The Complement System

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What is Complement?

- Major Arm of the Innate Immune System
- More than 30 plasma and cell surface proteins
- Numerous Functions in Inflammation and Immunity
- Activity is Heat Labile (56°C or greater)
Why should you care?

In the last 2 yrs >500 articles published on C3 alone

Disease associations

Gene defects in Complement proteins

SLE  MPGN  PNH
HUS  AMD  HANE

Clinically Useful
The Complement System

<table>
<thead>
<tr>
<th>Alternative Pathway</th>
<th>MBP Pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical Pathway</td>
<td></td>
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</tbody>
</table>

- C3
- C5

Opsonization
Phagocytosis/Clearance (C3b/iC3b)
B-lymphocyte Activation (C3d)

Complement Anaphylatoxins (C3a/C5a)
Membrane Attack Complex (C5b-C9)
The Complement System

Classical Pathway

Antigen-Antibody Complex (IgG or IgM) → Activated C1 → C1 → C4 → C4b → C2 → C4b2b → C3 → C3b → C5 → C5b → C5b-9 (Membrane Attack Complex)

Alternate Pathway

Mannose Binding Lectin → C4 → C2 → C3 → C3b → C3bBb → C5 → C5b → C5b-9 (Membrane Attack Complex)

C4b2b3b → C3a → C5a → C6 → C7 → C8 → C9
Classical Pathway
ACTIVATION OF CLASSICAL PATHWAY

- Dependent on formation of antigen-antibody complexes
- Either in the circulation or local tissue deposition
- Primarily by IgG and IgM immune complexes
  - IgM > IgG3 > IgG1 > IgG2
  - IgG4, IgA, IgD, and IgE do not activate
Also thought to bind to and clear apoptotic cells
Classical Complement Pathway

Ag-Ab Complexes → C1 → Activated C1

Cell Surface
Classical Complement Pathway

Ag-Ab Complexes

C1 ➔ Activated C1 ➔ C2a

C2 ➔ C2b ➔ C4 ➔ C4b ➔ C4a ➔ Cell Surface
Classical Complement Pathway

Ag-Ab Complexes → C1 → Activated C1 → C2b → C4 → C4b → C4b2b → C3 Convertase → Cell Surface
Classical Complement Pathway

Ag-Ab Complexes → C1 → Activated C1

C2b → C3 → C4b → C4b2b → C3 Convertase

Cell Surface
Classical Complement Pathway

Ag-Ab Complexes

C1 → Activated C1

C2b

C4 → C4b → C4b2b → C4b2b3b

C3 Convertase

C3a

C3b

C3 Convertase

C5 Convertase

Cell surface
Classical Complement Pathway

Ag-Ab Complexes

C1 → Activated C1

C1 → C4 → C4b → C4b2b → C3 Convertase

C2b → C4b2b → C4b2b3b → C5 Convertase

C3 Convertase

Cell Surface

C3 → C4 → C4b → C4b2b → C3b → C3a

C5 Convertase

C5 → C5b → C5a

C4b2b3b → C5b
Classical Complement Pathway

Ag-Ab Complexes

C1 → Activated C1

C1 → C4 → C4b → C4b2b

C2b

C3 → C3a

C3 Convertase

C4b2b3b → C5b → MAC

C5 Convertase

Cell Surface
Lectin (MBP) Pathway
MBL-MASP COMPLEX

- Mannose Binding Lectin (C₁q-like)
- MBL Associated Serine Protease (C₁r and C₁s-like)
- MBL-MASP Binds Polysaccharides on Gram-Neg Bacteria
- Initiates Classical Pathway Activation Independent of Ab
- MASP cleaves C₄ and C₂
MBP Pathway

MBL  MASP  (C1 like Enzyme)
Alternative Pathway
Alternative pathway activation

- Constant low level AP activation by hydrolysis of thioester bond on C3 “tickover”
- Primary activation via complex macromolecules on surface of pathogens
  - LPS
  - Bacteria
  - Viruses
  - Fungi
Alternative Pathway

C3 tick-over
Alternative Pathway

C3

\[ \text{O} = \text{C} \quad \text{S} \]

C3 b

\[ \text{H}_2\text{O} \]

\[ \text{HO} \]

\[ \text{O} = \text{C} \quad \text{S} \quad \text{H} \]

C3 tick-over

Acceptor Surface
Alternative Pathway

C3

C3b

Factor B

C3bB

Acceptor Surface
Alternative Pathway

O=C=S

C3

Factor B

Factor D

C3b → C3bB → C3bBb

Properdin

C3 Convertase

Acceptor Surface
Alternative Pathway

C3

O=C=S

C3b

Factor B

Factor D

C3bB

C3bBb

C3 Convertase

Accepter Surface

Properdin

C3

C3b

C3a
Alternative Pathway

C3

O=C=S

Factor B

Factor D

C3b

C3bB

C3bBb

C3bBb3b

C3a

Properdin

C3

C3 Convertase

Acceptor Surface

C5 Convertase
Alternative Pathway

C3 Convertase

C5 Convertase

O=C-S

C3

Factor B

Factor D

C3b

C3bB

C3bBb

C3 Convertase

C3 Convertase

Properdin

C3bBb3b

C3a

C5a

C5

C5b

C3bBb3b

Acceptor Surface
Alternative Pathway

C3

C3b

Factor B

C3bB

Factor D

C3bBb

Properdin

C3

C3a

C5

C5a

C5b

MAC

C5bBb3b

C3 Convertase

C5 Convertase

Acceptor Surface
Central role of the Convertases

Classical Pathway

MBP-MASP → C4b2b → C3bBb → C3

C3 Convertase

C5 convertase

C3bBb3b

C5b

Alternative Pathway

C5b

C5bBb3b
C3 Convertase

- Formation of C3 convertase is the critical step in complement activation
- All three activation pathways converge to form C3 convertase
- Tightly regulated
- Acts as an amplification step; 1 molecule of C3 convertase can cleave up to 1000 molecules of C3
LYTIC PATHWAY/BIG MAC ATTACK
What Regulates the Complement System?
Classical Pathway Regulators

- **C₁ inhibitor**
  - binds activated C₁r, C₁s, removes it from C₁q

- **C₄ binding Protein**
  - binds C₄b displacing C₂b, also cofactor for Factor I

- **Factor I**
  - protease cleaves C₃b and C₄b with cofactors: factor H, MCP, C₄bP and CR₁
Alternative Pathway Regulators

- **Factor H**
  - Binds C3b displaces Bb; cofactor for factor I

- **Factor I**
  - protease cleaves C3b; cofactors Factor H, CR1, DAF, MCP
Cell Surface Regulators

- **CR₁**
  - binds C₄b or C₃b, displaces C₂b or Bb: cofactor for Factor I

- **DAF** (decay accelerating Factor)
  - displaces C₂b from C₄b and Bb from C₃b

- **MCP** (membrane cofactor protein)
  - promotes C₃b and C₄b inactivation by Factor I
Terminal Pathway Regulators

Prevents formation of MAC on homologous cells

- CD59
- S-Protein
- Clusterin
Functions of Complement
Functions of Complement

- Cytolysis of pathogens (e.g. bacteria)
- Opsonization and phagocytosis of foreign organisms
- Activation and directed migration of leukocytes
- Solubilization and clearance of immune complexes
- Enhancement of humoral immune responses
CYTOLYSIS OF FOREIGN ORGANISMS
-C5B-9 MAC Complex

Membrane lesions—end on (rings)
Membrane lesions—side on (tubes)
C3 Cleavage/Degradation

C3 → C3b → iC3b → C3c → C3d → C3dg

- C3a
<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
<th>Function</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR1</td>
<td>C3b, C4b</td>
<td>Promotes decay of C3b/C4b</td>
<td>RBC, Mac/Mono, PMN, B-cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stimulates phagocytosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Immune complex</td>
<td></td>
</tr>
<tr>
<td>CR2</td>
<td>C3d, C3dg, iC3b</td>
<td>B-cell Receptor Complex; increase humoral responses</td>
<td>B-cells, FDC</td>
</tr>
<tr>
<td>CR3</td>
<td>iC3b</td>
<td>Stimulates phagocytosis</td>
<td>Mac/Mono, PMN, FDC</td>
</tr>
<tr>
<td>CR4</td>
<td>iC3b</td>
<td>Stimulates phagocytosis</td>
<td>Mono/Mac, PMN</td>
</tr>
<tr>
<td>C3aR/C5aR</td>
<td>C3a/C5a</td>
<td>Inflammatory</td>
<td>Leukocytes</td>
</tr>
</tbody>
</table>
Opsonization and Phagocytosis

- Dependent on C3b
- Complement receptors CR1 and CR3
Anaphylatoxins

- Chemotaxis
- Smooth Muscle Contraction
- Histamine release/degranulation
- Vascular Permeability
- Cytokine Induction

**C5a/C5aR**
- 100 x more potent than C3a
- Neutrophils
- Monocytes
- Macrophages
- Eosinophils

**C3a/C3aR**
- Eosinophils
- Not Neutrophils
Immune Complex Solubilization

- Antibody
- Antigen
- C3b
- Insoluble Immune Complex
- Soluble Immune Complexes
- CR1
- Transport to Liver and Spleen
- Erythrocyte
- Fixed Mφ
- CR1
- FcR
- CR3
Binding of CR2 induces CD19 phosphorylation

Potentiates BCR signaling beyond CD19 activation alone

CR2 expressed on B-cells

Binds C3d

Enhances Humoral Responses
# Complement Deficiencies

<table>
<thead>
<tr>
<th>Complement Component</th>
<th>Complement Function Abnormality</th>
<th>Clinical Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3</td>
<td>C3 Fragments not produced and terminal components not activated</td>
<td>Pyogenic Infections and immune complex disease (glomerulonephritis)</td>
</tr>
<tr>
<td>C1 (q, r, s); C4; C2</td>
<td>Classical Pathway not Activated</td>
<td>Autoimmune Diseases (SLE) and Pyogenic Infections</td>
</tr>
<tr>
<td>Properdin, Factor B, Factor D</td>
<td>Alternative Pathway not Activated</td>
<td>Pyogenic Infections</td>
</tr>
<tr>
<td>Mannose Binding Lectin</td>
<td>Lectin Pathway not Activated</td>
<td>Recurrent Bacterial Infections</td>
</tr>
<tr>
<td>C5, C6, C7, C8</td>
<td>No MAC Formation</td>
<td>Recurrent Neisserial Infections</td>
</tr>
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## Complement Deficiencies

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<th>Complement Function Abnormality</th>
<th>Clinical Condition</th>
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<tr>
<td>C1-Inhibitor</td>
<td>Deregulated Classical Pathway and C3 Consumption</td>
<td>Acute skin and mucosal edema (Hereditary Angioneurotic Edema-HANE)</td>
</tr>
<tr>
<td>DAF</td>
<td>Unregulated C3 Activation and C3 fragment deposition especially on RBCs</td>
<td>Hemolysis-Paroxysmal Nocturnal Hemoglobulinemia (PNH)</td>
</tr>
<tr>
<td>CD59</td>
<td>Unregulated Terminal Complement Activation</td>
<td>Hemolysis-Paroxysmal Nocturnal Hemoglobulinemia (PNH)</td>
</tr>
<tr>
<td>Factor H and Factor I</td>
<td>Unregulated C3 Activation and C3 Consumption</td>
<td>Pyogenic Infections and Immune Complex Disease (Glomerulonephritis)</td>
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<tr>
<td>CR3</td>
<td>Impaired Leukocyte Activation and Phagocytosis</td>
<td>Recurrent Bacterial Infections</td>
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Complement Summary
• Activation of complement occurs via 3 pathways
  • Classical pathway
  • MBP pathway
  • Alternative pathway

• All three pathways generate C3 convertase, and subsequently form C5 convertase

• Complement activation pathways converge to activate a common terminal pathway resulting in formation of the MAC
• C3 convertase formation is very tightly regulated both in the fluid phase and at the cell surface

• C3 convertase is a critical amplification step in complement activation

• Complement effector functions are mediated by C3 cleavage products acting via specific receptors and by MAC formation
Effector Functions of Complement

- Cytolysis of pathogens (e.g. bacteria)
- Opsonization and phagocytosis of foreign organisms
- Activation and directed migration of leukocytes
- Solubilization and clearance of immune complexes
- Enhancement of humoral immune responses
Complement Deficiencies and Associated Diseases

- Recurrent Pyogenic Bacterial Infections
- Autoimmune Conditions--Systemic Lupus Erythematosus
- Immune Complex Disease--Glomerulonephritis
- Acute Angioedema (HANE)
- Hemoglobinulinemia (PNH)
- Macular Degeneration