BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024,

Tamil Nadu, India

Programme: M.Sc., Biomedical Science

Course Code: BM35C6

Course Title: Immunology

Unit-III

Antigens

Dr. R. POORNIMA

Guest Faculty

Department of Biomedical Science

Unit III:

Antigens – Factors influence immunogenicity, Epitopes, haptens – Effector molecules of innate system -Acute phase proteins, complements- classical & alternative pathways of complement system. Effector molecules of cell-mediated and humoral immune responses - cytokines - Properties, receptors and antibodies / Immunoglobulins – Structure, antigenic determinants, immunoglobulin classes and functional significances.

PRESENTATION: 1

Antigens

- Antigens are **molecules or molecular structures** that are recognized by the immune system, specifically by antibodies, B cells, or T cells.
- The term "antigen" is derived from "**antibody generator**," reflecting its ability to stimulate the production of antibodies or to initiate an immune response.

1.Types of Antigens:

1. **Proteins:** Most common and **potent antigens**, including proteins from viruses, bacteria, and other pathogens.
2. **Polysaccharides:** Complex carbohydrates found on the surface of pathogens like **bacteria.**
3. **Lipids and Nucleic Acids:** Less common as antigens, but can become immunogenic when **associated with proteins**.

2. Sources of Antigens:

1. **Exogenous Antigens:** These come from **outside the body**, such as bacteria, viruses, fungi, and toxins. They are typically **processed by antigen-presenting cells (APCs)** and **presented on** major histocompatibility complex **(MHC) molecules to T cells**.
2. **Endogenous Antigens:** These originate from **within the body**, such as proteins produced by **cells infected with viruses, or cancer cells**. They are presented on MHC molecules to the immune system, often leading to the destruction of infected or malignant cells.
3. **Autoantigens:** These are normal proteins or molecules in the body that are **mistakenly targeted by the immune system**, leading to autoimmune diseases.

3. Antigen Presentation:

1. Antigens are presented to the immune system via MHC molecules on the surface of APCs.
2. **MHC Class I:** Presents **endogenous antigens to CD8+ cytotoxic T cells.**
3. **MHC Class II:** Presents **exogenous antigens to CD4+ helper T cells**.

4. Antigenic Determinants (Epitopes):

1. An antigen may have multiple epitopes, which are specific parts of the antigen recognized by the immune system.
2. Different antibodies or immune cells may recognize different epitopes on the same antigen.

Factors influence immunogenicity

1. Foreignness

- An antigen must be a foreign substance to the animal or recognized as non-self by the biological system to elicit an immune response.
- E.g. Bovine serum albumin, a common experiment antigen is not immunogenic when injected to the cow but strongly immunogenic when injected to the rabbit.

2. Molecular Size

- The most active immunogens tend to have a molecular mass of 14,000 to 6, 00,000 Da, **usually >1, 00,000 Da**. g. tetanus toxoid, egg albumin, thyroglobulin are highly antigenic.
- Generally, substances with molecular mass **less than 5,000 to 10,000 Da** are **poor immunogens**. E.g. Insulin (5,700 Da) is either non-antigenic or weakly antigenic.

3. Chemical Nature and Heterogeneity

- Antigens are **mainly proteins** and some are **polysaccharides.**
- In general, **chemically more complex** the substance is the **more immunogenic** it will be.
- Synthetic **homopolymers** (Multiple copies of single sugar or amino acids) tends to **lack immunogenicity** regardless of size while **heteropolymers** are usually **more immunogenic** than homopolymers.
- It is presumed that presence of an **aromatic radical is essential for rigidity and antigenicity of a substance**.

4. Physical form

- In general **particulate antigens** are **more immunogenic** than soluble ones.
- **Denatured antigens are more immunogenic** than the native form.
- **Large, insoluble or aggregated molecules are more immunogenic** than small, soluble ones.

5. Susceptibility to antigen processing and presentation

- Development of immune response (Both humoral and cell-mediated) requires interaction of T-cells and antigen that has been processed and presented together with MHC molecules.
- Antigens that **cannot be processed and presented by MHC are poor immunogens.**
- Antigens that are **easily phagocytosed** are generally **more immunogenic**.

6. Biological System of Host

1. Genotype of Recipient Animal:

- Some substances are immunogenic in one species but not in another .Similarly, some substances are immunogenic in one individual but not in others.

2. Dosage and Route of administration:

- An insufficient dosage of immunogen will not elicit immune response
- Conversely, excessively high dose will also lead to **unresponsiveness or tolerance**.
- There is a dose of antigen above or below which the immune response will not be optimal.

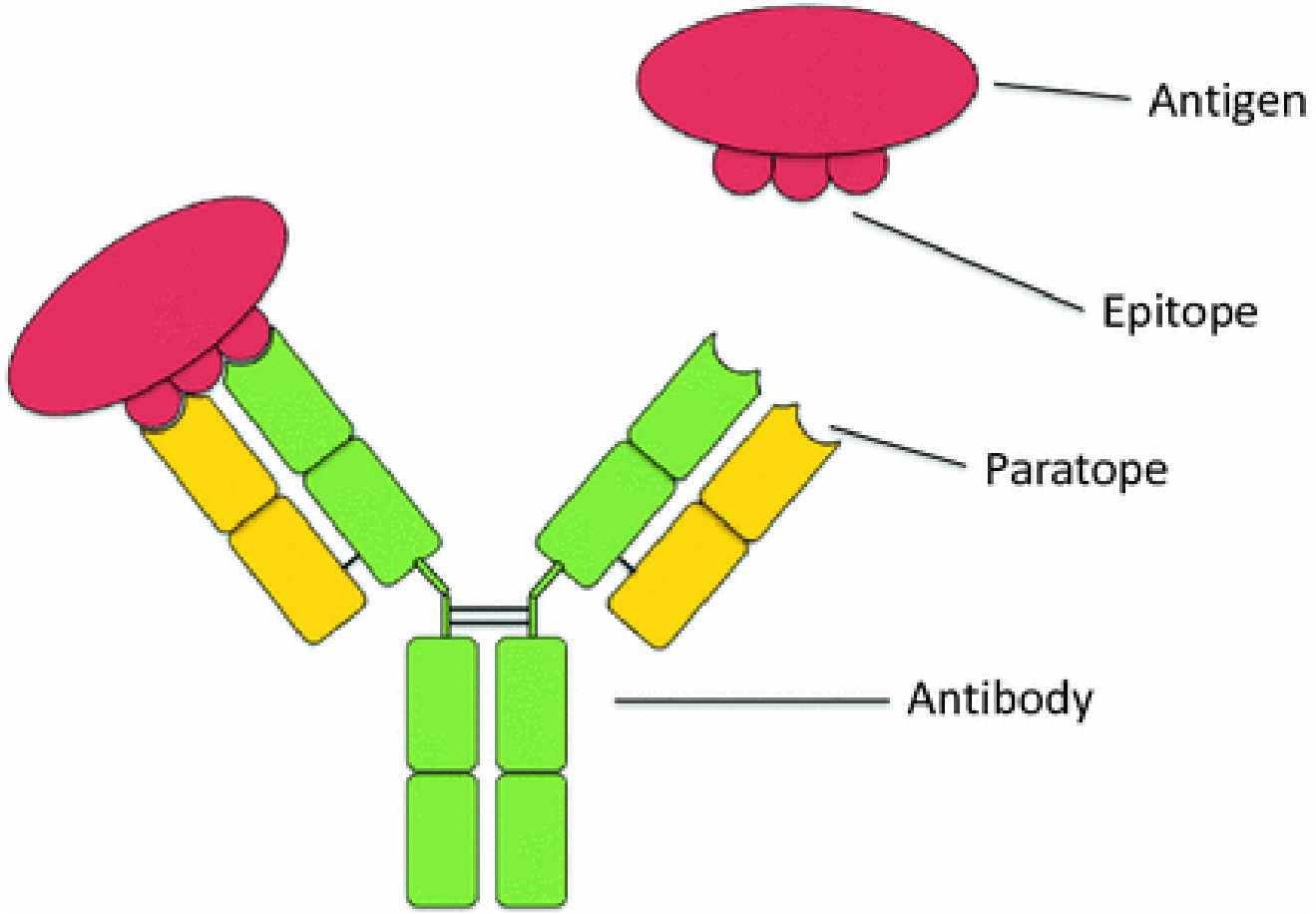
3. Adjuvants:

These are substances that when mixed and injected with antigen enhances the immunogenicity of antigen.

EPITOPES

- **Epitope** is a portion of a **foreign protein, or antigen**, that is capable of stimulating an immune response.
- An epitope is the part of the antigen that binds to a specific antigen receptor on the surface of a B cell.
- Binding between the receptor and epitope occurs only if their **structures are complementary**.

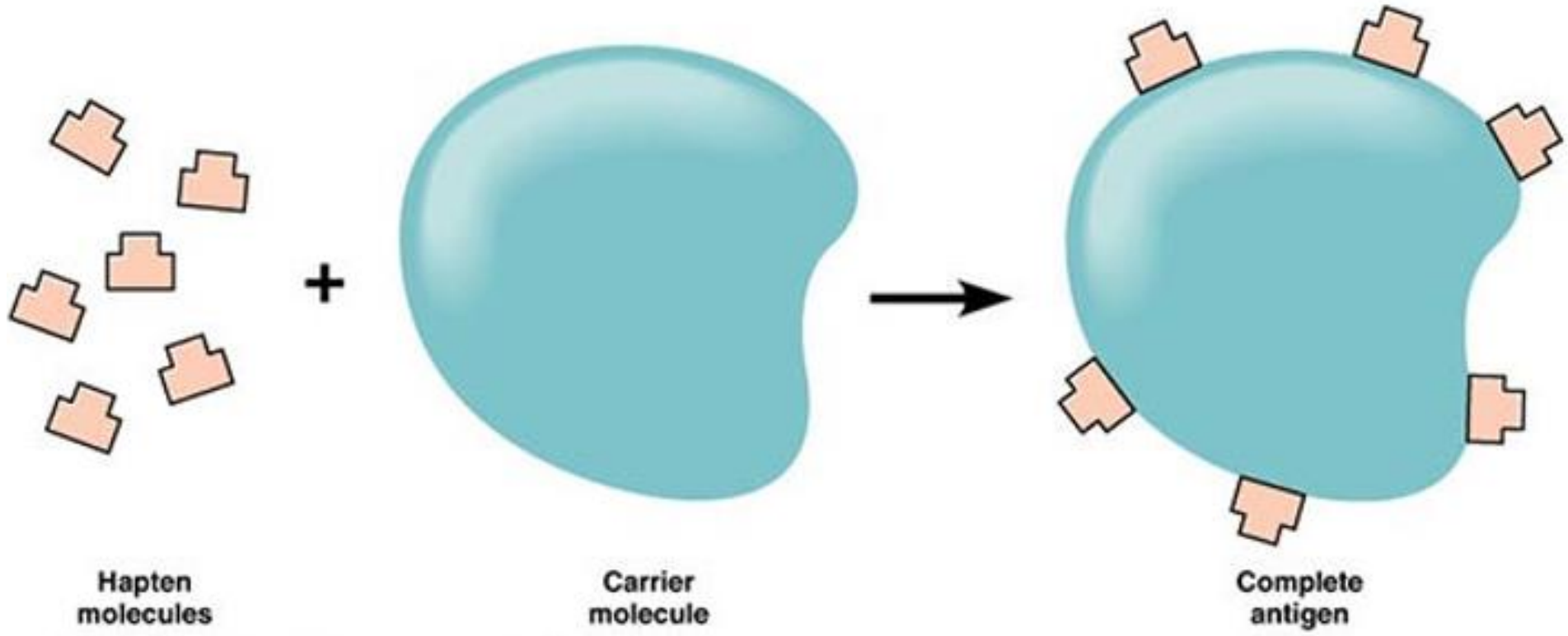
EPITOPE



HAPTENS

- **Hapten** is a small molecule that **stimulates the production of antibody** molecules **only when conjugated to a** larger molecule, called a **carrier molecule**.
- The term *hapten* is derived from the Greek *haptein*, meaning "**to fasten**."
- Haptens can become tightly fastened to a carrier molecule, most often a protein, by a covalent bond.

HAPTEN



Effector molecules of Innate system

- The innate immune system provides the first line of host defense against microbes before adaptive immune responses have had sufficient time to develop. The mechanisms of innate immunity exist before exposure to microbes.
- The cellular components of the innate immune system include **epithelial barriers** and **leukocytes (neutrophils, macrophages, NK cells, lymphocytes with invariant antigen receptors, and mast cells).**

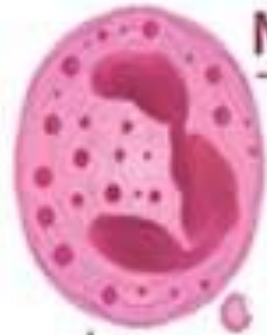
- The innate immune system uses **cell-associated PRRs,** present on plasma and endosomal membranes and in the cytosol, to recognize structures called **pathogen-associated molecular patterns (PAMPs).**
- In addition, these receptors recognize molecules made by the host but whose expression or location indicates cellular damage; these are called **DAMPs (Damage-associated molecular pattern molecules).**
- **Toll-like receptors (TLRs),** present on the cell surface and in endosomes, are the most important family of pattern recognition receptors, recognizing a wide variety of ligands, including bacterial cell wall components and microbial nucleic acids.

Mast cell

Function:
Allergy
Inflammation
Antigen presentation
Immunomodulation

Exosomes:

MHC class II	FcεRs
c-Kit	LFA1
esRNA	ICAM



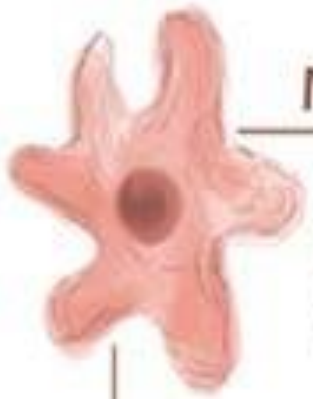
Neutrophil

Function:
Cytoxicity
Phenotypic plasticity
Vascular remodelling

Microvesicles:
Anti-bacterial

Ectosomes:

F-actin	MMPs
TGF β	Elastase
Myeloperoxidase	



Macrophage

Function:
Phagocytosis
Inflammation
Phenotypic plasticity

Eosinophil

Function:
Allergy
Cytotoxicity

Granules:

MBM	IFNγ
EPO	TNFα
Interleukins	LAMP2
	LAMP3

NK cell

Function:
Cytoxicity

Exosomes:
CD56
Perforin
FASL

Cytosolic pattern receptors :

- RIG-I-(**RLRs**) receptors, recognize viral RNA,
- Cystolic DNA Sensors (**CDSs)** recognize microbial DNA, and
- NOD-like receptors **(NLRs)** recognize bacterial cell wall constituents.

Pattern recognition receptors:

- **TLRs, NLRs, and RLRs**, signal to **activate the transcription factors NF-κB and AP-1**
- Stimulate expression of cytokines, co-stimulators, and other molecules involved in inflammation.
- IRF transcription factors, stimulate expression of the antiviral type I interferon genes.

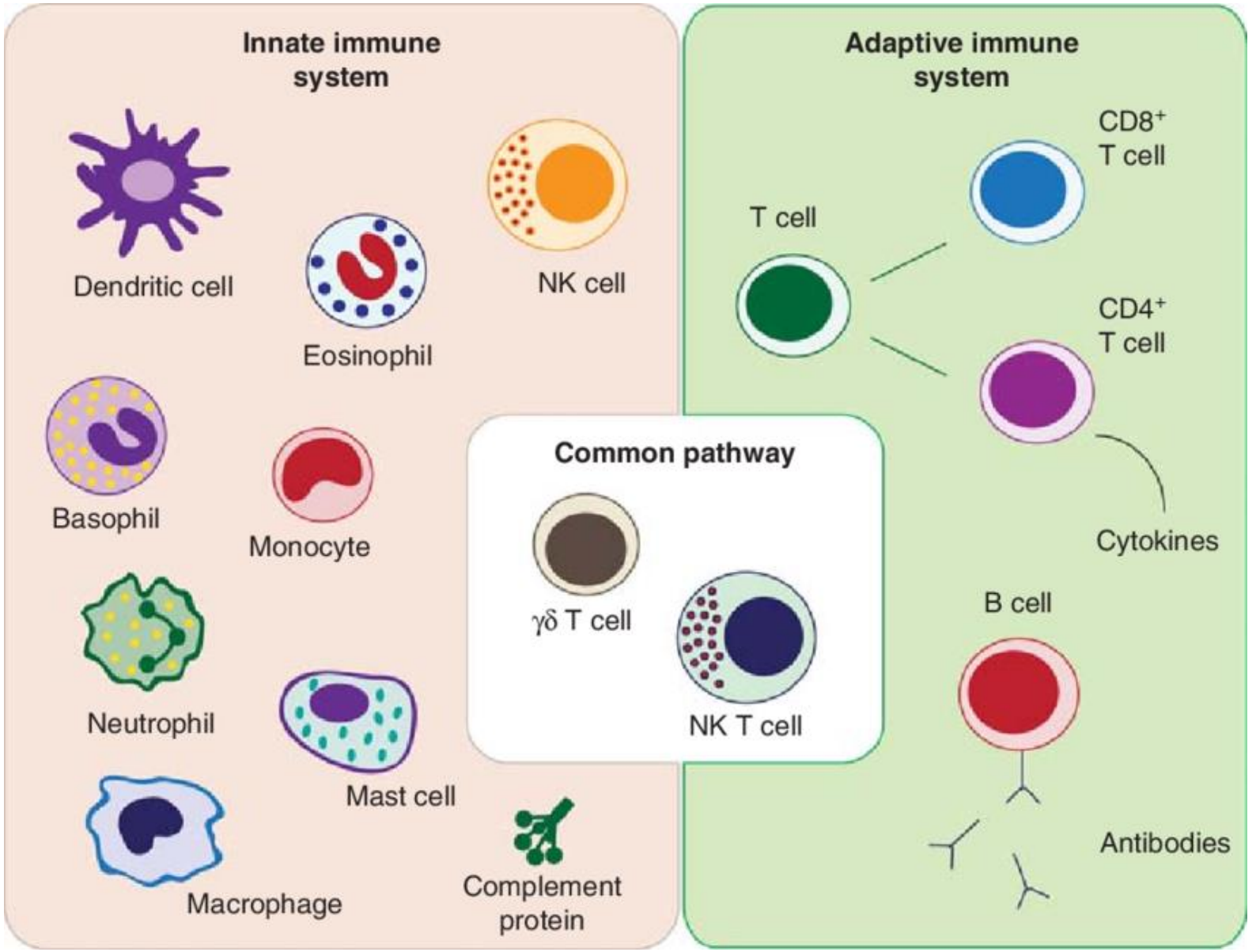
Effector Molecules and their functions

S. No	Effector Molecules	Functions
1	**Cytokines**	•**Interleukins**: communication between immune cells, inflammation, cell proliferation, and differentiation. •**Tumor Necrosis Factor:** Promotes inflammation and can induce apoptosis in infected or cancerous cells. •**Interferons**: antiviral response
2	**Chemokines (**subset of cytokines)	**Induce chemotaxis (**migration of leukocytes toward infected or damaged tissue**)**
3	**Complement System Proteins**	**Directly kill pathogens through membrane attack complexes.** Key components include C3, C5, and their fragments (C3a, C3b, C5a).
4	**Antimicrobial Peptides (AMPs)**	Small peptides such as defensins and cathelicidins that **directly kill bacteria, fungi, and viruses by disrupting their cell membranes or interfering with their metabolism.**

Effector Molecules and their functions

S. No	Effector Molecules	Functions
5	**Acute Phase Proteins (**Produced mainly by the liver in response to inflammation)	• **C-reactive protein (CRP):** Binds to microbial phosphocholine, aiding in opsonization. • **Mannose-binding lectin (MBL):** Binds to carbohydrate patterns on pathogens and activates the lectin pathway of complement activation.
6	**Pattern Recognition Receptors (PRRs)** (These are not effector molecules themselves)	but they **detect** (PAMPs) and trigger the release of effector molecules like TLRs, NLRs, and RLRs.
7.	**Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS)**	**Produced by phagocytes like neutrophils and macrophages**, these highly reactive molecules kill pathogens by damaging their DNA, proteins, and membranes.
8.	**Histamine**	•Released primarily by **mast cells and basophils**, it increases **vascular permeability and promotes the influx of immune cells to the site of infection.**
9	**Lysozyme**	An enzyme found in **saliva, tears, and other secretions**, lysozyme breaks down the peptidoglycan layer of bacterial cell walls, leading to their lysis.

Cell of Innate and Adaptive immunity



ACUTE PHASE PROTEINS

- Acute phase proteins (APPs) are defined as **proteins** that change their serum concentration by >25**% in response to inflammatory cytokines** (IL-1, IL-6, TNFα).
- The acute-phase response is considered part of the innate immune system, and APPs play a role in mediating such systemic effects as fever, leukocytosis, increased cortisol, decreased thyroxine, decreased serum iron, and many others.
- APPs can be categorized as positive (increasing serum concentration) or negative (decreasing serum concentration).
- Increased production of positive acute phase proteins is a sensitive indicator of inflammation

ACUTE PHASE PROTEINS

Positive APPs	Negative APPs
C-reactive protein (CRP)	Albumin
Serum Amyloid A (SAA)	Transferrin
Haptoglobin (Hp)	Transthyretin
Ceruloplasmin	Retinol-binding protein
α2-Macroglobulin	Adiponectin
α1-Acid glycoprotein (AGP)	
Fibrinogen	
Complement (C3, C4)	

Positive acute phase proteins

- Positive acute-phase proteins **increase in plasma concentration** in response to **inflammation** (usually within 1-2 days). Positive APPs are further categorized as major, moderate or minor, depending on the degree of increase.
- **Major APP:** A protein with a low concentration in the serum of healthy animals (often <0.1 μg/dL), but upon stimulation will increase over **100 – 1000 fold**, reaching a peak 24-48 hours after insult, then rapidly decreasing. An example of an major APP is Serum amyloid A.
- **Moderate APP:** Present in the blood of healthy animals, but increases **5 – 10 fold** upon stimulation, peaking around 48 – 72 hours after insult, then decreases at a slower rate than major APPs.
- **Minor APP:** Increase only by **50 – 100%** above resting levels and at a gradual rate.
- The rapidity and magnitude of the increase in each acute phase protein varies depending on the species.

Protein	Main function
Alpha-1-acid glycoprotein	Antiinflammatory and immunomodulatory agent: has antineutrophil and anticomplement activity and increases macrophage secretion of IL-1 receptor antagonist. Binds to lipophilic and acidic drugs.
C-reactive protein	On bacteria, it promotes the binding of complement, facilitating phagocytosis. Induction of cytokines Inhibition of chemotaxis and modulation of neutrophil function Neutralizes deleterious effects of histones
Ceruloplasmin	Copper transport (for wound healing, collagen formation and maturation) Antioxidant Reduces the number of neutrophils attaching to endothelium
Haptoglobin	Binds free hemoglobin (limiting Hb iron availability for bacterial growth) Natural antagonist for receptor-ligand activation of the immune system. Inhibition of granulocyte chemotaxis and phagocytosis
Serum amyloid A	Chemotactic recruitment of inflammatory cells to sites of inflammation Induction of inflammatory cytokines (via surface receptors, including Toll like receptor) Inhibition of myeloperoxidase release and lymphocyte proliferation Involved in lipid metabolism and transport immunomodulatory (via the inflammasome)

Negative acute phase proteins

- Negative acute phase proteins decrease in plasma concentration by greater than 25% in response to inflammation.
- This reduction can occur rapidly (within 24 hours) or may decrease gradually over a period of days.
- The two main negative acute phase proteins are **albumin and transferrin**.
- The mechanism by which their concentrations decrease is likely multifactorial, including decreased production by the liver in response to inflammatory cytokines, and possibly increased loss or increased proteolysis.

Albumin

- Reduced production of albumin allows **greater increase in the amount of amino acids available for positive APP production**
- Albumin concentration falls gradually and reduction in concentration is more **noticeable in chronic inflammatory disease**

Transferrin

- Usually measured to **assess iron status**

Adiponectin:

This protein, which is **produced in adipose tissue**, and promotes energy usage through increasing sensitivity to insulin, has anti-inflammatory properties.

Key Acute Phase Proteins

1. C-Reactive Protein (CRP):

1. One of the **most widely measured acute phase proteins**.
2. Binds to phosphocholine on the surface of dead or dying cells and some bacteria, promoting their clearance by immune cells (opsonization).
3. Activates the complement system, enhancing the immune response.

2. Serum Amyloid A (SAA):

1. Associates with high-density lipoprotein **(HDL) in plasma**.
2. Involved in recruiting immune cells to inflammatory sites and transporting cholesterol to the liver for secretion in bile.
3. Elevated levels are associated with chronic inflammatory conditions, such as **rheumatoid arthritis**.

3. Fibrinogen:

1. A **key protein in the coagulation cascade, promoting blood clotting**.
2. Increases blood viscosity and promotes wound healing.
3. Serves as a precursor to fibrin, which **forms a mesh that traps cells and pathogens at the site of injury.**

4. Haptoglobin:

1. Binds free hemoglobin released from erythrocytes, preventing iron loss and kidney damage.
2. Protects against oxidative damage by sequestering hemoglobin.

5. Mannose-Binding Lectin (MBL):

1. Recognizes carbohydrate patterns on the surfaces of pathogens and activates the lectin pathway of the complement system.
2. Enhances opsonization and phagocytosis.

6. Alpha-1 Antitrypsin (AAT):

1. Inhibits proteolytic enzymes such as neutrophil elastase, protecting tissues from enzymatic damage during inflammation.
2. Deficiency in AAT is associated with chronic obstructive pulmonary disease (COPD) and liver disease.

7. Ferritin:

1. An intracellular protein that stores and releases iron.
2. Increased levels can sequester iron, reducing its availability to pathogens and thus limiting their growth.

8. Ceruloplasmin:

1. An enzyme that carries copper in the blood and plays a role in iron metabolism.
2. Has antioxidant properties, protecting tissues from oxidative stress during inflammation.

9. Complement Proteins (e.g., C3, C4):

1. Components of the complement system that enhance the ability of antibodies and phagocytic cells to clear pathogens.
2. Their levels often increase during the acute-phase response to support immune functions.

Role and Function

- **Opsonization:** Marking pathogens for destruction by immune cells.
- **Complement Activation:** Triggering the complement cascade to enhance pathogen clearance.
- **Coagulation and Clotting:** Preventing the spread of infection and aiding in tissue repair.
- **Immune Cell Recruitment:** Attracting immune cells to the site of infection or injury.
- **Iron Sequestration:** Limiting the availability of iron to pathogens, thereby inhibiting their growth.

Clinical Relevance

- Acute phase proteins, particularly CRP and ESR (erythrocyte sedimentation rate, an indirect marker of fibrinogen), are commonly used in clinical settings as markers of inflammation.
- They help in diagnosing and monitoring various conditions, such as infections, autoimmune diseases, and malignancies.
- Elevated levels of these proteins often indicate an active inflammatory process, whereas changes in their levels can provide insights into the effectiveness of treatment or disease progression.

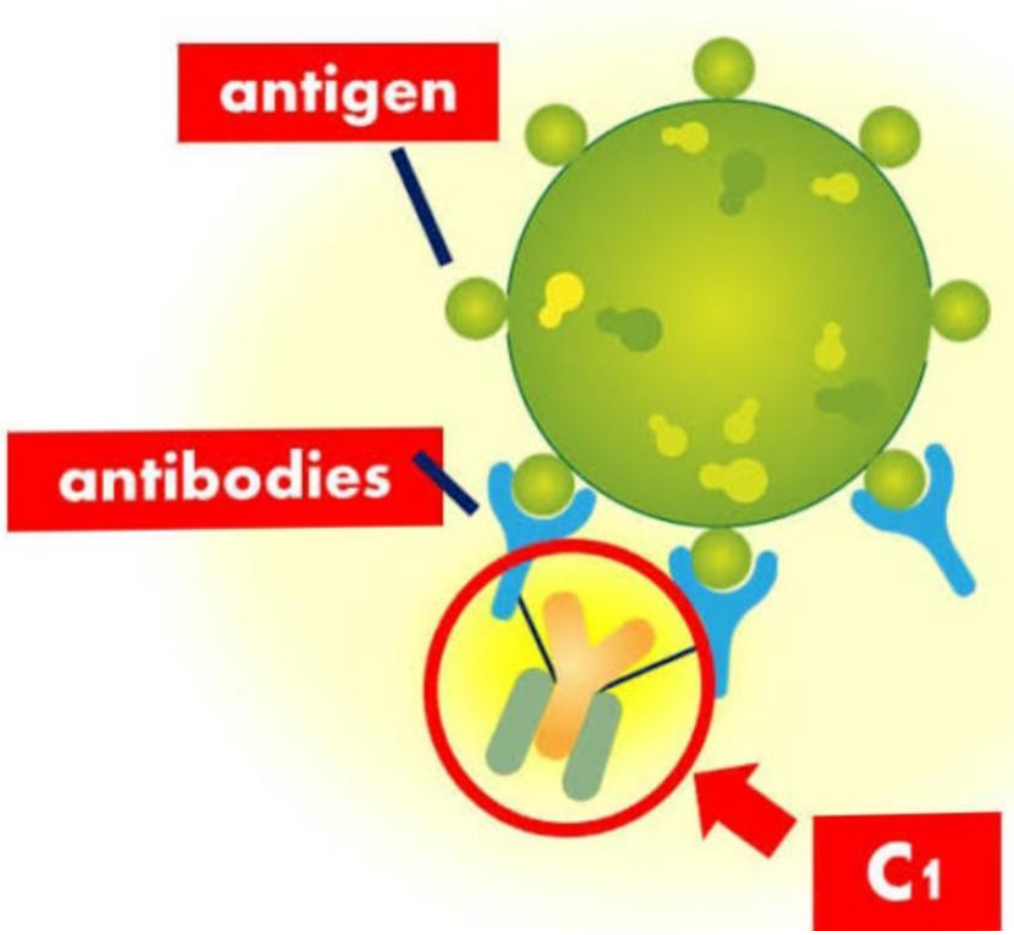

Complement
System

HISTORY

- Research on complement began in the 1890s, when **Jules Bordet** at the Institute Pasteur in Paris showed that sheep antiserum to the bacterium Vibrio Cholera caused lysis of the bacteria and that heating the antiserum destroyed its bacteria.
- He named those substances as **Alexins**.
- **Paul Ehrlich** coined the term complement.

COMPLEMENT SYSTEM

- **The complement system is also termed the complement cascade. It is basically a very important part of the immune system that complements or enhances the abilities of the phagocytic cells and the antibodies to clear the damaged cells and microbes from an organism.**
- **Other functions of the complement system are to promote inflammation and attack the cell membrane of the pathogens.**



Three pathway of complement activation

1.***Classical pathway:-***

- Is **antibody dependent pathway** and triggered by formation of soluble antigen-antibody complex or by binding of the antibody to the antigen present on the target cell surface.

2.***Alternative pathway:-***

Is **antibody independent pathway** stimulated by antigen directly eg. Bacterial cell surface components.

3.***Lectin Pathway:-***

Also **antibody independent** but resembles classical pathway.

Stages of complement activation

Three main stages in the activation of complement by any pathway are

- **Formation of C3 convertage**
- **Formation C5 convertage**
- **Formation of membrane attack complec(MAC)**

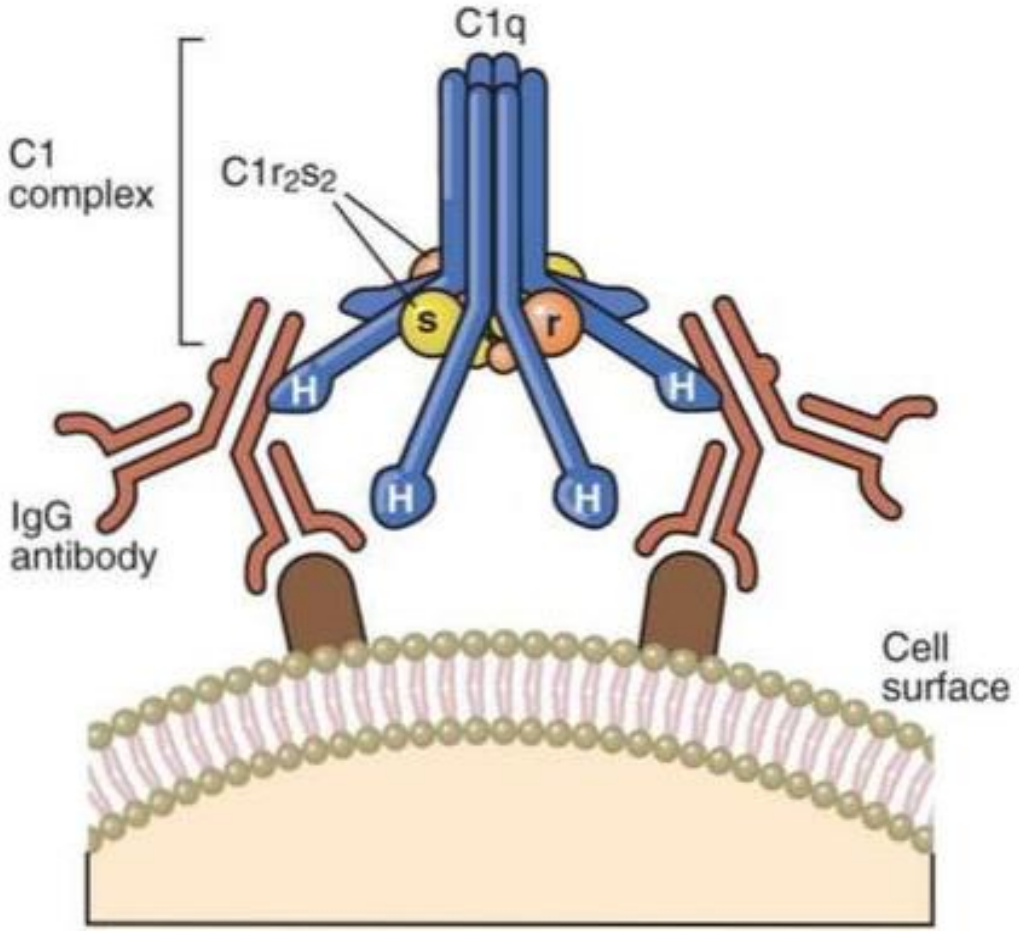
- The initiation and formation of C3 convertage are different in classical and alternative pathway . These then follow the parralel route to merge at C5 convertage stage and finally generate the MAC by a common route.

CLASSICAL PATHWAY

•The classical pathway is one of three activation pathways of the complement system, which is a major contributor to the **defense of infections, clearance of pathogens, removal of apoptotic/necrotic cells, and maintenance of homeostasis.**

•There are at least 21 different serum proteins have been confirmed as components of the classical pathway, in which 11 major protein play the most critical role, including **C1q, C1r, C1s, C2, C3, C4, C5, C6, C7, C8, C9.**

COMPLEMENT SYSTEM

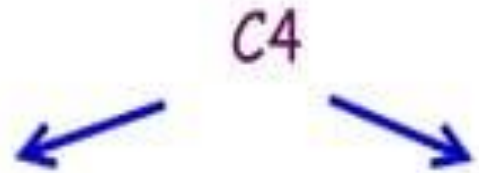


Components of the Classical Pathway		
Native component	Active component(s)	Function(s)
C1(q,r,s)	C1q	Binds to antibody that has bound antigen, activates C1r.
	C1r	Cleaves C1s to activate protease function.
	C1s	Cleaves C2 and C4.
C2	C2a	Unknown.
	C2b	Active enzyme of classical pathway; cleaves C3 and C5.
C3	C3a	Mediates inflammation; anaphylatoxin.
	C3b	Binds C5 for cleavage by C2b. Binds cell surfaces for opsonization and activation of alternate pathway.
C4	C4a	Mediates inflammation.
	C4b	Binds C2 for cleavage by C1s. Binds cell surfaces for opsonization.

Components of the Membrane-Attack Complex		
Native component	Active component(s)	Function(s)
C5	C5a	Mediates inflammation; anaphylatoxin, chemotaxin.
	C5b	Initiates assembly of the membrane-attack complex (MAC).
C6	C6	Binds C5b, forms acceptor for C7.
C7	C7	Binds C5b6, inserts into membrane, forms acceptor for C8.
C8	C8	Binds C5b67, initiates C9 polymerization.
C9	C9n	Polymerizes around C5b678 to form channel that causes cell lysis.

- Complement proteins: are proenzymes - activation by cleavage.

- Example:



C4a

C4b

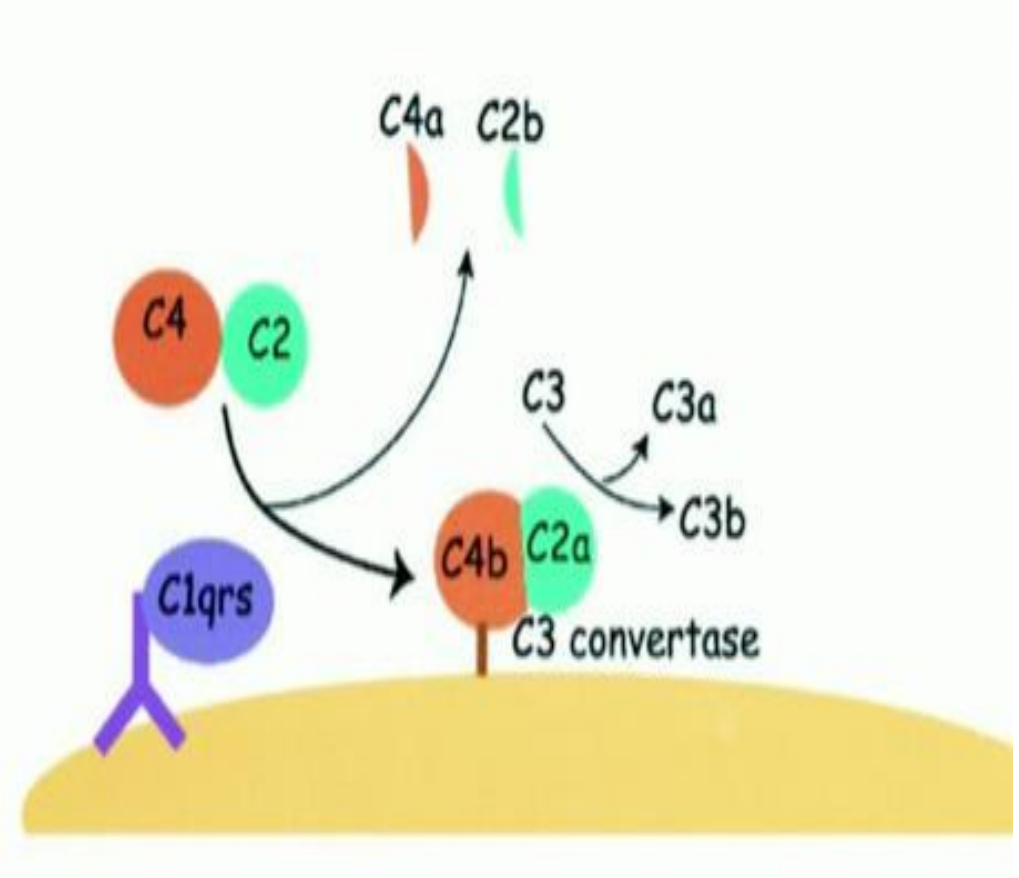
a = smaller fragment.
-Diffusion

b= larger fragment.
-remains bound to microbe

- Exception: C2: C2a = large fragment
C2b = small fragment

Formation of C3 convertase

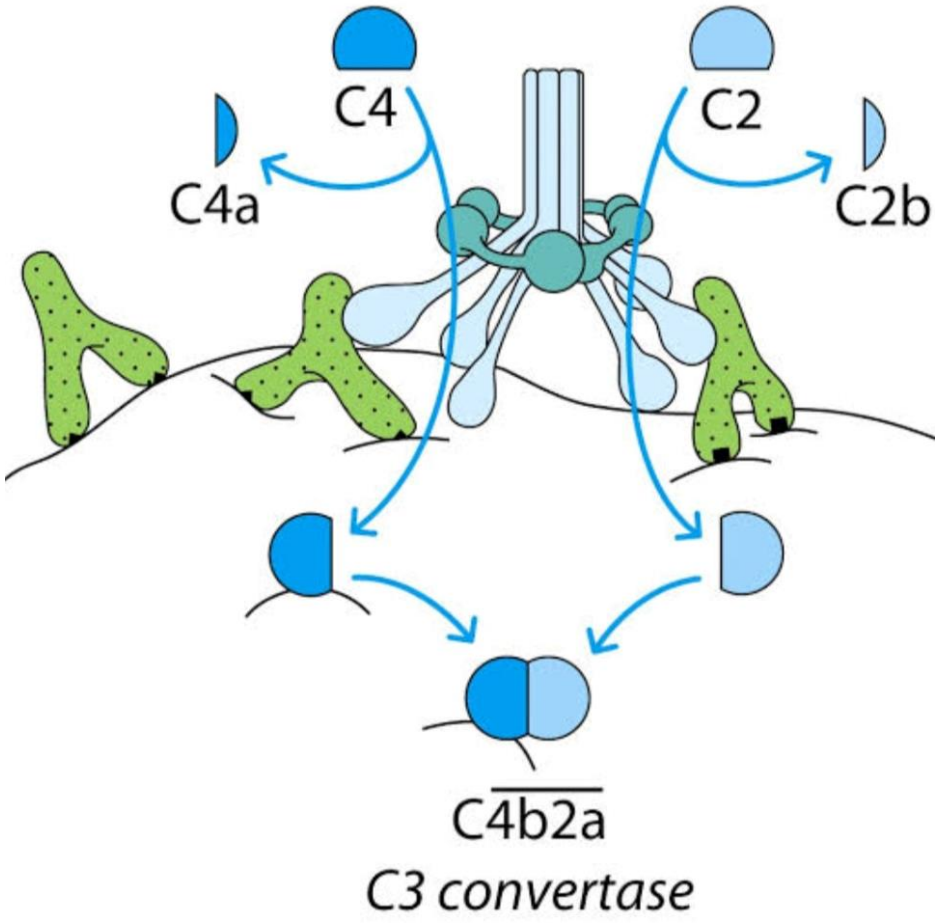
- Activated C1s binds C4 and enzymatically liberates C4a and C4b, which in turn promotes the cleavage of C2 into C2a and C2b.
- Surface-attached C4b serves as a platform for the formation of C3 convertase (C4b2a). C3 convertase can cleave C3 into C3a and C3b, which is essential for the next enzymatic reaction.

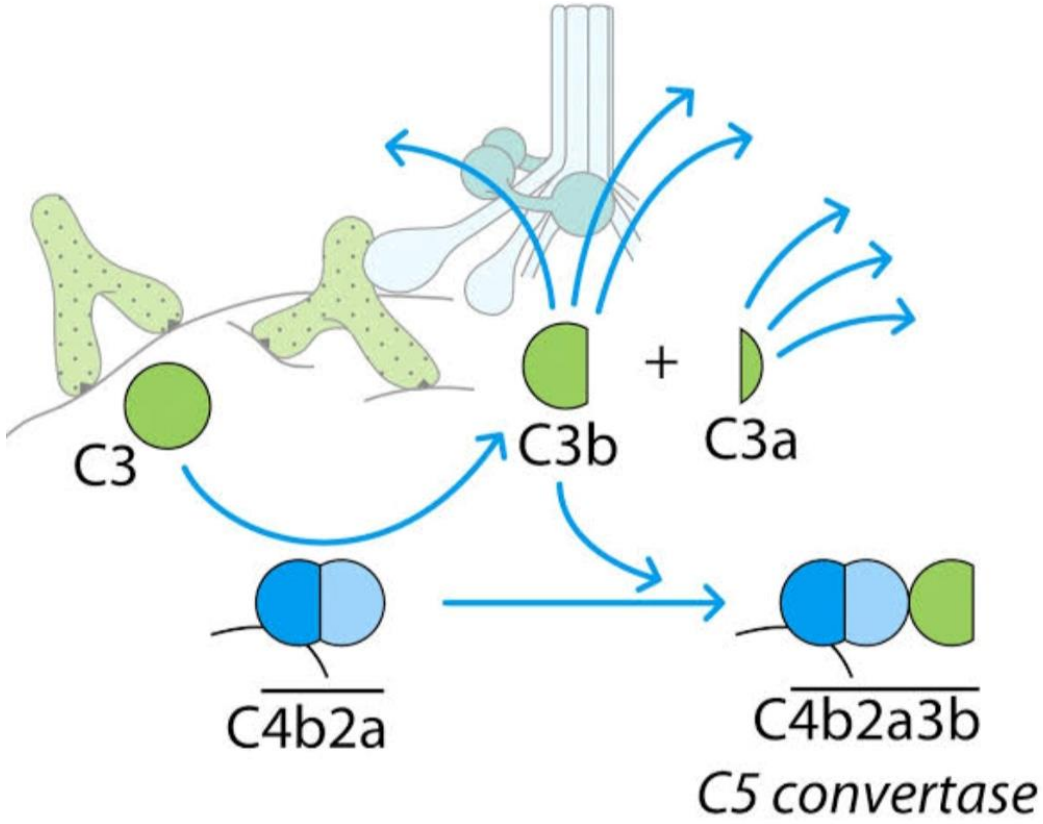


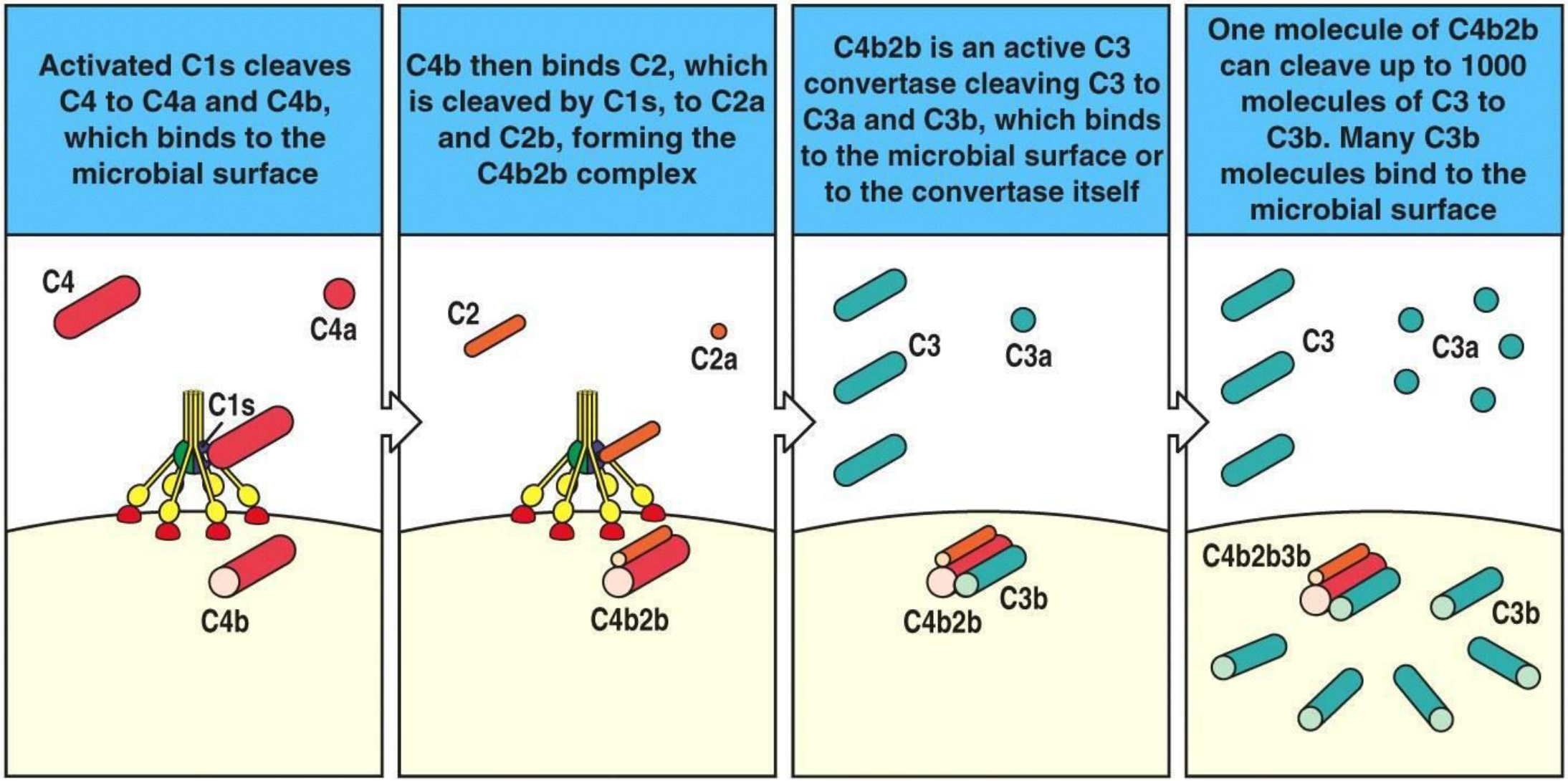
Formation of C5 convertase and MAC

- C3b binds to the C4b2a complex to form C5 convertase (C4b2a3b), while C3a plays role in the recruitment of inflammatory cells (anaphylatoxin).
- C5 convertase then cleaves C5 into C5a and C5b.
- .C5b combines with other terminal components C6, C7, C8, and C9 to form the Membrane Attack Complex (MAC), which leads to lysis of invasive bacteria through insertion into the target cell membranes to create functional pores.

Formation of C5 convertase and MAC






Activated C1s cleaves C4 to C4a and C4b, which binds to the microbial surface
C4
C4a
C1s
C4b
C4b then binds C2, which is cleaved by C1s, to C2a and C2b, forming the C4b2b complex
C2
C2a
C4b2b
C4b2b is an active C3 convertase cleaving C3 to C3a and C3b, which binds to the microbial surface or to the convertase itself
C3
C3a
C4b2b
C3b
One molecule of C4b2b can cleave up to 1000 molecules of C3 to C3b. Many C3b molecules bind to the microbial surface
C3
C3a
C4b2b3b
C3b

Alternative pathway

- When it comes to the alternative pathway, it is basically activated at a significantly low level. It is completely different from the classical pathway.
- The spontaneous hydrolysis of the C3 molecule results in the activation of this particular pathway. The hydrolysis occurs as a result of the breaking down of the internal thioester bond.
- Unlike other pathways, the alternative pathway doesn’t really rely on the antibodies that bind themselves to the pathogen.

Ab independent pathway:

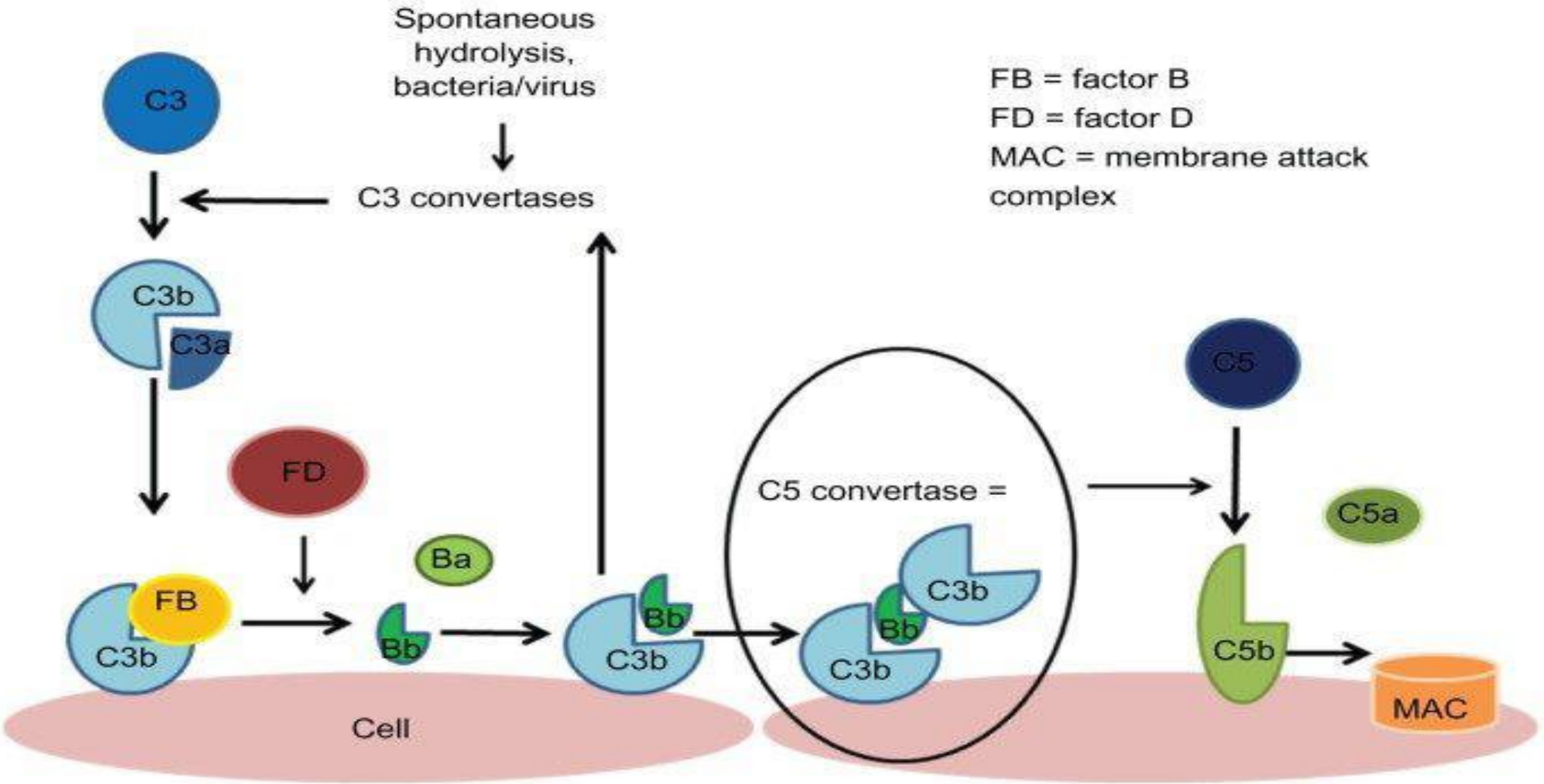
In the alternative pathway, many unrelated cell surface substances, e.g., bacterial lipopolysaccharides (endotoxin), fungal cell walls, and viral envelopes, can initiate the process by binding C3 and factor B.

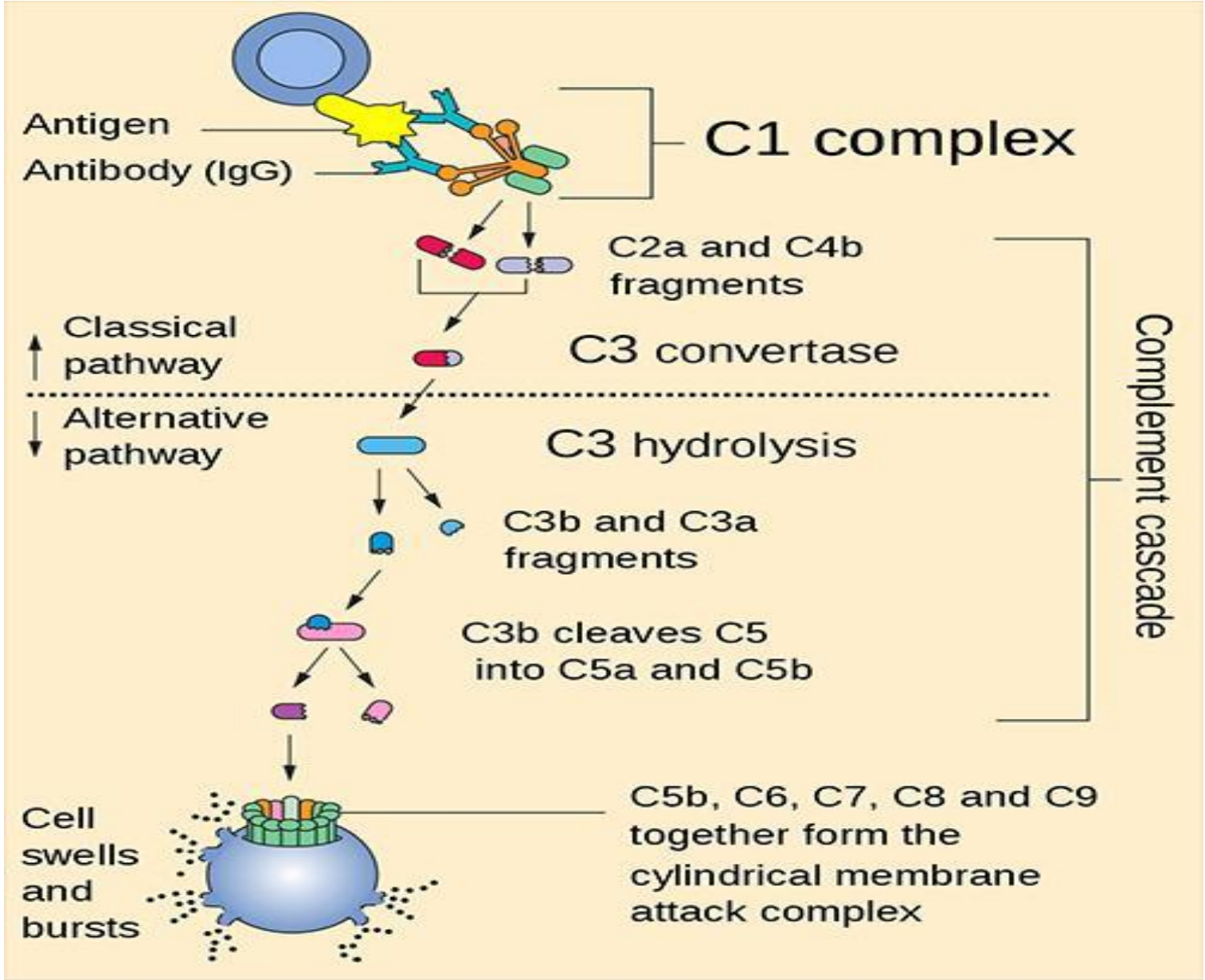
•This complex is cleaved by a protease, factor D, to produce C3bBb.

•This acts as a C3 convertase to generate more C3b.

Components of the Alternate Pathway		
Native component	Active component(s)	Function(s)
C3	C3a	Mediates inflammation; anaphylatoxin.
	C3b	Binds cell surfaces for opsonization and activation of alternate pathway.
Factor B	B	Binds membrane bound C3b. Cleaved by Factor D.
	Ba	Unknown.
	Bb	Cleaved form stabilized by P produces C3 convertase.
Factor D	D	Cleaves Factor B when bound to C3b.
Properdin	P	Binds and stabilizes membrane bound C3bBb.

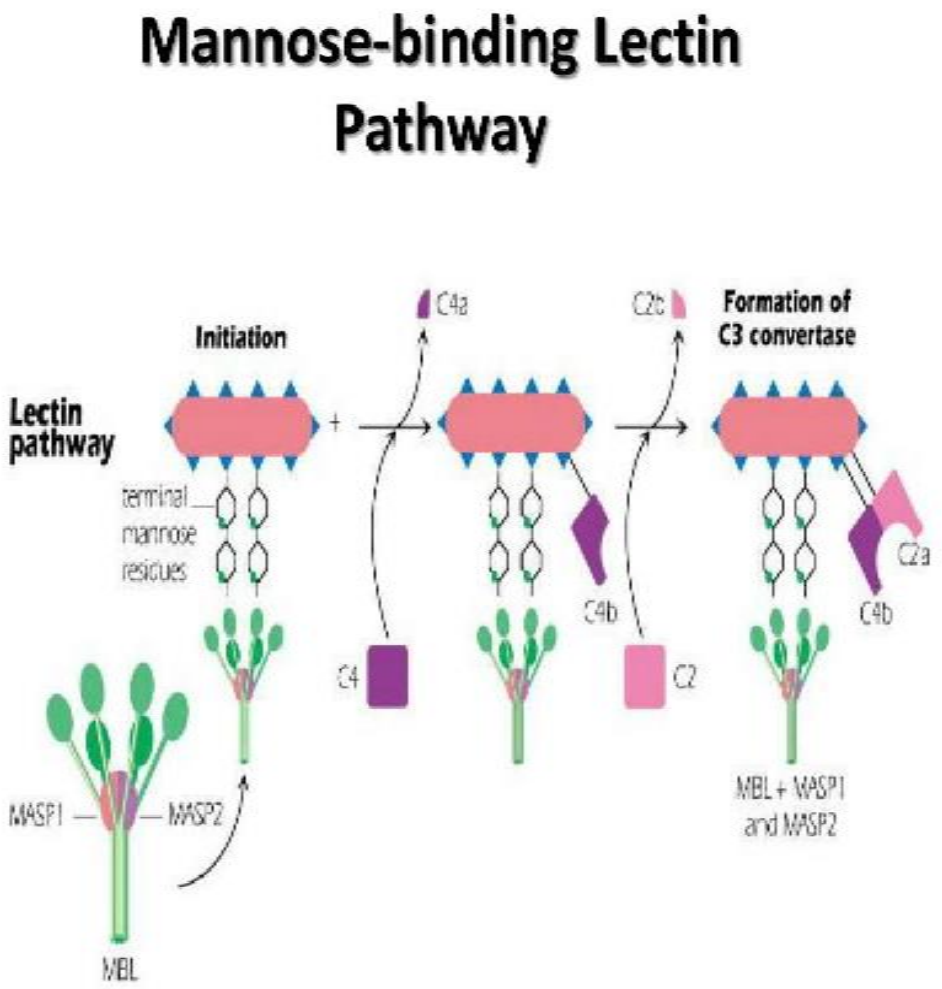
Alternative pathway



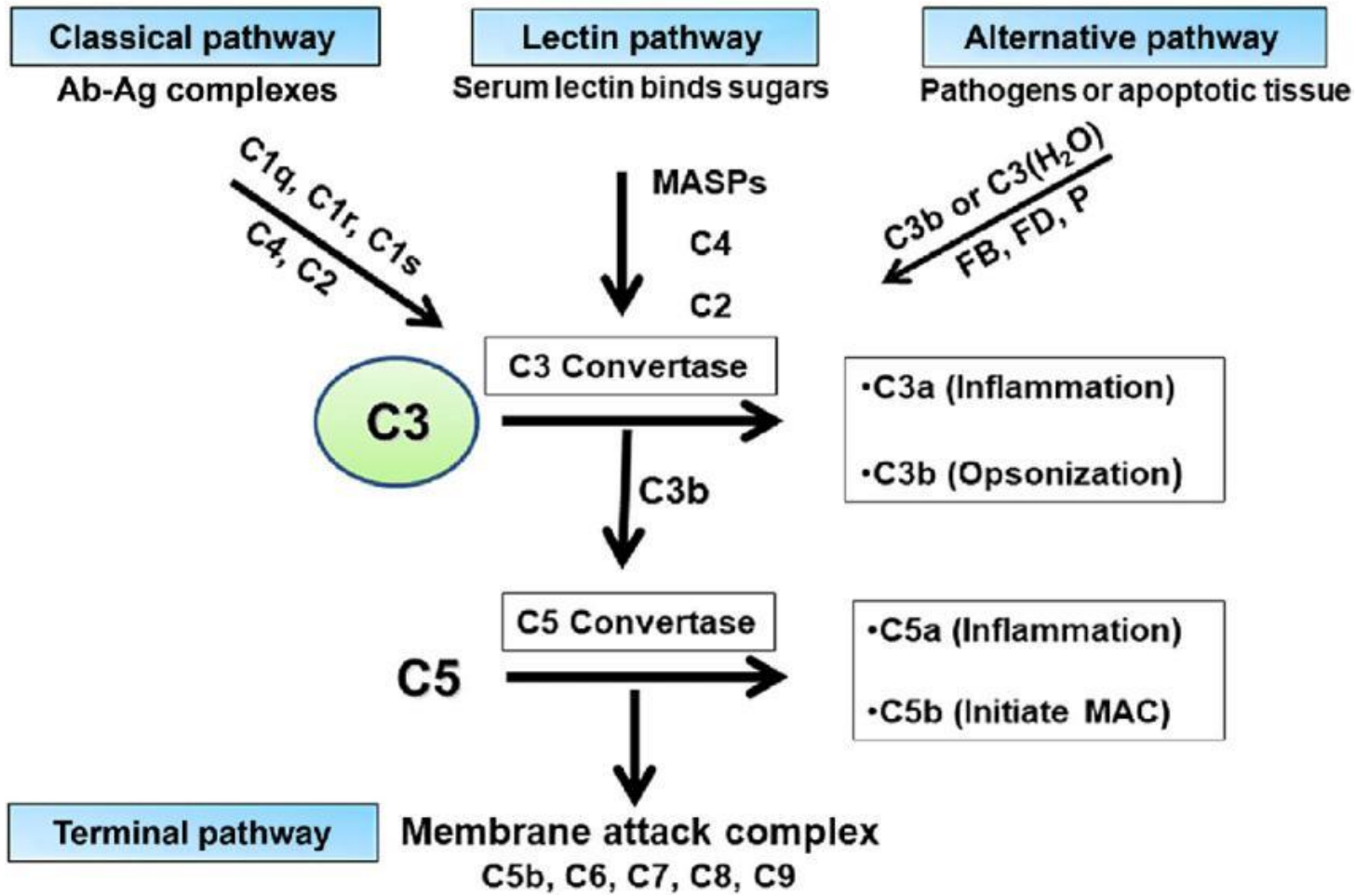

Antigen
Antibody (IgG)
C1 complex
C2a and C4b fragments
Classical pathway
C3 convertase
Alternative pathway
C3 hydrolysis
C3b and C3a fragments
C3b cleaves C5 into C5a and C5b
Complement cascade
Cell swells and bursts
C5b, C6, C7, C8 and C9 together form the cylindrical membrane attack complex

LECTIN PATHWAY

- MBL pathway resembles the classical pathway as it proceeds through the action of C4 and C2 to produce activated proteins of the complement system. MBL works the same as C1q which it resembles in structure.
- After the MBL binds to carbohydrate residues on the surface of a cell or pathogen, two components, MASP-1 and MASP-2 bind to MBL. MASP stands for MBL-associated serine proteases. Two proteases form a tetrameric complex similar to the one formed by C1r and C1s and cleaves C4 and C2 forming C3 convertase. The process now continues to form C5 convertase and the MAC as in the classical pathway.



Complement cascade



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