The Complement System

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Learning Objectives

By the end of this lecture you will be able to:

1. Recognize the biological functions of the complement cascade
2. Identify the components of the complement system
3. Describe the three pathways of complement activation
The Complement System

• A family of more than 20 plasma proteins that include enzymes, proenzymes (zymogens), enzyme inhibitors, and glycoproteins

• They interact in cascade and assist in resolution of microbial infection

• The name was coined because they were thought to “complement” the antibacterial activity of antibody
Complement Functions

LYSIS
- Target cell
- Complement

OPSONIZATION
- Phagocyte
- Bacteria
- Complement receptor
- Extravasation

ACTIVATION OF INFLAMMATORY RESPONSE
- Degranulation
- Tissue
- Blood

CLEARANCE OF IMMUNE COMPLEXES
- Phagocyte
- Ag-Ab complex

Figure 7-1
Kuby IMMUNOLOGY, Sixth Edition
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The Complement System

• Synthesized mainly by liver hepatocytes and other cell types (monocyte, macrophage, GI epithelial cells)

• Circulate as inactive proenzymes

• Proteolytic cleavage removes inhibitory fragment and exposes the active site of the complement molecule
Complement Nomenclature

1. Designated by numerals (C1-C9), letter symbols (factor D), or trivial names (homologus restriction factor)

2. Peptide fragments made by activation of a component are denoted:
   - For example, activation of C4 results in
     - “a” for smaller fragment – C4a
     - “b” for larger fragment – C4b
   - Exception: C2a fragment is larger than C2b
Complement Nomenclature

- Larger fragments bind to the target near the site of activation, while smaller fragments diffuse from the site of activation and can initiate localized inflammatory response.

③ Complexes with enzymatic activity have bar over the number or symbol: $C4b2a$
Complement Activation

CLASSICAL PATHWAY
Antigen:antibody complexes

LECTIN PATHWAY
Lectin binding to pathogen surfaces

ALTERNATIVE PATHWAY
Pathogen surfaces

Complement activation

Recruitment of inflammatory and immunocompetent cells

Opsonization of pathogens

Killing of pathogens

Figure 2-24 Immunobiology, 7ed. (© Garland Science 2008)
The Classical Pathway

• Begins with the formation of antigen-antibody complex (immune complex) or by binding of Ab on bacterial surface

• IgM and IgG can activate the classical complement pathway

• Early stage involves C1, C4, C2, and C3
The Classical Pathway

1. C1q binds antigen-bound antibody. C1r activates auto-catalytically and activates the second C1r; both activate C1s.
The Classical Pathway

2. C1s cleaves C4 and C2. Cleaving C4 exposes the binding site for C2. C4 binds the surface near C1 and C2 binds C4, forming C3 convertase.
The Classical Pathway

C3 convertase hydrolyzes many C3 molecules. Some combine with C3 convertase to form C5 convertase.
The Classical Pathway

4. The C3b component of C5 convertase binds C5, permitting C4b2a to cleave C5.
The Classical Pathway

C5b binds C6, initiating the formation of the membrane-attack complex.
C5b binds C6 and C7

C5b67 complexes bind to membrane via C7

C8 binds to the complex and inserts into the cell membrane

C9 molecules bind to the complex and polymerize

10–16 molecules of C9 bind to form a pore in the membrane

Membrane lesions—end on (rings)

Membrane lesions—side on (tubes)

Schematic representation of the membrane-attack complex pore

Figure 2.31 Janeway’s Immunobiology, 8ed. (© Garland Science 2012)
Membrane Attack Complex (MAC)

1. Activated complement proteins form complexes of proteins that create holes in the bacterial cell wall.

2. Water and salts diffuse into the bacterium through the holes.

3. The bacterium swells and eventually bursts.
The Lectin Pathway

• Lectins such as mannose-binding lectin (MBL) binds to mannose residues on the surface of microorganisms

• Early stage involve MASP1, MASP2, MBL, C2, C4, and C3

• Sugars recognized by MBL in human cells are covered with sialic acid
The Lectin Pathway

Activated MASP-2 associated with MBL or ficolin cleaves C4 to C4a and C4b, which binds to the microbial surface

C4b then binds C2, which is cleaved by MASP-2, to C2a and C2b, forming the C4b2a complex

C4b2a is an active C3 convertase cleaving C3 to C3a and C3b, which binds to the microbial surface or to the convertase itself

One molecule of C4b2a can cleave up to 1000 molecules of C3 to C3b. Many C3b molecules bind to the microbial surface

Figure 2.16 Janeway's Immunobiology, 8ed. (© Garland Science 2012)
Figure 2.31 Janeway's Immunobiology, 8ed. (© Garland Science 2012)
The Alternative Pathway

- It generates active products similar to those of the classical pathway but without antigen-antibody complex.

- Early stage involve C3, factor B, factor D, and properdin.

- Gram negative and gram positive bacterial cell wall can activate the alternative pathway.
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.


4. Convertase generates C3b; some binds to C3 convertase, activating C5 convertase. C5b binds to antigenic surface.

Figure 7-7
*Kuby IMMUNOLOGY,* Sixth Edition
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Figure 2.31 Janeway’s Immunobiology, 8ed. (© Garland Science 2012)
**LECTIN PATHWAY**
Mannose-binding lectin (MBL) and ficolins recognize and bind carbohydrates on pathogen surface

**CLASSICAL PATHWAY**
C1q interacts with pathogen surface or with antibodies bound to surface

**ALTERNATIVE PATHWAY**
C3 undergoes spontaneous hydrolysis to C3(H2O) to initiate eventual deposition of C3 convertase on microbial surfaces

- **ficolin**
- **MBL**
- **Pathogen surface**
- **MBL/ficolin, MASP-2**
  - C4
  - C2
- **C1q, C1r, C1s**
  - C4
  - C2
- **factor D**
- **factor B**
- **Properdin (factor P)**
- **C3bBb**

All pathways generate a C3 convertase, which cleaves C3, leaving C3b bound to the microbial surface and releasing C3a

**Figure 2.12 part 1 of 2 Janeway’s Immunobiology, 8ed. (© Garland Science 2012)**
All pathways generate a C3 convertase, which cleaves C3, leaving C3b bound to the microbial surface and releasing C3a.

C3a and C5a recruit phagocytic cells to the site of infection and promote inflammation.

Phagocytes with receptors for C3b engulf and destroy the pathogen.

Completion of the complement cascade leads to formation of a membrane-attack complex (MAC), which disrupts cell membrane and causes cell lysis.

Figure 2.12 part 2 of Janeway’s Immunobiology, 8ed. (© Garland Science 2012)
Activation Consequences

LYSIS
Complement
Target cell

OPSONIZATION
Bacteria
Phagocyte

ACTIVATION OF INFLAMMATORY RESPONSE
Complement receptor
Extravasation
Degranulation
Tissue
Blood

CLEARANCE OF IMMUNE COMPLEXES
Ag-Ab complex
Phagocyte

Figure 7-1
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Lysis

• MAC can lyse broad spectrum of cells

• Gram positive bacteria are generally more resistant to MAC because of their thick peptidoglycan layer

• Some cells have developed ways to evade MAC such as cancer cells
Lysis

Scanning electron micrograph of *E. coli* before and after complement lysis

Some microbes develop mechanisms to evade complement lysis
Inflammatory Response

- C3a and C5a (anaphylatoxins) bind to basophils and mast cells
Inflammatory Response

- C3a and C5a increase vascular permeability

- C3a and C5a mediate *chemotaxis* by inducing monocytes and neutrophils to adhere to vascular endothelium, extravasation, and migration to the site of complement activation in the tissue i.e. inflammation
Opsonization

- C3b and C4b binding facilitates phagocytosis

- Phagocytic cells express complement receptors CR1, CR3, and CR4

- Activation of phagocytic cells increase the number of expressed complement receptors
Virus Neutralization

- Binding of Ab to viral structures causes:
  - Complement fixation (classical, alternative, and lectin pathways)
  - Viral neutralization and aggregation by complement components e.g. C3b
  - Formation of thick protein coat around the virus
- These mechanisms blocks attachment to susceptible host cells
Clearing Immune Complexes

- Immune complexes can damage tissues
- C3b coats immune complexes
- RBC have capability of binding C3b coated complexes and carrying them to liver and spleen to be cleared
Activated Complement Regulation

• Activated complement components are able to harm normal tissues. Therefore, they get spontaneously inactivated if they are not stabilized by other components

• C3 convertase is a central amplification step in all pathways. Regulatory proteins control C3 convertase activity
Regulation of the Complement System

(a) Before assembly of convertase activity

1. C1 inhibitor (C1Inh) binds $C1r_2S_2$, causing dissociation from C1q.

2. Association of C4b and C2a is blocked by binding C4b-binding protein (C4bBP), complement receptor type I, or membrane cofactor protein (MCP).

3. Inhibitor-bound C4b is cleaved by factor I.

4. In alternative pathway, CR1, MCP, or factor H prevents binding of C3b and factor B.

5. Inhibitor-bound C3b is cleaved by factor I.
(b) After assembly of convertase

C3 convertases are dissociated by C4bBP, CR1, factor H, and decay-accelerating factor (DAF).

C4bBP, CR1, factor H, DAF → Dissociation of convertase; remaining C4b or C3b cleaved by factor I.
(c) Regulation at assembly of membrane-attack complex (MAC)

1. S protein prevents insertion of C5b67 MAC component into the membrane.

2. Homologous restriction factor (HRF) or membrane inhibitor of reactive lysis (MIRL or CD59) bind C5b678, preventing assembly of poly-C9 and blocking formation of MAC.

Figure 7-10
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Complement Deficiencies

- Paroxysmal Nocturnal Hemoglobinuria (PNH)
  - Somatic mutation in the pig-A gene that synthesizes GPI proteins
  - Mutated GPI cannot bind to DAF and CD59
  - This results in hemolytic anemia, iron deficiency, and thrombosis
**Impaired quality of life**
Disabling fatigue
Poor physical functioning
Pain
Dyspnea
Renal impairment

**Smooth muscle dystonia**
Abdominal pain
Dysphagia
Erectile dysfunction

**Thrombosis**
Venous
Liver, mesenteric, dermal, cerebral
Arterial
Myocardial infarction, cerebral vascular accident

**Anemia**
Transfusions
Fatigue
Dyspnea
Angina
## Complement Deficiencies

- **Paroxysmal Nocturnal Hemoglobinuria (PNH) - Treatment**

<table>
<thead>
<tr>
<th>Problem</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lack of RBC</td>
<td>RBC transfusion</td>
</tr>
<tr>
<td>Deficient erythropoietin</td>
<td>Recombinant erythropoietin</td>
</tr>
<tr>
<td>Increased thrombosis</td>
<td>Heparin and anticoagulants</td>
</tr>
<tr>
<td>Loss of iron</td>
<td>Iron supplementation</td>
</tr>
<tr>
<td>Persistent complement activation</td>
<td>Eculizumab to block C5</td>
</tr>
</tbody>
</table>
You are now able to:

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✓ Identify the components of the complement system
✓ Describe the three pathways of complement activation