Complement mediated glomerulopathies

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The complement system

- Essential component of the innate immunity
- Serum and cell-surface proteins
- React with one another in a cascade

Classical pathway

Immune complexes → C1 → C4bC2a → C3 convertase → C3 + C5
lectin pathway

mannans

\[ \text{mannans} \rightarrow \text{MASP} \rightarrow \text{C4bC2a} \rightarrow \text{C3 C5} \]
alternative pathway
gram+, gram-, LPS, bacterial toxin

C3 convertase  C3bBb  C3b

Amplification loop

C3  C5
alternative pathway

C3bBb

spontaneous

C3b

Amplification loop

C3
**Complement System**

- **CP (Classic Pathway)**: C1, MASP, C4C2, C3b, iC3b, C3d
- **lectin Pathway**: C1, MASP, C4C2, C3bBb
- **AP (Alternative Pathway)**: C3b, C3bBb, C5b-C9, C3a/C5a

**Key Reactions**:
- **C3 Activation**: C3b, iC3b, C3d
- **C5 Activation**: C5b-C9 (Membrane attack complex)
- **Inflammation**: C3a/C5a
- **Cell Lysis**: C5b-C9
- **Complement Activation and Cell Interaction**

**Diagram** shows the flow of complement activation, cell lysis, and inflammation processes.
Complement activation pathways involved in different types of glomerulonephritis

**Disease**
- MPGN
- Cryoglobulinemia
- Lupus Nephritis
- IgA Nephropathy
- Post-infectious glomerulonephritis
- ANCA associated vasculitis
- Atypical HUS
- C3 Glomerulopathy

**Mechanism**
- Glomerular Immune Complexes
- Ligands for MBL and ficolins displayed on injured glomerular cells (?)

**Pathway**
- Classical Pathway
- Mannose Binding Lectin Pathway
- Alternative Pathway

Thurman J, Nephrol Dial Transplant 2017
C3 glomerulopathy

- C3 glomerulopathy designate a disease process
- Accumulation of the C3 component within or along the GBM
- Acquired and hereditary anomalies of regulation of alternative pathway

<table>
<thead>
<tr>
<th>IF deposits</th>
<th>C3 glomerulopathy</th>
<th>Post infectious GN</th>
<th>IC-MPGN</th>
</tr>
</thead>
<tbody>
<tr>
<td>IF deposits</td>
<td>C3</td>
<td>C3</td>
<td>C3+C4+Ig</td>
</tr>
<tr>
<td>chronic</td>
<td>chronic</td>
<td>acute</td>
<td>chronic</td>
</tr>
<tr>
<td>DDD</td>
<td>C3GN</td>
<td>PIGN</td>
<td>IC-MPGN</td>
</tr>
</tbody>
</table>

Pickering et al, Kidney Int, 2013
C3 deposits

• The C3 fragments can be detected by immunohistochemistry/immunofluorescence

• The C3 fragments are detected in routine by an antibody directed against C3c

• Dominant C3 of X2 orders of magnitude of intensity by IF greater than any other immune reactant (using a scale of 0 to 3)

Pickering et al, Kidney Int, 2013
• Suspected by light microscopy
• Gold standard: electron microscopy
• Deposits within the GBM, mesangium, Bowman’s capsule, TBM
• Form « sausage-like » shapes
• Transform the lamina densa
Histological forms of DDD

1. membranoproliferative DDD
   Thickened capillary loops and endocapillary proliferation

2. mesangial proliferative DDD
   Focal, segmental mesangial hypercellularity

3. crescentic DDD
   Crescents involving 45% of glomeruli

4. acute proliferative and exudative DDD
   Endocapillary proliferation with neutrophilic infiltration

5. unclassified DDD
   Intermediate electron dense transformation by electron microscopy

Walker et al., Modern Pathology, 2007
2/3 cases:
- MPGN pattern
- Mesangial proliferation
- Subendothelial, mesangial, epimembranous deposits
- Double contours aspect
- Accumulation of mesangial matrix (nodular aspect)

1/3 cases:
- Mesangial and epimembranous deposits, without subendothelial deposits
- No proliferation

Post-infectious glomerulonephritis

• Distinction from C3 glomerulopathy depends on the presence of typical features on light microscopy and EM
• Endocapillary hypercellularity
• Involves all the glomeruli: homogenous distribution
• Many neutrophiles
• Subepithelial deposits
• Crescents
Post-infectious glomerulonephritis

- Typical clinical course: complete clinical and biological resolution.
- The presence of any atypical clinical or histological features in a case of apparent PIGN should raise suspicion of C3 glomerulopathy
IC-MPGN

- Cases of C3 GN with a membranoproliferative pattern with dominant C3 staining but with some Ig are frequent
IC-MPGN

• Features of complement dysregulation are frequent
  – investigation for an underlying cause, such as autoimmunity or infection
  – after secondary causes are excluded, consider complement dysregulation

Servais et al, Kidney Int, 2012;
Composition of deposits

Laser microdissection
Mass spectrometry

Sethi et al., Kidney Int, 2009
Mass spectrometry

C3dg is the predominant cleavage product detected.
The C3 spectra for both C3GN and DDD are similar.

Sethi et al., NDT 2017
C3b amplification loop of the complement pathways

Control of C3b in fluid phase

Control of C3b on cell surfaces

Figure Ph Lesavre
Clinical presentation

- Age at diagnosis: young patient
  - DDD more frequent in children
  - C3GN later onset, mean age 30 yrs
- Asymptomatic PU and HU or acute presentation, sometimes nephrotic syndrome, hypertension and acute kidney injury

Servais et al, Kidney Int, 2012
• Low C3 bas and normal C4: alternative pathway activation
  – 60% of DDD
  – 40% of C3GN

– 20% of DDD and C3GN have C3<20%

Servais et al, Kidney Int, 2012
C3NeF

C3NeF auto-antibodies which fix and stabilize the C3 convertase.
C3NeF

- Fluctuation during follow up in 1/3 of patients
- No correlation with clinical evolution

**Table 1** | C3 nephritic factor prevalence in DDD and C3GN

<table>
<thead>
<tr>
<th>Reference</th>
<th>n</th>
<th>DDD (%)</th>
<th>C3GN (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Servais et al. (2007)</td>
<td>17</td>
<td>N/A</td>
<td>41</td>
</tr>
<tr>
<td>Nasr et al. (2009)</td>
<td>9</td>
<td>78</td>
<td>N/A</td>
</tr>
<tr>
<td>Zhang et al. (2012)</td>
<td>32</td>
<td>78</td>
<td>N/A</td>
</tr>
<tr>
<td>Servais et al. (2012)</td>
<td>75</td>
<td>86</td>
<td>45</td>
</tr>
<tr>
<td>Sethi et al. (2012)</td>
<td>10</td>
<td>N/A</td>
<td>50</td>
</tr>
</tbody>
</table>

Abbreviations: C3GN, C3 glomerulonephritis; DDD, dense deposit disease; N/A, not applicable.

Bomback et al, Nat Rev Nephrol, 2012
Anti FB and anti C3b antibodies

10% of des patients, 50% of IC-MPGN
Infectious triggers

Marinozzi et al. JASN, 2017
Anti FH antibodies

- 17 C3GN patients positive for anti-FH IgG
  - In contrast to the aHUS, in C3GN patients
    - no circulating FH containing immune complexes
    - anti-FH IgG : weaker affinity for FH
    - no homozygous deletions of CFHR1 and CFHR3

- Associated with C3Nef in children and with monoclonal gammopathy in adults

Blanc et al, J Immunol, 2015
C5 nephritic factors

- Cohort 120 patients C3GP
- C3 NeF 75% and C5 NeF 49% of patients
- Only C5 NeF correlated with sC5b9 levels
- C5 NeF positive patients more likely to have C3GN than DDD

Marinozzi et al, Kidney Int 2018
Genetic factors

- **25% rares variants** in complement-related genes:
  - *CFH, CFI, MCP, C3, CFB, CFHR5, THBD*
- 11% familial forms
- *CFHR* rearrangements or duplications: creation of *CFHR* fusion genes
- *CFHR5* gene variant that creates a FHR5-FHR5 fusion protein
  - Endemic in Cyprus
  - Microscopic hematuria
  - 40% proteinuria, CKD
- Association with specific haplotypes

Investigations

C3, C4, FH, C3NeF

Serum paraprotein detection

Mutation screening of complement regulatory genes: CFH, CFI, C3, CFB, CFHR5, CFH-CFHR locus

C3NeF, C5NeF, C4NeF, auto-antibodies anti FH, anti FB, anti C3

CFB, C5, soluble C5b9
Treatment

• ACE inhibitors
  – Better renal survival

![Graph showing survival rates]

P<0.0001

• Association ACE inhibitors+IS

Servais et al, KI, 2012
Nasr et al, CJASN, 2009
Mycophenolate mofetil in combination with steroids

Avasare et al, CJASN, 2018
Eculizumab

- Monoclonal antibody anti C5

Wong et al, Mol Immunol, 2013
Eculizumab

• 26 patients (13 children/adolescents)

• After eculizumab treatment (median, 14 months),
  – 6 (23%) global clinical response
    • lower eGFR, more rapidly progressive course, more extracapillary proliferation on kidney biopsy
  – 6 (23%) partial clinical response
  – 14 (54%) no response

Lequintrec et al, AJKD, 2018
## New drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target</th>
<th>Mechanism</th>
<th>Clinical trial number</th>
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<tbody>
<tr>
<td>ACH0144471</td>
<td>Factor D</td>
<td>Prevents formation of C3 and C5 convertases</td>
<td>NCT03369236, NCT03459443 and NCT03124368</td>
</tr>
<tr>
<td>LNP023</td>
<td>Factor B</td>
<td>Prevents formation of C3 and C5 convertases</td>
<td>Not yet registered</td>
</tr>
<tr>
<td>APL2</td>
<td>C3</td>
<td>Prevents formation of C3 and C5 convertases</td>
<td>NCT03453619</td>
</tr>
<tr>
<td>AMY101</td>
<td>C3</td>
<td>Prevents formation of C3 and C5 convertases</td>
<td>NCT03316521</td>
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<tr>
<td>OMS721</td>
<td>MASP2</td>
<td>Blocks initiation of lectin pathway</td>
<td>NCT02682407</td>
</tr>
<tr>
<td>Eculizumab</td>
<td>C5</td>
<td>Blocks progression of terminal pathway</td>
<td>Off-label use</td>
</tr>
<tr>
<td>Avacopan</td>
<td>C5aR1</td>
<td>Blocks anaphylatoxin formation (C3a, C4a and/or C5a)</td>
<td>NCT03301467</td>
</tr>
</tbody>
</table>

Smith et al, Nat Rev Nephrol, 2019
Renal survival

10 years renal survival: 63.5%

N  49  38  18  3
44  33  17  3
26  22  11  2

Servais et al, KI, 2012
Renal survival in adults

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>34</th>
<th>19</th>
<th>8</th>
<th>2</th>
<th>0</th>
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<tbody>
<tr>
<td></td>
<td>23</td>
<td>14</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Servais et al, KI, 2012
Both total activity and total chronicity scores emerged as the strongest predictors of progression.

Bomback et al, Kidney Int, 2018
Monoclonal gammopathy and C3GP

Lambda light chain:
anti Facteur H antibody

fixation on CFH SCR 3 and
inhibition of the interaction of facteur H
with C3b
MIg-C3G

- Anti complement antibodies > 50% patients but different target compare to C3GN
- Purified total IgG of MIg-C3G patients were able to enhance C3 convertase activity in 65%
- In 5 cases, the C3 convertase enhancement was mostly due to the monoclonal immunoglobulin

**TABLE 1 | Comparison of immunological findings in 41 MIg-C3G patients and 107 C3GN adults patients without MIg.**

<table>
<thead>
<tr>
<th>IMMUNOLOGICAL FINDINGS</th>
<th>MIg-C3G N = 41</th>
<th>Adults C3GN N = 107</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3 (mg/L)</td>
<td>703 (78-1220)</td>
<td>781 (67-1760)</td>
<td>0.86</td>
</tr>
<tr>
<td>Low C3 level, n(%)</td>
<td>18 (44%)</td>
<td>56 (40%)</td>
<td>0.71</td>
</tr>
<tr>
<td>C4 (mg/L)</td>
<td>250 (104-575)*</td>
<td>252 (94-751)*</td>
<td>1</td>
</tr>
<tr>
<td>sC5b-9 (ng/mL)</td>
<td>848 (164-2880)</td>
<td>478 (94-2582)</td>
<td>0.005</td>
</tr>
<tr>
<td>Elevated sC5b9 (upper 420ng/mL)</td>
<td>29/37 (78%)</td>
<td>47/76 (62%)</td>
<td>0.09</td>
</tr>
<tr>
<td>Elevated sC5b-9 (upper twice the normal)</td>
<td>15/37 (41%)</td>
<td>13/76 (17%)</td>
<td>0.01</td>
</tr>
<tr>
<td>C3NeF, n(%)</td>
<td>3 (7%)</td>
<td>44/98 (45%)</td>
<td>0.0001</td>
</tr>
<tr>
<td>C5NeF, n(%)</td>
<td>0/12 (0%)</td>
<td>11/21(52%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Anti-FH Abs, n(%)</td>
<td>9 (17%)</td>
<td>10/91 (11%)</td>
<td>0.09</td>
</tr>
<tr>
<td>Anti-Fi Abs, n(%)</td>
<td>2 (5%)</td>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>Anti-CR1 Abs, n(%)</td>
<td>11 (27%)</td>
<td>3/84 (4%)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**GENETIC ANALYSIS**

- Pathogenic variants: 2/28(7%) vs 27/99 (27%)** | **p = 0.02**

Chauvet et al, Front Immunol, 2018
Atypical hemolytic uremic syndrome is a rare disease characterized by acute kidney injury, thrombocytopenia, and microangiopathic hemolytic anemia.
• **Thrombosis:**
  - intraluminal fibrin or fibrin-platelet plugging

• **Non thrombotic features:**
  - Endothelial swelling and denudation, mesangiolysis, double contours, subendothelial accumulation of flocculent material
  - In arteries and arterioles: intraluminal fibrin, myxoid intimal thickening and concentric myointimal proliferation

Goodship et al, Kidney Int, 2017
Defect of protection of endothelial cells surfaces

Activation of endothelial cells

Defective FH

C3b, iC3b, C3b, B, C3b, Bb

C5b, C5b-9

C5a, C5a

Lesions by the Membrane Attack Complex

TMA

Hemolytic anemia

Thrombopenia

Karpman et al, 2008; Licht et al, 2009
Atypical HUS and complement

- Atypical HUS ≠ secondary forms
- Anomalies of alternative pathway
  - Main cause of aHUS: 60-70%
  - Heterozygous mutations in complement regulatory genes
  - Incomplete penetrance (50%)
  - 20-30% familial
Renal survival

Frémeaux-Bacchi et al, CJASN, 2013

Number of aHUS patients at risk

<table>
<thead>
<tr>
<th></th>
<th>Pediatric onset</th>
<th>Adult onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pediatric onset</td>
<td>89</td>
<td>34</td>
</tr>
<tr>
<td>Adult onset</td>
<td>125</td>
<td>18</td>
</tr>
</tbody>
</table>

5 yrs
64% in children
36% in adults
Eculizumab

Increase of eGFR of 32 ml/min at 26 sem
80% of renal recovery

Conclusion

- The complement plays a crucial role to maintain the integrity of renal cells and GBM
- Acquired and hereditary complement anomalies are found in 70% of patients with C3GP and aHUS
- Renal biopsy is required to establish diagnosis and samples must show typical predominant C3 staining
- Patients with C3GP aged >50 years should be evaluated for the presence of a monoclonal gammopathy