# COMPLEMENT SYSTEM

**Dr Dinesh Kumar Sharma**
**Department of Microbiology**
**Ch. Charan Singh University, Meerut**

# INTRODUCTION

Complement refers to a collection of over 30 heat-labile proteins found in human blood plasma. The proteins act in a cascading fashion to lyse cell membranes, augment phagocytosis, and produce inflammatory peptides.

These activities were said to "complement“ the other antibacterial activities of the host; hence the name complement.
Complement activation can be initiated by three different signals, but all three lead to a common final pathway that:
(1) defends against bacterial infections by facilitating and enhancing phagocytosis, chemotaxis, activation of leukocytes, and lysis of bacteria;
(2) bridges innate and specific immune functions by enhancing antibody responses and immunologic memory; and
(3) disposes of wastes such as dead host cells and immune complexes, the products of inflammatory injury.

**Note:** Discovered in 1893 by the Belgian scientist **JULES BORDET**, who called it ‘alexine’. **PAUL EHRLICH** later introduced the rival term ‘complement’.

The name “complement” is derived from experiments performed by Jules Bordet shortly after the discovery of antibodies. He demonstrated that if fresh serum containing an antibacterial antibody is added to the bacteria at physiologic temperature (37°C), the bacteria are lysed. If, however, the serum is heated to 56°C or more, it loses its lytic capacity. This loss of lytic capacity is not due to decay of antibody activity because antibodies are relatively heat stable, and even heated serum is capable of agglutinating the bacteria. Bordet concluded that the serum must contain another heat-labile component that assists, or *complements*, *the lytic function of antibodies*, and this component was later given the name complement.

# The Functions of Complement

The complement system is one of the major effector mechanisms of humoral immunity and is also an important effector mechanism of innate immunity.

The complement system consists of serum and cell surface proteins that interact with one another and with other molecules of the immune system in a highly regulated manner to generate products that function to eliminate microbes. Complement proteins are plasma proteins that are normally inactive; they are activated only under particular conditions to generate products that mediate various effector functions of complement.

Several features of complement activation are essential for its normal function.

After initial activation, the various complement components interact, in a highly regulated cascade, to carry out a number of basic functions including:

- Lysis of cells, bacteria, and viruses

- Opsonization, which promotes phagocytosis of particulate antigens

- Binding to specific complement receptors on cells of the immune system, triggering specific cell functions, inflammation, and secretion of immunoregulatory molecules.

- Immune clearance, which removes immune complexes from the circulation and deposits them in the spleen and liver

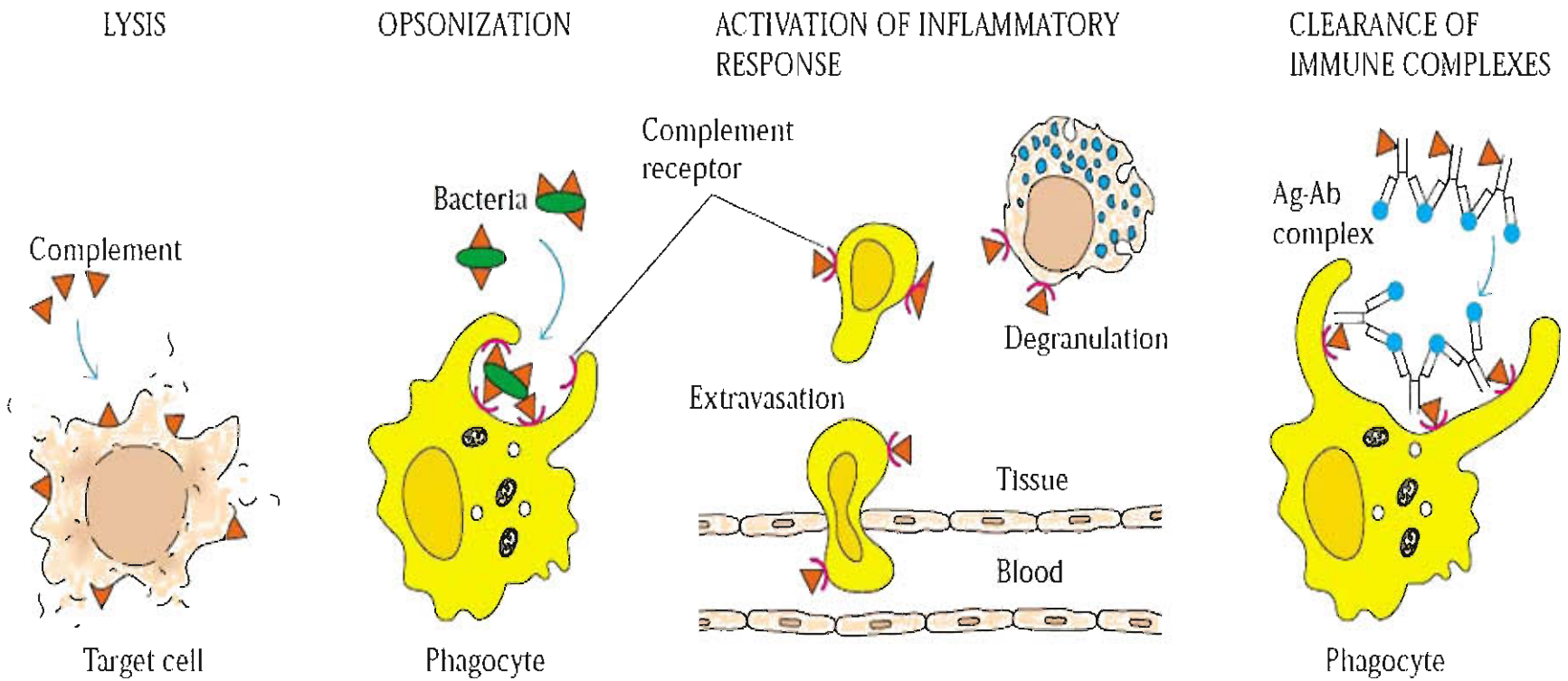



**FIGURE 13-1** The multiple activities of the complement system. Serum complement proteins and membrane-bound complement receptors partake in a number of immune activities: lysis of foreign cells by antibody-dependent or antibody-independent pathways; opsonization or uptake of particulate antigens, including bacteria, by phagocytes; activation of inflammatory responses; and clearance of circulating immune complexes by cells in the liver and spleen. Soluble complement proteins are schematically indicated by a triangle and receptors by a semi-circle; no attempt is made to differentiate among individual components of the complement system here.

# THE COMPLEMENT COMPONENTS

The proteins and glycoproteins that compose the complement system are synthesized mainly by liver hepatocytes, although significant amounts are also produced by blood monocytes, tissue macrophages, and epithelial cells of the gastrointestinal and genitourinary tracts. These components constitute 5% (by weight) of the serum globulin fraction. Most circulate in the serum in functionally inactive forms as proenzymes, or *zymogens*, which are inactive until proteolytic cleavage, which removes an inhibitory fragment and exposes the active site. The complement-reaction sequence starts with an enzyme cascade.

Complement components are designated by numerals (C1–C9), by letter symbols (e.g., factor D). Peptide fragments formed by activation of a component are denoted by small letters. In most cases, the smaller fragment resulting from cleavage of a component is designated “a” and the larger fragment designated “b” (e.g., C3a, C3b; note that C2 is an exception: C2a is the larger cleavage fragment). The larger fragments bind to the target near the site of activation, and the smaller fragments diffuse from the site and can initiate localized inflammatory responses by binding to specific receptors.

# PATHWAYS OF COMPLEMENT ACTIVATION

There are three major pathways of complement activation:

The **lectin pathway** is initiated by soluble carbohydrate-binding proteins—mannose-binding lectin (MBL) and the ficolins—that bind to particular carbohydrate structures on microbial surfaces. Specific proteases, called MBL-associated serine proteases (MASPs), that associate with these recognition proteins then trigger the cleavage of complement proteins and activation of the pathway.

The **classical pathway** is initiated when the complement component C1, which comprises a recognition protein (C1q) associated with proteases (C1r and C1s), either recognizes a microbial surface directly or binds to antibodies already bound to a pathogen.

Finally, the **alternative pathway** can be initiated by spontaneous hydrolysis and activation of the complement component C3, which can then bind directly to microbial surfaces.

# The Complement System

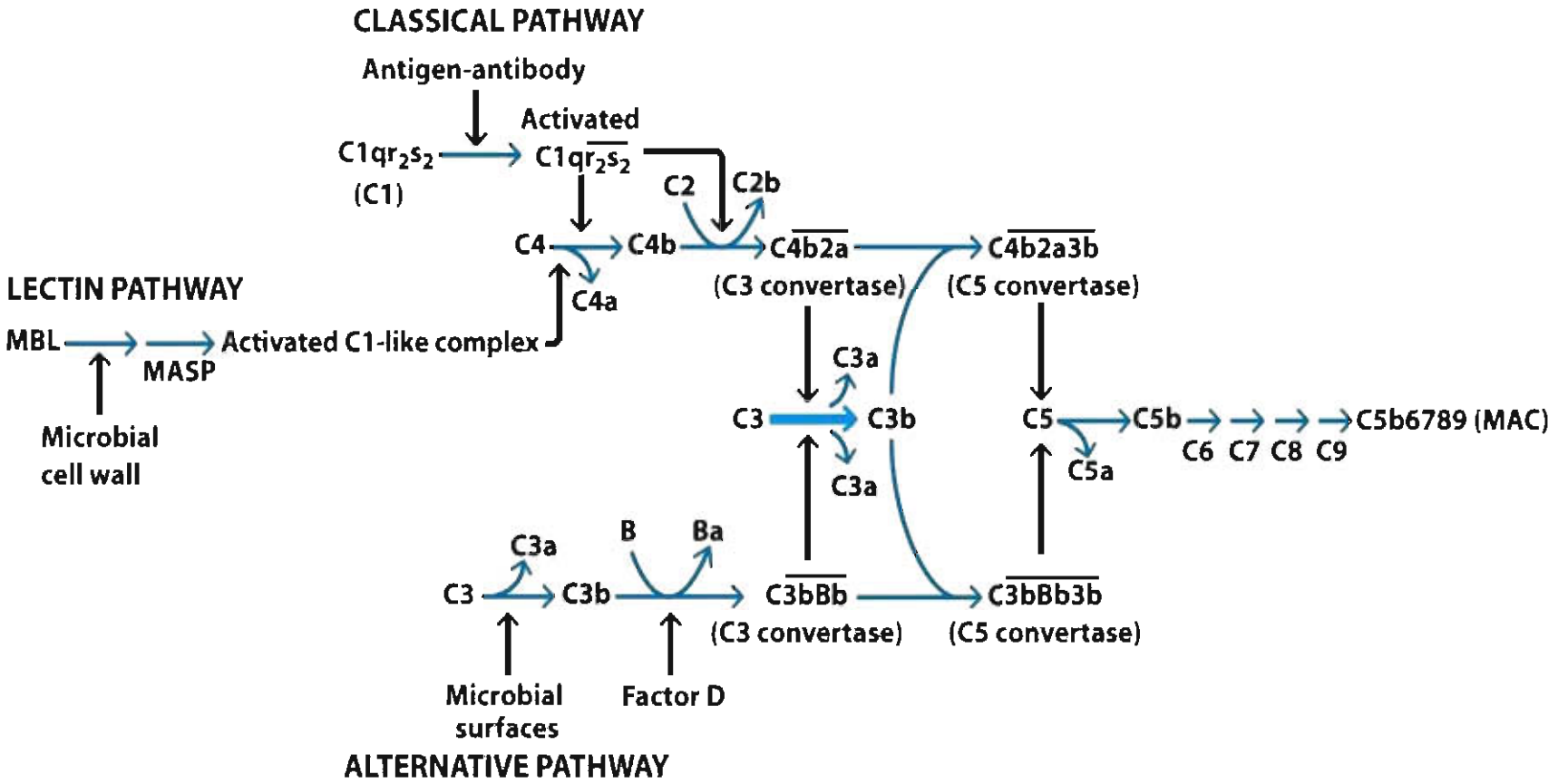
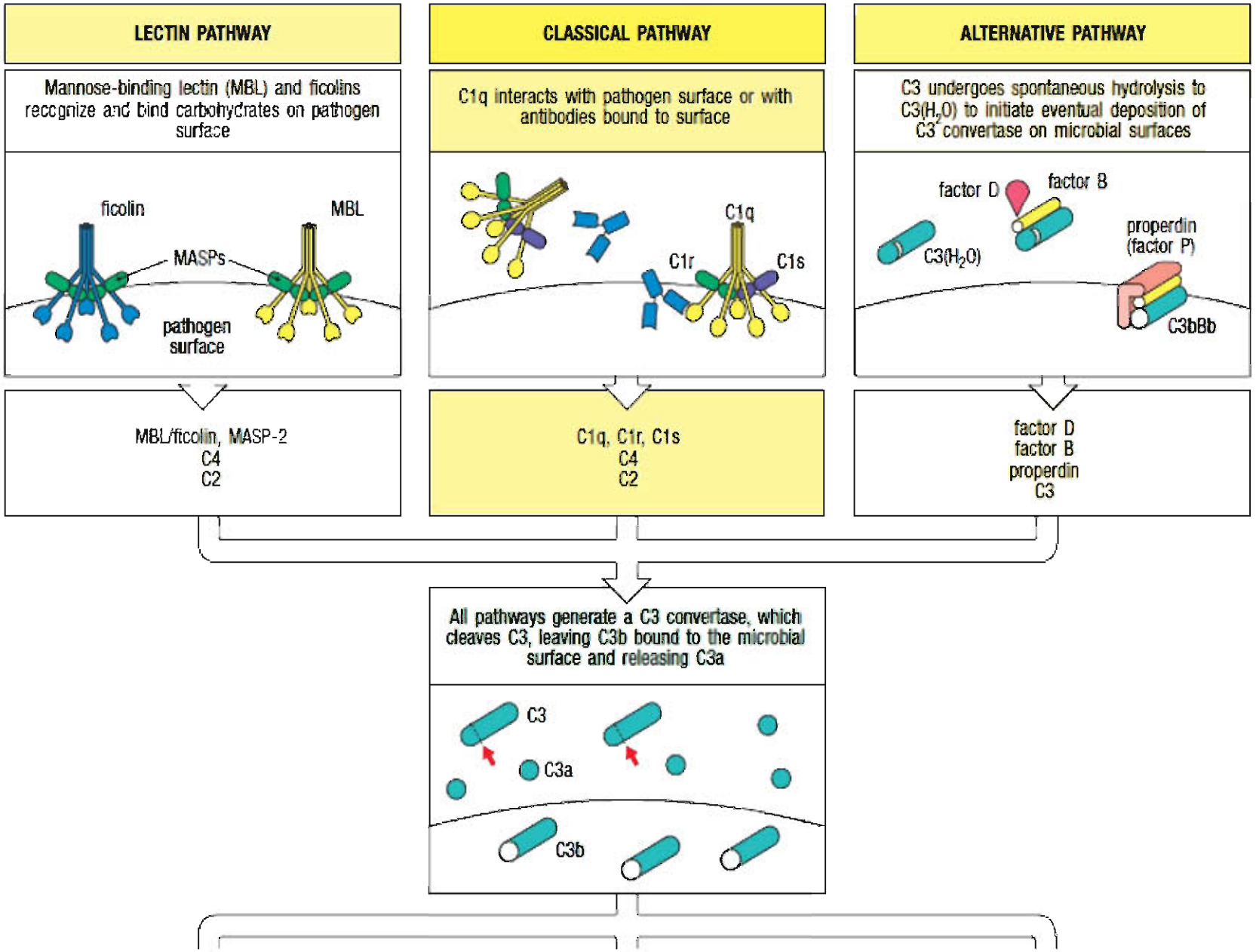



**Figure 7-2**
*Kuby IMMUNOLOGY, Sixth Edition*


LECTIN PATHWAY
Mannose-binding lectin (MBL) and ficolins recognize and bind carbohydrates on pathogen surface
ficolin
MBL
MASPs
pathogen surface
MBL/ficolin, MASP-2
C4
C2
CLASSICAL PATHWAY
C1q interacts with pathogen surface or with antibodies bound to surface
C1q
C1r
C1s
C1q, C1r, C1s
C4
C2
ALTERNATIVE PATHWAY
C3 undergoes spontaneous hydrolysis to C3(H2O) to initiate eventual deposition of C3 convertase on microbial surfaces
factor D
factor B
C3(H2O)
properdin (factor P)
C3bBb
factor D
factor B
properdin
C3
All pathways generate a C3 convertase, which cleaves C3, leaving C3b bound to the microbial surface and releasing C3a
C3
C3a
C3b

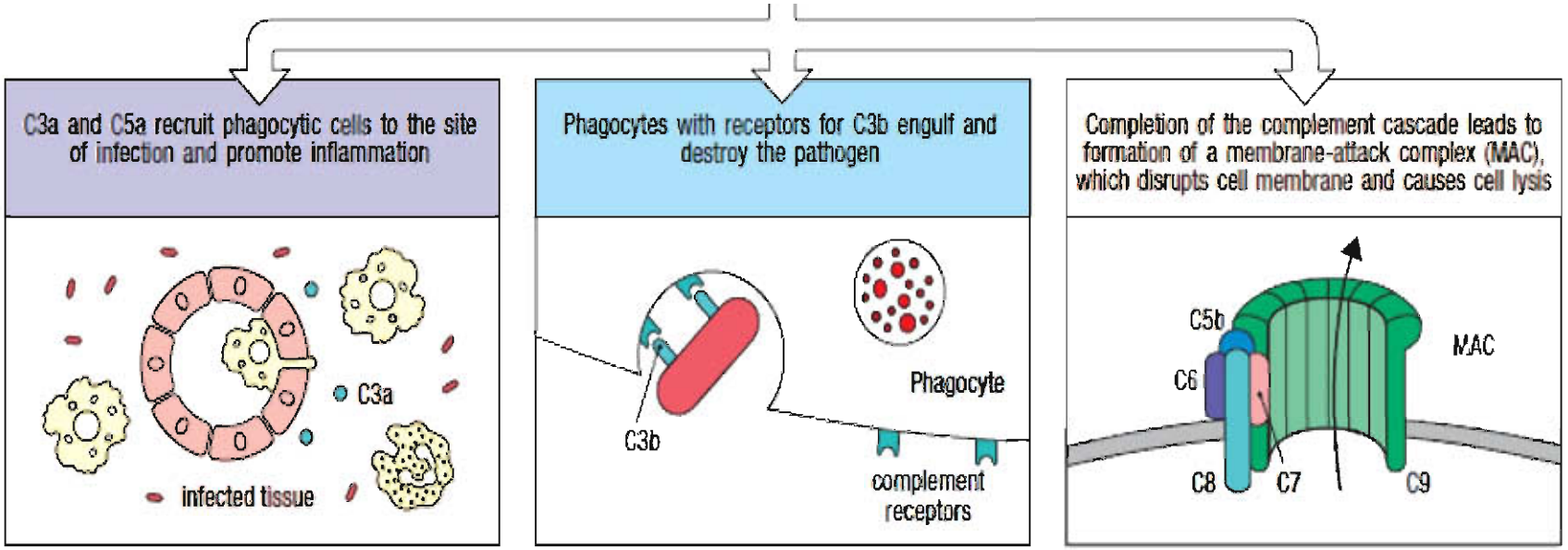



**Fig. 2.15 Complement is a system of soluble pattern recognition receptors and effector molecules that detect and destroy microorganisms.** The pathogen-recognition mechanisms of the three complement-activation pathways are shown in the top row, along with the complement components used in the proteolytic cascades leading to formation of a C3 convertase. This enzyme activity cleaves complement component C3 into the small soluble protein C3a and the larger component C3b, which becomes covalently bound to the pathogen surface (middle row). The components are listed by biochemical function in Fig. 2.14 and are described in detail in later figures. The lectin pathway of complement activation (top left) is triggered by the binding of mannose-binding lectin (MBL) or ficolins to carbohydrate residues in microbial cell walls and capsules. The classical pathway (top center) is triggered by binding of C1 either to the pathogen surface or to antibody bound to the pathogen. In the alternative pathway (top right), soluble C3 undergoes spontaneous hydrolysis in the fluid phase, generating C3($H_2O$), which is augmented by the action of factors B, D, and P (properdin). All pathways thus converge on the formation of C3b bound to a pathogen and lead to all of the effector activities of complement, which are shown in the bottom row. C3b bound to a pathogen acts as an opsonin, enabling phagocytes that express receptors for C3b to ingest the complement-coated microbe more easily (bottom center). C3b can also bind to C3 convertases to produce another activity, a C5 convertase (detail not shown here), which cleaves C5 to C5a and C5b. C5b triggers the late events of the complement pathway in which the terminal components of complement—C6 to C9—assemble into a membrane-attack complex (MAC) that can damage the membrane of certain pathogens (bottom right). C3a and C5a act as chemoattractants that recruit immune-system cells to the site of infection and cause inflammation (bottom left).

## *Rapid Reference Box 4*

**alternative pathway** – the activation pathways of the complement system involving C3 and factors B, D, P, H, and I, which interact in the vicinity of an activator surface to form an alternative pathway C3 convertase ($C\overline{3bBb}$).

**amplification loop** – the alternative complement activation pathway that acts as a positive feedback loop when C3 is split in the presence of an activator surface.

**anaphylatoxins** – complement peptides (C3a and C5a) that cause mast cell degranulation and smooth muscle contraction.

**bystander lysis** – complement-mediated lysis of cells in the immediate vicinity of a complement activation site that are not themselves responsible for the activation.

**C1–C9** – the components of the complement pathways responsible for mediating inflammatory reactions, opsonization of particles, and lysis of cell membranes.

**C3 convertases** – the enzyme complexes $C\overline{3bBb}$ and $C\overline{4b2a}$ that cleave C3.

**classical pathway** – the pathway by which antigen–antibody complexes can activate the complement system, involving components C1, C2, and C4, and generating a classical pathway C3 convertase ($C\overline{4b2a}$).

**complement** – a group of serum proteins involved in the control of inflammation, the activation of phagocytes, and the lytic attack on cell membranes.

**complement receptors (CR1–CR4)** – a set of four cell surface receptors for fragments of complement C3.

**decay accelerating factor (DAF)** – a cell surface molecule on mammalian cells that limits activation and deposition of complement C3b.

**lectin pathway** – a pathway of complement activation, initiated by mannan-binding lectin (MBL), that intersects the classical pathway.

**membrane attack complex (MAC)** – the assembled terminal complement components C5b–C9 of the lytic pathway that becomes inserted into cell membranes.

**zymogen** – pro-enzyme that requires proteolytic cleavage to become active; the enzymatically active form is distinguished from its precursor by a bar drawn above.

# THE CLASSICAL PATHWAY

The classical pathway is initiated by binding of the complement protein C1 to the CH2 domains of IgG or the CH3 domains of IgM molecules that have bound antigen.

- The pathway is activated through antigen-antibody complexes: initially, C1 component binds to a site on the Fc fragment of Ig (IgG (but not $IgG_4$) or IgM); however, native Ig molecules do not interact with C1.

- C1 component – contains three polypeptides (C1q, C1r, C1s); C1q attaches first to Ig (for initiation of complement activation, C1q has to interact with two or more Ig monomers) → C1q activates proenzyme C1r → C1r cleaves proenzyme C1s → C1s is able to cleave C4 component .

- Activated C1s cleaves C4 to C4a (an anaphylatoxin) + C4b → C4b binds to cell membranes → the next component becomes susceptible to enzymatic attack by activated C1
- C4b + C2 + C1s → removal of C2a → enzymatically active molecular complex C4b2a (= **C3 convertase of the classical pathway**).
- Formation of C3 convertase represents the nodal point for all pathways of complement system activation .

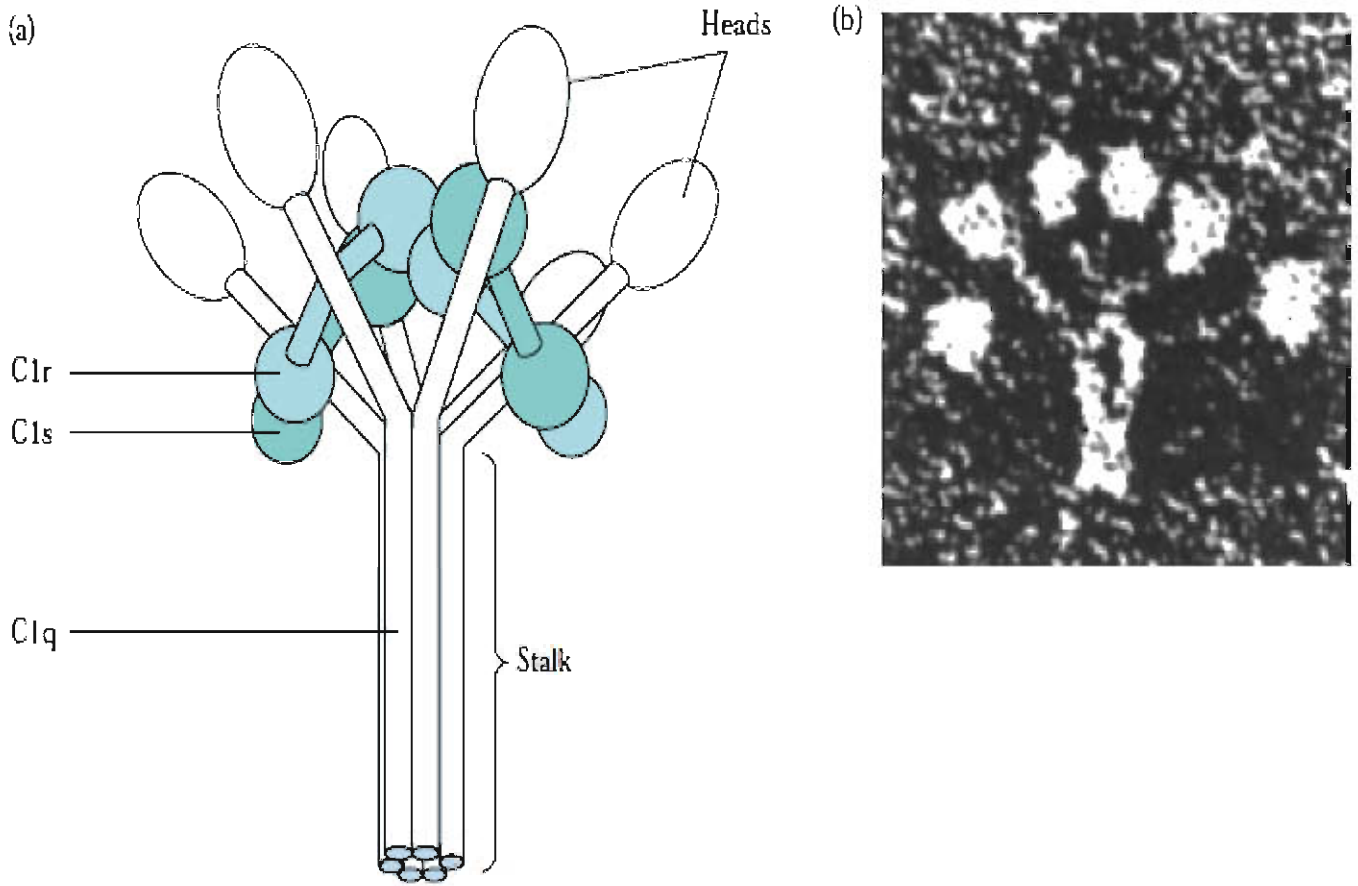



**FIGURE 13-3** Structure of the C1 macromolecular complex. (a) Diagram of $C1qr_2s_2$ complex. A C1q molecule consists of 18 polypeptide chains arranged into six triplets, each of which contains one A, one B, and one C chain. Each C1r and C1s monomer contains a catalytic domain with enzymatic activity and an interaction domain that facilitates binding with C1q or with each other. (b) Electron micrograph of C1q molecule showing stalk and six globular heads. *[Part (b) from H. R. Knobel et al., 1975,* Eur. J. Immunol. ***5:****78.]*

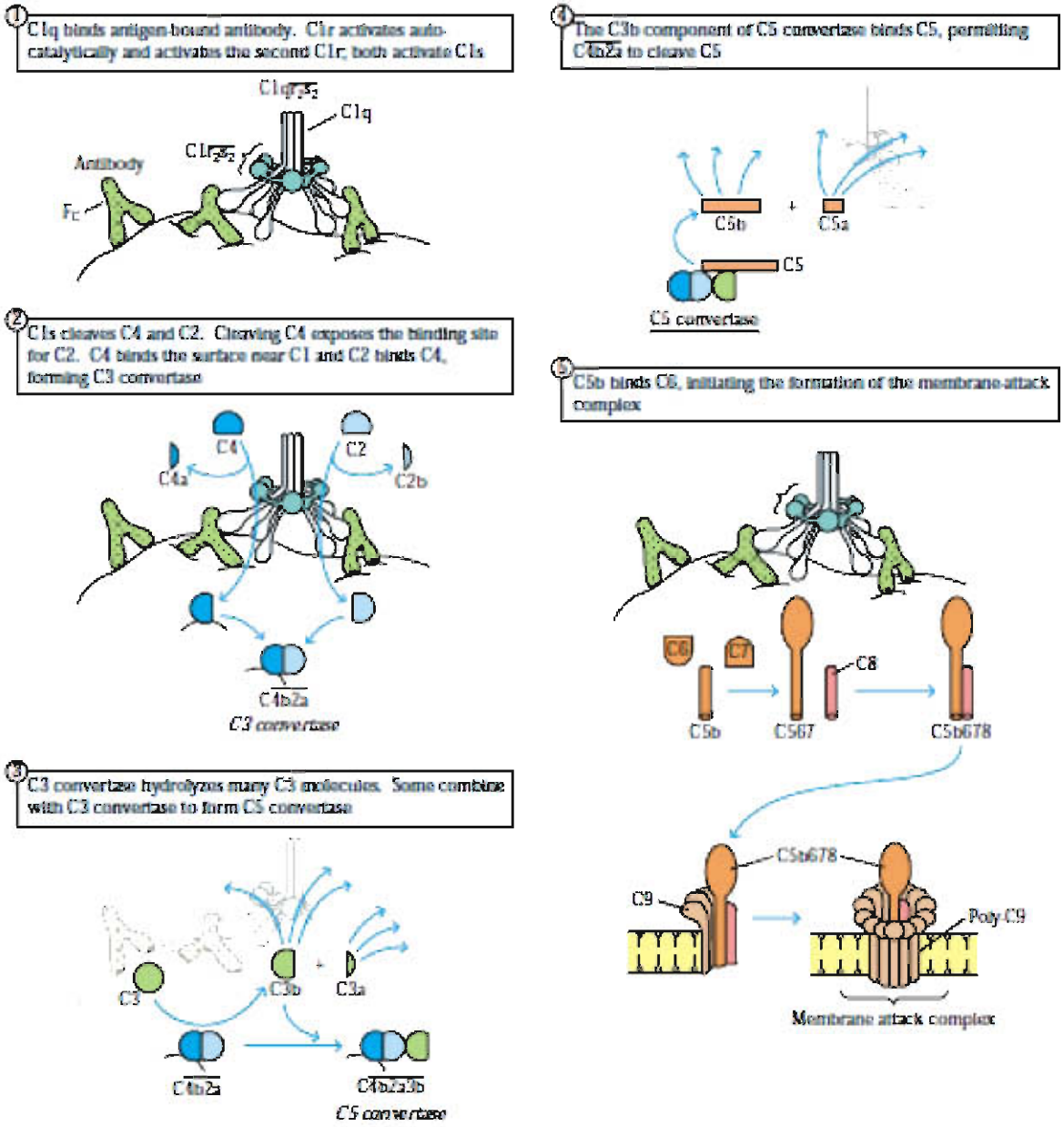



**FIGURE 13-5** Schematic diagram of intermediates in the classical pathway of complement activation. The completed membrane-attack complex (MAC, bottom right) forms a large pore in the membrane.

# THE ALTERNATIVE PATHWAY

**The alternative pathway is antibody-independent.**

➢The alternative pathway is considered to be a primitive „bypass“ mechanism, that does not require C1, C2 and C4.

➢ The pathway is activated through reaction of the complement system and some substances of microbial origin (polysaccharides – e.g. lipopolysaccharides of Gram negative bacteria, teichoic acid of G positive bacteria, zymosan from yeast cell walls, surface components of some animal parasites) or other foreign materials.

➢ C3 cleaves into C3a + C3b spontaneously; however, these are inactive under standard conditions.

➢ In this case, C3b binds to microbial surface → it reacts with factor B → removal of Ba (it is chemotactic for neutrophils) → C3bBb (= C3 convertase of the alternative pathway) – it is stabilized by properdin (P).

## TABLE 13-1 Initiators of the alternative pathway of complement activation

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

SOURCE: Adapted from M. K. Pangburn, 1986, in *Immunobiology of the Complement System*, Academic Press.

VISUALIZING CONCEPTS

1. C3 hydrolyzes spontaneously, C3b fragment attaches to foreign surface
2. Factor B binds C3a, exposes site acted on by Factor D. Cleavage generates C3bBb, which has C3 convertase activity
3. Binding of properdin stabilizes convertase
4. Convertase generates C3b; some binds to C3 convertase activating C5' convertase. C5b binds to antigenic surface

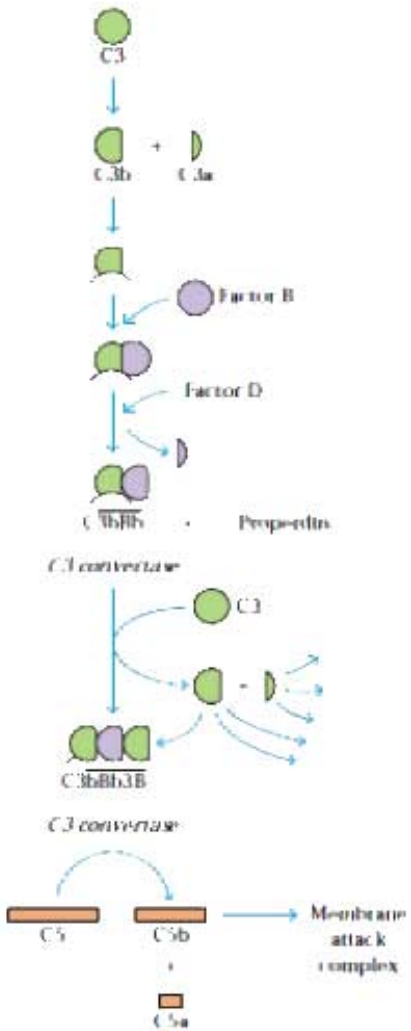



**FIGURE 13-7** Schematic diagram of intermediates in the formation of bound C3b by the alternative pathway of complement activation. The $\overline{C3bBb}$ complex is stabilized by binding of properdin. Conversion of bound C5 to the membrane attack complex occurs by the same sequence of reactions as in the classical pathway (see Figure 13.5).

# THE LECTIN PATHWAY

The lectin pathway, like the classical pathway, proceeds through the activation of a C3 convertase composed of C4b and C2a. However, instead of relying on antibodies to recognize the microbial threat and to initiate the complement activation process, this pathway uses lectins—proteins that recognize specific carbohydrate components primarily found on microbial surfaces—as its specific receptor molecules.

- **Mannose-binding lectin (MBL),** the first lectin demonstrated to be capable of initiating complement activation, binds close-knit arrays of mannose residues that are found on microbial surfaces such as those of *Salmonella, Listeria, and Neisseria strains of bacteria; Cryptococcus neoformans and Candida albicans strains* of fungi; and even the membranes of some viruses such as HIV-1 and respiratory syncytial virus.

- The complement pathway that it initiates is referred to as the **lectin pathway of complement activation.**

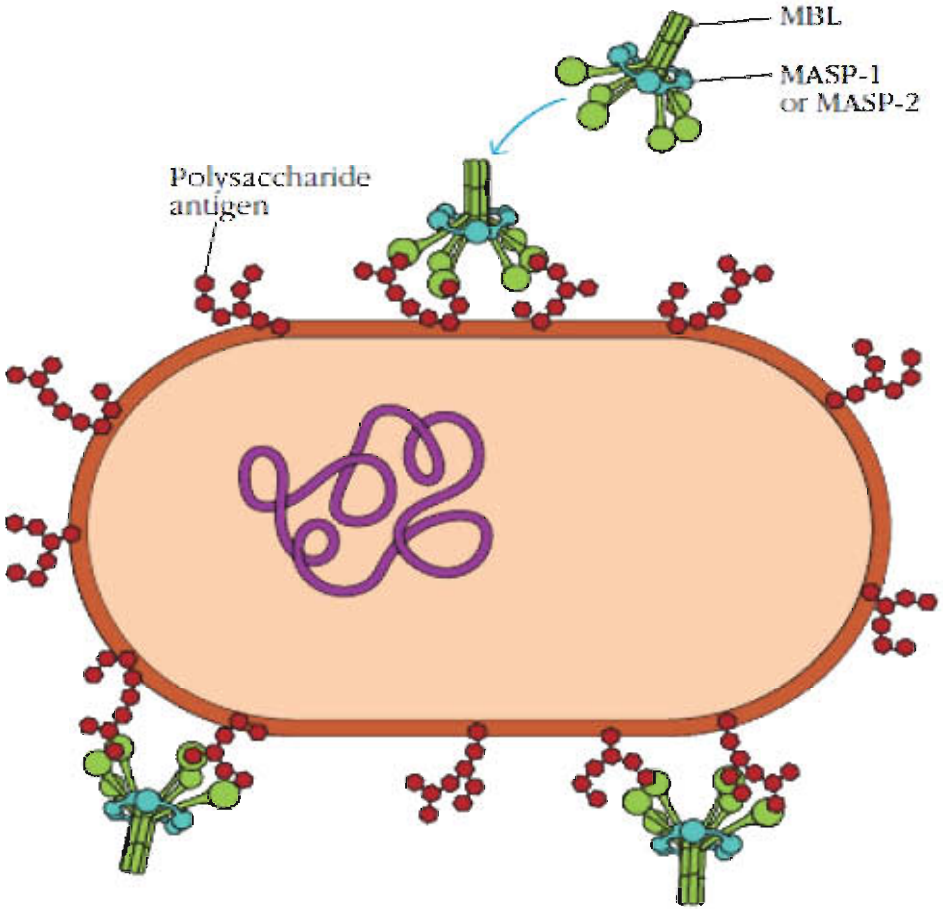



**FIGURE 6-7 Initiation of the lectin pathway relies on lectin receptor recognition of microbial cell surface carbohydrates.** Lectin receptors, such as MBL, bind microbial cell surface carbohydrates. Once attached to the carbohydrates, they bind the MASP family serine proteases, which cleave C2 and C4 with the formation of a lectin-pathway C3 convertase.

# REGULATION OF THE COMPLEMENT SYSTEM

Because many elements of the complement system are capable of attacking host cells as well as foreign cells and microorganisms, elaborate regulatory mechanisms have evolved to restrict complement activity to designated targets.

**TABLE 13-2 Proteins that regulate the complement system**

| Protein | Type of protein | Pathway affected | Immunologic function |
|---|---|---|---|
| C1 inhibitor (C1Inh) | Soluble | Classical | Serine protease inhibitor: causes $C1r_2s_2$ to dissociate from C1q |
| C4b-binding protein (C4bBP)* | Soluble | Classical and lectin | Blocks formation of C3 convertase by binding C4b; cofactor for cleavage of C4b by factor I |
| Factor H* | Soluble | Alternative | Blocks formation of C3 convertase by binding C3b; cofactor for cleavage of C3b by factor I |
| Complement-receptor type 1 (CR1)*<br>Membrane-cofactor protein (MCP)* | Membrane bound | Classical, alternative, and lectin | Block formation of C3 convertase by binding C4b or C3b; cofactor for factor I-catalyzed cleavage of C4b or C3b C3bBb |
| Decay-accelerating factor (DAE or CD55)* | Membrane bound | Classical, alternative, and lectin | Accelerates dissociation of $C\overline{4b2a}$ and $C3\overline{bBb}$ (classical and alternative C3 convertases) |
| Factor-I | Soluble | Classical, alternative, and lectin | Serine protease: cleaves C4b or C3b using C4bBP, CR1, factor H, DAE, or MCP as cofactor |
| S protein | Soluble | Terminal | Binds soluble C5b67 and prevents its insertion into cell membrane |
| Homologous restriction factor (HRF)<br>Membrane inhibitor of reactive lysis (MIRL or CD59)* | Membrane bound | Terminal | Bind to C5b678 on autologous cells, blocking binding of C9 |
| Anaphylatoxin inactivator | Soluble | Effector | Inactivates anaphylatoxin activity of C3a, C4a, and C5a by carboxypeptidase N removal of C-terminal Arg |

*An RCA (regulator of complement activation) protein. In humans, all RCA proteins are encoded on chromosome 1 and contain short consensus repeats.

# BIOLOGICAL CONSEQUENCES
## (COMPLEMENT ACTIVATION)

- Complement serves as an important mediator of the humoral response by amplifying the response and converting it into an effective defense mechanism to destroy invading microorganisms.

- The MAC mediates cell lysis, while other complement components or split products participate in the inflammatory response, opsonization of antigen, viral neutralization, and clearance of immune complexes

## TABLE 13-3 Summary of biological effects mediated by complement products

| Effect | Complement product mediating* |
|---|---|
| Cell lysis | C5b–9, the membrane-attack complex (MAC) |
| Inflammatory response | |
| Degranulation of mast cells and basophils† | C3a,C4a, and C5a (anaphylatoxins) |
| Degranulation of eosinophils | C3a, C5a |
| Extravasation and chemotaxis of leukocytes at inflammatory site | C3a, C5a, C5b67 |
| Aggregation of platelets | C3a, C5a |
| Inhibition of monocyte/macrophage migration and induction of their spreading | Bb |
| Release of neutrophils from bone marrow | C3c |
| Release of hydrolytic enzymes from neutrophils | C5a |
| Increased expression of complement receptors type 1 and 3 (CR1 and CR3) on neutrophils | C5a |
| Opsonization of particulate antigens, increasing their phagocytosis | C3b, C4b, iC3b |
| Viral neutralization | C3b, C5b–9 (MAC) |
| Solubilization and clearance of immune complexes | C3b |

*Boldfaced component is most important in mediating indicated effect.

†Degranulation leads to release of histamine and other mediators that induce contraction of smooth muscle and increased permeability of vessels.

# COMPLEMENT RECEPTORS

Fragments of complement components can bind to complement receptors, which are expressed on the surface of different cells.

**TABLE 13-4 Complement-binding receptors**

| Receptor | Major ligands | Activity | Cellular distribution |
|---|---|---|---|
| CR1 (CD35) | C3b, C4b | Blocks formation of C3 convertase; binds immune complexes to cells | Erythrocytes, neutrophils, monocytes, macrophages, eosinophils, follicular dendritic cells, B cells, some T cells |
| CR2 (CD21) | C3d, C3dg,* iC3b | Part of B-cell coreceptor; binds Epstein-Barr virus | B cells, follicular dendritic cells, some T cells |
| CR3 (CD11b/18)<br>CR4 (CD11c/18) | iC3b | Bind cell-adhesion molecules on neutrophils, facilitating their extravasation; bind immune complexes, enhancing their phagocytosis | Monocytes, macrophages, neutrophils, natural killer cells, some T cells |
| C3a/C4a receptor | C3a, C4a | Induces degranulation of mast cells and basophils | Mast cells, basophils, granulocytes |
| C5a receptor | C5a | Induces degranulation of mast cells and basophils | Mast cells, basophils, granulocytes, monocytes, macrophages, platelets, endothelial cells |

*Cleavage of C3dg by serum proteases generates C3d and C3g.

# FUNCTIONS OF THE COMPLEMENT SYSTEM - OVERVIEW

- **Inflammation** (mast cell degranulation, chemotaxis, increases vascular permeability, margination and diapedesis of polymorphonuclears, smooth muscle contraction, activation of polymorphonuclears, NK cells and macrophages)
- **Clearance of immune complexes**
- **Cell lysis** (G negative bacteria, *Protozoa*, some viruses)
- **Viral neutralization**
- **Opsonization**

# THE COMPLEMENT SYSTEM - OVERVIEW

- The alternative and lectin pathways are clear components of innate immune system, whereas the classical pathway depends on addaptive immune response (it is triggered through antigen-antibody reaction)
- **Three functions of the complement system**:

1. C3b coats microbes and promotes the binding of these microbes to phagocytes (by receptors for C3b).

2. Some breakdown products of complement proteins are chemoattractants for neutrophils and monocytes and promote inflammation at the site of complement activation.

3. Complement activation results to the formation of a polymeric protein complex (MAC), causing osmolysis or apoptosis of microbes.

## Role of Complement in Disease

The complement system plays a critical role in inflammation and defence against some bacterial infections. Complement may also be activated during reactions against incompatible blood transfusions, and during the damaging immune responses that accompany autoimmune disease. Deficiencies of individual complement components or inhibitors of the system can lead to a variety of diseases (**Table 1**), which gives some indication of their role in protection against disease.

**Table 1**. Diseases associated with complement deficiencies

| Complement Deficiency | Disease |
|---|---|
| C3 and Factor B | Severe bacterial infections |
| C3b-INA, C6 and C8 | Severe Neisseria infections |
| Deficiencies of early C components C1, C4, C2. | Systemic lupus erythematosus (SLE), glomerulonephritis and polymyositis |
| C1-inhibitor | Hereditary angioedema |

# SUMMARY

• **Complement is central to the development of inflammatory reactions** and forms one of the major immune defense systems of the body.

• **Complement activation pathways have evolved to label pathogens for elimination.** The classical pathway links to the adaptive immune system. The alternative and lectin pathways provide non-specific 'innate' immunity, and the alternative pathway is linked to the classical pathway.

• **The complement system is controlled to protect the host.** C1 inhibitor controls the classical and lectin pathways. C3 and C5 convertase activity are controlled by decay and enzymatic degradation.

• **The membrane attack pathway results in the formation of a transmembrane pore.** Regulation of the membrane attack pathway reduces the risk of 'bystander' damage to adjacent cells.

• **Many cells express one or more membrane receptors for complement products.** Receptors for fragments of C3 are widely distributed on different leukocyte populations. Receptors for C1q are present on phagocytes, mast cells, and platelets.

• **Complement has a variety of functions.** Its principal functions are chemotaxis including opsonization and cell activation, lysis of target cells, and priming of the adaptive immune response.

• **Complement deficiencies illustrate the homeostatic roles of complement.**
Classical pathway deficiencies result in tissue inflammation. Deficiencies of mannan-binding lectin (MBL) are associated with infection in infants.
Alternative pathway and C3 deficiencies are associated with bacterial infections.
Terminal pathway deficiencies predispose to Gram-negative bacterial infections.
C1inhibitor deficiency leads to hereditary angioedema. Deficiencies in alternative pathway regulators produce a secondary loss of C3.

# THANKS