Komplementsystemet
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ny behandling

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Vårkonferanse i medisinsk immunologi
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Bordet (1895): Complement lysis

1. Bacteria + Antiserum $\rightarrow$ Lysis
2. Bacteria + Antiserum $(56^\circ C, 30\,') \rightarrow$ No lysis
3. Experiment 2 + Normal serum $\rightarrow$ Lysis
4. Bacteria + Normal serum $\rightarrow$ No lysis

Conclusion:
Heat stable (Ab) and heat labile (C) factor

“Many immunologists hold that complement is baffling or irrelevant or, most conveniently, both but a recent meeting emphasized that complement is interesting and that it may be important, even only as an elegant model system.

Complement in 2015
30 years later

Complement therapeutics is in the clinic!

No way back!
Interaction between cascades

Complement

Coagulation

Fibrinolysis

Kallikrein-Kinin

C1-INHIBITOR
Cascade Principles

“The point of no return”

“Undetonated bombs”

Local vs systemic

Biological effects

Inhibition

Amplification

P = Proenzyme
E = Enzyme
THE COMPLEMENT SYSTEM

Classical Pathway
- C1-INH
- C1qrs
- CRP
- SAP

Lectin Pathway
- Ficolins
- MBL
- Collectins
- MASP

Alternative Pathway
- Host surface
- Foreign surface

Anaphylatoxins
- C5a
- C3a

Regulation
- MCP
- DAF
- CR1
- CR1
- CR1
- CR3
- CR4

Phagocytosis
- CD59

Inflammation
- TCC
- MAC
- Lysis

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Inflammatory effects mediated by C5a

**Chemotaxis**
- Lysosomal enzyme release
- Neutrophil aggregation

**Histamin release**
- Smooth muscle contraction
- Increased permeability

**Cell adhesion**
- CR3 (CD11b/18)

**Cytokines**
- IL-1, IL-6, IL-8, TNF

**CR1 and FcR expression**

**B- and T-cell responses**

**Reactive oxygen metabolites**

**Arachidonic acid metabolites (LT, PG)**

**Platelet activating factor (PAF)**
What is inflammation?

Heat  Redness  Swelling  Pain  Reduced function
Reasons to analyse complement

• **Complement deficiencies**
  - are associated with certain diseases

• **Complement activation**
  - clinical: reflects ongoing disease processes
  - experimental: animal and *in vitro* models

• **Monitoring complement inhibition**
  - is already in the clinic (eculizumab, C5 inhibition)
What is serum and what is plasma?

A traditional question I get is:

“We have a unique serum biobank - can you do complement analyses of this material?

My answer is:
- Do you have plasma and in case what kind of samples do you have? - how where they obtained, prepared and stored etc.

The traditional answer I get back is: “I am not quite sure, but I think is serum obtained from the Central Laboratory after they finished their routine analyses”.
What is serum and what is plasma?

**SERUM:**

- **Coagulated**
  - Fibrinogen converted to fibrin and coagulation activation completed

- **Fresh frozen:** after 1 hr preparation safely stored at -70 degrees.

- **Aged:** serum from the routine lab - nobody knows how it has been stored (1 day, 1 week; on bench, in fridge).

- **Heat inactivated:** 56 degrees, 30 min. Not complement specific.
What is serum and what is plasma?

**PLASMA:**

- **Heparin:** Affects different coagulation factors. Influence the complement system (low doses activates, high doses inhibits).
- **Citrate:** Calcium blocker, needed for coagulation analyses. Inhibits complement, but not sufficient to block it *in vitro.*
- **EDTA:** Ca/Mg blocker and one the best complement inhibitors and should be used for sampling to detect complement activation *in vivo.* Cannot be use to study complement experimentally *in vitro.*
- **Lepirudin:** blocks thrombin specifically. Keeps all other inflammatory arms open - including complement (“serum-like” or even better).
Complement tests

- **Protein quantification (Serum)**
  - C3, C4, C1-inhibitor (and others)

- **Complement function (Fresh serum)**
  - Screening for complement deficiency
    - Total complement system Screen (Wieslab®) ("CH50")
    - Monitoring clinical inhibition

- **Activation products (EDTA-plasma)**
  - E.g. sC5b-9 (soluble TCC)
  - Detects activation of the whole cascade
Manifestations of Complement Deficiency

- Hereditary angioedema
  - C1-inhibitor

- Infections
  - Neisseria: Alternative and terminal components
  - Recurrent infections early in life: MBL, others

- Autoimmune diseases (SLE)
  - C1q, other classical and terminal components

- PNH
  - GPI-anchor defect (DAF, CD59)

- Kidney: aHUS, MPGN II, Tx-rejection
  - Factor H, I, MCP (or gain of function: C3, fB)

- Eye: Adult macula degeneration
Hereditary Angioedema

- **Etiology:** C1-inhibitor deficiency
  - Genetic low protein conc. (Type 1) or dysfunction (Type 2)
  - Acquired (malignancy, autoimmunity)

- **Pathophysiology:**
  - C4, C2 and kallikrein activation
  - Increased vascular permeability and EDEMA (bradykinin)

- **Clinical features:** Attacks of local edema in any organ
  - Duration: 2-5 days. No effect of anti-allergic treatment
  - Edema of larynx may be lethal

- **Diagnosis:** low C1-inhibitor antigen or function (and C4)

- **Treatment:** Danazol, C1-inhibitor, Bradykinin receptor antagonist
Total Complement System Activity

Serum added to microtiter wells (Wieslab®)

Classical pathway

- IgM
  - C1qrs
  - C2
  - C3
  - C4
  - C5

Lectin pathway

- Mannan
  - MBL
  - MASP-2
  - C2
  - C4

Alternative pathway

- LPS
  - FB
  - FD
  - P
Antigenic changes during complement activation

Native component

Activation products

Neoepitopes:
  - Detection
  - Manipulation

Native-restricted epitope

Activation independent epitope
Enzyme immunoassay (EIA) for quantification of TCC (neoepitope)
Complement activation products

Classical pathway
C1rs-C1-INH
C4a, C4bc, C4d
C3
C3a, C3bc, C3dg

Lectin pathway

Terminal pathway
C5a
TCC/sC5b-9

Alternative pathway
Ba, Bb
C3bPBb
TCC as complement activation marker

- **TCC = sC5b-9**
  - Reflects activation through all pathways and release of C5a

- **In vitro stability**
  - Relatively resistant to spontaneous activation
  - Relatively resistant to freezing and thawing

- **In vivo**
  - Relatively long half-life (60 min) compared with C5a (1 min)
  - Low physiological concentration
Complement activation and prognosis in patients with meningococcal disease

Activation products measured in samples obtained on admission to hospital

Terminal Complement Complex (TCC)

< 12.7 AU/mL: 1 of 32 died
≥ 12.7 AU/mL: 6 of 7 died

p < 0.0001

Brandtzaeg et al. J Infec Dis 1989
Spontaneous \textit{in vitro} complement activation

Stability of TCC in EDTA-plasma at room temperature

![Graph showing the increase in C3dg and TCC over days. The y-axis represents the increase (fold) and the x-axis represents days.]
Spontaneous in vitro activation in serum

Consequences for different assays

C3 antigen conc.

C3 activation (C3bc)

C3 hemolytic activity

%
Factors influencing the amount of native components (e.g. C3 and C4)

- **Synthesis**
  - Reduced amounts in liver failure
- **Acute phase reaction**
  - Most components increase
- **In vivo activation**
  - Consumption leads to decreased amounts
- **Hemodilution**

The sum of these factors determines the level
### Native components and activation products in two patients

<table>
<thead>
<tr>
<th></th>
<th>Patient 1 (F24)</th>
<th>Patient 2 (F35)</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C3</strong></td>
<td>0.20</td>
<td>0.30</td>
<td>0.50 - 1.00 g/L</td>
</tr>
<tr>
<td><strong>C4</strong></td>
<td>0.09</td>
<td>0.06</td>
<td>0.10 - 0.50 g/L</td>
</tr>
<tr>
<td><strong>C3dg</strong></td>
<td>25</td>
<td>126</td>
<td>20 - 45 AU/mL</td>
</tr>
<tr>
<td><strong>TCC</strong></td>
<td>3.9</td>
<td>15</td>
<td>2.2 - 6.6 AU/mL</td>
</tr>
</tbody>
</table>

**Diagnosis:**
- Liver failure
- Chronic active hepatitis
Complement activation products: Treatment of samples

Due to rapid in vitro activation it is crucial that the samples are obtained and stored properly

- EDTA tubes (10-20 mM final conc.)
  - turn tube gently x 3 to ensure good mixing
- Store on crushed ice or at +4°C
  - immediately (< 10 min)
- Separate plasma cool and store at -70°C
  - within a few hours (4-6)
Effect of eculizumab

Eculizumab

Inflammation

C1r, C1s

MASPs

C4
C2

C3

C4b2a (C3 convertases)

C3bBbP

Amplification

C3(H$_2$O) B P

Terminal pathway

C5a

C5

C5b-9(m)

TCC

sC5b-9

Membrane damage

Eculizumab
Indications for use of eculizumab (Soliris®)

- **Paroxysmal nocturnal hemoglobinuria (PNH)**
  - Clonal defect in bone marrow cells (PIG-A)
  - Red cells lack CD55 and CD59
  - Spontaneous complement-mediated lysis

- **aHUS**
  - Atypical hemolytic uremic syndrome

- **Others: MPGN II?**
aHUS

- Thrombotic microangiopathy
  - No pathogens or known external “danger signals”
  - Caused by internal complement dysfunction
  - Severe disease affecting children - kidney failure

- Imbalance in complement activation
  - Mutations in complement proteins and regulators
    - Reduced efficacy of complement regulators
      - Factor H, I, MCP, DAF
    - “Gain on function” of ordinary components
      - C3, factor B
Complement deposition in kidney tissue

MPGN II (DDD) in factor H dysfunction
  “C3G”
TCC (C5b-9) in glomeruli

Acute Ab-mediated rejection
  C4d in peritubular capillaries
**COMPLEMENT DISEASES**

**Acute**
- Adult respiratory distress syndrome
- Ischemia-reperfusion injury:
  - Myocardial infarct
  - Skeletal muscle
  - Lung inflammation
- Hyperacute rejection (transplantation)
- Sepsis
- Cardiopulmonary bypass
- Burns, wound healing
- Asthma
- Restenosis
- Multiple organ dysfunction syndrome
- Trauma, hemorrhagic shock
- Guillain-Barré syndrome

**Chronic**
- Paroxysmal nocturnal hemoglobinuria
- Glomerulonephritis
- Systemic lupus erythematosus
- Rheumatoid arthritis
- Infertility
- Alzheimer’s disease
- Organ rejection (transplantation)
- Myasthenia gravis
- Multiple sclerosis

**Biomaterials incompatibility**
- Platelet storage
- Hemodialysis
- Cardiopulmonary bypass equipment

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**PNH treated with eculizumab**

Normal erythrocyte protected against complement

Hemolysis of a PNH erytrocyte

PNH erytovyte protected by eculizumab

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*Complement in the future: Therapeutic aspects*