Komplement och immunbrist - en uppdatering

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Complement is part of the immune system

Immunity

Innate immunity
- cellular
- Granulocytes
- NK cells
- Macrophages

Acquired immunity
- humoral
- Complement system
- Antibodies
- Cytokine
- Cellular
- Lymphocytes
- Dendritic Cells

The Complement System

- ~ 35 plasma proteins and membrane proteins
- Several complement proteins present in proenzyme form
- Three main activation pathways
Spitzer et al, J Immunol, 2007

Complement activation

MBL and ficolins

Teizo Fujita, Nature Reviews Immunology 2, 346-353 (May 2002)
Lectin Pathway activation

Fujita et al, Immunol Rev, 2004

Molecules forming complexes with MASPs:
- MBL
- Ficolin-1
- Ficolin-2
- Ficolin-3
- Collectin-11
**The complement system: activation and regulation**

**Classical pathway**

**Mathematical pathway**

**Complement protein synthesis**

- Monocytes are able to produce most (all) of the complement proteins.
- Hepatocytes in the liver produce the majority of the complement protein molecules found in serum (e.g. C4, C3, factor B and C1-INH).
- Epithelial cells, fibroblasts are able to produce several complement proteins e.g. C3.
- C1q is produced by bone-marrow derived cells
- Factor D (adipsin) is produced by fat cells

**Biological effects of complement**

- Scavenger effect: Clearance of apoptotic cell material by binding to C1q
- Lymphocyte activation: Binding of antigen-bound C3d to CR2 on B cells enhances antibody production
- Virus neutralisation: Coating of virus with e.g. C3b prevents cell contact
Biological effects of complement

- **Opsonisation**
  Coating of microbes with mainly C3b and/or iC3b increases phagocytosis

- **Elimination of immune complexes**
  Coating of IC with C3b and/or C4b, and binding to CR1 on erythrocytes and transport to the liver and the spleen

- **Inflammatory response**
  Anaphylatoxins (C5a, C3a), Chemotaxins (C5a, C3a).

- **Cytolysis**
  Activation and formation of the Membrane attack complex (MAC)

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Biological effects of complement

- **Protective**
  Scavenger effect, opsonisation, inflammatory response, lysis …

- **Harmful**
  Scavenger effect, opsonisation, inflammatory response, lysis …

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Complement deficiencies

- **Inherited complement deficiencies**
  - Complete or subtotal deficiency or dysfunction
  - Autosomal recessive or X-linked basis
  
  *One exception: C1 inhibitor deficiency or dysfunction, which causes hereditary angioedema (HAE) in the heterozygous state.*

- **Acquired complement deficiencies**
  - Autoantibodies to complement proteins
Complement deficiencies

Infections in complement deficiency: principal reasons

- Impaired antibody responses
- Impaired serum bactericidal activity
- Impaired opsonization

Classical pathway deficiencies and association with SLE

- Decreased capacity for immune complex handling
- The waste-disposal hypothesis (C1q)
- Aberrant tolerance induction
- Influence on cytokine regulation (C1q)
### Complement deficiencies in Sweden 2014

<table>
<thead>
<tr>
<th>Deficiency (n)</th>
<th>Identified (n)</th>
<th>Estimated (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1q</td>
<td>8</td>
<td>&lt;5</td>
</tr>
<tr>
<td>C4</td>
<td>1</td>
<td>&lt;5</td>
</tr>
<tr>
<td>C2</td>
<td>63</td>
<td>450</td>
</tr>
<tr>
<td>C3</td>
<td>1</td>
<td>&lt;5</td>
</tr>
<tr>
<td>C6</td>
<td>2</td>
<td>?</td>
</tr>
<tr>
<td>C7</td>
<td>18</td>
<td>?</td>
</tr>
<tr>
<td>C8</td>
<td>3</td>
<td>?</td>
</tr>
<tr>
<td>C9</td>
<td>1</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Properdin</td>
<td>9</td>
<td>?</td>
</tr>
<tr>
<td>MBL</td>
<td>&gt;200</td>
<td>&gt;500,000</td>
</tr>
<tr>
<td>MASP-2</td>
<td>1</td>
<td>?</td>
</tr>
<tr>
<td>C1 inhibitor</td>
<td>~130</td>
<td>180</td>
</tr>
<tr>
<td>Factor I</td>
<td>2</td>
<td>?</td>
</tr>
<tr>
<td>Factor H</td>
<td>1</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

Population 8 milj.

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### Complement deficiencies

**Table 3** Summary of complement deficiencies in humans

<table>
<thead>
<tr>
<th>Component</th>
<th>Frequency</th>
<th>Role in disease association</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1q</td>
<td>50–100</td>
<td>SLE, glomerulonephritis, infections</td>
<td>Shafii et al.1</td>
</tr>
<tr>
<td>C2</td>
<td>20–50</td>
<td>SLE, glomerulonephritis</td>
<td>Shafii et al.1</td>
</tr>
<tr>
<td>C3</td>
<td>20–50</td>
<td>SLE, glomerulonephritis, infections</td>
<td>Shafii et al.1</td>
</tr>
<tr>
<td>C4</td>
<td>25–50</td>
<td>SLE, glomerulonephritis</td>
<td>Shafii et al.1</td>
</tr>
<tr>
<td>Factor B</td>
<td>&lt;20</td>
<td>Neutrophil dysfunction</td>
<td>Sluiter et al.2</td>
</tr>
<tr>
<td>Factor H</td>
<td>5–10</td>
<td>Usually healthy</td>
<td>Shafii et al.1</td>
</tr>
<tr>
<td>C1 inhibitor</td>
<td>~130</td>
<td>Usually healthy</td>
<td>Shafii et al.1</td>
</tr>
<tr>
<td>MBL</td>
<td>~130</td>
<td>Neutrophil dysfunction</td>
<td>Weiss et al.3</td>
</tr>
<tr>
<td>MASP-2</td>
<td>~130</td>
<td>Neutrophil dysfunction</td>
<td>Weiss et al.3</td>
</tr>
<tr>
<td>Properdin</td>
<td>20–100</td>
<td>SLE, glomerulonephritis</td>
<td>Sturfelt et al.4</td>
</tr>
</tbody>
</table>

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### The MBL gene

- **Chrom. no. 10**
- **Promotor region variants:** L/H
- **Structural gene variants:** A/B/C/D

![Diagram of the MBL gene](image-url)
MBL deficiency – a disease modifier

MBL def.: Serum conc. <100 ng/mL.
Increased risk for infections reported in several disease states:

- Hypogammaglobulinemia
- Severe immunosuppression
- Cystic fibrosis
- SLE
- Possible association with atherosclerosis
- May modify inflammatory disease such as rheumatoid arthritis

Complement deficiencies

Table 3 (Summary of complement deficiencies in humans)

<table>
<thead>
<tr>
<th>Complement component involved</th>
<th>Frequency</th>
<th>Main disease association(s)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1q, C2, C4a, C4b, C5a, C5b-9</td>
<td>5–10% reported cases</td>
<td>SLE, glomerulonephritis, infections</td>
<td>Skattor et al.</td>
</tr>
<tr>
<td>C2, C4</td>
<td>5–15% reported cases</td>
<td>SLE, glomerulonephritis</td>
<td>Wu et al.19</td>
</tr>
<tr>
<td>C3, C5</td>
<td>20–30% reported cases</td>
<td>SLE, glomerulonephritis, recurrent infections</td>
<td>Skattor et al.</td>
</tr>
<tr>
<td>C9</td>
<td>20–30% reported cases</td>
<td>SLE, glomerulonephritis, infections</td>
<td>Skattor et al.</td>
</tr>
<tr>
<td>Factor B</td>
<td>5 reported cases</td>
<td>Hemorrhagic disease</td>
<td>Fajgenbaum et al.</td>
</tr>
<tr>
<td>Factor C1</td>
<td>5–10% reported cases</td>
<td>Hemorrhagic disease</td>
<td>Fajgenbaum et al.</td>
</tr>
<tr>
<td>Factor H</td>
<td>5–10% reported cases</td>
<td>Hemorrhagic disease</td>
<td>Fajgenbaum et al.</td>
</tr>
<tr>
<td>Factor I</td>
<td>5–10% reported cases</td>
<td>Hemorrhagic disease</td>
<td>Fajgenbaum et al.</td>
</tr>
<tr>
<td>Factor D</td>
<td>5–10% reported cases</td>
<td>Hemorrhagic disease</td>
<td>Fajgenbaum et al.</td>
</tr>
</tbody>
</table>

Adapted from: Sturfelt & Truedsson, Nature Rheumatol., 2012
Nearly all cases of C2D (90%) are caused by a 28-bp deletion in the C2 gene, a mutation associated with the HLA-B*18, S042, DRB1*15 haplotype – C2 deficiency type I

Principal manifestations of C2D in a Swedish study

- Invasive infections 57%
- Systemic lupus erythematosus 25%
- Cardiovascular disease 15%

C2 deficiency type I

- The predominant form of C2 deficiency (>95%)
- No C2 synthesis due to a 28 bp deletion in the C2 gene located in the MHC haplotype: HLA-A*25, HLA-B*18, S042, DRB1*15
- Prevalence of heterozygous carriers about 1.4% in Western countries
- Prevalence of homozygous deficiency about 1/20,000 in Western countries
C2 deficiency type II

- C2 secretion defect
- Small amounts of circulating C2 can be found
- Rare variants have been described
- Various MHC haplotypes

Phagocytosis response in C2 deficiency

Increased phagocytosis of pneumococci by neutrophils in post-vaccination serum

Complement activation in C2 deficiency

Blocked pathways:
- Classical pathway
- MBL and ficolin pathways (C4b2a)

Available pathways:
- Alternative pathway
- C1q-dependent C2 bypass
- MBL-dependent C2 bypass

C2 bypass activation

Lysis of sensitized sheep erythrocytes in human sera deficient in the second component of complement.

Knutzen Steuer I., Sloan J.B., Ogleby T.J., Farries T.C., Nickolls M.W., Dennis P., Harley J.B., Atkinson J.P.


Mannan-binding lectin activates C3 and the alternative complement pathway without involvement of C2

Bartlem-Battey N., Pålsson-Mohr J., Anders Hedqvist P., Ewa Hedin, Inge E. Malmborg, Magnus Mårtensson, Stellan Thunberg, and Anders G. Sjöblom

Complement deficiencies

Adapted from: Sturfelt & Truedsson, Nature Rheumatol., 2012

Properdin deficiency

Three deficiency types
- type I: complete deficiency
- type II: low serum concentration (~10%)
- type III: dysfunctional properdin molecule
  - X-linked inheritance
  - >80 cases from 24 families are reported
  - Increased risk for Neisserial infection

Properdin deficiency – heterogenous genetic background

- Type I – 10 different mutations found (exons 4, 5, 6, 7 and 8)
- Type II – 2 different mutations found (exons 4 and 8)
- Type III – 1 mutation in a Dutch family (exon 9)
Alternative pathway deficiencies

- Properdin, factor D, factor B
- Increased risk for Neisseria infection but not for bacterial infections in general
- Explanation - properdin as activation initiator?

Complement deficiencies

<table>
<thead>
<tr>
<th>Complement component</th>
<th>Frequency</th>
<th>Role</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1q</td>
<td>50-100</td>
<td>SLG, glomerulonephritis</td>
<td>Stoll et al.</td>
</tr>
<tr>
<td>C2 at C1s</td>
<td>20-40</td>
<td>SLG, glomerulonephritis</td>
<td>We et al.</td>
</tr>
<tr>
<td>C3</td>
<td>2-70%</td>
<td>SLG, glomerulonephritis</td>
<td>Stoll et al.</td>
</tr>
<tr>
<td>C4</td>
<td>25-50%</td>
<td>SLG, glomerulonephritis</td>
<td>Stoll et al.</td>
</tr>
<tr>
<td>Factor B</td>
<td>1-20%</td>
<td>Neisseria infections</td>
<td>Sado et al.</td>
</tr>
<tr>
<td>Properdin</td>
<td>0-10%</td>
<td>Neisseria infections</td>
<td>Pip et al.</td>
</tr>
<tr>
<td>C2a, C2b, C2c</td>
<td>2-10%</td>
<td>Usually healthy, occasional infections</td>
<td>Stoll et al.</td>
</tr>
<tr>
<td>Factor B</td>
<td>1-20%</td>
<td>Usually healthy</td>
<td>Stoll et al.</td>
</tr>
<tr>
<td>Properdin</td>
<td>1-50%</td>
<td>Usually healthy</td>
<td>Wang et al.</td>
</tr>
</tbody>
</table>

Assembly of C1q

- C1q 460 kD
- C1q chains A, B and C
- A/B heterodimers and C/C homodimers
- Heterotrimeric unit
- C1q with 18 chains
C1q deficiency in Sweden

8 known cases
5 children with C1q deficiency
Infection proneness and SLE-like disease
Varying clinical symptoms
Treatment by hematopoietic stem cell transplantation has been used (2 cases)

A 9-year-old boy and a 12-year-old girl with refractory SLE.
C1q production was restored and decreased the severity of SLE symptoms decreased post-transplant.
The boy developed post-transplant lymphoproliferative disease and died from multiple organ failure four months post-transplant.
The girl is alive and well 24 months post-transplant, and all clinical symptoms of SLE have resolved.

Conclusions: Allo-HSCT can cure SLE in human C1q deficiency and should be considered early in patients with severe disease.

Olsson RF et al.: Allogeneic Haematopoietic Stem Cell Transplantation in the Treatment of Human C1q Deficiency: the Karolinska Experience. Transplantation, in press.
Successful cure of C1q deficiency in human subjects treated with hematopoietic stem cell transplantation

By the Editor:

Hematopoietic stem cell transplantation (HSCT) is useful in curing a number of primary immunodeficiency diseases that have never been treated for patients with complement deficiencies. Additional treatment modalities are provided in this issue. Further details on the success rate of HSCT in children with C1q deficiency and the impact on outcomes are provided.

J Allergy Clin Immunol 2014
Jan;133(1):265-7

Marked variability in clinical presentation and outcome of patients with C1q immunodeficiency

OBJECTIVES: Globally, approximately 25 cases of C1q deficiency have been described with early presentation of systemic lupus erythematosus (SLE) and hemoptysis. It has been reported that the clinical presentation of C1q deficiency is not attributed to C1q deficiency alone and is due to other activation of the alternate pathway of complement and possibly other factors. The main objective of this study was to evaluate the clinical presentation, long-term outcomes, and impact of C1q deficiency on quality of life in C1q-deficient patients who are at increased risk of developing lymphoma.

METHODS: We performed a cohort study of patients with C1q deficiency and patients with other autoimmune diseases. A high-resolution image analysis was performed on all patients with C1q deficiency, and a detailed analysis of the clinical presentation and course of disease was performed.

RESULTS: Follow-up data of 41 patients from 14 facilities was obtained. At diagnosis, 17 patients (41%) suffered from SLE, 10 (24%) from RA, and 14 (34%) from other autoimmune diseases. The incidence of SLE in the general population is 1/1000, and the incidence of RA is 1/1000, so the proportion of patients with C1q deficiency with SLE and RA is significantly higher than in the general population.

CONCLUSION: We report the largest study to date on C1q deficiency and its association with high-risk malignancies. The treatment of C1q deficiency requires a combination of approaches.

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Treatment of infection susceptibility in complement deficiency

- Increased awareness (antibiotics etc).
- Substitution therapy:
  - Plasma infusion (C1q, C2 and C3)
  - Purified plasma MBL has been tried as treatment (cystic fibrosis).
  - Recombinant MBL has been developed (tested in volunteers).
  - Recombinant C2 has been developed, not tested in humans.
- Vaccination.
How to find the complement deficient patients

- Clinical symptoms indicating immunodeficiency
- Complement analysis with screening for deficiency

Analysis of complement function to detect deficiency

- CH50, AP50
- Hemolysis in gel (HIG)
- ELISA

Analysis of complement function

[Diagram showing complement activation ELISA with Classical, Lectin, and Alternative pathways]
Analysis of complement function

Table 1
Complement deficiency and detection by complement activation ELISA

<table>
<thead>
<tr>
<th>Component</th>
<th>Classical</th>
<th>Lectin</th>
<th>Alternative</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1, C4</td>
<td>Low</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>C2, C5</td>
<td>Low</td>
<td>Low</td>
<td>Normal</td>
</tr>
<tr>
<td>MBL, MASP2</td>
<td>Normal</td>
<td>Low</td>
<td>Normal</td>
</tr>
<tr>
<td>B, D, P</td>
<td>Normal</td>
<td>Normal</td>
<td>Low</td>
</tr>
<tr>
<td>C3, C5, C6, C7, C8, C9</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
</tbody>
</table>

Andra metoder –
arrays?

Miniaturization of multiplexed planar recombinant antibody arrays for serum protein profiling.
Petersson L, Coen M, Amro NA, Truedsson L, Borrebaek CA, Wingren C.

SLIFE 2015
A) Parallel detection of complement factors is enabled by using antibody bead arrays and direct labelling of serum or plasma proteins.

B) A preanalytical heating of diluted and labelled serum was found to influence complement measurements.

C) Sera from patients with complement deficiencies were analysed and used to evaluate the assay.

D) Eluates from dried blood spots was labelled and profiled in the assay which enables large-scale neonatal screening for complement deficiencies.

Works with blood spots from Guthrie cards.

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D) Eluates from dried blood spots was labelled and profiled in the assay which enables large-scale neonatal screening for complement deficiencies.

Works with blood spots from Guthrie cards.
Complement deficiency – take home message

- The consequence varies from almost none as in C9D to serious disease/infections as in e.g. C1q D.
- Classical pathway def. but no other complement def. are associated with SLE and SLE-like disorders.
- Classical pathway def. confer increased susceptibility for infections with encapsulated bacteria.
- Alternative and terminal pathway def. confer increased susceptibility for infections with Neisseria species.
- Lectin pathway def. is associated with many types of infections in presence of a concomitant immune deficiency.
- The identification of complement def. states is important to ensure optimal prevention and treatment.
- Vaccination against the bacteria commonly causing the infections is important.
- Supplementation of the missing component has so far been tried in very few cases, but should hopefully have a place in the treatment of these patients in the future.
- HSCT may be an option in treatment of complete C1q deficiency.
Factor H deficiency

Results in decreased C3 concentration
Associated with atypical hemolytic uremic syndrome (aHUS)
Recurrent pyogenic infections (*N. meningitidis, H. influenzae*)

Typ I phenotype
  - Factor H mutation causing a structural defect - low factor H concentration.

Typ II phenotype
  - Factor H mutation giving rise to a functional defect.

Factor I deficiency

Results in decreased C3 concentration
Recurrent infections mainly of the respiratory tract
Various immune-complex related diseases
  (glomerulonephritis, vasculitis, SLE)
Clearance of apoptotic cells and SLE

Complement activation -> C3

Infections in C2 deficiency: prophylaxis

- Long-term antibiotic treatment
- Vaccination against infection with encapsulated bacteria (S. pneumoniae, N meningitidis and H. influenzae type b).
### Complement genes

Genes for complement proteins in different chromosomes, examples

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Complement protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p</td>
<td>C1q (β chains C1qα, C1qβ, C1qγ)</td>
</tr>
<tr>
<td>1q (RCA gene cluster)</td>
<td>C8 (ααβ chain)</td>
</tr>
<tr>
<td>6p (MHC class III)</td>
<td>B, C2, C4 (exists as two isotypes, C4A and C4B)</td>
</tr>
<tr>
<td>9q</td>
<td>C8 (gamma chain)</td>
</tr>
<tr>
<td>X</td>
<td>Properdin</td>
</tr>
</tbody>
</table>

- Several of the complement proteins exist in different genetic variants.
- The complement proteins encoded by genes located in the MHC class III region display a high degree of polymorphism.