Lecture 3: Complement II

Phase II – Formation of C5 Convertase & the Membrane Attack Complex.

1. Fixed C3 convertase (C3bBb) binds soluble C3b, forming C5 convertase = \([C3b]_2Bb\).
2. C5 converted to C5a + C5b (soluble), C5b binds to membrane.
3. C5b binds to soluble C6 + C7, forming the initial membrane complex.
4. C5b-C6-C7 complex binds C8.
5. C5b-C6-C7-C8 complex causes polymerization of \(~16\) molecules of C9.
6. C5b-C6-C7-C8-C9\(_{16}\) is the MAC, leading to cell lysis.

Activation of other Branches of the Complement Pathway:

Lectin pathway:

1. Recognition of mannose residues by mannose binding lectin (MBL).
   This is an example of recognition of a PAMP.
   \(\text{PAMP} = \text{Pathogen Associated Molecular Pattern}\).
2. MASP-1 (MBL associated serine protease) is activated.
3. MASP-1 activated, cleaves itself and other MASP-1 (auto-activation)
4. MASP-2 activated by cleavage by MASP-1
5. MASP-2 cleaves C4, C4b is fixed on surface
6. MASP-2 cleaves C2, forming C2a & C2b
7. C2a binds to C4b, generating C3 convertase.
Classical Pathway:
Triggered:
- by antigen-antibody (Ab) complexes on microbial surfaces
- by c-reactive protein, which is produced by the liver during the innate response (acute phase response). This binds to phosphocholine on the surface of pathogens (another example of a PAMP)

C1q: Recognizes IgM or c-reactive protein
C1r: Activated by C1q, autocleavage and cleavage of other C1r.
C1s: Cleaved by activated C1r, producing active protease.

Formation of C3 convertase of classical pathway:

Homology between Lectin & Classical Pathways
MLB C1q
Masp-1 C1r
Masp-2 C1s

Biological Consequences of Complement Activation
1. Opsonization:

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Cells bearing receptor</th>
<th>Ligands</th>
<th>Consequences of ligand binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR1</td>
<td>RBC</td>
<td>C3b/iC3b</td>
<td></td>
</tr>
<tr>
<td>CR2</td>
<td>B-cell</td>
<td>C3b/iC3b/C3d</td>
<td></td>
</tr>
<tr>
<td>CR3</td>
<td>Neutrophil Macrophage</td>
<td>iC3b</td>
<td></td>
</tr>
</tbody>
</table>

(iC3b is an inactive form of C3b, C3d is yet another split product of C3.)

2. Inflammation:
3. Direct killing: formation of functional MAC on cell surfaces causes cell lysis
4. Removal of necrotic and apoptotic cells: C3b deposits on dying cells and released organelles, signaling rapid clearance by phagocytic cells

5. Clearance of immune complexes: C3b deposits on antibody-antigen complexes and causes their dissociation and clearance.

6. Neutralization of viral infection: Complement on antibody-antigen complexes forms a thick coat and blocks viral entry into cells.

7. Enhances activation of B-cells during an infection (via CR2, see above table).

Discussion: In the genetic disease *paroxysmal nocturnal hemoglobinuria*, the urine contains high levels of hemoglobin at various times of the day. Can you suggest what might be causing this disease?
Regulation of Complement Activity (Alternative pathway)
- Factor H and MCP (membrane co-factor) prevent B binding to fixed C3b.
- Factor I (protease) converts fixed C3b to iC3b, an inactive form. Cleavage enhanced by H and MCP.
- DAF (decay accelerating factor) disrupts C3 convertase by causing loss of Bb.
- CD59 (protectin) inhibits formation of the MAC.

Summary/Learning Goals
- Distinguish between the activation of each pathway (innate versus acquired)
- Describe the role of C3b.
- Describe the role of C3a, C5a.
- State the composition of the C3 and C5 convertase in each pathway.
- Describe the formation of the MAC complex.
- Provide examples of regulation of complement.
- Describe the outcome of C3b binding to complement receptors & biological functions of complement (1-7 above).
- Two examples of PAMP receptors – MBL and C1q, recognizing mannose and phosphorylcholine.

Overview of Complement Pathways and Regulation

Alternative Pathway
Activation - Spontaneous (iC3)

Lectin Pathway
Activated by:
MBL + mannose residues.

Classical Pathway
Activated by:
C1q + c-reactive protein
C1q + Ab