The Complement System

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Lecture series Master Biochemistry | Moleculare Medicine | Immunology | SS 17

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The Complement System

PART I

- general features and facts
- central role of C3 convertases

PART II

- complement cascades
  - classical pathway
  - lectin pathway
  - alternative pathway
The starting point of complement

Historically:

1890s  Jules Bordet (nobel prize 1919)

*Complement:* a heat-labile protein component of plasma

➢ ‘complement‘ the action of antibodies to opsonize and kill bacteria

Functionally:

- *Complement:* A part of the humoral innate immune response
- becomes activated, when a pathogen enters the organism and breaches the
  epithelial barriers
  ➢ the presence of antibodies is not absolutely necessary
The humoral immune defence

Killing of pathogens directly/indirectly

humoral innate immunity
Complement
Lysozyme
IFN-α/β
Cytokine
TNF-α

A common goal!

humoral adaptive immunity
Antibodies
IFN-γ
IL

Pinterest (2017): Hands together; 350084571007758711
Profile of the complement system

- **Starting point:** early phase of an infection
- **Structure:** triggered enzyme cascade
- **Components:** >30 interacting plasma proteins (mostly proteases)
- **Location:** blood, other body fluids
- **Activation:** by pathogens or apoptotic cells
  - via Ag-Ab complexes, lectin, pathogen surface
- **Goal:** to generate effector molecules that allow the...
- **Features:**
  1. opsonization of pathogens
  2. chemoattraction of phagocytes
  3. direct bacteriolysis by pore formation
  4. activation of the adaptive immune system
The cascade principle

Activation of zymogens:

**Zymogens:** Inactiv pro-enzymes, activated by proteolysis

An inactive zymogen (C[Y]) is cleaved in two peptide fragments (C[Y]a, C[Y]b) by the respective active protease and becomes an active protease itself that can trigger the following proteolysis...etc...

\[ C[Y] \rightarrow C[Y]a + C[Y]b \]

**C[Y]a:** small fragment

- mediates inflammation, chemoattraction and activation of phagocytes

**C[Y]b:** large fragment

- an active serine protease, catalysing the next step of the cascade
- Exception: C2a is a large fragment
Nomenclature of the complement proteins

1. **Complement proteins of the classical pathway:**
   - **native proteins** are named with „C“ + number, e.g. C1, C2
   - named in the order of their discovery, not due to the reaction process!
     - C1, C4, C2, C3, C5, C6, C7, C8, C9
   - **cleavage products** are named with „C“ + number + small letter (a,b),
     - e.g. C3a, C3b
   - !Exception: C1q, C1r, C1s are not cleavage products of C1 (=subunits of C1)

2. **Complement proteins of the alternative pathway:**
   - **proteins:** B, C, D
   - **cleavage products** (see above), e.g. Ba, Bb
# Protein classes in the complement system

<table>
<thead>
<tr>
<th>Protein class and function</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding to Ag-Ab complexes and pathogen surfaces</td>
<td>C1q</td>
</tr>
<tr>
<td>Binding to carbohydrate structures (mannose, GlcNAc on microbial surface)</td>
<td>MBL, Ficolins, C1q, Properdin (factor P)</td>
</tr>
<tr>
<td>Activating enzymes</td>
<td>C1r, C1s, C2a, Bb, D, MASP-1, MASP-2</td>
</tr>
<tr>
<td>Membrane-binding proteins and opsonins</td>
<td>C4b, C3b</td>
</tr>
<tr>
<td>Peptide mediators of inflammation</td>
<td>C5a, C3a, C4a</td>
</tr>
<tr>
<td>Membrane-attack proteins</td>
<td>C5b, C6, C7, C8, C9</td>
</tr>
<tr>
<td>Complement receptors</td>
<td>CR1, CR2, CR3, CR4, C1qR</td>
</tr>
<tr>
<td>Complement-regulatory proteins</td>
<td>C1INH, C4BP, CR1, MCP, DAF, H, I, P, CD59</td>
</tr>
</tbody>
</table>

Pathways of complement activation

At least one of this pathway gets active in the early phase of an infection

Classical pathway
Antigen-antibody-complex

MB-lectin pathway
Lectin binds on the surface of the pathogen

Alternative pathway
Pathogen surfaces

Complement activation
C3 convertases

Attraction of inflammatory cells
Opsonisation of pathogens
Destruction of pathogens

Murphy K., Travers P., Walport M. (2009): The complement caskade; Janeway Immunology, 7. ed., p. 82, Spektrum
Components and effects

Murphy K., Travers P., Walport M. (2009): The components of the complement system; Janeway Immunology, 7. ed., p. 84, Spektrum
**Key role of C3 convertases**

- C3 convertases are involved in the terminal cascade of all three pathways
  - *generate effector proteins, when any of the pathways interact with a pathogen surface*

<table>
<thead>
<tr>
<th>C3 convertases</th>
<th>C5 convertase</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Diagram" /></td>
<td></td>
</tr>
</tbody>
</table>

- **C3 convertases:** C4b2a
  - Classical and lectin pathway
  - C3bBb, C3(H2O)Bb: Alternative pathway
  - *Hydrolysed C3 in C3a + C3b*

- **C3b**
  - Binds to C3 convertase, forming a C5-convertase (C4b2a3b)

- **C5 convertase**
  - Cleaves C5 in C5a + C5b

<table>
<thead>
<tr>
<th>C3a: peptide mediator, chemoattractor</th>
<th>C5a: Most important inflammatory peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3b: most important opsonin</td>
<td>C5b: Part of the membrane attack complex</td>
</tr>
</tbody>
</table>
Key feature of C3b

- the opsonin C3b form a covalent bond with pathogen surface (C4b too)
- allows the innate recognition of pathogens to be translated into effector responses

### The classical pathway

- **Initial step:** Activation of the C1 complex
  - **Activation:** Binding of C1q to the pathogen surface
  - **Binding:** direct to CRP, PAMPs
    - indirect by antibodies (IgM, IgG)

#### C1 complex (C1q:C1r₂:C1s₂)

- **C1q:** (Hexamer, „pathogen sensor“)
  - „bridge“ between the humoral innate and the humoral adaptive immune system
  - C1q can bind to Ag-Ab complexes
  - Δconf. of C1r
  - enables autoactivation of C1r

- **C1r:** cleaves C1s to an active serine protease

- **C1s:** cleaves C4 and C2

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The classical pathway

Activated C1s cleaves C4 to C4a and C4b, which binds to the microbial surface.

C4b then binds C2, which is cleaved by C1s, to C2a and C2b, forming the C4b2a complex.

C4b2a is an active C3 convertase that cleaves C3 in C3a and C3b; C3b binds to the pathogen surface or to the convertase itself.

A C4b2a molecule can cleave up to 1000 molecules C3 in C3b; many C3b molecules bind to the pathogen surface.


C3 convertase: C4b2a
# Proteins of the classical pathway

<table>
<thead>
<tr>
<th>Native component</th>
<th>Active form</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 (C1q, C1r, C1s)</td>
<td>C1q</td>
<td>Binds directly to pathogen surface or indirectly to antibody bound to pathogens, thus allowing autoactivation of C1r</td>
</tr>
<tr>
<td></td>
<td>C1r</td>
<td>Cleaves C1s to active serine protease</td>
</tr>
<tr>
<td></td>
<td>C1s</td>
<td>Cleaves C2 and C4</td>
</tr>
<tr>
<td>C4</td>
<td>C4a</td>
<td>Peptide mediator on inflammation (weak activity)</td>
</tr>
<tr>
<td></td>
<td>C4b</td>
<td>Covalently binds to pathogen and opsonizes it. Binds C2 for cleavage by C1s</td>
</tr>
<tr>
<td>C2</td>
<td>C2a</td>
<td>Active enzyme of classical pathway C3/C5 convertase: cleaves C3 and C5</td>
</tr>
<tr>
<td></td>
<td>C2b</td>
<td>Precursor of vasoactive C2 kinin</td>
</tr>
<tr>
<td>C3</td>
<td>C3a</td>
<td>Peptiede mediator of inflammation (intermediate activity)</td>
</tr>
<tr>
<td></td>
<td>C3b</td>
<td>Many molecules of C3b bind covalent to pathogen surface and act as opsonins. Initiates amplification via the alternative pathway. Bind C5 for cleavage by C2a</td>
</tr>
</tbody>
</table>

The lectin pathway

- homolog to the classical pathway except for the activation
- both pathways use similar proteins:
  - $C1q \approx \text{MB-lectin}$, $C1r \approx \text{*MASP-1}$, $C1s \approx \text{*MASP-2}$
- **Activation**: Binding of a pathogen-recognition molecule (MB-lectin, ficolins) to respective carbohydrates on the pathogen surface (mannose, $N$-Acetylglucosamin)

*MASP: MB-lectin-associated serine protease

_C3 convertase_: C4b2a

The lectin pathway

Pattern-recognition receptors:

1. Mannose-binding lectin (MBL)
   - oligomer, synthesized in liver, presents in blood
   - 2-6 MBL trimeric monomers bind the serine proteases MASP-1 and MASP-2
   - binds mannose-binding carbohydrates on pathogen surface
   - on vertebrate cells there is no complement activation by MBL (mannose is covered by other carbohydrates e.g. sialic acid)

2. Ficolins
   - types: L, M, H
   - bind N-Acetylglucosamin-binding carbohydrates on pathogen surface
Regulation of the classical and lectin pathway

Complement activation is confined to the surface on which it is initiated.

How to protect endogenous cells against the complement?

Remember:

- C3b/C4b bind covalent to the pathogen surface
  - Interaction between the thioester of the opsonin and a hydroxyl/amino group on the pathogen surface

- Missing covalent binding results in a fast irreversible hydrolysis of C3b/C4b
  - Prevents endogenous cells to become attached from the opsonins C3b/C4b
The alternative pathway

Two different ways of activation, two different C3 convertases:

1. by the action of the classical and lectin pathway
   - amplification loop to increase C3b production

C3 convertase: C3bBb (contains C3b itself>can generate more of itself!)

The alternative pathway

Two different ways of activation:

2. spontaneous hydrolysis of the C3 thioester


**fluid-phase C3 convertase:** C3(H₂O)Bb
# The alternative pathway

<table>
<thead>
<tr>
<th>Native component</th>
<th>Active fragments</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3</td>
<td>C3b</td>
<td>Binds to pathogen surface, binds B for cleavage by D, C3b, Bb is C3 convertase and C3b₂, Bb is C5 convertase</td>
</tr>
<tr>
<td>Factor B (B)</td>
<td>Ba</td>
<td>Small fragment of B, unknown function</td>
</tr>
<tr>
<td>Factor D (D)</td>
<td>Bb</td>
<td>Bb is active enzyme of the C3 convertase C3b, Bb and C5 convertase C3b₂, Bb</td>
</tr>
<tr>
<td>Factor P (properdin)</td>
<td>P</td>
<td>Plasma protein with affinity for the C3b,Bb convertase on bacterial cells</td>
</tr>
</tbody>
</table>
Regulation of the alternative pathway

How to differentiate between endogenous cells and pathogens?

1. endogenous cells:
   - express complement-regulatory proteins (*CR1, *MCP, *DAF)
   - C3b becomes inactivated by factor I
   - no complement activation

2. pathogens:
   - no expression of complement-regulatory proteins
   - factor P (properdin) binds and stabilizes the C3 convertase (C3bBb)
   - accumulation of C3b on the pathogen surface

* CR1: Complement Receptor1; MCP: Membrane Cofactor Protein; DAF: Decay Accelerating Factor
summary

Complement system:

- part of the humoral innate immune system
- early warning system to detect and kill extracellular pathogens
- protein cascade enables the transformation of zymogens into serine proteases
- activation can be directly, indirectly or spontaneous

C3 convertases:

- trigger the production of effector molecules to recognize and kill pathogens
- types: C4bC2b classical, lectin pathway
  - C3bBb, C3b(H₂O)Bb alternative pathway
summary

Pathways:

- the classical and lectin pathway are homolog (except for activation)
- the classical pathway connects the innate and the adaptive immune system
- the lectin pathway is independent of antibodies and thereby of the adaptive immune system too
- the alternative pathway acts as an amplification loop to increase C3b
- the alternative pathway does not require specific recognition of antigens
Thank you for your attention!

Don`t worry, the complement system won`t leave us now!

The next steps:

➢ membrane attacking complex
➢ complement receptors
➢ regulation of complement factors