Chapter 13
Complement

History
• Jules Border in 1890’s discovered complement
• Paul Ehrlich coined the term “complement”
• “The activity of blood serum that completes the action of antibody”
• Now: “Set of serum proteins that act in a cascade fashion to increase the immune response”

The Functions of Complement

Complement Components
• Over 20 serum proteins
• Cascade fashion
• Components designated: C1 – C9
• Proteolysis results in: Large fragments “b” and smaller fragments “a” → C3a, C5b
• The only exception is C2, where C2a is the large fragment and C2b is the smaller fragment and diffuses away

Complement Pathways
• 1) Classical Pathway – activated by antigen-antibody interaction. Best Ab for complement activation: IgM, IgG1, IgG2
• 2) Alternative pathway – activated by C3b binding to microbial cell surfaces
• 3) Lectin pathway – binding of the lectin mannose-binding lectin to the surface of pathogens.
Structure of C1

- C1q
- 2 molecules of C1r
- 2 molecules of C1s

Binding of C1 complex to Ab leads to activation of C1r and C1s

- Substrate for C1s is C4 and C2

C1 complex (C1s) hydrolysis C4, resulting in C4a and C4b. C4b binds to the cell surface.

C1 complex (c1s) hydrolysis C2 resulting in C2b (small) and C2a which binds to C4b on the cell surface to form the C3 convertase (C4b2a)

Amplification Step

C3 + C4b2a → C4b2a3b

C4b2a3b C5 convertase
• ALTERNATIVE PATHWAY

- C3 in serum undergoes spontaneous hydrolysis → C3a, C3b
- The half life of these products is very short, except…
- C3b can bind to host and bacterial cell surfaces
- Mammals have high levels of sialic acid → inactivation of C3b
- This is not the case in bacteria, longer half life

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

<table>
<thead>
<tr>
<th>PATHOGENS AND PARTICLES OF MICROBIAL ORIGIN</th>
<th>NONPATHOGENS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Many strains of gram-negative bacteria</td>
<td>Human IgG, IgA, and IgE in complexes</td>
</tr>
<tr>
<td>Lipopolysaccharides from gram-negative bacteria</td>
<td>Rabbit and guinea pig IgG in complexes</td>
</tr>
<tr>
<td>Many strains of gram-positive bacteria</td>
<td>Cobra venom factor</td>
</tr>
<tr>
<td>Teichoic acid from gram-positive cell walls</td>
<td>Heterologous erythrocytes (rabbit, mouse, chicken)</td>
</tr>
<tr>
<td>Fungal and yeast cell walls (zymosan)</td>
<td>Anionic polymers (dextran sulfate)</td>
</tr>
<tr>
<td>Some viruses and virus-infected cells</td>
<td>Pure carbohydrates (agarose, insulin)</td>
</tr>
</tbody>
</table>
- C3b bound to cell surfaces is stabilized by Factor B
- Factor B cleaves C3bB and generates large fragment (Bb) and small fragment (Ba)
- C3bBb is the C3 convertase

**Lectin Pathway**
- Lectins are carbohydrate-binding proteins
- Does not require antibody
- Recognizes mannose residues on glycoproteins
- The mannose binding lectin (MBL) is an acute phase protein that increases during inflammation
- Plays a similar role to that of C1q
- After binding of C1q to cell surfaces, associates with MBL-associated serine proteases (MASP-1 and MASP-2).
- This complex activates C4 and C2 just as in the classical pathway
- MASP-1 and MASP very similar to C1r and C1s

**Biological Effects of Complement Components**
1) Cell Lysis -------------------------- C5-C9
2) Inflammation
   - Degranulation of mast cells/basophils ---- C3a, C4a, C5a
   - Chemotactic for leukocytes -------------- C3a, C5a
3) Opsonization ----------------------- C3b, iC3b
4) Solubilization and clearance
   of Immune complexes ------------------ C3b

**Regulation of Complement**

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**Table 13.2**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Type of protein</th>
<th>Pathway affected</th>
<th>Immunological function</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 inhibitor (C1inh)</td>
<td>Soluble</td>
<td>Classical</td>
<td>Serine protease inhibitor; C1 inhi</td>
</tr>
<tr>
<td>C8-binding protein (C8bp)</td>
<td>Soluble</td>
<td>Classical and lectin</td>
<td>Blocks formation of C8 convertase by binding C8b; inhibition of C8 activation</td>
</tr>
<tr>
<td>Factor H</td>
<td>Soluble</td>
<td>Alternative</td>
<td>Blocks formation of C3 convertase by binding C3b; inhibition of C3 activation</td>
</tr>
</tbody>
</table>
| Complement receptor type 1 (CR1) | Membrane bound | Classical, alternative, and lectin |...

*Some of the regulatory components are encoded on chromosome 1 and certain short anonymous repeats.*
**I. EARLY**

1. C1inh \(\rightarrow\) Classical \(\rightarrow\) Dissociation of C1r2S2
2. C4BP \(\rightarrow\) Classical & Lectin \(\rightarrow\) X C3 Convertase (C4 to C2)
3. MCP (Membrane-cofactor protein) \(\rightarrow\) Classical, Lectin & Alternative \(\rightarrow\) X C3 Convertase by binding to C4b or C3b

**II. DAF (Decay Accelerating Factor) \(\rightarrow\) Classical, Alternative & Lectin \(\rightarrow\) Accelerates dissociation of C3 convertase (C4b2a, C3bBb)**

(b) After assembly of convertase

\[ \text{C4bBP, CR2, Factor H, DAF} \rightarrow \text{Dissociation of convertase; remaining C4b or C3b cleaved by Factor 1} \]

**III. LATE: S protein \(\rightarrow\) binds to C5b67 and prevents insertion into cell membrane**

HRF (Homologous restriction factor) \(\rightarrow\) binds to C5b678 and block binding of C9

**Biological Effects of Complement Components**

- 1) Cell Lysis -------------------------- C5-C9
- 2) Inflammation
  - Degranulation of mast cells/basophils ---- C3a, C4a, C5a
  - Chemotactic for leukocytes ------------------ C3a, C5a
- 3) Opsonization ---------------------- C3b, iC3b
- 4) Solubilization and clearance of Immune complexes ---------- C3b
What is the point of all this?? …3 major functions of complement activation:

1. Phagocytic cells have receptors for C3b and iC3b. Phagocytosis of cells coated with C3b is enhanced. (i.e. C3b is an opsonin)

2. C3a (and C5a) are anaphylatoxins i.e. they act on macrophages, neutrophils, basophils and mast cells to promote chemotaxis of these cells (particularly neutrophils) to the site, degranulation and the respiratory burst. This creates a local inflammatory response that damages any pathogens in the vicinity (and also host tissue).

2. Further enzyme reactions produce a complex (the membrane attack complex, MAC) that creates pores in the microbial cell membrane, resulting in lysis and death of the cell.

### Complement Receptors

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Ligand</th>
<th>Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR1</td>
<td>C3b</td>
<td>RBC – Phagocytes</td>
</tr>
<tr>
<td>CR2*</td>
<td>C3d, iC3b</td>
<td>B cells*</td>
</tr>
<tr>
<td>CR3</td>
<td>iC3b</td>
<td>Phagocytes, NK cells</td>
</tr>
<tr>
<td>CR4</td>
<td>iC3b</td>
<td>Phagocytes, NK cells</td>
</tr>
<tr>
<td>C3a/C4a</td>
<td>C3a, C4a</td>
<td>Mast cells, Basophils</td>
</tr>
<tr>
<td>C5a</td>
<td>C5a</td>
<td>Mast cells, Basophils, Phagocytes</td>
</tr>
</tbody>
</table>

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![Kuby Figure 13-11](image-url)
Microbial Evasion

- Gram negative bacteria:
  - Long LPS
  - Outer membrane
  - Elastase (C3a and C5a are inactivated)

- Gram positive bacteria:
  - Peptidoglycan in cell wall
  - Capsule

Deficiencies:

- Systemic lupus erythomatosus (SLE) is an autoimmune disease that results in tissue damage due to complement activation by Ag-Ab complexes
  - C1, C2, C4 and CR1 predispose to SLE
  - Lack of C4 results in 90% of SLE
  - Deficiencies in C1, C2 and C4 results in low levels of C3b required for clearance of Ag-Ab complexes.

What is the point of all this?? ...3 major functions of complement activation:

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3. Further enzyme reactions produce a complex (the membrane attack complex, MAC) that creates pores in the microbial cell membrane, resulting in lysis and death of the cell.
C3bBb is usually rapidly inactivated, but it is stabilized if it comes into contact with microbial surfaces.

C3bBb bound to microbial surfaces has enzyme activity – it is a C3 convertase:

\[ C3 \xrightarrow{C3bBb} C3b + C3a \]

The additional C3b formed by this enzyme binds to the microbial surface, where it is progressively broken into smaller fragments.

What is the point of all this? …3 major functions of complement activation:

1. Phagocytic cells have receptors for C3b (CR1) and iC3b (CR3, CR4). Phagocytosis of cells coated with C3b is enhanced. (i.e. C3b is an opsonin)

2. C3a (and C5a) are anaphylatoxins i.e. they act on macrophages, neutrophils, basophils and mast cells to promote chemotaxis of these cells (particularly neutrophils) to the site, degranulation and the respiratory burst. This creates a local inflammatory response that damages any pathogens in the vicinity (and also host tissue).

When activated, mast cells and basophils can release a large number of inflammatory mediators, and also produce leukotrienes, prostaglandins and thromboxanes.

These compounds contribute to the characteristic features of inflammatory responses:

**Vasodilation** – results in redness at the site

**Increased capillary permeability** results in swelling at the site.
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