Chapter 7
Complement

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

The Functions of Complement
- Anaphylotoxins
- Lysis
- Opsonization
- Activation of inflammatory response
- Clearance of immune complexes
- Complement receptor
- Degranulation
- Target cell
- Phagocyte
- Extravasation
- Tissue
- Blood
- Phagocyte
- Antibody complex
- Large fragments "b"
- Smaller fragments "a" → C3a, C5b
- The exception is C2, where C2a is the large fragment and C2b is the smaller fragment and diffuses away

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 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 mannose-binding lectin (MBL) to the surface of pathogens

Pathways of complement activation
- Classical Pathway
- Lectin Pathway
- Alternative Pathway
- Activation of C3 and generation of C5 convertase
- LYTIC ATTACK PATHWAY
1. Classical Pathway

- Activated by Ag-Ab complexes
- Complement bind to Fc region
- Antibodies: IgM, IgG (IgG3>IgG1>IgG2)
- Divided into two steps:
  - A) Pre-C3 (C1 to C4)
  - B) Post-C3 (C5 to C9)
2. 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 to mannose-residues on the 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-2 very similar to C1r and C1s

3. Alternative Pathway

- Activated by microbial cell wall and cell wall components
- C3 in serum undergoes spontaneous hydrolysis → C3a, C3b

- The half life of these products is very short, except... if C3b can bind to host and bacterial cell surfaces!!!!

- In mammals there is high levels of sialic acid → inactivation of C3b

- This is not the case in bacteria and fungi → longer half life

**Summary**

**CLASSICAL PATHWAY**
- Antigen:antibody complexes (pathogen surfaces)
- C1q, C1r, C1s, C4, C2

**MB-LECTIN PATHWAY**
- Mannose-binding lectin binds mannose on pathogen surfaces
- MBL, MASPs-1, MASPs-2

**ALTERNATIVE PATHWAY**
- C3
- B, D

- C3b bound to cell surfaces is stabilized by **Factor B**

- **Factor D** is recruited and acts on Factor B to generate a large (Bb) and small fragment (Ba)

- **C3bBb** is the C3 convertase

- **Properdin** (small protein) stabilizes the C3 convertase

**Generation of C5 convertase leads to the activation of the Lytic pathway**
Components of the lytic pathway

Lytic pathway
C5-activation

Lytic pathway:
assembly of the lytic complex

Lytic pathway:
insertion of lytic complex into cell membrane

Biological Effects of Complement Components

• 1) Cell Lysis --------------- C5b-C9
• 2) Inflammation
  – Degranulation of mast cells/basophils --- C3a, C5a (C4a)
  – Chemotactic for leukocytes ----------- C3a, C5a
• 3) Opsonization --------------- C3b, iC3b
• 4) Solubilization and clearance
  of Immune complexes ------- C3b
Overall Picture!!!!

1) Alternative Pathway
2) Amplification Step
3) Opsonization
4) Anaphylactic Factors
5) Vascular Effect
6) Cell Recruitment
7) Neutrophil Recruitment
8) Cell Activation

Regulation of Complement

<table>
<thead>
<tr>
<th>Protein Type</th>
<th>Pathway Affected</th>
<th>Immune Modulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 inhibitor (C1inh)</td>
<td>Soluble Classical</td>
<td>Serine protease inhibitor causes C1a, C1b to dissociate from C1</td>
</tr>
<tr>
<td>C1q binding protein (C1qBP)</td>
<td>Soluble Classical and lectin</td>
<td>Blocks formation of C1 convertase by binding C1r/C1s complex in C3b/C4b inactivator</td>
</tr>
<tr>
<td>Factor H</td>
<td>Soluble Alternative</td>
<td>Blocks formation of C3 convertase by binding C1r/C1s complex in C3b/C4b inactivator and C3 convertase in inactive form</td>
</tr>
<tr>
<td>Complement receptor type 1 (CR1), Membrane cofactor protein (MCP)</td>
<td>Membrane bounded Classical, Alternative, and lectin</td>
<td>Accelerates dissociation of C3b-C3bi and complements C1 convertase</td>
</tr>
<tr>
<td>Decay-accelerating factor (DAF)</td>
<td>Membrane bounded Classical, Alternative, and lectin</td>
<td>Accelerates dissociation of C3b-C3bi and complements C1 convertase</td>
</tr>
</tbody>
</table>

Figure 2-37 part 2 of 4

Complement Regulatory Protein (CR1), Factor H, Membrane Cofactor Protein (MCP) and Decay-Accelerating Factor (DAF) Target C3 convertase (C3b)

CD59 and S protein – MAC complex

Stages at which complement activity is regulated

The terminal components of complement form a membrane pore—the membrane-attack complex

CD59 prevents final assembly of the membrane-attack complex at the C8 to C9 stage

CD59 and S protein – MAC complex

Figure 2-37 part 4 of 4

CD59 – Targets C9

S Protein – Targets C7

Figure 2-37 part 6 of 6

Overall Picture!!!!

CD59 and S protein – MAC complex
THE GOOD SIDE OF THE STORY: Factor H and Factor I actively neutralize C3b generating smaller products (iC3b). However, if C3b binds to a surface this mechanism is less effective. The chances of C3b to bind to a surface are higher if hydrolysis occur near a cell surface (Classical and Lectin Pathways).

THE ALSO GOOD SIDE OF THE STORY: CR1 and Factor I

- C3b ---- Binds to CR1
- CR1 recruits Factor I (C3b protease)

\[ \text{CR1} \rightarrow \text{C3b} \rightarrow \text{iC3b} \rightarrow \text{C3d} \]
- iC3b – Opsonin; C3d – Antibody production

What is the point of all this?? …3-4 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. They bind to mast cells and basophils through specific receptors. They also act on macrophages, neutrophils, basophils and mast cells to promote chemotaxis of these cells (particularly neutrophils) to the site of injury, degranulation and the respiratory burst. This creates a local inflammatory response that damages any pathogens in the vicinity (and also host tissue). (Cell degranulation)

Opsonization and phagocytosis

Biological effects of C5a
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.

**Cytokines** – IL-6, TNF-α

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**What is the point of all this?? …**

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).

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.

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**TABLE 7.3** Summary of biological effects mediated by complement products

| Effect | Complement product mediating
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory response</td>
<td>C3b, C5b-9 (the membrane attack complex, MAC)</td>
</tr>
<tr>
<td>Phagocytosis of microorganisms</td>
<td>C3b, C5b-9 (the membrane attack complex, MAC)</td>
</tr>
<tr>
<td>Regulation of eicosanoid synthesis</td>
<td>C5a, C3a</td>
</tr>
<tr>
<td>Aggregation of platelets</td>
<td>C5a, C3a</td>
</tr>
<tr>
<td>Activation of monocytes/macrophages</td>
<td>C5a, C3a</td>
</tr>
<tr>
<td>Promotion of chemotaxis of neutrophils</td>
<td>C5a, C3a</td>
</tr>
<tr>
<td>Increased expression of adhesion receptors</td>
<td>C5a</td>
</tr>
<tr>
<td>Phagocytosis of particulate antigens, increasing their phagocytosis</td>
<td>C3b, C5b-9 (MAC)</td>
</tr>
<tr>
<td>Viral neutralization</td>
<td>C3b, C5b-9 (MAC)</td>
</tr>
<tr>
<td>Stabilization and clearance of immune complexes</td>
<td>C3b</td>
</tr>
</tbody>
</table>

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**What is the point of all this?? …**

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.

4. Removal of immune complexes in the spleen or liver

**Presence of CR1 on RBC**

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**Figure 7.17**
Complement Receptors

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Ligand</th>
<th>Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR1 (CD35)</td>
<td>C3b, C4b</td>
<td>RBC – Phagocytes</td>
</tr>
<tr>
<td>CR2* (CD21)</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>• C3aR/C4aR</td>
<td>C3a, C4a</td>
<td>Mast cells, Basophils</td>
</tr>
<tr>
<td>• C5aR</td>
<td>C5a</td>
<td>Mast cells, Basophils, Phagocytes</td>
</tr>
</tbody>
</table>

Microbial Evasion

- **Gram negative bacteria:**
  - Long LPS
  - Outer membrane
  - Elastase (C3a and C5a are inactivated)
- **Gram positive bacteria:**
  - Peptidoglycan in cell wall
  - Capsule
- **Viruses, Bacteria, Parasites**
  - Incorporation or microbe production of regulatory components of the complement cascade

![E. coli – some strains!!!](Kuby Figure 13-11)

C1-inhibitor deficiency: angioedema

![C1-inhibitor deficiency: angioedema](Kuby Figure 13-11)
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 C1q or C4 results in 90% of SLE.
- Deficiencies in C1q, C1r, C1s, C2 or C4 results in low levels of C3b required for clearance of Ag-Ab complexes (glomerulonephritis, vasculitis).

Deficiencies:

- Deficiency in C3 → Severe bacterial infections with *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*.
- Deficiency in C5, C6, C7, C8 or C9 results in high risk for bacterial meningitis caused by *Neisseria meningitidis*.

The End