Foundations of Public Health
Immunology

Complement
Objectives

• Identify functions of complement
• Recognize the similarities and differences of the three complement pathways
• Identify important complement components & their functions
• Identify deleterious effects of complement activation and deficiency
Complement (C’)

- Series of approximately 30 heat-labile proteins
- Normally inactive in the serum
  - Inactive complement proteins known as zymogens
- Can be **sequentially activated** in a controlled sequence
  - Amplification of the reaction occurs at each step
- Production of **biologically active** fragments for lysis or killing of the target
Complement Proteins

- Synthesized in the liver and by several cell types (splenic macrophages)
- Plays an **essential role in inflammation** and in facilitating antibody effectiveness
- Severe infections or autoimmune diseases can result from complement deficiencies (rare in the population)
Functions of Complement

- Essential role in inflammation
- Assists antibodies in **effector functions** (Antibody Dependent Cell-mediated Cytotoxicity – ADCC)
- Assist in clearing immune complexes
- **Opsonization and facilitation of phagocytosis**
- **No antigen specificity**
Functions of Complement

- **Lysis**: Complement, Target cell
- **Opsonization**: Bacteria, Phagocyte
- **Activation of Inflammatory Response**: Complement receptor, Extravasation, Degranulation
- **Clearance of Immune Complexes**: Ag-Ab complex, Phagocyte
3 Pathways of Activation

- **Classical**
  - Triggered when IgM or certain IgG subclasses bind antigens

- **Alternative (Properdin)**
  - Triggered by the deposition of complement protein, C3b, onto microbial surfaces
  - No antibodies required for activation

- **Lectin**
  - Triggered by the attachment of plasma mannose-binding lectin (MBL) to microbes
  - No antibodies required for activation
**Complement Activators**

<table>
<thead>
<tr>
<th>Activators of complement</th>
<th>Immunoglobulins</th>
<th>Microorganisms</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>complexes containing IgM, IgG1, IgG2 or IgG3</td>
<td>murine retroviruses, vesicular stomatitis virus</td>
<td>Mycoplasma</td>
</tr>
<tr>
<td><strong>Classic pathway</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>polyanions, esp. when bound to cations. PO₄²⁻ (DNA, lipid A, cardiolipin) SO₄²⁻ (dextran sulphate, heparin, chondroitin sulphate)</td>
</tr>
<tr>
<td><strong>Lectin pathway</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>complexes containing IgG, IgA or IgE (less efficient than the classic pathway)</td>
<td>some virus-infected cells (e.g. EBV)</td>
<td>arrays of terminal mannose groups</td>
</tr>
<tr>
<td><strong>Alternative pathway</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>complexes containing IgG, IgA or IgE (less efficient than the classic pathway)</td>
<td>some virus-infected cells (e.g. EBV)</td>
<td>trypanosomes, Leishmania, many fungi</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>dextran sulphate, heterologous erythrocytes, carbohydrates (e.g. agarose)</td>
</tr>
</tbody>
</table>

Fig. 4.3 This table summarizes the activators of the classical lectin and alternative pathways.

**Activators start the domino effect . . .**
Early Steps

- The initial steps vary between pathways
- Dependent on activating substance
- C3 convertase quickly forms in all paths to cleave C3
Late Steps

- Late steps (after C5 or convertase) are **same in all pathways**
- Lead to formation of MAC
All Step Together ...
**Important Complement Proteins**

**C3**
- **Most abundant** complement protein
- **Common to all three pathways**
- A & b fragments have **important biologic effects**

**C3a**
- Anaphylatoxin that **promotes inflammation**

**C3b**
- **Binds** the microbial surface thus **acting as an opsonin**
- C3b also a component of the C3 & C5 convertases
Important Complement Proteins

- C5a

**Biological effects of C5a and C5a-des-Arg**

1. Neutrophil activation
2. Neutrophil adhesion
3. Neutrophil emigration and chemotaxis
4. Monocyte activation
5. Mast cell degranulation

Soluble C5a

- Smooth muscle contraction and increased vascular permeability
- IL-1
- IL-6

**Fig. 4.18** C5a causes (1) neutrophil activation, (2) increased expression of adhesion molecules, (3) emigration of neutrophils and chemotaxis, (4) monocyte activation and (5) mast cell degranulation, which in turn causes smooth muscle contraction and increased vascular permeability.
Important Complement Proteins

- **C5b**
  - *Initiates the late steps of complement activation (Common Pathway)*
  - C6, C7, C8, and C9 sequentially bind C5 in the Common Pathway
  - **C9 polymerizes to form the Membrane Attack Complex (MAC)** which forms a pore in the target cell and causes cell lysis

Another view of the membrane attack complex creating a pore in the cell membrane.

Complement membrane attack complexes (above) punch holes in the membranes of microbial invaders.
Membrane Attack Complex
Functional Proteins

- Overview of how complement effects innate immunity & inflammation
Complement Effects

- Lysis (destruction of target cell)
  - Membrane Attack Complex
  - **Antibody makes complement more efficient and guides complement deposition**

- Opsonization (enhanced phagocytosis)
  - Due to the formation of C3b and C5b (larger fragments that “a” fragments)
  - **C3b and C5b bind substrate**
  - **Attract phagocytes** to microbes coated with complement
Complement Effects

• **Activation of the inflammatory response**
  • Anaphylatoxins C3a and C5a promote inflammation
  • C3a and C5a are freely soluble in solution
  • Increased cellular attraction and activation
  • Increased vascular permeability

• **Induction and enhancement of antibody response**
  • Complement receptors on APC and B lymphocytes enhance antigen presentation
  • Accessory role in antibody response
Complement Effects

- Viral neutralization
- Clearance of immune complexes
  - Efficient removal from tissue by phagocytic cells
  - Solubilize immune complexes
Especially note the Complement Mediated Inflammatory Responses!!

### TABLE 13-3 Summary of biological effects mediated by complement products

<table>
<thead>
<tr>
<th>Effect</th>
<th>Complement product mediating*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell lysis</td>
<td>C5b–9, the membrane-attack complex (MAC)</td>
</tr>
<tr>
<td>Inflammatory response</td>
<td></td>
</tr>
<tr>
<td>Degranulation of mast cells and basophils†</td>
<td>C3a, C4a, and C5a (anaphylatoxins)</td>
</tr>
<tr>
<td>Degranulation of eosinophils</td>
<td>C3a, C5a</td>
</tr>
<tr>
<td>Extravasation and chemotaxis of leukocytes at inflammatory site</td>
<td>C3a, C5a, C5b67</td>
</tr>
<tr>
<td>Aggregation of platelets</td>
<td>C3a, C5a</td>
</tr>
<tr>
<td>Inhibition of monocyte/macrophage migration and induction of their spreading</td>
<td>Bb</td>
</tr>
<tr>
<td>Release of neutrophils from bone marrow</td>
<td>C3c</td>
</tr>
<tr>
<td>Release of hydrolytic enzymes from neutrophils</td>
<td>C5a</td>
</tr>
<tr>
<td>Increased expression of complement receptors type 1 and 3 (CR1 and CR3) on neutrophils</td>
<td>C5a</td>
</tr>
<tr>
<td>Opsonization of particulate antigens, increasing their phagocytosis</td>
<td>C3b, C4b, iC3b</td>
</tr>
<tr>
<td>Viral neutralization</td>
<td>C3b, C5b–9 (MAC)</td>
</tr>
<tr>
<td>Solubilization and clearance of immune complexes</td>
<td>C3b</td>
</tr>
</tbody>
</table>

*Boldfaced component is most important in mediating indicated effect.
†Degranulation leads to release of histamine and other mediators that induce contraction of smooth muscle and increased permeability of vessels.
Deleterious Effects of Complement

- Systemic Activation
  - Triggered by Gram Negative organisms
  - Leads to septicemia, anaphylatoxins and shock

- Activation by unrelated tissue necrosis
  - Ischemia (myocardial infarction)
Regulation of Complement

- Tight regulation of complement system necessary to prevent autoimmunity
- Opsonization by binding to complement receptors on cells
- Recognition of “non-self” by C3b which doesn’t bind to self or is limited in formation
- C3 convertase enzyme also produces inhibition of complement activity (feedback loop)
<table>
<thead>
<tr>
<th>Protein</th>
<th>Type of protein</th>
<th>Pathway affected</th>
<th>Immunologic function</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 inhibitor (C1Inh)</td>
<td>Soluble</td>
<td>Classical</td>
<td>Serine protease inhibitor; causes C1r, C2 to dissociate from C1q</td>
</tr>
<tr>
<td>C4b-binding protein (C4bBP)*</td>
<td>Soluble</td>
<td>Classical and lectin</td>
<td>Blocks formation of C3 convertase by binding C4b; cofactor for cleavage of C4b by factor I</td>
</tr>
<tr>
<td>Factor H*</td>
<td>Soluble</td>
<td>Alternative</td>
<td>Blocks formation of C3 convertase by binding C3b; cofactor for cleavage of C3b by factor I</td>
</tr>
<tr>
<td>Complement-receptor type 1 (CR1)*</td>
<td>Membrane bound</td>
<td>Classical, alternative, and lectin</td>
<td>Block formation of C3 convertase by binding C4b or C3b; cofactor for factor I-catalyzed cleavage of C4b or C3b C3bBb</td>
</tr>
<tr>
<td>Membrane cofactor protein (MCP)*</td>
<td>Membrane bound</td>
<td>Classical, alternative, and lectin</td>
<td>Accelerates dissociation of C4b2a and C3bBb (classical and alternative C3 convertases)</td>
</tr>
<tr>
<td>Decay-accelerating factor (DAE or CD55)*</td>
<td>Membrane bound</td>
<td>Classical, alternative, and lectin</td>
<td>Serine protease: cleaves C4b or C3b using C4bBP, CR1, factor H, DAE, or MCP as cofactor</td>
</tr>
<tr>
<td>Factor-I</td>
<td>Soluble</td>
<td>Classical, alternative, and lectin</td>
<td>Bind to C5b678 on autologous cells, blocking binding of C9</td>
</tr>
<tr>
<td>S protein</td>
<td>Soluble</td>
<td>Terminal</td>
<td>Binds soluble C5b67 and prevents its insertion into cell membrane</td>
</tr>
<tr>
<td>Homologous restriction factor (HRF)</td>
<td>Membrane bound</td>
<td>Terminal</td>
<td>Bind to C5b678 on autologous cells, blocking binding of C9</td>
</tr>
<tr>
<td>Membrane inhibitor of reactive lysis (MIRL or CD59)*</td>
<td>Membrane bound</td>
<td>Terminal</td>
<td>Bind to C5b678 on autologous cells, blocking binding of C9</td>
</tr>
<tr>
<td>Anaphylatoxin inactivator</td>
<td>Soluble</td>
<td>Effector</td>
<td>Inactivates anaphylatoxin activity of C3a, C4a, and C5a by carboxypeptidase N removal of C-terminal Arg</td>
</tr>
</tbody>
</table>

*An RCA (regulator of complement activation) protein. In humans, all RCA proteins are encoded on chromosome 1 and contain short consensus repeats.*
Regulation of the Complement System

(a) Before assembly of convertase activity

- C1r2s2
- C1q2s2
- Antibody
- C1r2s2

(b) After assembly of convertase

- C4bBP, CR2, Factor H, DAF
- Dissociation of convertase; remaining C4b or C3b cleaved by Factor I

(c) Regulation at assembly of membrane-attack complex (MAC)

- C4bBP, CR1, or MCP
- Factor I
- C4c
- C4d

- C3b
- C3 convertase

- C3f
- iC3b

- C5b67
- C5b678
- S protein
- Cannot attack nearby cells
- Membrane attack complex

- C5b67
- C8
- HRF, MIRL
- C9
- Poly-C9
Complement Deficiencies

- Clinical symptoms are determined by the Complement Pathway affected
- Can be acquired or inherited
- No specific treatments
  - Antibiotics and immunizations used to reduce risk of disease
Complement Pathways and Deficiencies

**Classical (C1 q,r,s, C4 and C2) deficiencies**: are associated with an increased predisposition for developing immune complex diseases such as SLE.

**MBL deficiencies**: are associated with an increased risk of infection with the yeast Saccharomyces cerevisiae and with encapsulated bacteria.

**Alternative Pathway (Factor D, B, Properdin and C3)**: are associated with decreased opsonization ability and a subsequent increased risk of infection, especially with encapsulated bacteria.

**C3 deficiencies**: are associated with defective opsonization, deficient leucocyte chemotaxis, and decreased bactericidal killing activity (because of decreased MAC formation). These deficiencies are associated with overwhelming infections with encapsulated bacteria. There is also a 79% association with the development of immune complex disease.

**MAC deficiencies**: are associated with an increased risk of infection, especially with the bacteria N. meningitidis, but have decreased morbidity and mortality rates than C3 deficiencies.

C2 & C4 Deficiencies

- **C2** deficiency is most widely reported deficiency of all components in complement pathways.
- Immune complex disorders are the main problem with a deficiency of C2.
- Complete **C4** deficiency is rare.
- Almost all the patients with complete C4 deficiency have discoid or systemic lupus erythematosus (with or without associated glomerulonephritis).
**C3 Deficiencies**

- C3 is central to all three complement pathways!!
- *Usually rare* and leads to an inability to form the membrane attack complex (MAC)
- Predisposes person to frequent bouts of pyogenic **bacterial infection** such as meningococci and pneumococci
Meningococcal disease

- *Neisseria meningitidis* is the most frequently isolated pathogen from patients with bacterial meningitis
- Only humans can harbor *N. meningitidis*
- Susceptibility to meningococcal disease is highest in children aged 3-24 months
- Meningococcal meningitis occurs worldwide
  - Prevalent serotypes vary according to the geographic region
  - ‘African Meningitis Belt’ in sub-Saharan Africa
    - In 1996, Africa experienced the largest recorded outbreak of epidemic meningitis in history, with over 250,000 cases and 25,000 deaths recorded
Summary of the actions of complement and its role in the acute inflammatory reaction

1. Increased vascular permeability
2. Smooth muscle contraction
3. Mast cell degranulation
4. Opsonization and phagocytosis of bacteria
5. Neutrophil activation and chemotaxis
6. Lysis of bacteria
7. Lysis of foreign cells
In Summary

• Identify the similarities and differences of the 3 complement pathways
• Identify the functions (effects) of complement
• Identify deleterious effects of complement activation
• Identify deleterious effects of complement deficiency
Self-Test Questions

• What is complement?
• What are the 3 pathways of activation?
• Which pathways are not activated by antibodies?
• Which complement components stimulate inflammation?
• Name 2 effects of complement.
• What disease or infection may a person deficient in C3 be pre-disposed to?