Lecture 5 **Dr. Basim Mohammed Hanon**

COMPLEMENT:-

Historically the term **complement** (C) was used to refer to a heat labile serum protein component that was **able to lyse bacteria.**

Complement is actually composed of over **20 different serum proteins** produced by a variety of cells, **hepatocytes, macrophages** and **gut epithelial cells.**

Complement functions:

- Complement can **opsonize bacteria** for enhanced **phagocytosis**; it can recruit and PMNs and macrophages, it can **participate in regulation of antibody responses** and it can aid in the **clearance of immune complexes** and **apoptotic cells.**
- Complement can contributes to **inflammation** and **tissue damage** and it can trigger **anaphylaxis C3a and C5a cause degranulation of mast cells** with release of mediators, eg. Histamine, leading to increased **vascular permeability and smooth muscle contraction.**
- **Chemotaxis:** C5a and C567 complex attract neutrophils. They migrate especially well to ward C5a. C5a also **enhances the adhesiveness** of neutrophils to endothelium.
- **Cytolysis:** Insertion of the C5b6789 complex into the cell membrane leads to **killing or lysis of many types of cells** including erythrocytes, bacteria and tumor cells.
- **Enhancement of Antibody Production:** The binding of C3b to its receptors on the surface of **activated B cells** greatly **enhances antibody production** compared with that by B cells that are activated by antigen alone.

C-Activation: alteration of C proteins, enabling them to interact with another component.

C-Fixation: utilization of C by antigen-antibody complexes.

C-Inactivation: denaturation (usually by heat) of an early complement component resulting in **loss of hemolytic activity**

Convertase/esterase: altered C protein which acts as a **proteolytic enzyme** for another C component.

Activated components of complement:

- When enzymatically cleaved, the **larger fragment binds to the activation complex** or membrane and the **smaller fragment is released into the microenvironment**.
- **The letter "b"** is usually added to name of the **larger membrane-binding fragment** and the **letter "a" to the smaller fragment** (e.g. C3b and C3a).
- **The exception is for C2** in which the **larger membrane-binding fragment** is given **the "a"** designation and the **smaller fragment is given the "b"** designation.

Complement pathways

- The complement system is divided into **four pathways** each of which requires different protein components (Figure 1).
- **The classical pathway,** named because it was the first described, is **dependent upon antibody** to be operational.
- **The lectin and alternative pathways** are **antibody independent** for their function.
- All three of these pathways ultimately result in the **activation of C3 and the formation of a C5 convertase**, which leads to the activation of C5 and the **lytic pathway.**

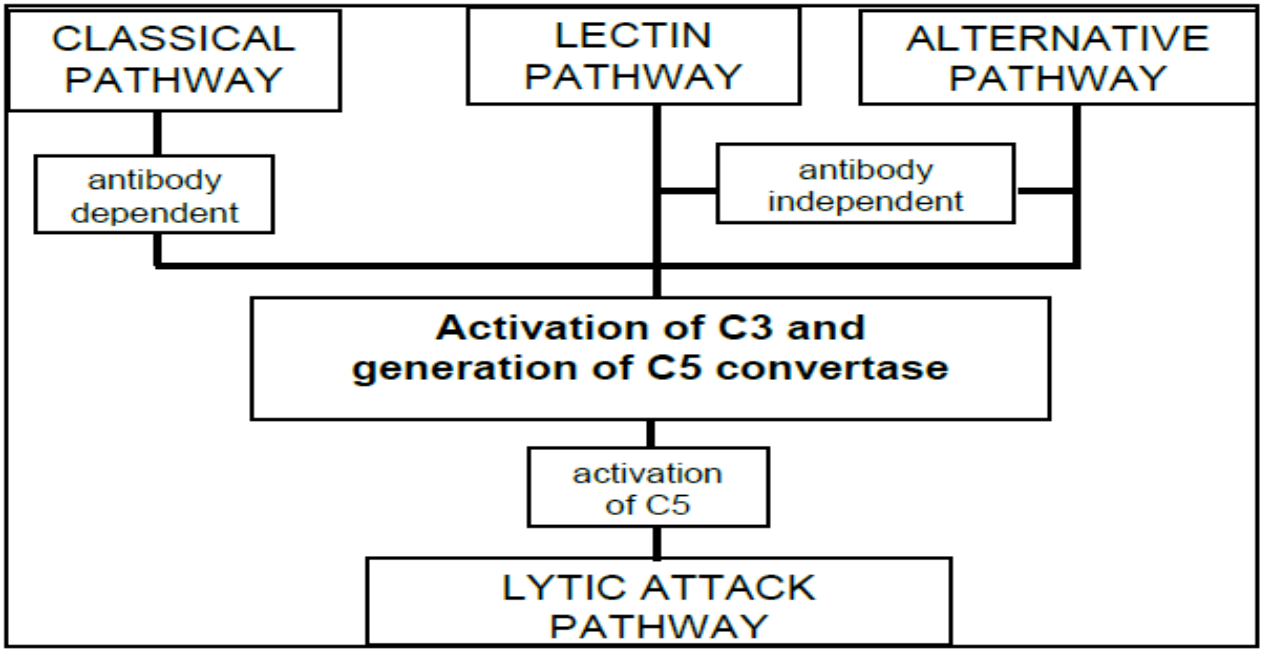


Figure 1. Pathways of complement activation

A. Classical Pathway:

- C1 a multi-subunit protein containing three different proteins, **C1q, C1r and C1s**.
- Binds to the **Fc region of IgG and IgM antibody** molecules that have interacted with antigen.
- C1 binding does not occur to antibodies that have not complexed with antigen and binding **requires calcium and magnesium ions**.
- In some cases C1 can bind to **aggregated immunoglobulin** [e.g. aggregated IgG] or to certain pathogen surfaces in the absence of antibody).
- The binding of C1 to antibody is **via C1q** and C1q must cross link at **least two antibody molecules before it is firmly fixed.**
- The binding of **C1q** results in the **activation of C1r** which in turn **activates C1s**.
- The result is the formation of an **activated "C1qrs",** which is an enzyme that cleaves C4 into two fragments **C4a and C4b**.
- The **C4b fragment binds** to the membrane and the C4a fragment is **released into the microenvironment.**
- Activated "C1qrs" also cleaves C2 into **C2a and C2b**. C2a binds to the membrane in association with C4b and C2b is released into the microenvironment.
- **The resulting C4bC2a complex is a C3 convertase,** which cleaves C3 into C3a and C3b. C3b binds to the membrane in association with C4b and C2a and C3a is released into the microenvironment.
- **The resulting C4bC2aC3b is a C5 convertase.** The generation of C5 convertase is the end of the classical pathway.
- If the classical pathway were not regulated there would be continued production of **C2b, C3a, and C4a**.
- Thus, there must be some way to regulate the activity of the classical pathway. Table 3 summarizes the ways in which the classical pathway is regulated.

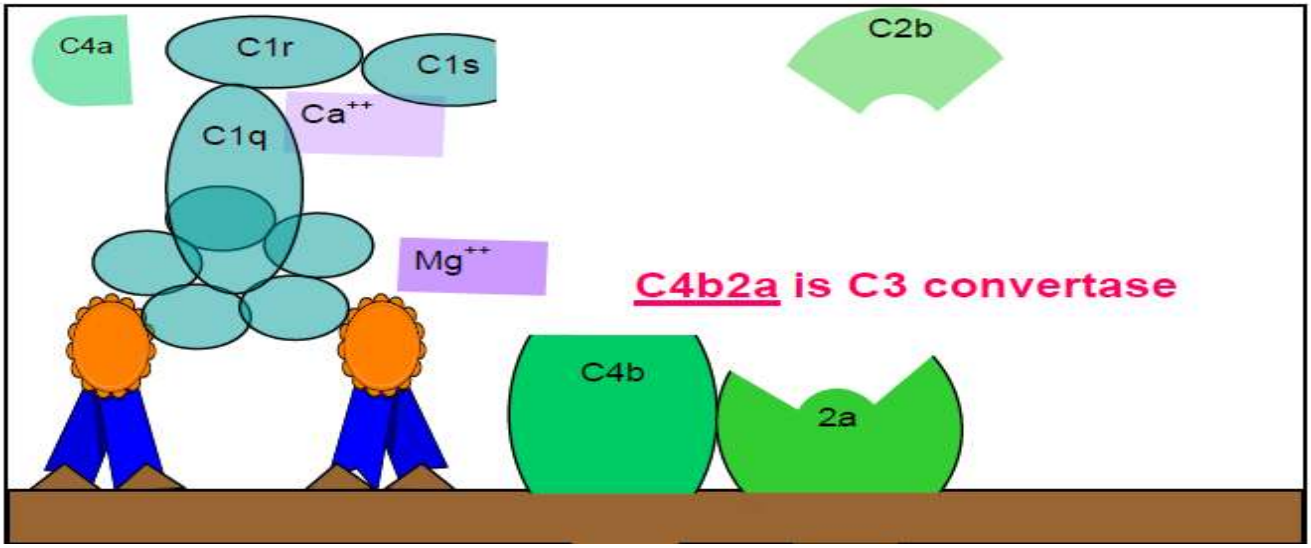


Figure 2. Generation of C3 convertase in the classical pathway

B. Lectin Pathway:

- The lectin pathway is very similar to the classical pathway. It is initiated by the binding of **mannose binding lectin (MBL) to bacterial surfaces with mannose-containing polysaccharides.**
- Binding of MBL to a pathogen results in the association of two **serine proteases**, **MASP-1 and MASP-2 (MBL-associated serine proteases).**
- **MASP-1 and MASP-2 are similar to C1r and C1s, respectively and MBL is similar to C1q.**
- Formation of the **MBL/MASP-1/MASP-2 complex** results in the activation of the MASPs and subsequent cleavage of **C4 into C4a and C4b.**
- The **C4b fragment** binds to the membrane and the **C4a fragment** is released into the microenvironment.
- Activated MASPs also cleave **C2 into C2a and C2b**. **C2a binds** to the membrane in association with C4b and **C2b is released** into the microenvironment.
- The resulting **C4bC2a complex** is a **C3 convertase**, which cleaves C3 into C3a and C3b. **C3b binds to the membrane in association with C4b and C2a** and C3a is released into the microenvironment.
- The resulting **C4bC2aC3b is a C5 convertase**. The generation of C5 convertase is the end of the lectin pathway.

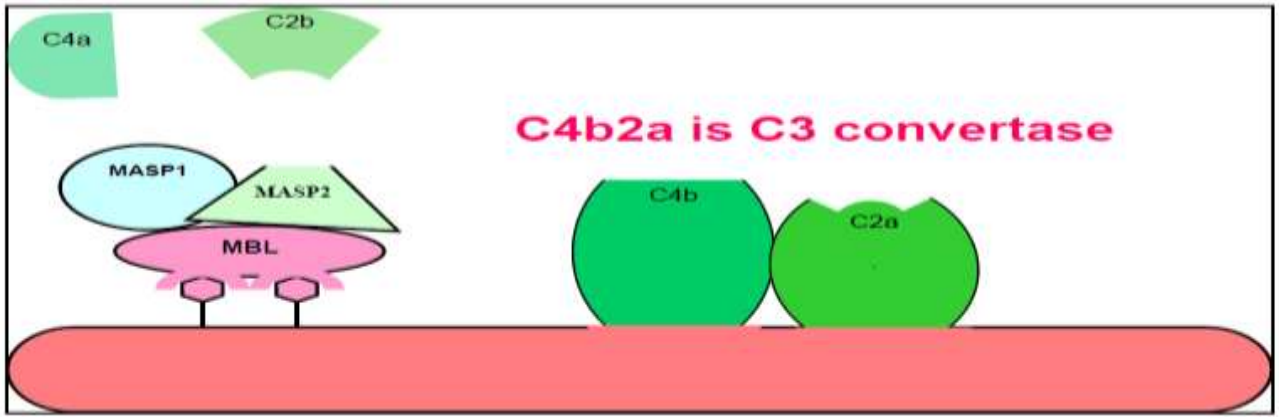


Figure 3. generation of C3 convertase in the lactin pathway

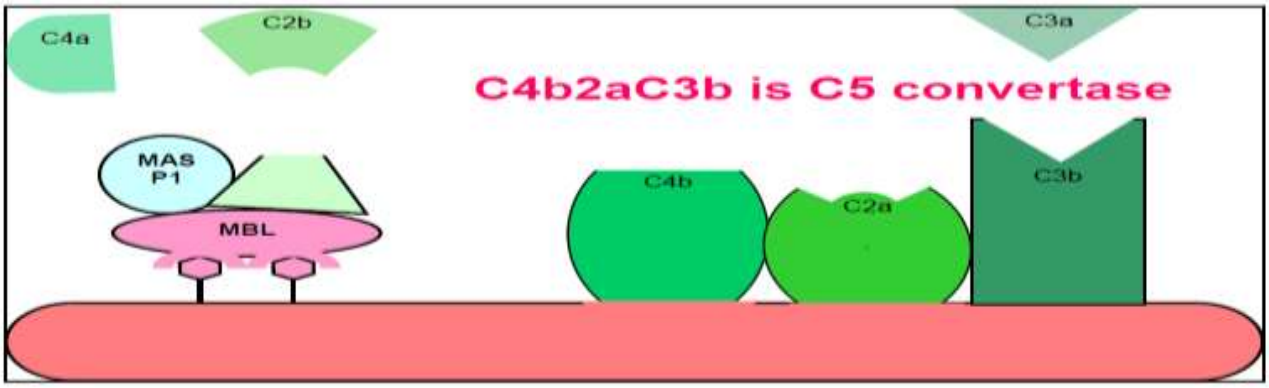


Figure 4. Generation of C5 convertase in the lactin pathway

C. Alternative Pathway:

- In serum there is **low level spontaneous hydrolysis of C3** to produce **C3i**.
- **Factor B** binds to C3i and becomes susceptible to **Factor D**, which cleaves Factor B into **Bb**.
- **The C3iBb complex acts as a C3 convertase** and cleaves C3 into C3a and C3b. Once C3b is formed, **Factor B** will bind to it and becomes susceptible to cleavage by **Factor D.**
- The resulting **C3bBb complex is a C3 convertase** that will continue to generate more C3b, thus amplifying **C3b production**.
- If this process continues unchecked the result would be the consumption of all C3 in the serum. Thus, the spontaneous production of C3b is tightly controlled.

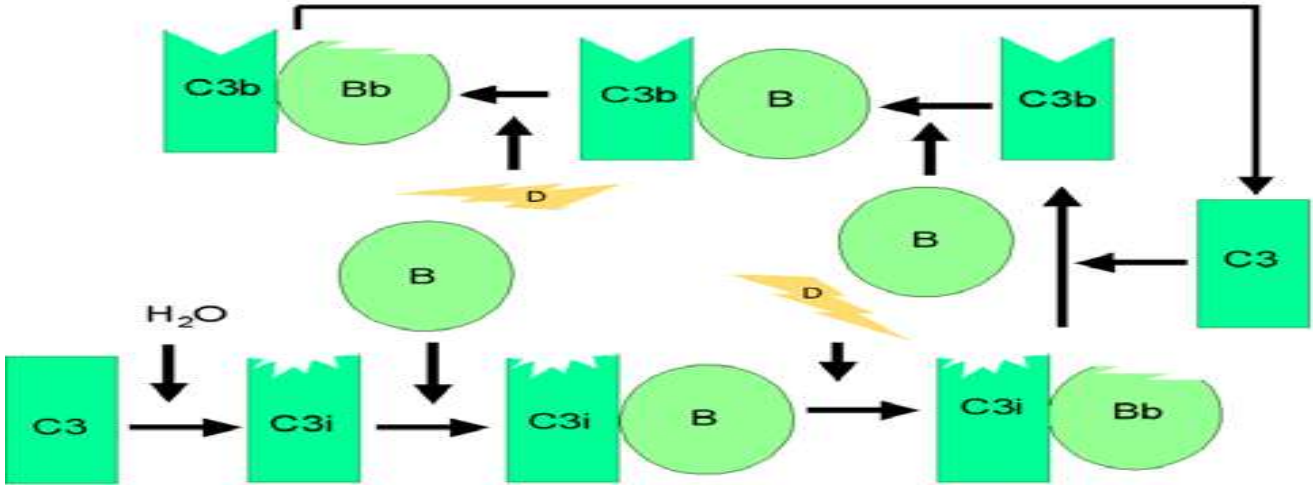


Figure 5. Spontaneous activation of C3

Membrane attack pathway: (Figure 11)

- **C5 convertase** from the **classical (C4b2a3b), lectin (C4b2a3b)** or **alternative (C3bBb3b)** pathway cleaves C5 into C5a and C5b.
- **C5a remains in the fluid phase** and the C5b rapidly associates with C6 and C7 and inserts into the membrane.
- **The C5b67 complex** is referred to as the **membrane attack complex (MAC).** Subsequently C8 binds, followed by several molecules of C9.
- The C9 molecules form a pore in the membrane through which the cellular contents leak and lysis occurs.
- Lysis is not an enzymatic process it is thought to be due to **physical damage** to the membrane.

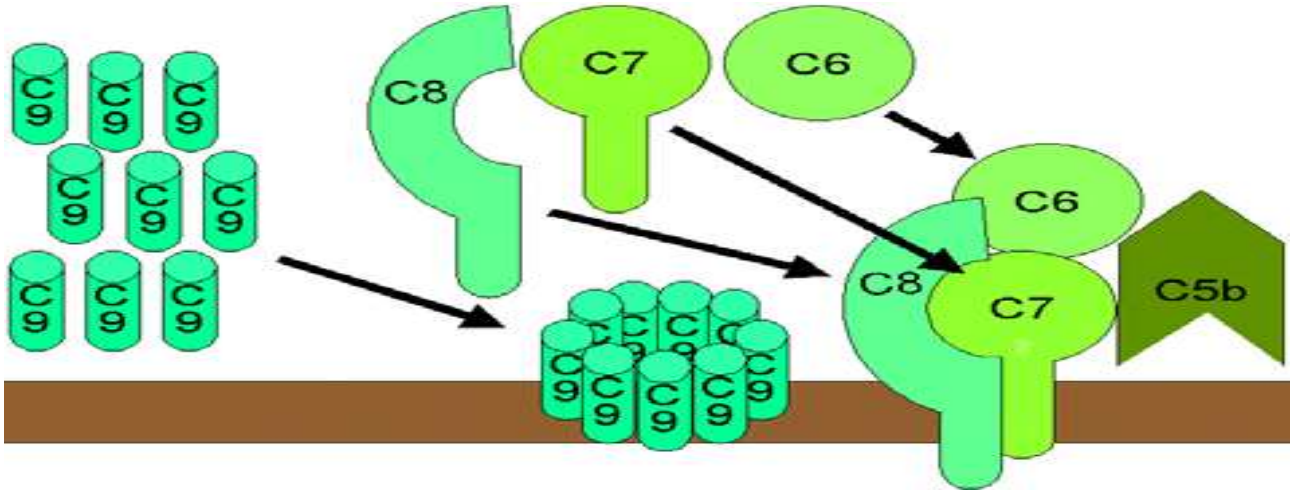


Figure 6. The lytic pathway

Table 1. Complement proteins

C1 (q r s), C2, C3, C4, C5. C6, C7, C8 and C9
Factors B, D, H, I and Properdin (P)
Mannose binding lectin (MBL), MBL-associated serine proteases (MASP-1 and MASP-2)
C1 inhibitor (C1-INH, serpin), C4-binding protein (C4-BP), decay accelerating factor (DAF)
Complement receptor 1 (CR1) protein S (vitronectin)

Activities of Complement Activation Products and their Control Factors

Fragment	Activity	Effect
C2b	Prokinin, accumulation of fluids	Edema
C3a	Basophil and mast cells degranulation; enhanced vascular permeability, smooth muscle contraction	Anaphylaxis
C3b	Opsonin, phagocyte activation	Phagocytosis
C4a	Basophil and mast cells degranulation; enhanced vascular permeability, smooth muscle contraction	Anaphylaxis (least potent)
C4b	Opsonin	Phagocytosis
C5a	Basophil and mast cells degranulation; enhanced vascular permeability, smooth muscle contraction	Anaphylaxis (most potent)
	Chemotaxis, stimulation of respiratory burst, activation of phagocytes, stimulation of inflammatory cytokines	Inflammation
C5bC6C7	Chemotaxis	Inflammation
	Attaches to other membranes	Tissue damage

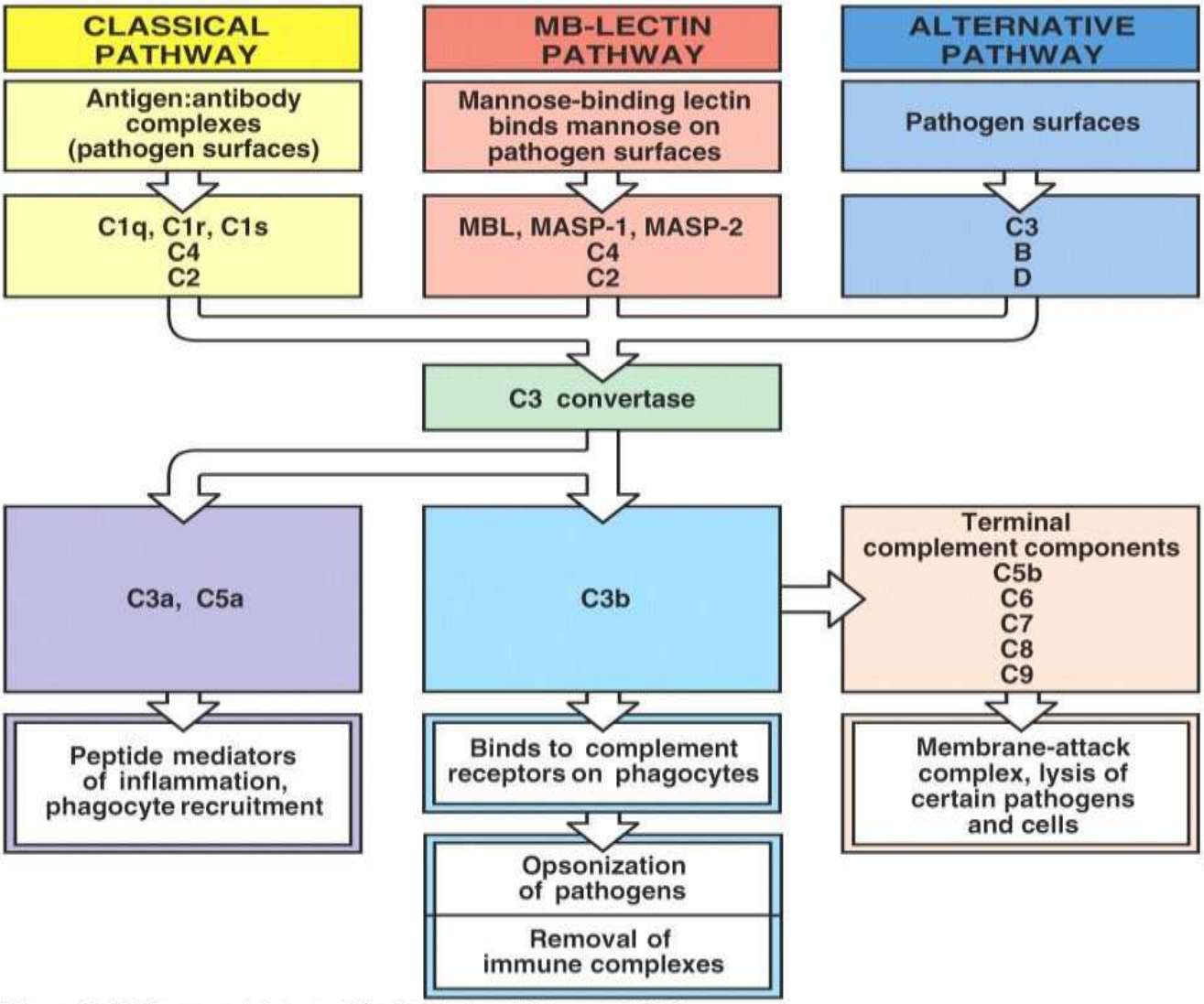
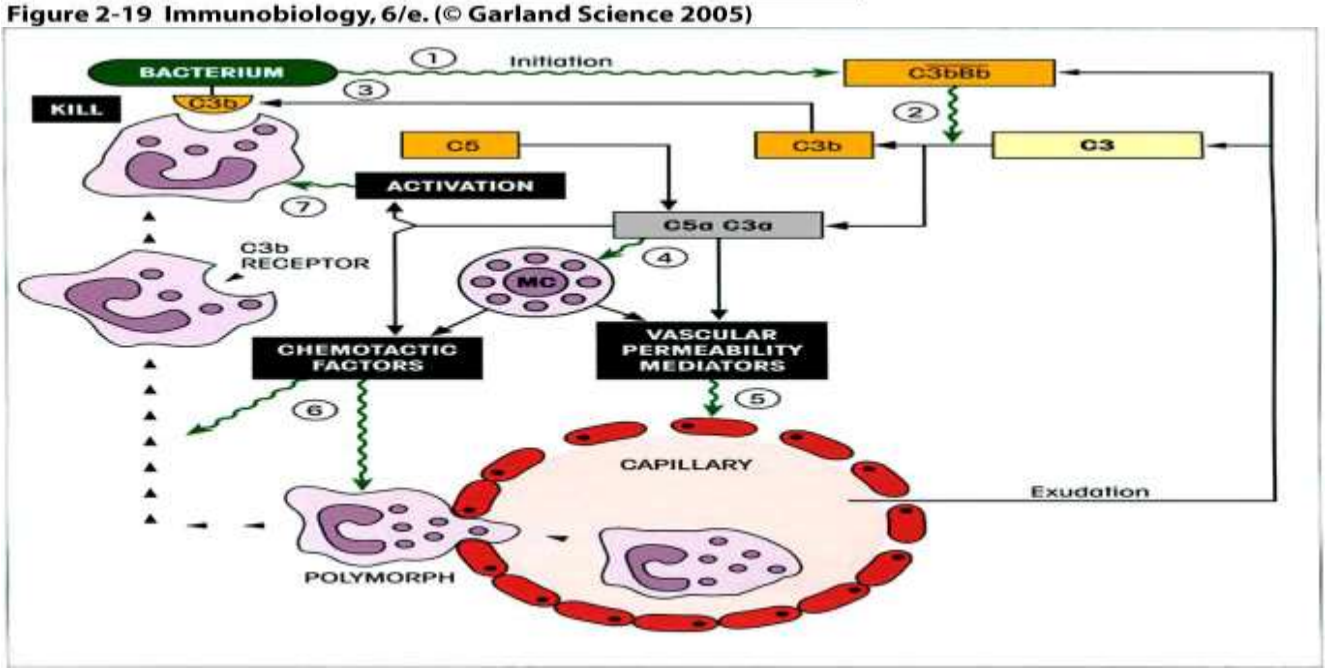
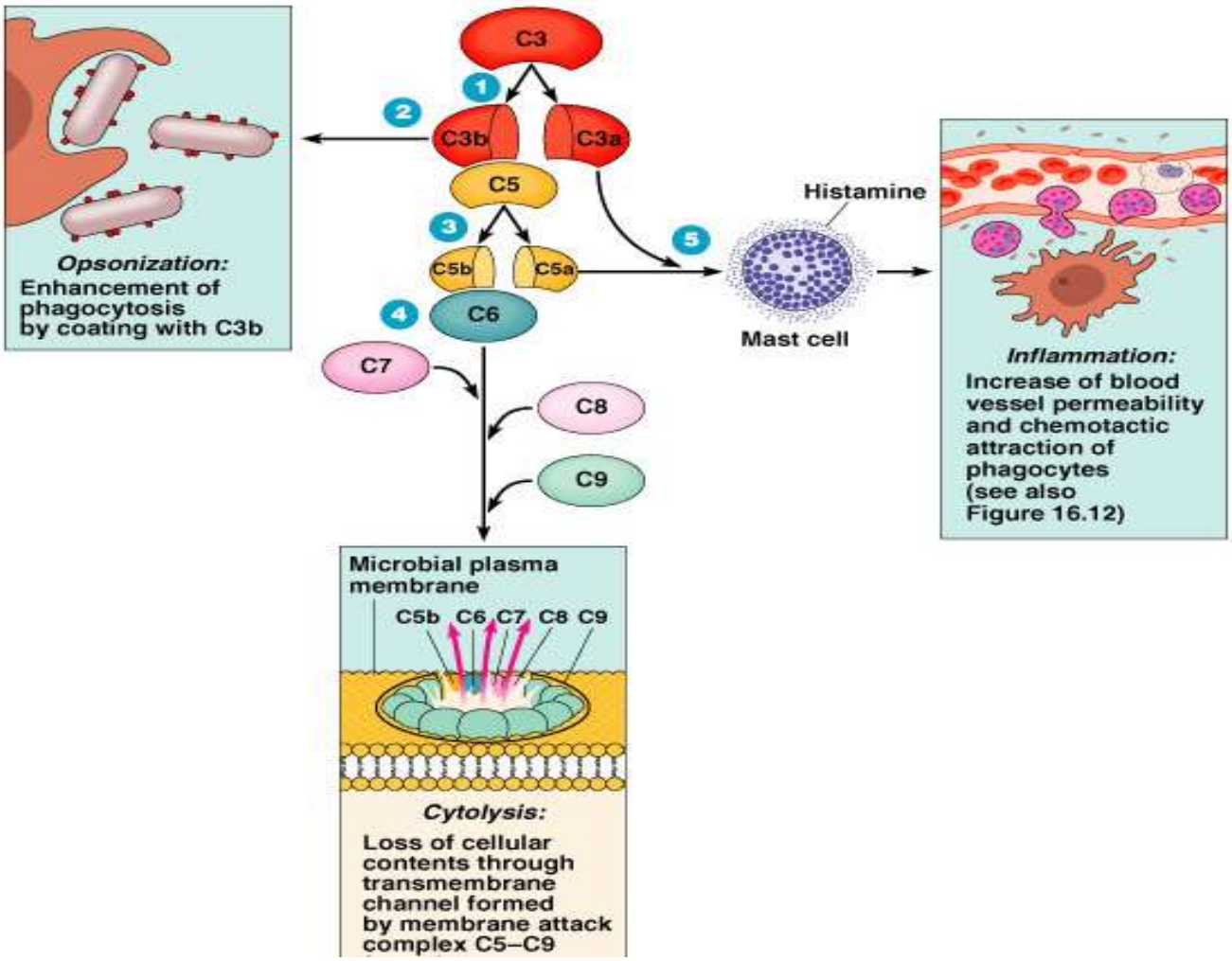


Figure 2-19 Immunobiology, 6/e. (© Garland Science 2005)




C3
1
2
C3b
C3a
C5
3
C5b
C5a
4
C6
C7
C8
C9
5
Histamine
Mast cell
Opsonization:
Enhancement of phagocytosis by coating with C3b
Inflammation:
Increase of blood vessel permeability and chemotactic attraction of phagocytes (see also Figure 16.12)
Microbial plasma membrane
C5b C6 C7 C8 C9
Cytolysis:
Loss of cellular contents through transmembrane channel formed by membrane attack complex C5–C9