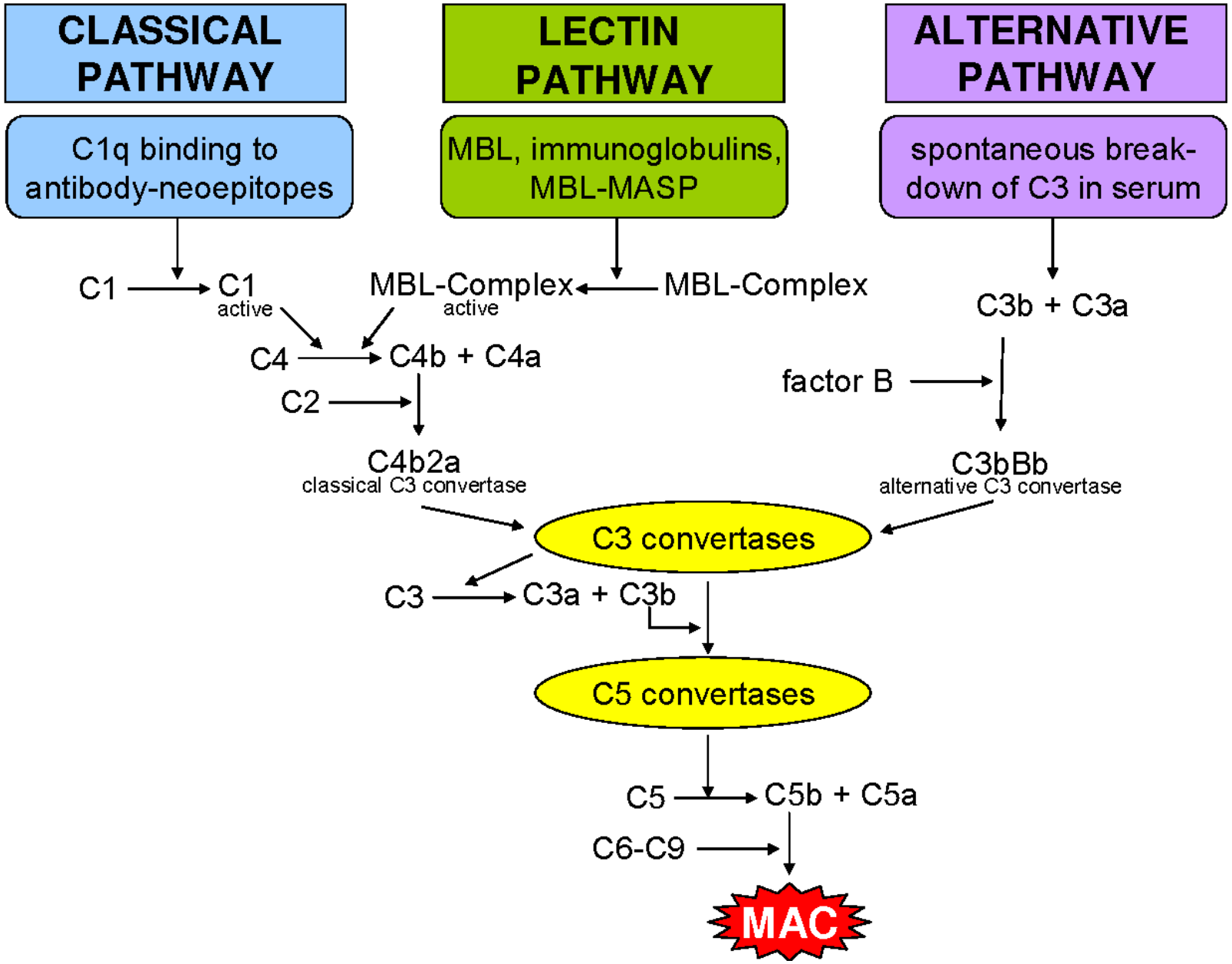
Classical pathway

- C1 exists in blood serum as a molecular complex containing:
  - C1q
  - C1r
  - C1s
- The IGM and IGG that bound by antigen, contain a binding site for C1q. (A single molecule of IgM is enough to initiate the pathway. IgG is far less efficient, requiring many molecules to do so.)
- Binding of C1q activates **C1s** and **C1r**.
- Activated C1s (a protease) cleaves two serum proteins:
  - **C4** is cleaved into a large fragment
    - **C4b**, which binds covalently to surface of antigen (opsonisation) and
    - **C4a,** smaller, inactive, which diffuses away.
  - **C2** is cleaved into
    - **C2b**, which binds to a site on **C4b**,
    - **C2a** a smaller, inactive, fragment of which diffuses away.
  - The complex of C4b2a is called "**C3 convertase**" because it catalyzes the cleavage of **C3**.

Complement

- **The alternative pathway**, is triggered when a complement protein called C3 simultaneously degraded to C3b that recognizes certain microbial surface structures, such as bacterial LPS.
- C3b undergoes its post-cleavage conformational change, a binding site for a plasma protein called Factor B is also exposed. Factor B then binds to the C3b protein that is now covalently tethered to the surface of a microbial or host cell. Bound factor B is in turn cleaved by a plasma serine protease called Factor D, releasing a small fragment called Ba and generating a larger fragment called Bb that remains attached to C3b.
- The C3bBb complex is the alternative pathway **C3 convertase**,


B
C3
C3b
C3b
Bb
C3 convertase


CLASSICAL PATHWAY
LECTIN PATHWAY
ALTERNATIVE PATHWAY
C1q binding to antibody-neoepitopes
MBL, immunoglobulins, MBL-MASP
spontaneous break-down of C3 in serum
C1
C1 active
MBL-Complex active
MBL-Complex
C3b + C3a
C4
C4b + C4a
C2
factor B
C4b2a
classical C3 convertase
C3bBb
alternative C3 convertase
C3 convertases
C3
C3a + C3b
C5 convertases
C5
C5b + C5a
C6-C9
MAC

Lectin pathway

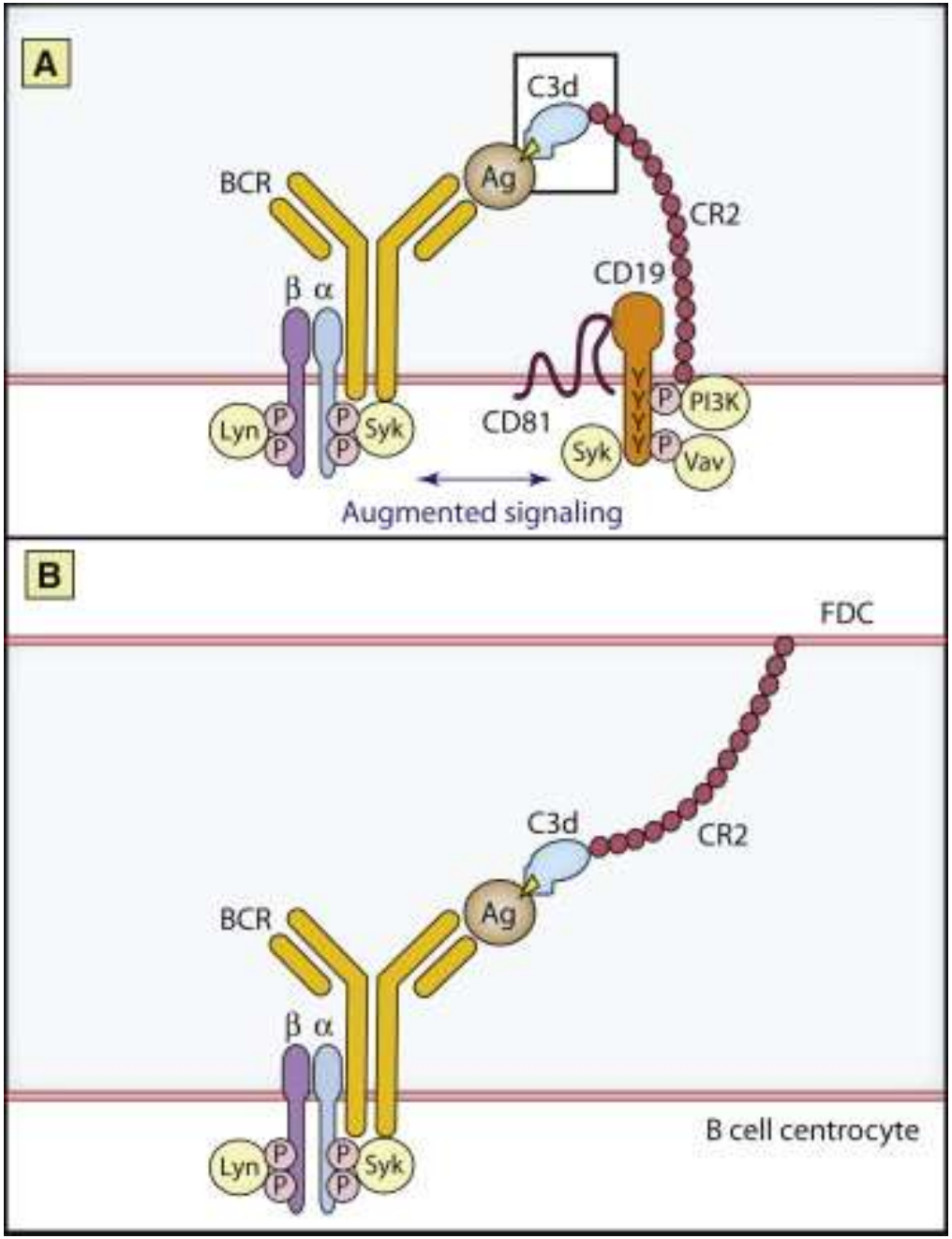
- Lectin pathway. The lectin (proteins macromolecules in blood that are highly specific to mannose on pathogen) pathway is homologous to the classical pathway, but with mannose-binding lectin (MBL) instead of C1q, and in the absence of antibody
- This pathway is activated by binding of lectin to mannose residues on the pathogen surface, which can then split C4 into C4a and C4b and C2 into C2a and C2b the rest pathway is similar to classical

C3

- Recognition of microbes by any of the three complement pathways results in sequential recruitment and assembly of additional complement proteins
- **C3** is the most abundant protein of the complement system. Because of its abundance and its **ability to activate itself** (as described later), it greatly magnifies the response.
  - **C3 convertase** cuts **C3** into major fragments:
    - **C3b**, which binds covalently to glycoproteins scattered across the microbial cell surface. Macrophages and neutrophils have receptors for **C3b** and can bind the C3b-coated cell or particle preparatory to phagocytosis. This effect qualifies C3b as an **opsonin**.
    - **C3a** This small fragment is released into the surrounding fluids. It can bind to receptors on basophils and mast cells triggering them to release their vasoactive contents (e.g., histamine). Because of the role of these materials in anaphylaxis and inflammation, C3a is called an **anaphylatoxins**.
    - C3d:

C3d link innate to humoral immunity

- antigen gets “tagged” with the appropriate C3d product via the classical or lectin complement pathways.
- C3d bind both the antigen receptor on B cell (IGM) and complement receptor CR2 (CD21)on B cells, which forms with CD19 a coreceptor on B cells during antigen-induced activation regulating humoral immunity and enhancing signaling through the B cell Ag receptor
- CR2 on B cell enhance entrance and infection of B cells by by Epstein–Barr virus

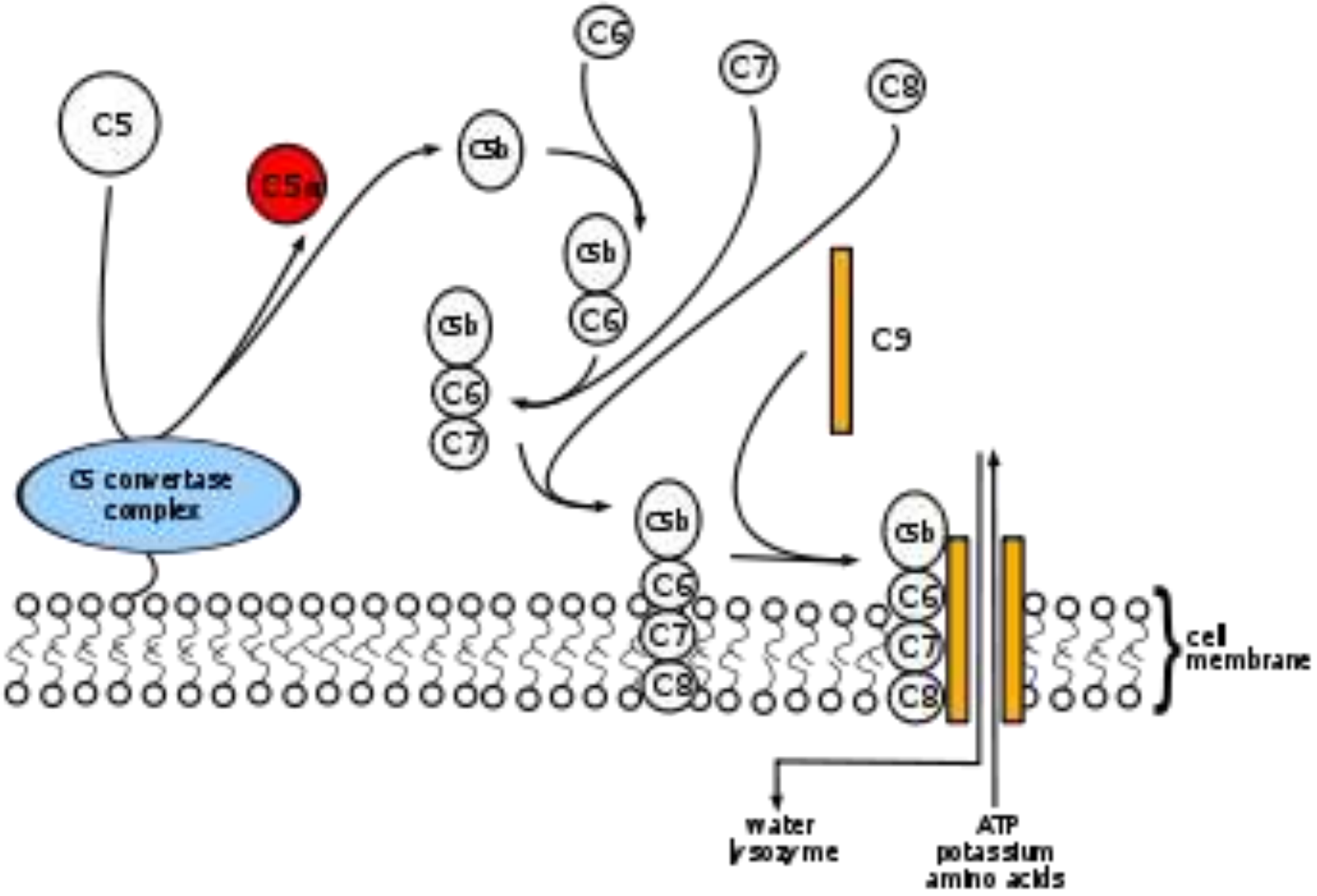

A
C3d
Ag
BCR
CR2
CD19
β α
CD81
Lyn
P
Syk
PI3K
Vav
Augmented signaling
B
FDC
C3d
CR2
Ag
BCR
B cell centrocyte

Membrane attack complex (MAC

-**C5 convertase formed by joining C3 convertase to C3b**

- Cleavage of C5 by the (C3bBb3b)and other complements (C4b•2a.3b)(which is thus a "C5 convertase".) produces:
- **C5a**, which is released into the fluid surroundings where it
  - is a potent anaphylatoxin
  - is a chemotactic attractant
- **C5b**, which serves as the anchor for the assembly of a single molecule each of
  - C6;
  - C7, and
  - C8.

- **The Membrane Attack Complex (MAC)**
  - The resulting complex **C5b•6•7•8** guides the polymerization of as many as 18 molecules of **C9** into a tube inserted into the lipid bilayer of the plasma membrane. This tube forms a channel allowing the passage of ions and small molecules. Water enters the cell by osmosis and the cell lyses.


C5
C5a
C5b
C6
C7
C8
C9
C5b
C6
C5b
C6
C7
C5 convertase complex
C5b
C6
C7
C8
C5b
C6
C7
C8
cel membrane
water
lysozyme
ATP
potassium
amino acids

Summary of complement functions

- **Opsonization** by C3b, C4b and C5b targets foreign particles for phagocytosis.
- **Chemotaxis** by C5a, C4a and C3a attracts phagocytic cells to the site of damage.
- This is aided by the **increased permeability (anaphylatoxins)** they cause smooth muscle contraction, vasodilation, histamine release from mast cells, and enhanced vascular permeability.mediated by **C3a, C5a, C4a**.
- C3b ; are also important for **solubilizing** antigen-antibody complexes and elimination from the body (by binding the immune complex to CR1 on erythrocyte). otherwise aggregation of the complexes lead to immune complex disorder (SLE, diabetes mellitus, RA)
- **Lysis** of target cells (C5b-9).
- Promoting B cell activation and antibody formation. Breakdown of C3b generates a fragment (C3d) that binds to antigens enhancing their uptake by B cells.

Complement receptors

- Type 1 receptor(CR1); bind C3b, and C4b. Expressed in erythrocytes, macrophages, neutrophil
  - Do opsonization and Induce phagocytosis (with antibody)
  - Help to remove immune complexes from blood to liver and spleen (erythrocytes)
- Type 2 receptor (CR2), bind C3d, and, expressed on B lymphocytes and DC
  - With other proteins enhance B cell response to antigen
  - Receptor for epstein barr virus on B cells
- Type 3 and 4 receptors on phagocytes
  - bind opsonizing C3b and lead to phagocytosis. Found on macrophages and neutrophils

Regulation of complement activity

- The explosive potential of the complement system requires that it be kept under tight control. At least 12 proteins are known that do this. Three examples:
- **Factor H and Decay-accelerating factor (DAC),** removes Bb from the alternative pathway C3 convertase.
- **Factor I** inactivates C3b.
- **C1 inhibitor** (**C1INH**) binds to sites on activated C1r and C1s shutting down their proteolytic activity.
- **CD59** on normal tissue cells which inhibit association of C9 with C5b-8

Disorders of the complement system

- With so many proteins involved, it is not surprising that inherited deficiencies of one or another are sometimes encountered in humans. Four examples:
  - **C3**. An inherited deficiency of C3 predisposes the person to frequent bouts of bacterial infections mainly gram negative bacteria.
  - **C2 , C1, C3 or C4**. immune complex disorders are the main problem with a deficiency of C2 , C1 or C4. This emphasizes the important role of the complement system in clearing away antigen-antibody complexes. A deficiency of C2 is frequently found in patients with lupus erythematosus (SLE).
  - **C9**. most people who cannot make C9 have no problem with bacterial infections. Laboratory studies suggest that the **C5b•6•7•8** complex by itself is able to lyze bacteria although not as efficiently as C9.
  - **C1INH**. A deficiency of C1INH produces **hereditary angioedema**. The massive release of anaphylatoxins (C3a, C5a) may cause dangerous swelling (edema) of the airways, as well as of the skin and intestine.
  - **CD59** deficiency in its expression lead to inadequate control of MAC assembly results in intravascular red cell lyses called **paroxysmal nocturnal haemoglobinuria**

Formation Of Membrane Attack Complex (MAC)

Membrane Attack Complex (MAC)
C5b6789 complex