BHARATHIDASAN UNIVERSITY

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Programme: M.Sc., Biotechnology (Marine)

Course Title : Immunology

Course Code : 21 CC7

Unit : I

Three dimensional structure of IgM & IgG

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Immunoglobulin

Immunoglobulins (Ig) are glycoproteins that your immune cells make to fight off bacteria, viruses, fungi, parasites, cellular antigens, chemicals, and synthetic substances.

- B cells are produced in stem cells in bone marrow and play an important role in the humoral immune response.
- Immunoglobulins constitute about 20% of the protein in plasma.
- The antibody immune response is highly complex and exceedingly specific.

Types

Based on structure and antigenic nature, the five primary classes of immunoglobulins are

- IgG, (gamma)
- IgM, (mu)
- IgA, (alpha)
- IgD,(delta)
- IgE.(Epsilon)

Basic structure of immunoglobulins

Different immunoglobulins can differ structurally, they all are built from the same basic units.

- Heavy and Light Chains:

All immunoglobulins have a four chain structure as their basic unit. They are composed of two identical light chains (23kD) and two identical heavy chains (50-70kD).

- Disulfide bonds-

➢ Inter-chain disulfide bonds -The heavy and light chains and the two heavy chains are held together by inter-chain disulfide bonds and by non-covalent interactions The number of inter-chain disulfide bonds varies among different immunoglobulin molecules.

➢ Intra-chain-

disulfide binds within each of the polypeptide chains there are also intra-chain disulfide bonds.

Variable (V) and Constant (C) Regions

When the amino acid sequences of many different heavy chains and light chains were compared, it became clear that both the heavy and light chain could be divided into two regions based on variability in the amino acid sequences.

These are the:

Light Chain - VL (110 amino acids) and CL (110 amino acids)

Heavy Chain - VH (110 amino acids) and CH (330-440 amino acids).

Hinge Region -This is the region at which the arms of the antibody molecule forms a Y. It is called the hinge region because there is some flexibility in the molecule at this point.

Domains

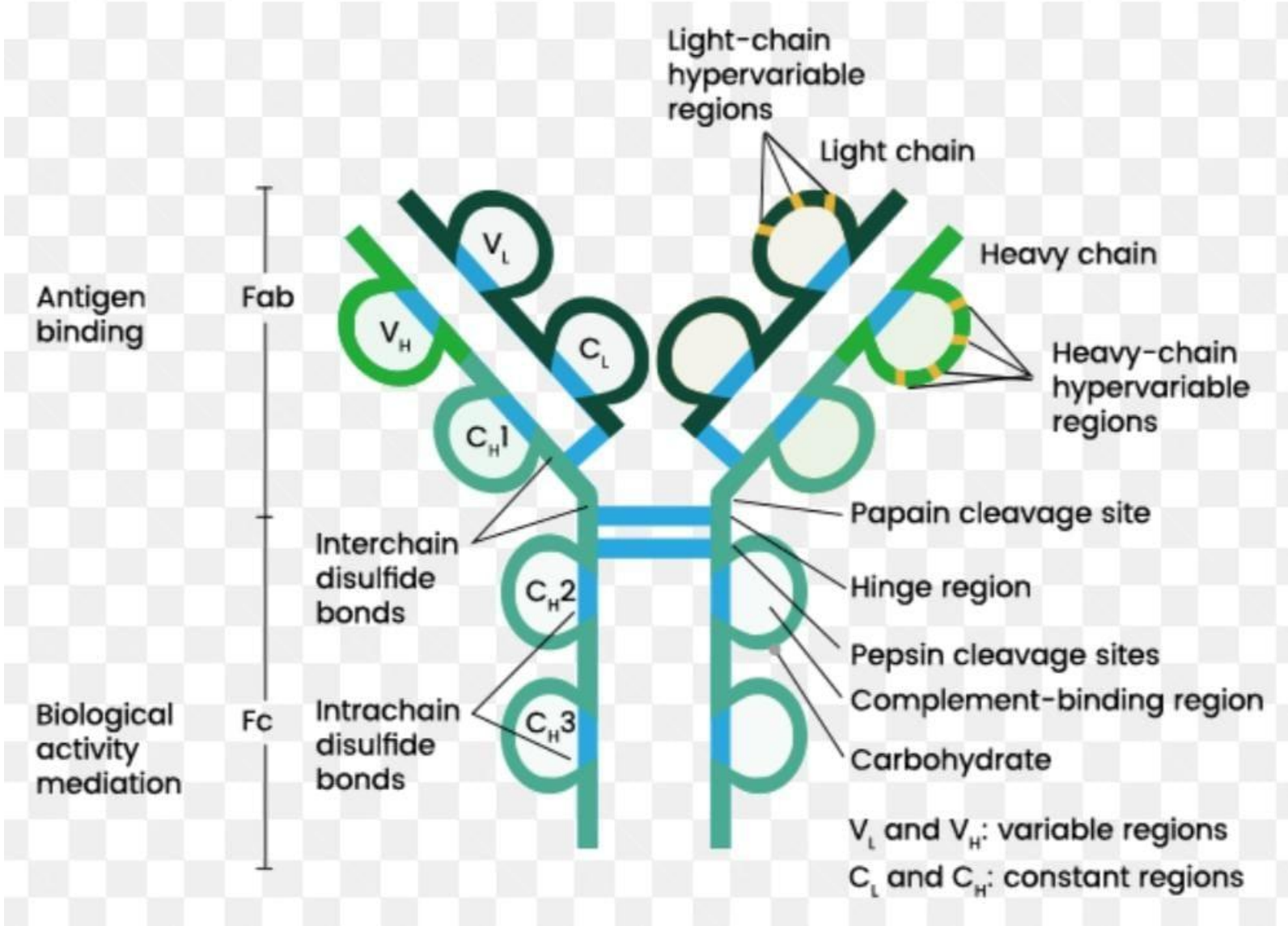
Three dimensional images of the immunoglobulin molecule show that it is not straight . Rather, it is folded into globular regions each of which contains an intra-chain disulfide bond . These regions are called domains.

Light Chain Domains - VL and CL

Heavy Chain Domains - VH, CH1 - CH3 (or CH4)

Oligosaccharide

Carbohydrates are attached to the CH2 domain in most immunoglobulins. However, in some cases carbohydrates may also be attached at other locations.


Light-chain hypervariable regions
Light chain
Heavy chain
Heavy-chain hypervariable regions
Antigen binding
Fab
Fc
Biological activity mediation
V_L
V_H
C_L
C_H1
C_H2
C_H3
Interchain disulfide bonds
Intrachain disulfide bonds
Papain cleavage site
Hinge region
Pepsin cleavage sites
Complement-binding region
Carbohydrate
V_L and V_H: variable regions
C_L and C_H: constant regions

Three dimensional structure of Ig-M

- IgM immunoglobulin is the first isotype expressed during B cell development and following B cell activation.
- Naïve B cells express IgM monomers on their surface.
- Antigenic stimulation results in IgM secretion in a pentameric form, linked by disulfide bonds in the constant heavy regions.
- IgM is produced before class switching and has a low affinity.

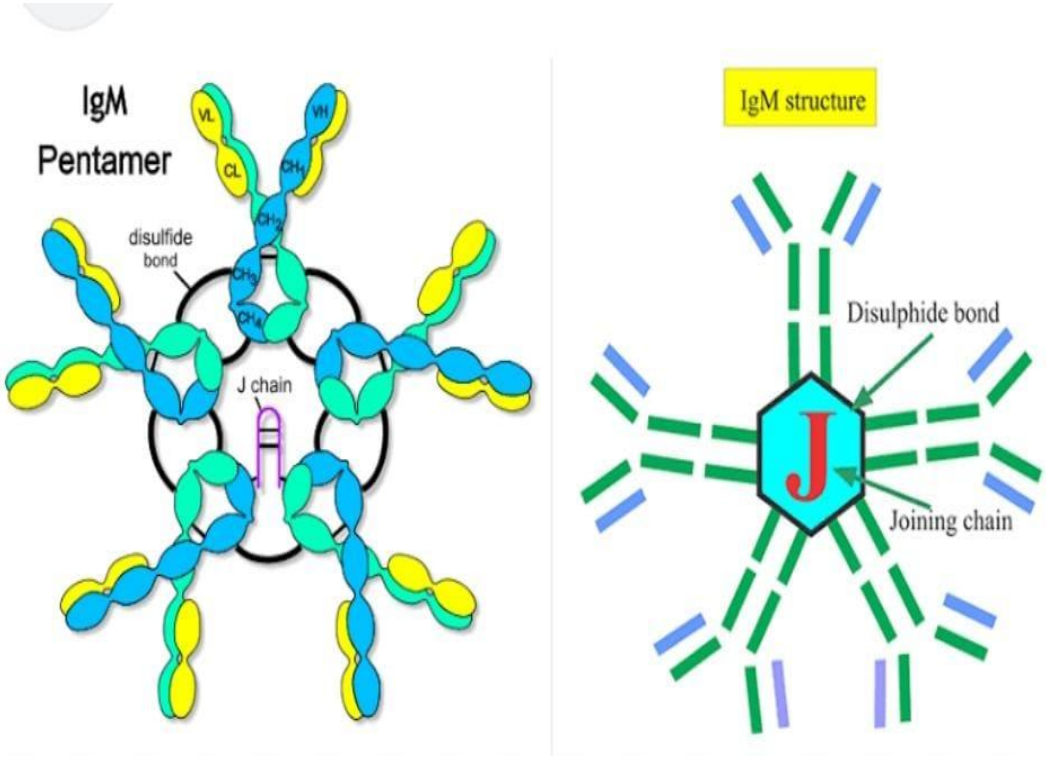
Three dimensional structure of Ig-M

- However, the secreted pentameric form provides 10 antigen-binding sites and thus has high overall avidity.
- Due to the large size of the IgM pentamers, IgM is primarily found in the bloodstream, although it is present in the lymph to a lesser extent.
- IgM is especially effective at activating the classical complement system, leading to opsonization of antigens and cytolysis.
- Clinically, IgM is frequently used in diagnostic assays for acute infections since it is involved in the primary immune response.

Three dimensional structure of Ig-M

- Size: 900 kDa
- Light chain: λ or κ
- Heavy chain: μ – variable domain and 4 constant domains.
- Number of antigen binding sites: 10
- Percent of total Ig: 10%
- Serum half-life: 5 days
- Distribution: mainly in bloodstream

- **Structure**: IgM normally exists as a pentamer (19S immunoglobulin) but it can also exist as a monomer. In the pentameric form all heavy chains are identical and all light chains are identical.
- Thus, the valence is theoretically 10. IgM has an extra domain on the mu chain (CH4) and it has another protein covalently bound via a S-S bond called the J chain.
- This chain functions in polymerization of the molecule into a pentamer.



Three dimensional structure of IgG

IgG is the most predominant isotype found in human serum, has the longest serum half-life, and controls infections in tissues.

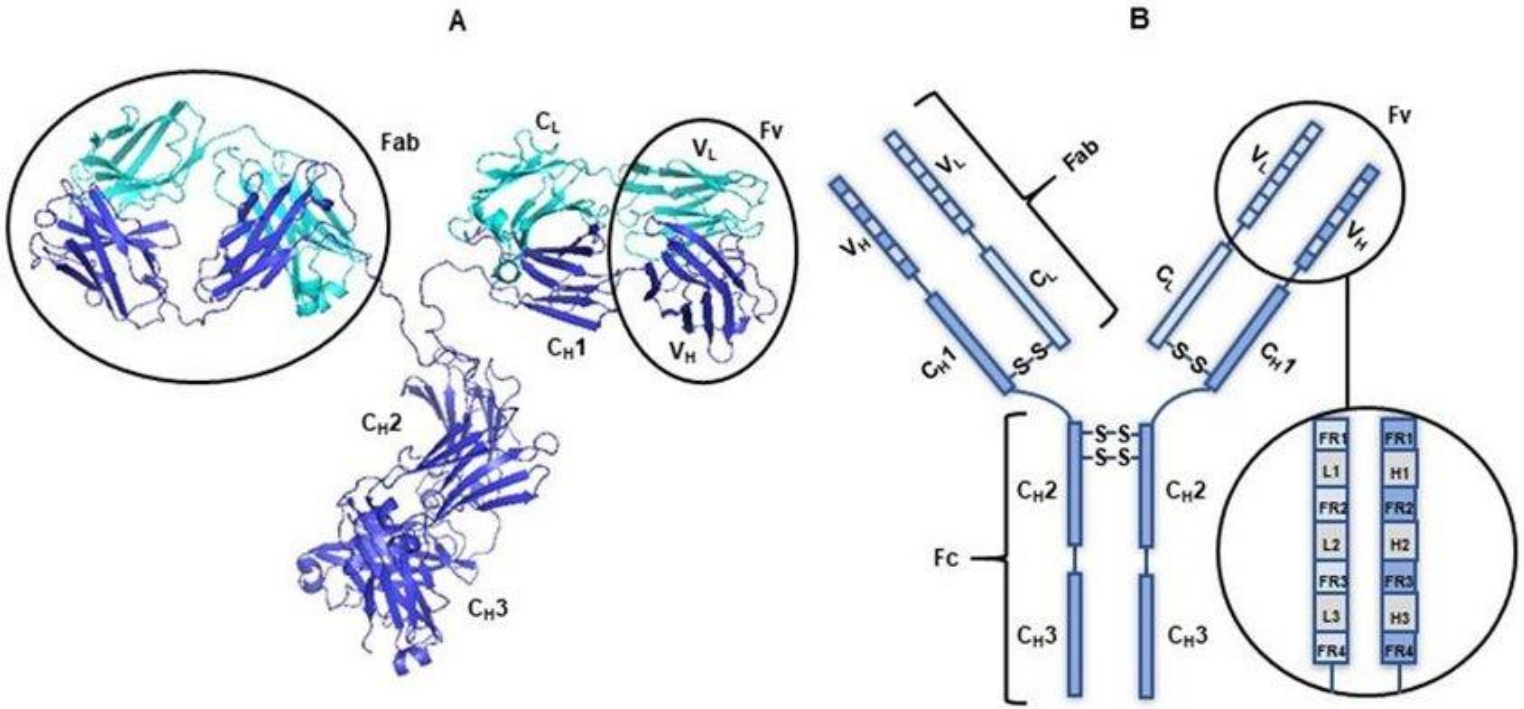
- IgG has many functions, including activation of the classical complement pathway, opsonization, neutralization of toxins, and antibody-dependent cell-mediated cytotoxicity (ADCC). IgG is the only isotype that can cross the human placenta and enter the fetal circulation, protecting the fetus and newborn.
- There are 4 subclasses of IgG named in order of relative abundance (IgG1, IgG2, IgG3, and IgG4) that have different biological properties.

Three dimensional structure of IgG

- IgG1 and IgG3 antibodies typically recognize protein antigens, while IgG2 recognizes polysaccharide antigens.
- IgG4 can bind to allergens and chronically persistent antigens and is considered anti-inflammatory.
- Due to its specificity, abundance, and longevity, IgG is the most commonly used isotype in research and diagnostics.

Structure

- All IgG's are monomers (7S immunoglobulin). The subclasses differ in the number of disulfide bonds and length of the hinge region.



Source: https://www.eurekaselect.com/article/92086

- Distribution: bloodstream and extracellular fluid
- Size: 150 kDa
- Light chain: λ or κ
- H-chain: γ1, γ2, γ3, γ4 – variable domain, 3 constant domains, and a hinge domain.
- Number of antigen binding sites: 2
- Percent of total Ig: 75%
- Serum half-life: 7-23 days, depending on the subclass.

Extracellular killing and complement mediated lysis

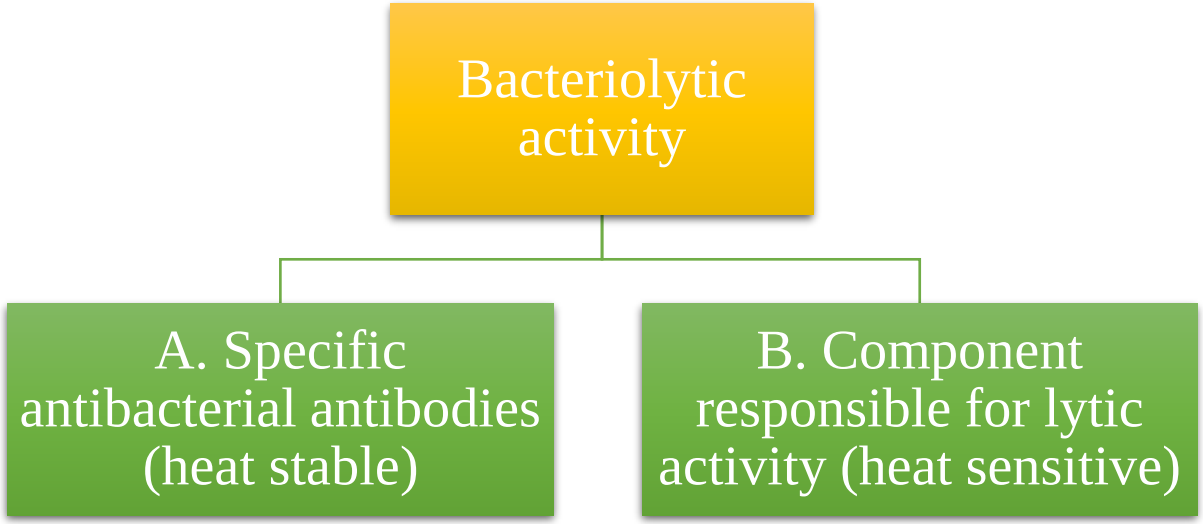
The Complement System

- **The complement system** refers to a group of proteins that play a crucial role in the body's immune response.
- These proteins circulate in the blood and tissues, remaining inactive until they are triggered by pathogens or antibody-antigen complexes.
- Once activated, the complement system follows a cascade of reactions that enhance the ability of antibodies and phagocytic cells to clear pathogens from an organism.

Discovery

1890s – Jules Bordet – Institute Pasteur, Paris

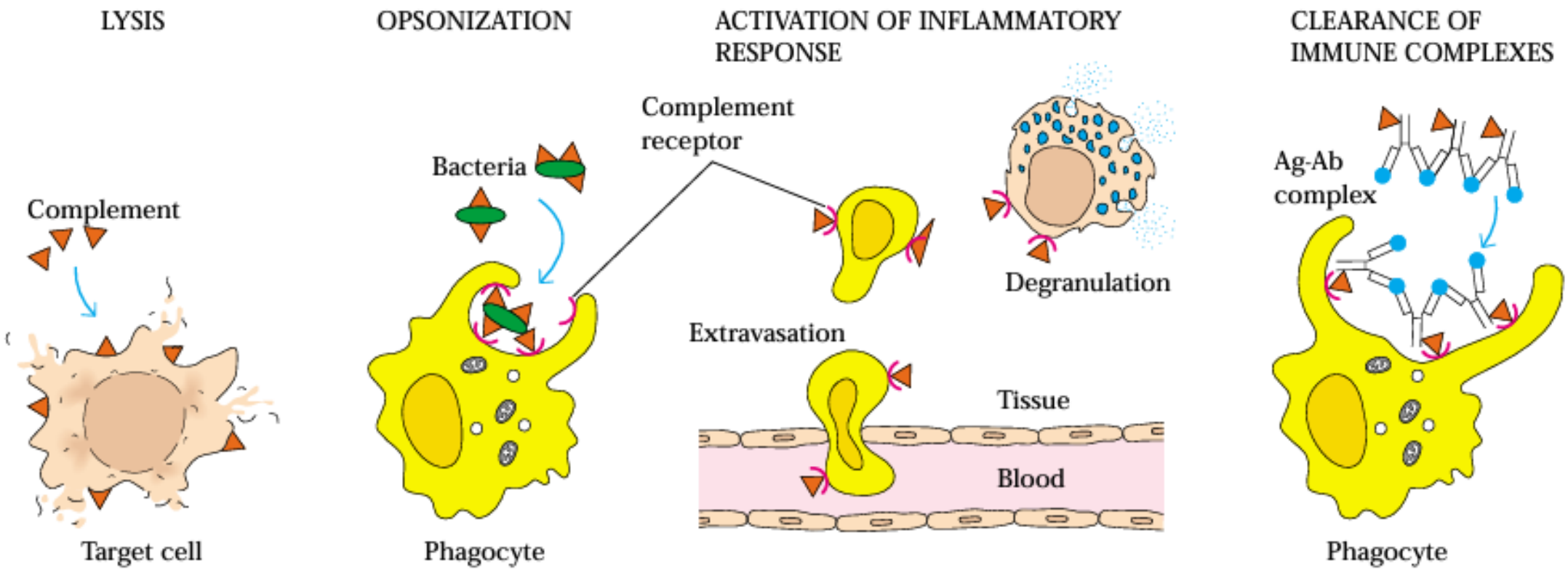
1. Sheep Antiserum + Vibrio cholera = Lysis of bacteria
2. Sheep Antiserum + Heat = Loss of bacteriolytic activity
3. Heated sheep antiserum + Fresh serum (without any antibody in itself)
   = Bacteriolytic property of heated serum restored



Paul Ehrlich – in Berlin
– Carried out independent experiments

- *Coined the term* " Complement "
- *Defined it as* : " the activity of blood serum that completes the action of antibody."

Functions of the Complement Pathways



Source: https://biosiva.50webs.org/complements.htm

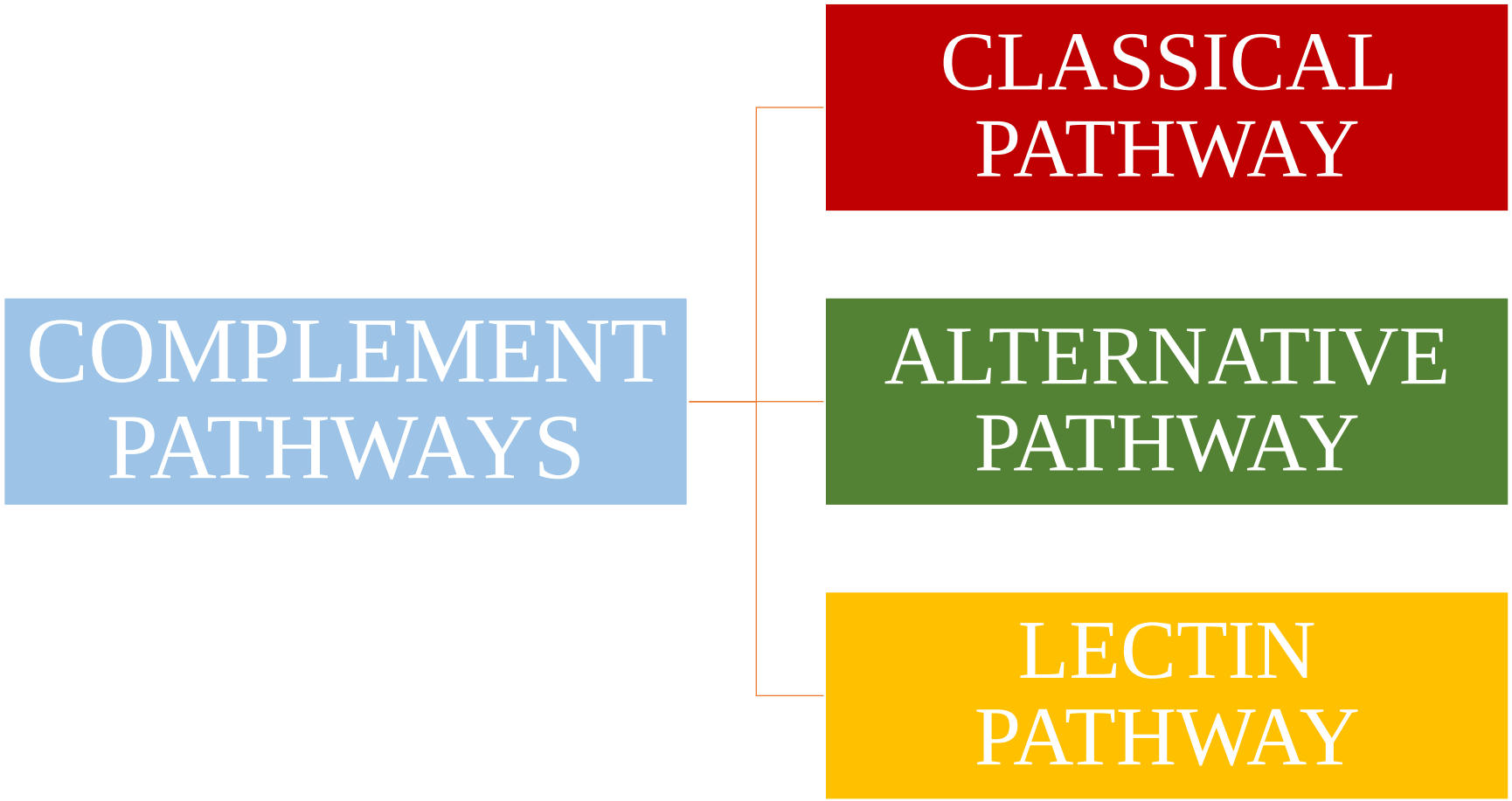
Extracellular Lysis Property

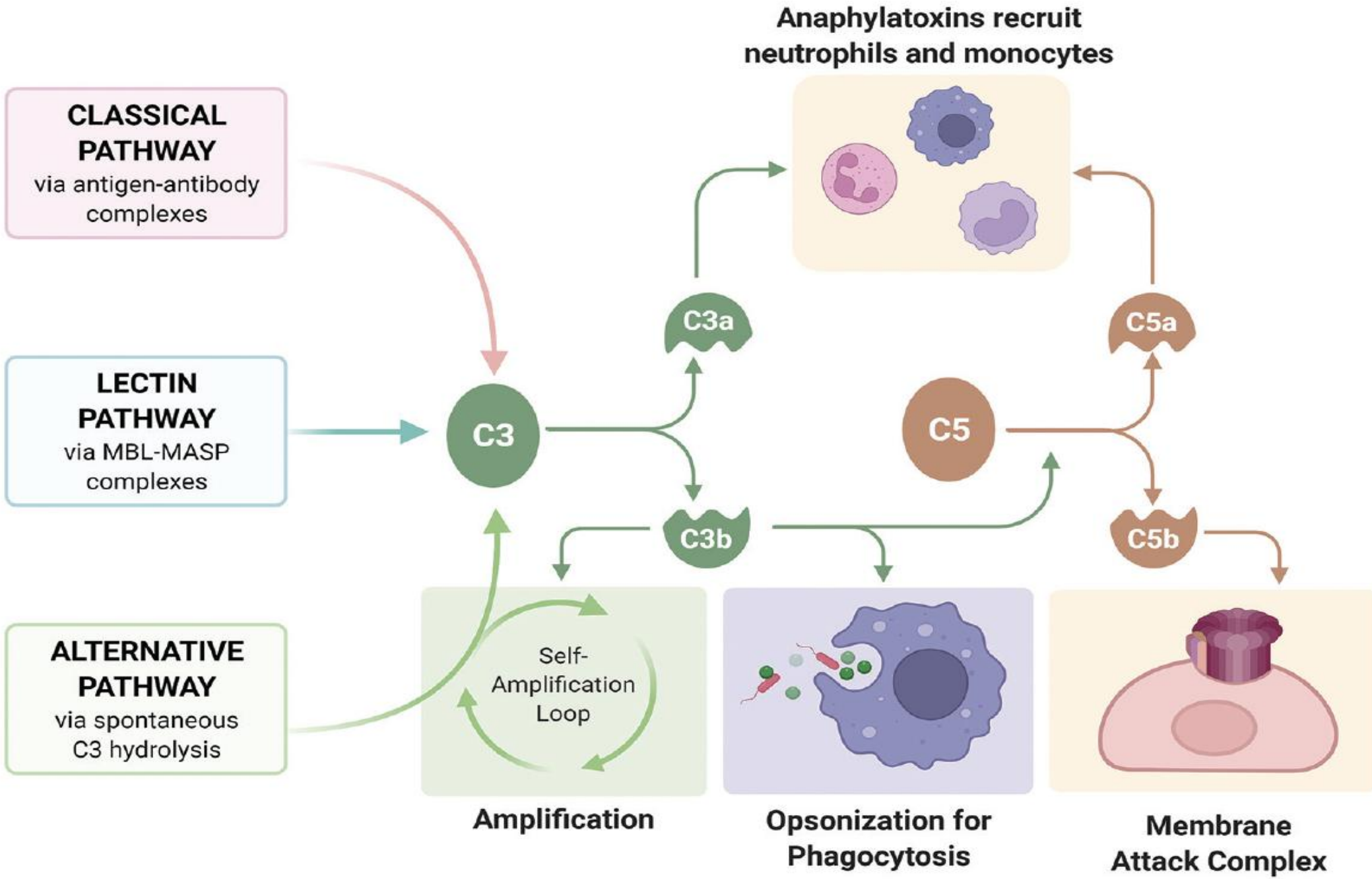
Membrane Attack Complex (MAC): The MAC forms on the surface of pathogens, creating pores in their cell membranes, leading to lysis and death. This entire process happens outside the cells of the host organism.

Opsonization: Complement proteins, such as C3b, bind to the surface of pathogens, marking them for recognition by phagocytes. Phagocytosis occurs when immune cells (like macrophages and neutrophils) engulf these opsonized pathogens in the extracellular space.

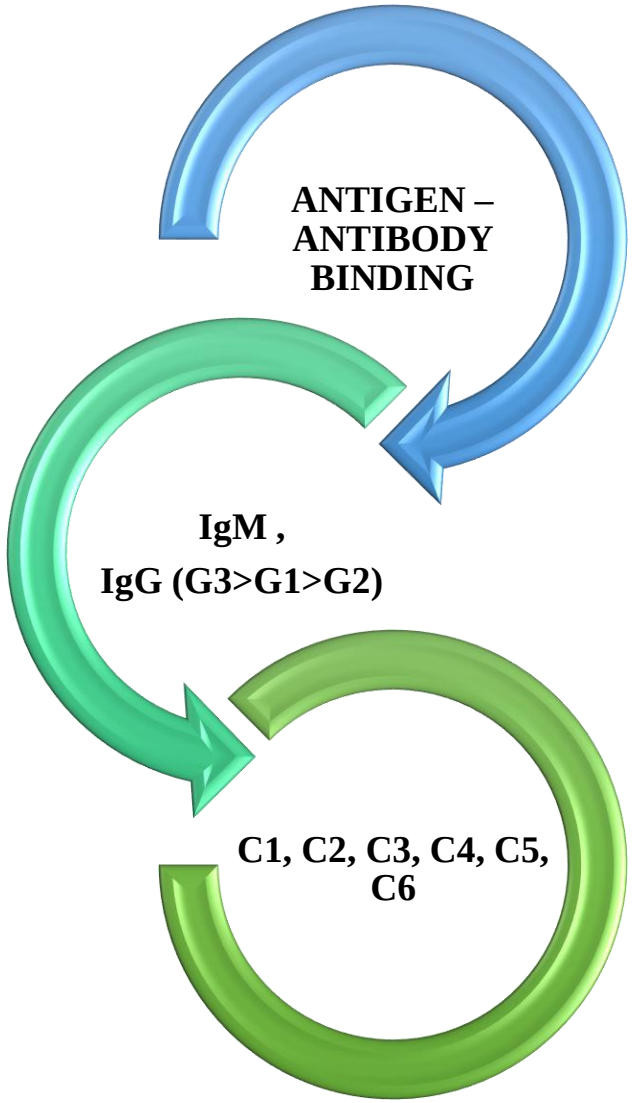
Inflammation and Chemotaxis: Complement proteins (e.g., C3a, C5a) act as chemoattractants, drawing immune cells to the site of infection or injury. These processes occur in the extracellular matrix and tissues.

Immune Clearance: The complement system facilitates the removal of immune complexes (antigen-antibody complexes) and apoptotic cells from the bloodstream and tissues. This clearance happens through binding and tagging in the extracellular space.

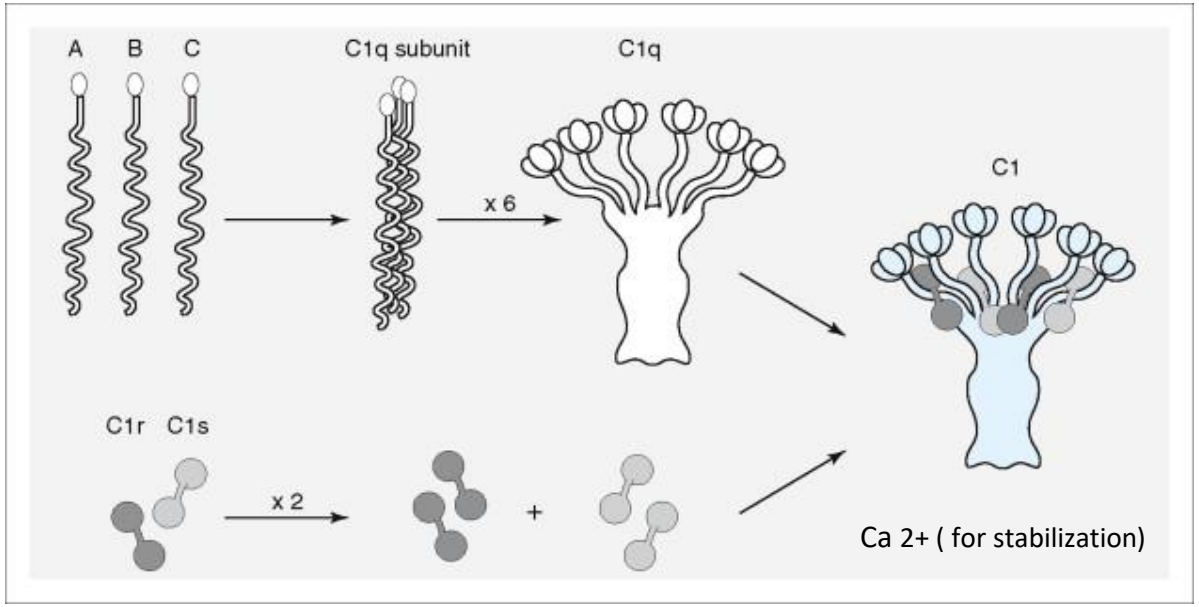

COMPLEMENT PATHWAYS
CLASSICAL PATHWAY
ALTERNATIVE PATHWAY
LECTIN PATHWAY

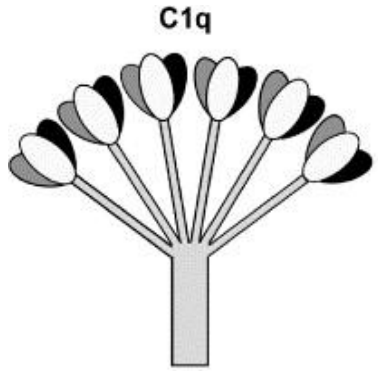

Anaphylatoxins recruit neutrophils and monocytes
CLASSICAL PATHWAY
via antigen-antibody complexes
LECTIN PATHWAY
via MBL-MASP complexes
ALTERNATIVE PATHWAY
via spontaneous C3 hydrolysis
C3
C3a
C3b
C5
C5a
C5b
Self-Amplification Loop
Amplification
Opsonization for Phagocytosis
Membrane Attack Complex

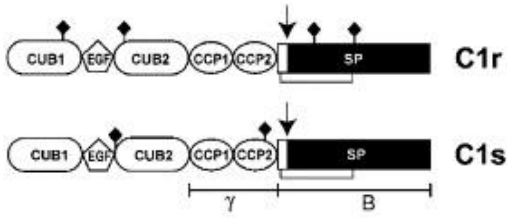
Classical pathway

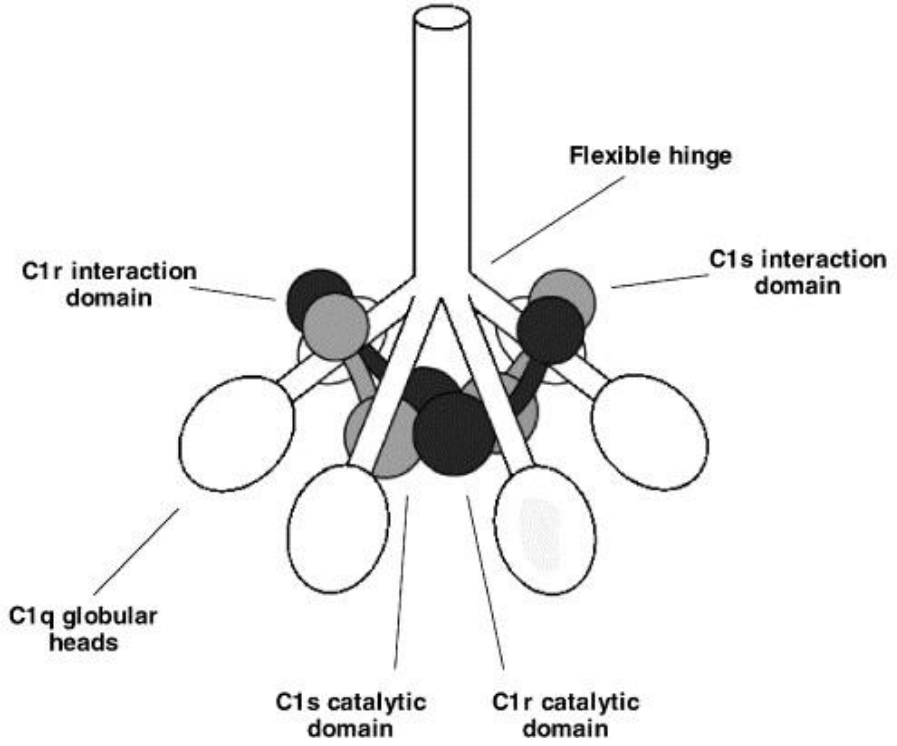


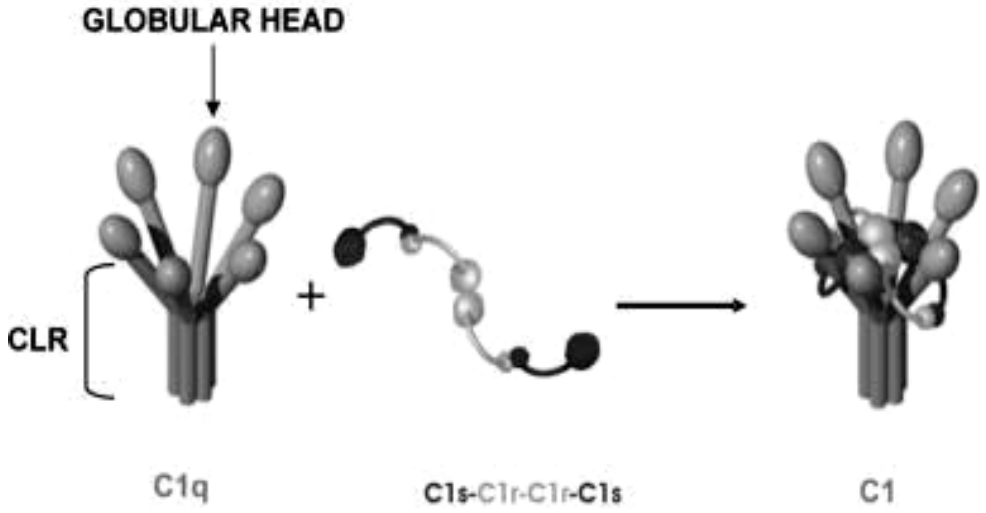
C1 : A Macromolecular Complex in Serum



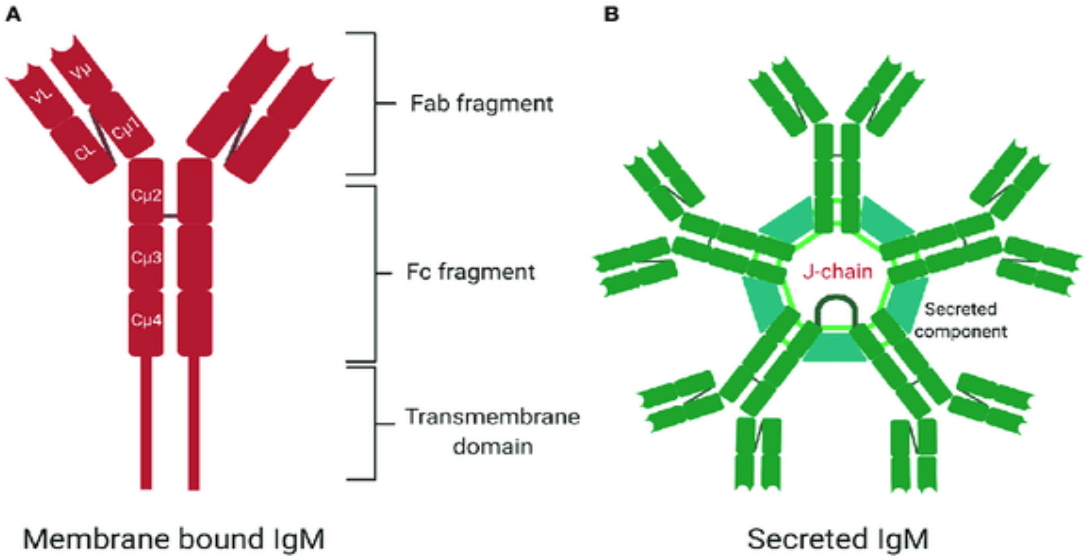


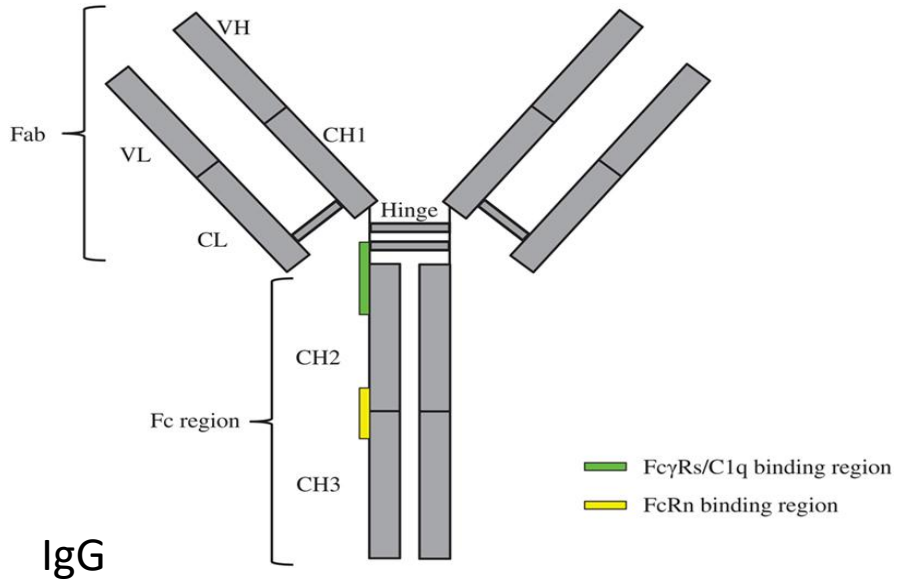






IgM & IgG interaction with C1q





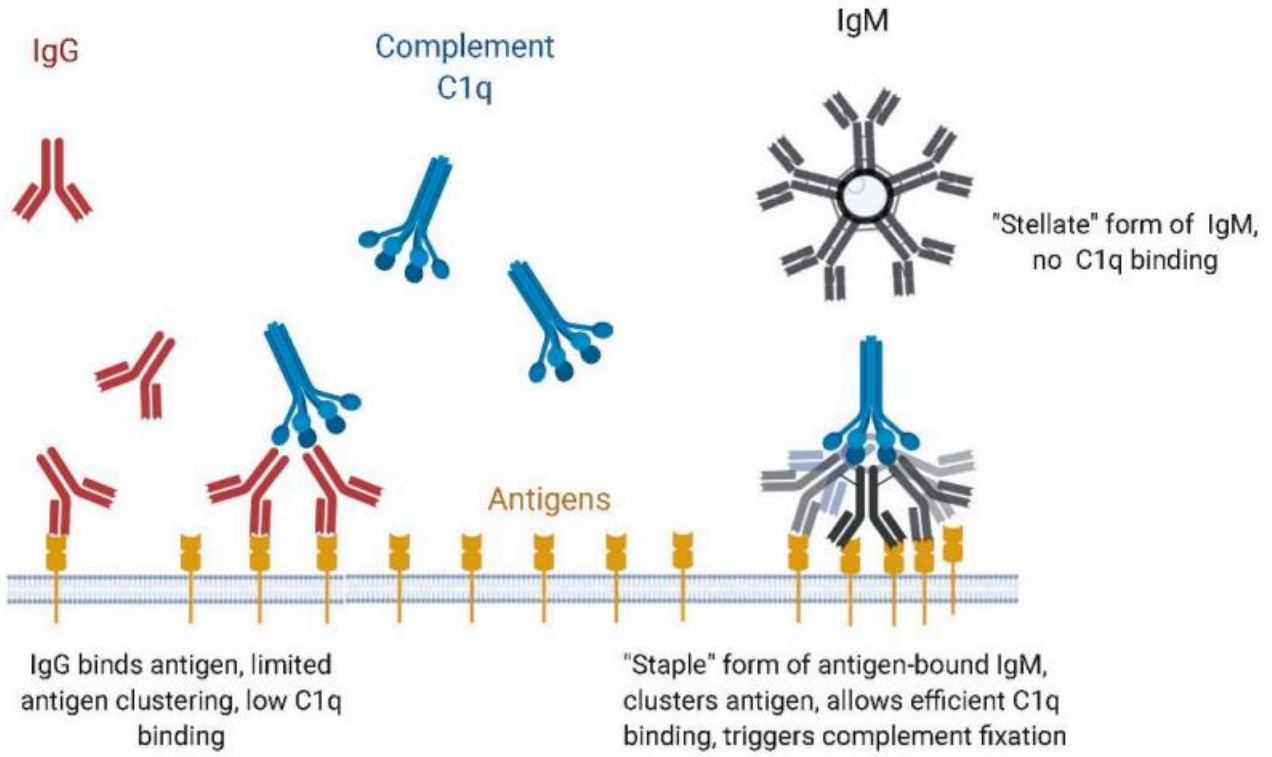
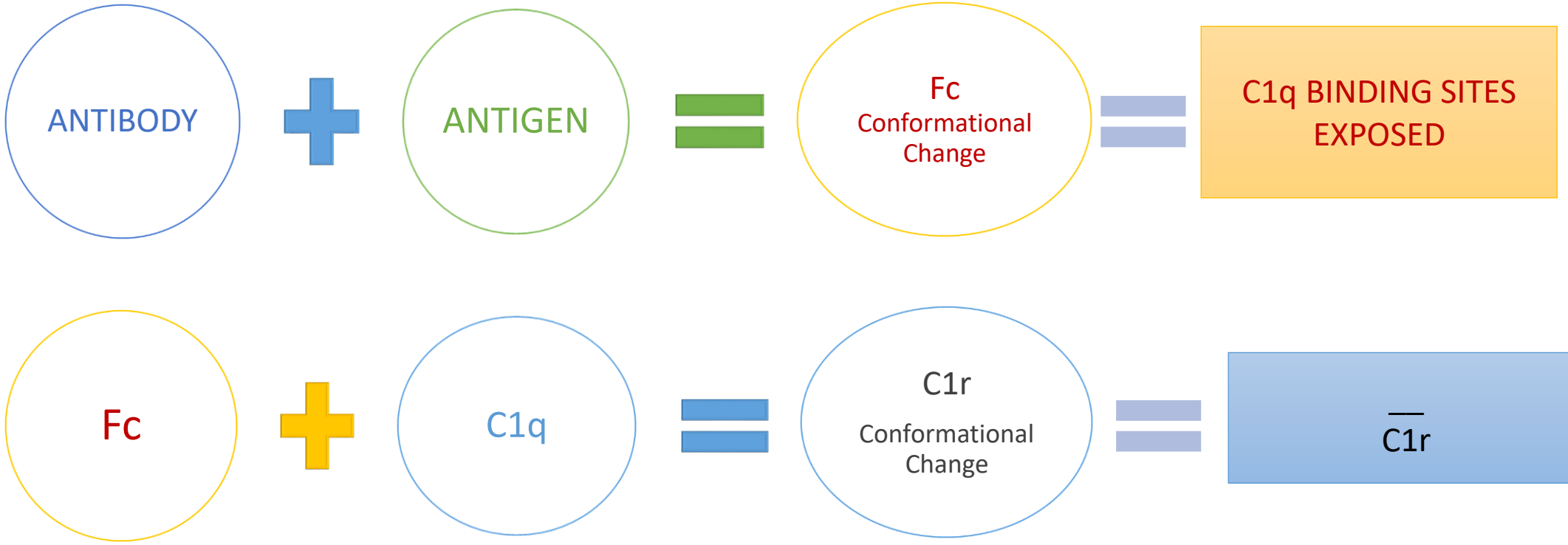
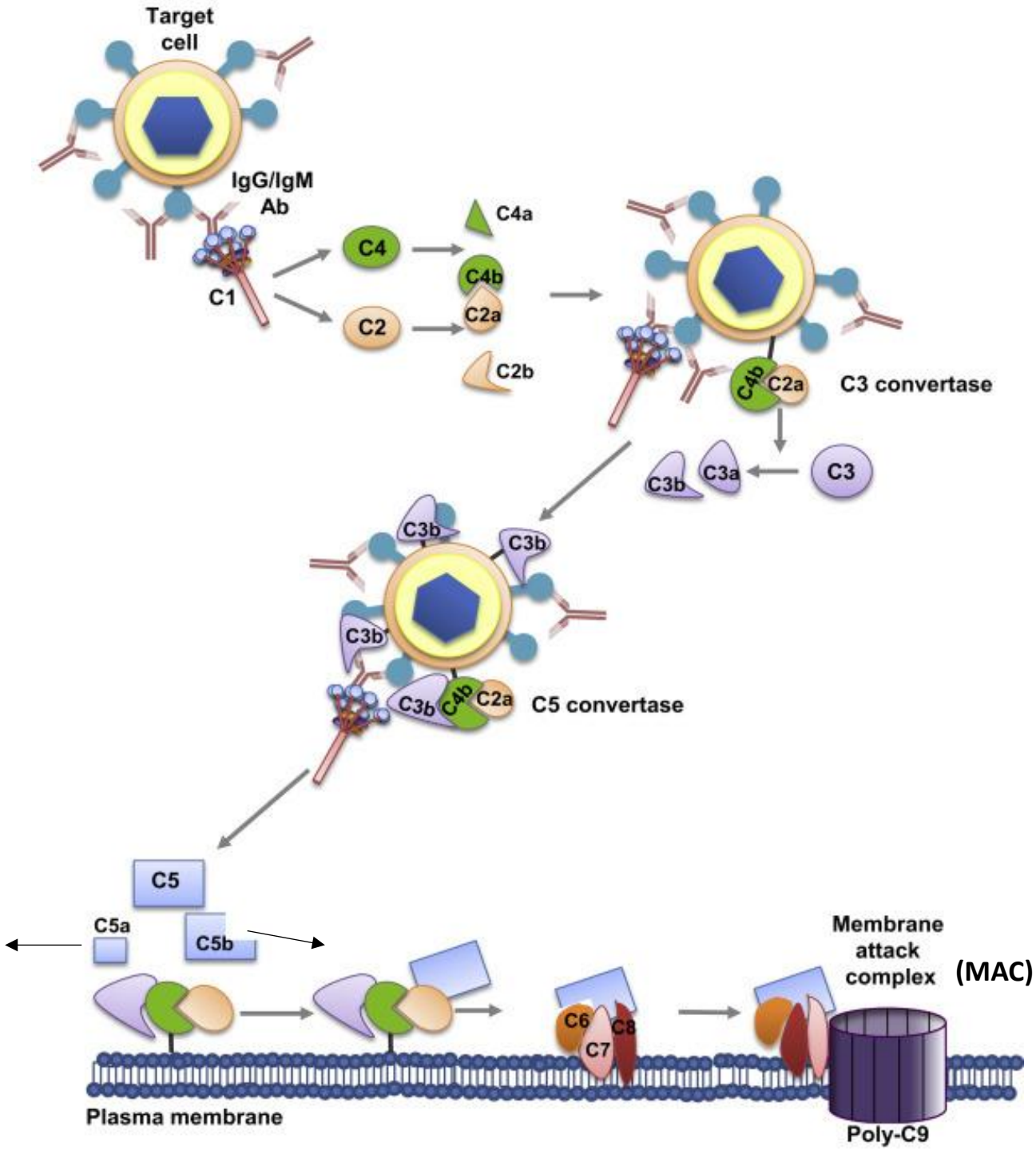


Figure 7. Complement binding and activation with IgM as compared with IgG.

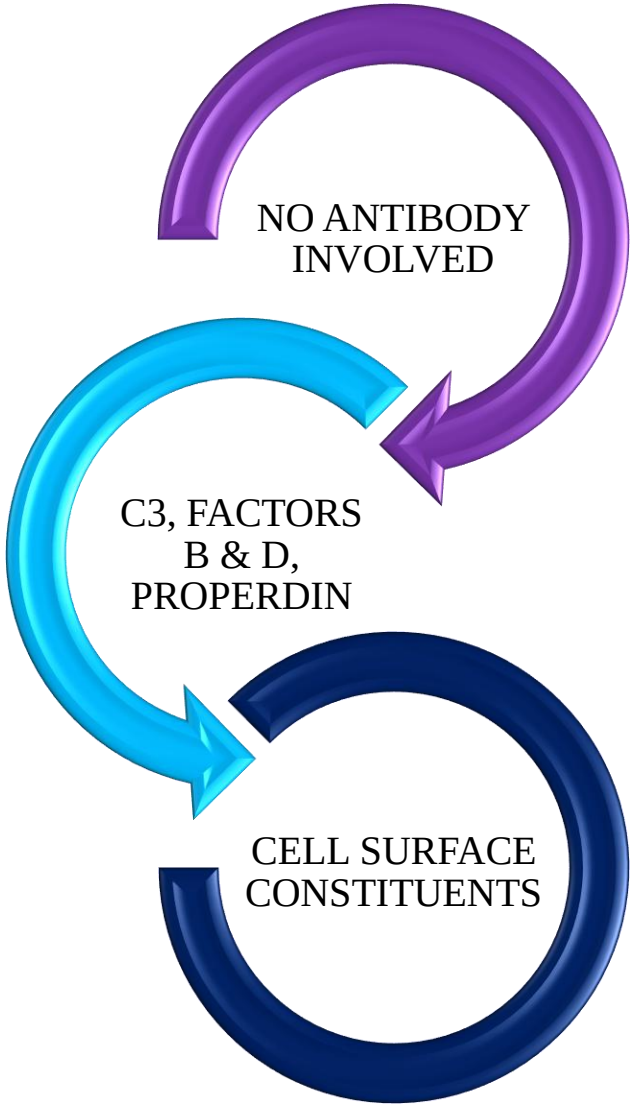
Source: https://www.slideshare.net/slideshow/complement-system-by-rupesh-trivedi/241468891

Initial Steps :




Target cell
IgG/IgM Ab
C1
C4
C2
C4a
C4b
C2a
C2b
C4b
C2a
C3 convertase
C3b
C3a
C3
C3b
C3b
C3b
C3b
C4b
C2a
C5 convertase
C5
C5a
C5b
C6
C7
C8
Membrane attack complex
(MAC)
Plasma membrane
Poly-C9

Alternative pathway



Initiators of the Alternative Pathway of Complement Activation

PATHOGENS ANS PARTICLES OF MICROBIAL ORIGIN	NONPATHOGENS
Many strains of gram-negative bacteria	Human IgG, IgA, and IgE in complexes
Lipopolysaccharides from gram-negative bacteria	Rabbit and guinea pig IgG in complexes
Many strains of gram-positive bacteria	Cobra venom factor
Teichoic acid from gram-positive cell walls	Heterologous erythrocytes (rabbit, mouse, chicken)
Fungal and yeast cell walls (zymosan)	Anionic polymers (dextran sulfate)
Some viruses and virus-infected cells	Pure carbohydrates (agarose, inulin)
Some tumor cells	
Parasites (trypanosomes)	

Steps :

1. It does not involve early complement component (C1, C2, C4)

C3b + Factor B —Mg2+→ C3bB complex

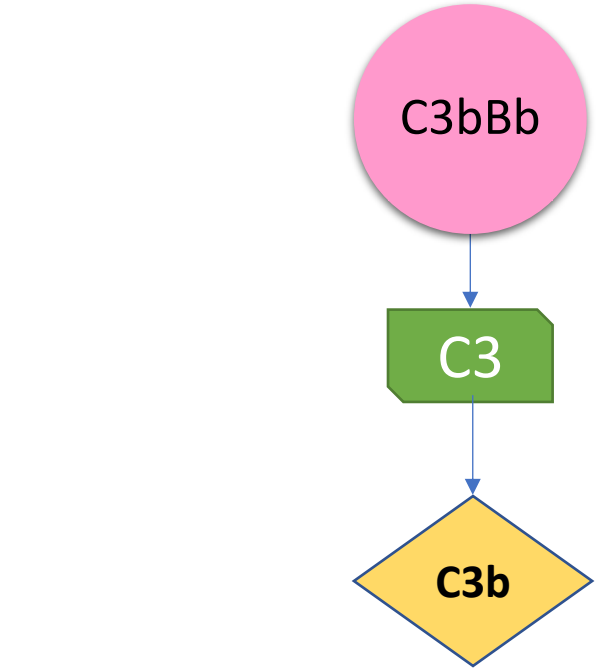
2. Interaction of C3bB with factor D

Factor D (OR C3 PROACTIVE CONVERTASE) → C3bB complex → Ba + Bb

- Ba is released into the medium.
- Bb binds to C3b forming the C3bBb complex

C3b + Bb → C3bBb complex (C3 CONVERTASE)

C3bBb + Properdin = Stabilizing complex (extending half life from 5 mins to 30 mins.)



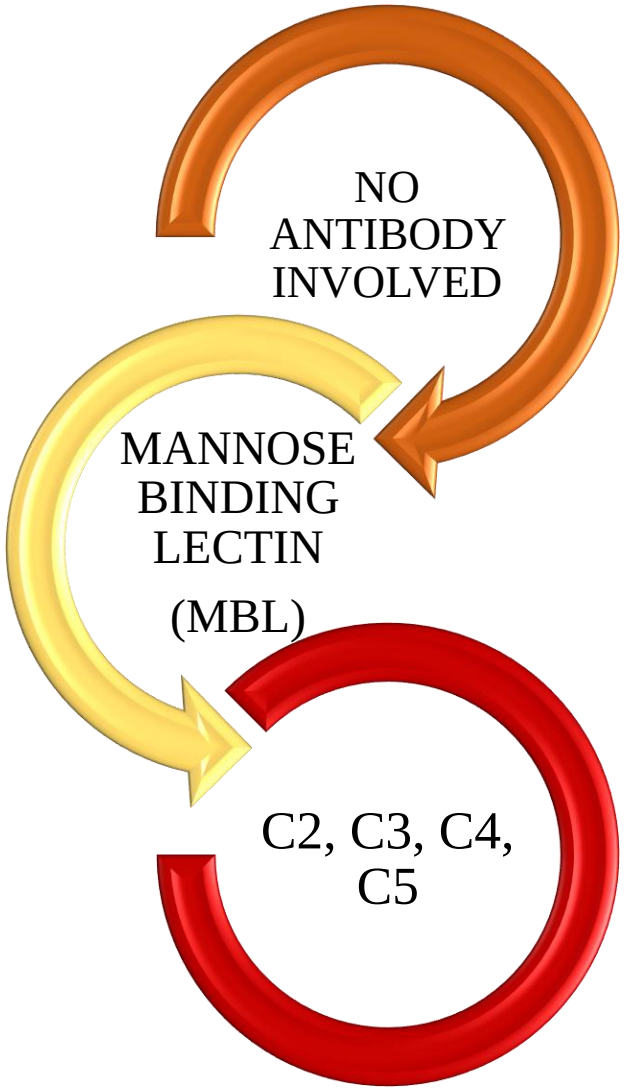
C3bBb + C3b = C3bBb3b

C3bBb3b: C5 convertase

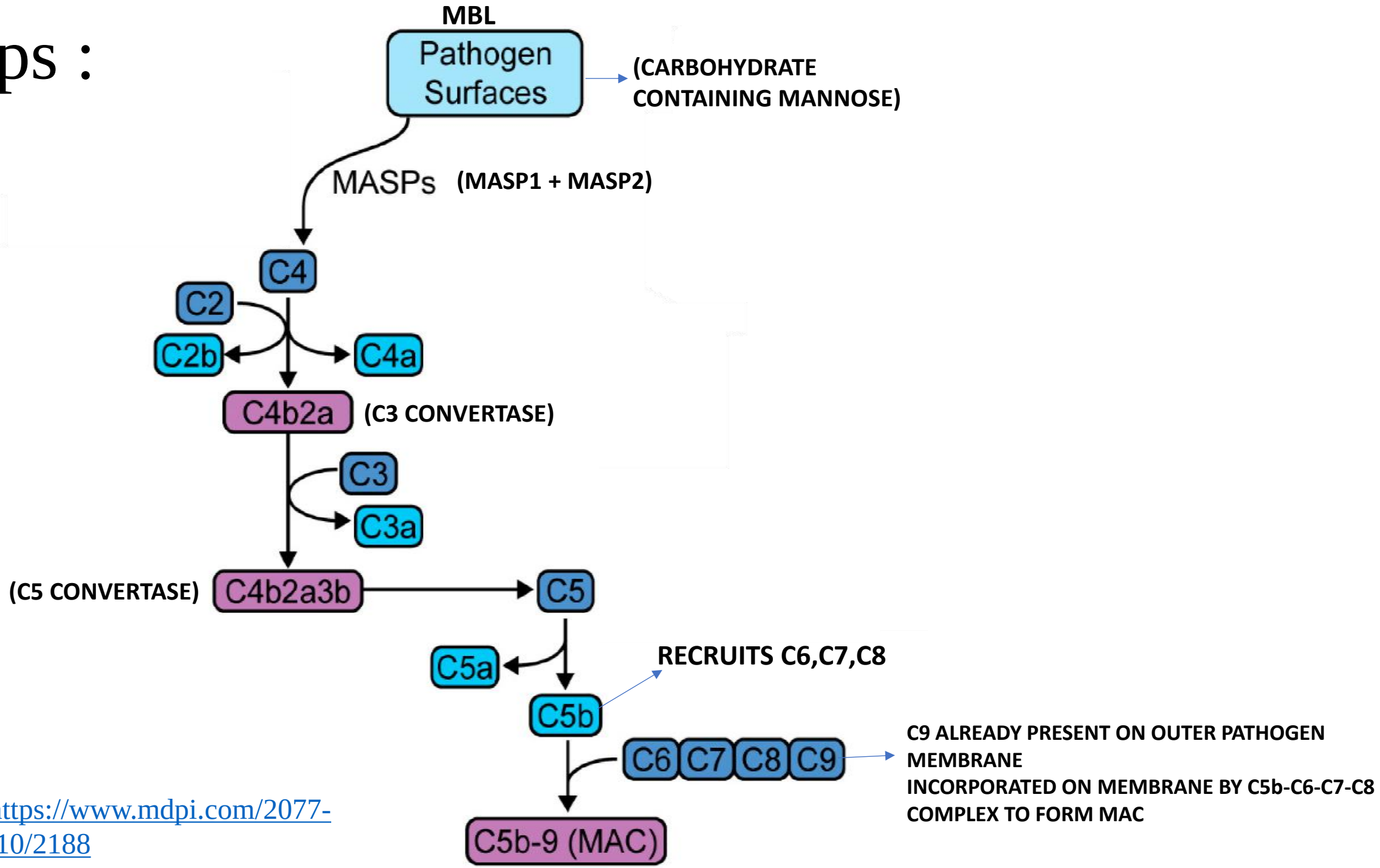
C3bBb3b + C5 → C5a + C5b

C5b → Target membrane (MAC)

Lectin pathway

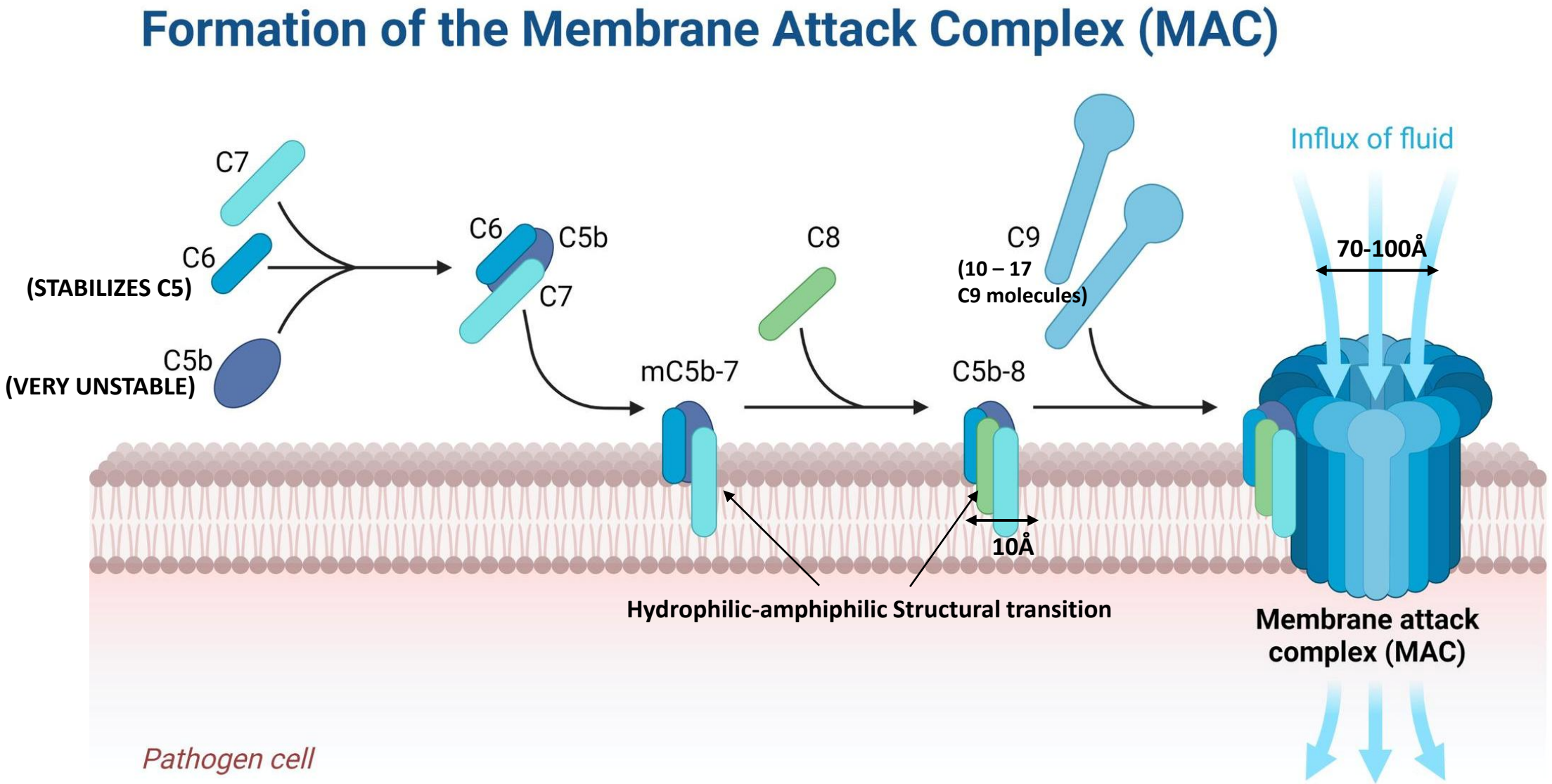


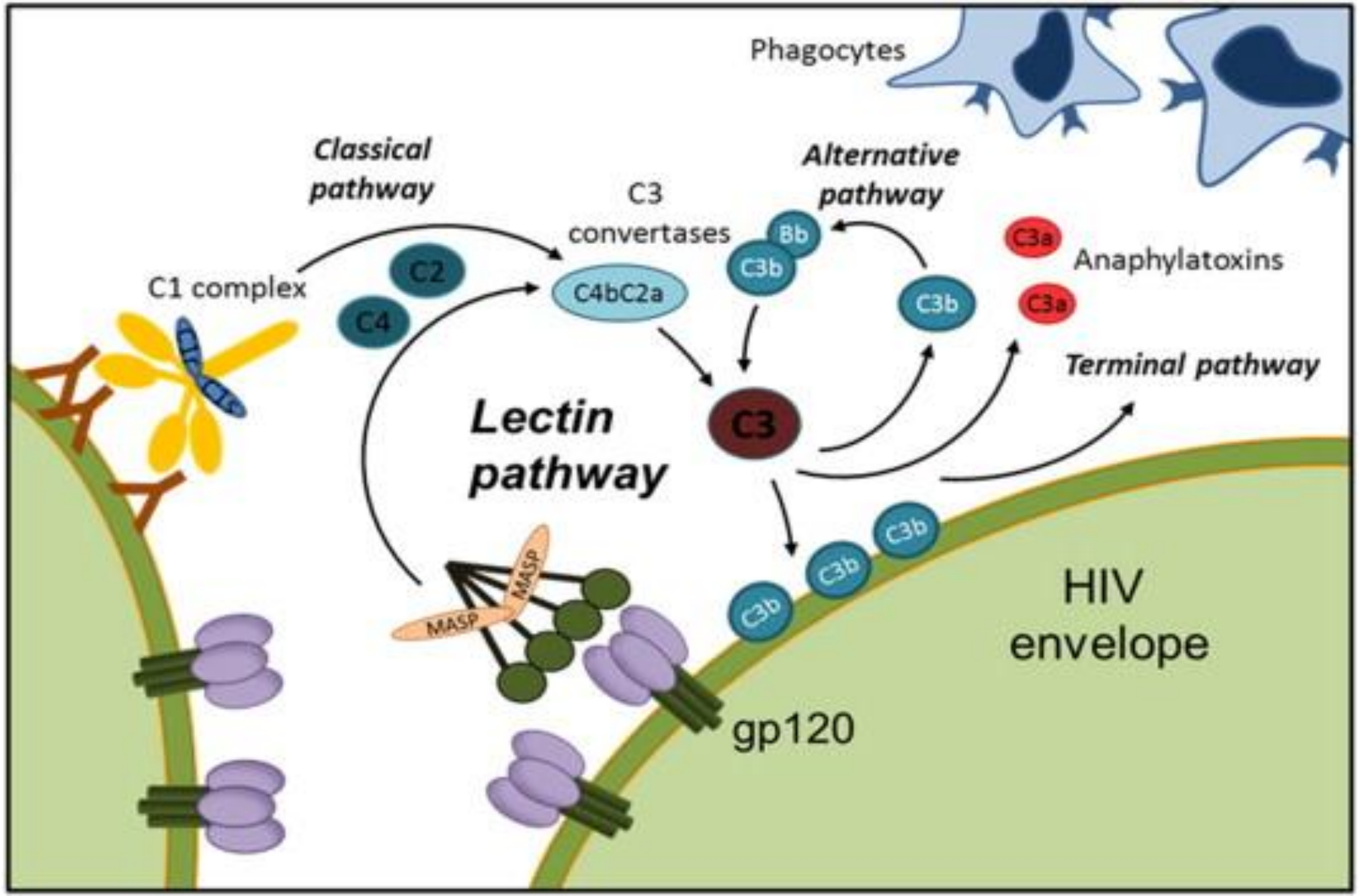
Steps :



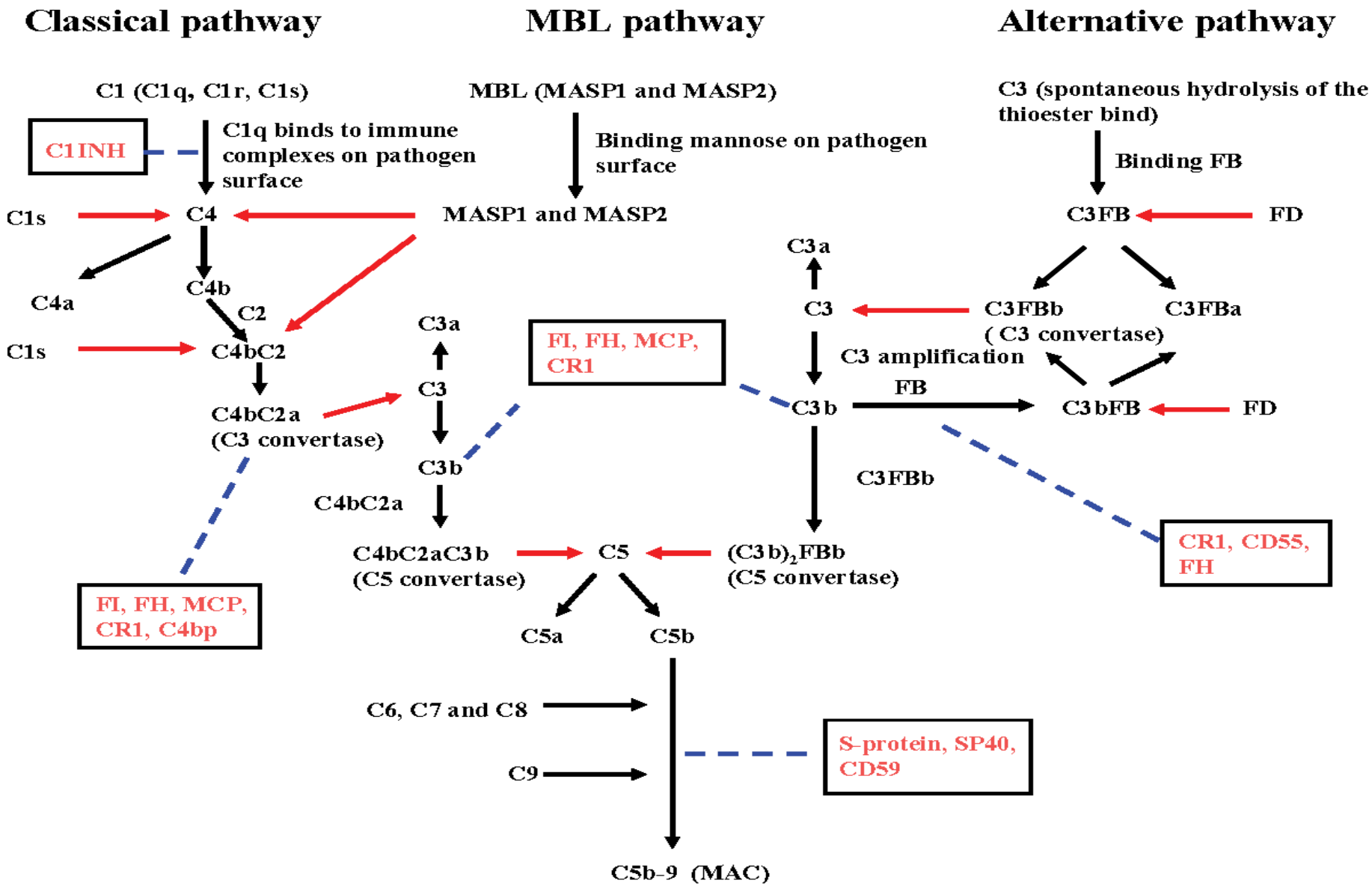
Source: https://www.mdpi.com/2077-0383/10/10/2188

The 3 Complement Pathways Converge at the MAC




Phagocytes
Classical pathway
C3 convertases
Alternative pathway
C1 complex
C2
C4
C4bC2a
Bb
C3b
C3a
Anaphylatoxins
C3a
C3b
Terminal pathway
Lectin pathway
C3
MASP
MASP
C3b
C3b
C3b
HIV envelope
gp120

Regulation of the Complement Pathways



Importance of Regulating the Complement Pathways

- Prevents damage to host tissues by ensuring complement activation occurs only on pathogens.
- Maintains immune homeostasis by avoiding unnecessary or excessive inflammation.
- Protects self-cells from being targeted by the immune system.
- Ensures efficient clearance of immune complexes and apoptotic cells.
- Modulates the immune response to avoid autoimmunity.
- Balances pro-inflammatory and anti-inflammatory signals.
- Preserves the integrity of the vascular endothelium.
- Facilitates proper immune regulation and tolerance.