The Complement System

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What is Complement?

Term dates to the 1890’s, where a heat labile component of serum “complemented” a heat resistant factor (antibody) in mediating bacteriolysis.
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
Complement system

Complex network of proteins (>30)

- Activation Cascade
  - Serum proteins
- Regulatory proteins
  - Serum proteins
  - Cell surface proteins
- Cell surface receptors
The Complement System

- Alternative Pathway
- MBP Pathway
- Classical Pathway

C3 → C5

- Opsonization
- Phagocytosis/Clearance (C3b/iC3b)
- B-lymphocyte Activation (C3d)
- Complement Anaphylatoxins (C3a/C5a)
- Membrane Attack Complex (C5b-C9)
Complement Cascade

Serum proteins

• Activation pathways
  – Classical pathway
  – Alternative pathway
  – Manose-binding protein/Lectin pathway

• Terminal pathway
  – Membrane Attack Complex
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
BINDING AND ACTIVATION OF C1

Diagram showing the interaction between C1q, C1r2, C1s2 tetramer, antibody, and antigen on the cell surface, with a distance of less than 40 nm.
Classical Complement Pathway

Ag-Ab Complexes

C1 ➔ Activated C1

Cell Surface
Classical Complement Pathway

Ag-Ab Complexes

C1 → Activated C1

C2 → C2a

C2b

C4 → C4b

C4a

Cell Surface
Classical Complement Pathway

Ag-Ab Complexes

C1 → Activated C1

C1 → C2b

C4 → C4b → C4b2b

C3 Convertase

Cell Surface
Classical Complement Pathway

Ag-Ab Complexes → C1 → Activated C1 → C4 → C4b → C4b2b → C3 Convertase

C3 → C3a, C3b

Cell Surface
Classical Complement Pathway

Ag-Ab Complexes

\[ \text{C1} \rightarrow \text{Activated C1} \]

\[ \text{C4} \rightarrow \text{C4b} \rightarrow \text{C4b2b} \]

C3 Convertase

\[ \text{C3} \rightarrow \text{C3b} \rightarrow \text{C4b2b3b} \]

C5 Convertase

Cell surface
Classical Complement Pathway

Ag-Ab Complexes → C1 → Activated C1 → C4 → C4b → C4b2b → C3 Convertase → C3 → C3b C3a → C5 C5b → C5a → C5 Convertase

Cell Surface
Classical Complement Pathway

Ag-Ab Complexes

C1 → Activated C1

C1 → C4 → C4b → C4b2b

C2b → C3 → C4b2b3b

C5 → C5b → C5a → MAC

C3 Convertase

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)

Cell surface
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 tick-over

Acceptor Surface
Alternative Pathway

O=C=S

C3

Factor B

C3b -> C3bB

Acceptor Surface
Alternative Pathway

C3 Convertase

Factor B → C3bB → C3bBb

Factor D

Properdin

O=C=S

C3

Acceptor Surface
Alternative Pathway

C3 → C3b → C3bB → C3bBb

Factor B  Factor D  Properdin

C3 Convertase

Acceptor Surface

C3a  C3b
Alternative Pathway

C3

O=C=S

C3

Factor B

C3b

Factor D

C3bB

Properdin

C3

C3bBb

C3 Convertase

C3bBb3b

C5 Convertase

Acceptor Surface
Alternative Pathway

- C3
- Factor B
- Factor D
- Properdin
- C3 Convertase
- C5 Convertase
- C5a
- C5b
- C3b
- C3bB
- C3bBb
- C3bBb3b
- C5
- Acceptor Surface
Alternative Pathway

C3 Convertase

C5 Convertase

Acceptor Surface
Central role of the Convertases

Classical Pathway

MBP-MASP → C4b2b → C3 → C3b → C3bBb → C3bBb3b

C3 Convertase

C5 convertase

C4b2b3b → C4b2b3b

C5 → C5b

C3bBb3b

Alternative Pathway
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
STRUCTURE OF MEMBRANE ATTACK COMPLEX (MAC)
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

- CD59
- S-Protein
- Clusterin

Prevents formation of MAC on homologous cells
Complement Pathways And Regulatory Proteins

Classical Pathway
- C1inh

Manose Pathway
- C4BP
- Factor I

Alternative Pathway
- C3
- C3b
- C5
- C5b
- MAC
- C4b2b
- C4b2b3b
- C3b
- C3bBb
- C3bBb3b

- S-protein
- Clusterin
- Factor H
- Factor I
- DAF
- CD59
- CR1
- CD59

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
<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&lt;br&gt;Stimulates phagocytosis&lt;br&gt;Immune complex</td>
<td>RBC&lt;br&gt;Mac/Mono&lt;br&gt;PMN&lt;br&gt;B-cells</td>
</tr>
<tr>
<td>CR2</td>
<td>C3d, C3dg, iC3b</td>
<td>B-cell Receptor Complex; increase humoral responses</td>
<td>B-cells&lt;br&gt;FDC</td>
</tr>
<tr>
<td>CR3/CD11b/CD18</td>
<td>iC3b</td>
<td>Stimulates phagocytosis</td>
<td>Mac/Mono&lt;br&gt;PMN&lt;br&gt;FDC</td>
</tr>
<tr>
<td>CR4/CD11c/CD18</td>
<td>iC3b</td>
<td>Stimulates phagocytosis</td>
<td>Mono/Mac&lt;br&gt;PMN</td>
</tr>
<tr>
<td>C3aR/C5aR</td>
<td>C3a/C5a</td>
<td>Inflammatyory</td>
<td>Leukocytes</td>
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Opsonization and Phagocytosis
Mediated by C3 Cleavage Products

C3b, iC3b coated microorganisms

CR1 and CR3 expressed by Neutrophils and Macrophages

Diagram:
- Bacterium is coated with complement and IgG antibody
- When C3b binds to CR1 and antibody binds to Fc receptor, bacteria are phagocytosed
- Lysosomes fuse with vesicles, delivering enzymes that degrade the bacteria
Anaphylatoxins (C3a, C4a, and C5a)

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

Chemotaxis of Leukocytes

C5a/C5aR
100 x more potent than C3a

- Neutrophils
- Monocytes
- Macrophages
- Eosinophils

C3a/C3aR

- Eosinophils
- Not Neutrophils
Complement augments immune complex solubilization
CR2 expressed on B-cells, and FDC
Binds C3d
Delivers Antigen to germinal centers
Activates B-cells

Binding of CR2 induces CD19 phosphorylation
Potentiates BCR signaling beyond CD19 activation alone

Complement Enhances Humoral Immune Responses
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
Summary

• C3 convertase formation in 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
Summary
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