Complement in vasculitis and glomerulonephritis

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The complement system

An evolutionary conserved pathway central to maintenance of host defense

Destruction of pathogens
Elimination of virally infected cells
Removal of immune complexes and cell debris
Facilitating efficient B and T cell responses
Sensing danger signals

Prevention of autoimmune disease
The complement system

- Alternative pathway
- Classical pathway
- Lectin pathway

a. Initiation

b. C3 convertase

b. C3 convertase

- C3 convertase
- C3a
- C3b
- C5a
- C5
- C5b
- C5b-C9

- Opsonization
- Inflammation

- MASP

Terminal complement complex (lysis)
Control of C3 activation

A. Loss of CFH Inhibition
- C3
  - C3bBbP
  - CFH
- C3b
  - iC3b

B. CFH Deregulation
- C3
  - C3bBbP
  - CFH
- C3b
  - iC3b
  - CFHR proteins

C. Stabilization of the C3 Convertase
- C3
  - C3bBbP
- C3b
  - iC3b

D. Impaired Inactivation of C3b to iC3b
- C3
  - C3bBbP
  - CFH
  - CFI
  - MCP
- C3b
  - iC3b

De Vriese et al JASN 2015
The complement system selectively targets microbial surfaces
## Autoimmunity in complement deficiency

<table>
<thead>
<tr>
<th>Complement deficiency</th>
<th>Frequency</th>
<th>SLE (%)</th>
<th>Recurrent bacterial Infections</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C1q</strong></td>
<td>75 cases</td>
<td>90%</td>
<td>88%</td>
</tr>
<tr>
<td><strong>C1r</strong></td>
<td>12 cases</td>
<td>65%</td>
<td>85%</td>
</tr>
<tr>
<td><strong>Cis</strong></td>
<td>8 cases</td>
<td>65%</td>
<td>65%</td>
</tr>
<tr>
<td><strong>C4</strong></td>
<td>Rare</td>
<td>75%</td>
<td>Common</td>
</tr>
<tr>
<td><strong>C2</strong></td>
<td>1 in 20,000</td>
<td>10%</td>
<td>Rare</td>
</tr>
<tr>
<td><strong>C3</strong></td>
<td>Very rare</td>
<td>Rare</td>
<td>Very common</td>
</tr>
</tbody>
</table>

Data from Vignesh Clin Chim Acta 2017
Complement and glomerulonephritis
an overview

Complement and predisposition to glomerulonephritis
- C1q, C4 and C2 deficiency and SLE

Complement abnormalities as a cause of glomerulonephritis
- C3 glomerulopathy
  Dense deposit disease
  C3 glomerulonephritis
- C4 glomerulonephritis

Complement proteins as effectors of injury
- Membranous nephropathy
- Focal necrotizing glomerulonephritis
Dense Deposit Disease
The Prototypic C3 nephropathy

Identified as a unique entity in 1962 by Berger and Galle

- Progressive glomerulonephritis presenting in the young
- Membrano-proliferative appearance on light microscopy
- Glomerular C3 deposition in absence of IgG
- Band-line dense deposit within the GBM
Dense Deposit Disease

- Low C3 with alternative pathway activation
- Unrestrained intravascular complement activation
- Associated with autoantibodies to C3Bb convertase (C3NeF)
- Frequent recurrence in renal allografts

Subsumed into membranoproliferative glomerulonephritis more generally
Membranoproliferative glomerulonephritis

MPGN Type 1 – Subendothelial and subepithelial deposits
Deposition IgG and complement (C3)

MPGN Type 2 - Intramembranous dense deposits
Deposition of complement without IgG

(MPGN Type 3 - As Type 1 but prominent subepithelial deposits)
Membranoproliferative glomerulonephritis

**MPGN Type 1** – Subendothelial and subepithelial deposits
  Deposition IgG and complement (C3)
  Some MPGN type 1 have
    - no IgG deposits
    - low C3 concentrations
    - C3Nef

**MPGN Type 2** – Intramembranous dense deposits
  Deposition of complement without IgG
  Some dense deposit disease - don’t have MPGN
  Gn with isolated C3 deposition not invariable MPGN
Membranoproliferative glomerulonephritis

MPGN Type 1 – Subendothelial and subepithelial deposits
Deposition IgG and complement (C3)
Some MPGN type 1 have - no IgG deposits
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MPGN Type 2 - Intramembranous dense deposits
Deposition of complement without IgG
Some dense deposit disease - don’t have MPGN
Gn with isolated C3 deposition not invariable MPGN

New pathogenetically informed terminology
C3 Nephropathy
C3 glomerulopathy: a new classification

Fadi Fakhouri, Véronique Frémeaux-Bacchi, Laure-Hélène Noël, H. Terence Cook and Matthew C. Pickering

C3 glomerulopathy: consensus report

Matthew C. Pickering¹, Vivette D. D’Agati², Carla M. Nester³,⁴, Richard J. Smith³,⁴, Mark Haas⁵, Gerald B. Appel⁶, Charles E. Alpers⁷, Ingeborg M. Bajema⁸, Camille Bedrosian⁹, Michael Braun¹⁰, Mittie Doyle⁹, Fadi Fakhouri¹¹, Fernando C. Fervenza¹², Agnes B. Fogo¹³, Véronique Frémeaux-Bacchi¹⁴, Daniel P. Gale¹⁵, Elena Goicoechea de Jorge¹, Gene Griffin⁹, Claire L. Harris¹⁶, V. Michael Holers¹⁷, Sally Johnson¹⁸, Peter J. Lavin¹⁹, Nicholas Medjeral-Thomas¹, B. Paul Morgan¹⁶, Cynthia C. Nast⁵, Laure-Hélène Noel²⁰, D. Keith Peters²¹, Santiago Rodríguez de Córdoba²², Aude Servais²³, Sanjeev Sethi²⁴, Wen-Chao Song²⁵, Paul Tamburini⁹, Joshua M. Thurman¹⁷, Michael Zavros²⁶ and H. Terence Cook¹
C3 glomerulopathy: a process not a disease

A disease process due to abnormal control of complement activation, deposition, or degradation

Characterized by predominant glomerular C3 fragment deposition with electron-dense deposits on EM

Caused by unrestrained alternative pathway activation within the circulation either due to mutations of or autoantibodies to complement regulatory proteins

Results in a spectrum of glomerular appearances separated into Dense Deposit Disease and C3 glomerulonephritis
C3 glomerulopathy - features

**Morphology**
variable and includes mesangial proliferative Gn, MPGN and endocapillary Gn ± crescents

**Electron microscopy**
Dense intra membranous deposits ± subendothelial deposits (DDD) or varied subendothelial and mesangial deposits

**Immunohistology/fluorescence**
Predominant C3 with or without lesser amounts of IgG – many with MPGN type I have C3Nef
Control of C3 activation

A. Loss of CFH Inhibition

B. CFH Deregulation

C. Stabilization of the C3 Convertase

D. Impaired Inactivation of C3b to iC3b
Factor H deficient Yorkshire strain pigs
Spontaneous early onset DDD

Høgåsen K et al JCI 1995,
Immuno-EM very early C3 and C5-9 deposition followed by dense intramembranous deposits
Uncontrolled C3 activation causes membranoproliferative glomerulonephritis in mice deficient in complement factor H

Matthew C. Pickering¹, H. Terence Cook², Joanna Warren¹, Anne E. Bygrave¹, Jill Moss³, Mark J. Walport¹ & Marina Botto¹

Rescued by infusions of human CFH
CFI essential for GBM localisation but not mesangial
C5 deficiency is protective but C6 deficiency is not
Aggravated by coincident CR3 deficiency
Trials of potential therapies CR2-FH and truncated CFH

Nature Genetics 2002
C3 glomerulopathy – consensus report

Morphological appearance

Glomerulonephritis with dominant C3

Disease category

C3 glomerulopathy
Post-infectious GN
Other

DDD
Specific genetic forms and/or autoantibodies
Not otherwise specified

C3 GN
Specific genetic forms for example CFHR5 nephropathy and/or autoantibodies
Not otherwise specified

Monoclonal gammopathy
Atypical postinfectious glomerulonephritis is associated with abnormalities in the alternative pathway of complement

Sanjeev Sethi¹, Fernando C. Fervenza², Yuzhou Zhang³, Ladan Zand², Nicole C. Meyer³, Nicolò Borsa³, Samih H. Nasr¹ and Richard J.H. Smith³,⁴,⁵

<table>
<thead>
<tr>
<th>Patient</th>
<th>CFH</th>
<th>CFHRS</th>
<th>FH antibodiesa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>c.2171delC, p.Thr724fsX, 725</td>
<td>No mutations</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>No mutations</td>
<td>c.646-647, AA&gt;TT, p.Asn216Phe</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>No mutations</td>
<td>No mutations</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>No mutations</td>
<td>No mutations</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>No mutations</td>
<td>No mutations</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>No mutations</td>
<td>No mutations</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>c.3350A&gt;G, p.Asn1117Ser</td>
<td>No mutations</td>
<td>Negative</td>
</tr>
<tr>
<td>8</td>
<td>No mutations</td>
<td>No mutations</td>
<td>Negative</td>
</tr>
<tr>
<td>9</td>
<td>No mutations</td>
<td>No mutations</td>
<td>Negative</td>
</tr>
<tr>
<td>10</td>
<td>c.1699A&gt;G, p.Arg567Gly</td>
<td>No mutations</td>
<td>Negative</td>
</tr>
<tr>
<td>11</td>
<td>No mutations</td>
<td>No mutations</td>
<td>Negative</td>
</tr>
</tbody>
</table>

In addition 7 had C3NeF (including 6 without mutations)
C3 Glomerulopathy and post-infectious glomerulonephritis define a disease spectrum

Badria Al-Ghaithi1 - Rahul Chanchlani1,2,3 - Magdalena Riedl4 - Paul Thorner1,5 - Christoph Licht1,2,7

Post infectious GN

Complete recovery %

Group A
N=14

67 %

Group B
N=9

86 %

Group C
N=6

25 %

Group D
N=2

Pediatric Nephrology 2016
CLINICAL TRIALS AND OBSERVATIONS

Treatment of B-cell disorder improves renal outcome of patients with monoclonal gammopathy–associated C3 glomerulopathy

Sophie Chauvet,1-3 Véronique Frémeaux-Bacchi,2,4 Florent Petitprez,5 Alexandre Karras,1 Laurent Daniel,6 Stéphane Burtey,7 Gabriel Choukroun,8 Yalous Delmas,9 Dominique Guerrot,10 Arnaud François,11 Moglie Le Quintrec,12 Vincent Javaux,13,14 David Ribes,15 Laurence Vignaud,16 Bertrand Arnulf,17 Jean Michel Goujon,14,18 Pierre Ronco,19 Guy Touchard,13,14 and Frank Bridoux13,14

French cohort of C3 glomerulopathy
N = 201 adult patients

without monoclonal gammopathy
N = 141
29/141 were aged over 50

C3G+ Monoclonal Ig
N = 60

Cryoglobulinemia or unavailable clinical data
N = 10

N = 50 patients
Renal prognosis of C3 nephropathy worse with coincident monoclonal glomerulopathy

Chauvet et al Blood 2017
Treatment of monoclonal glomerulopathy improves renal prognosis in C3 nephropathy

C3 concentrations returned to normal with complete remission

Chauvet et al Blood 2017
C3 glomerulopathy – consensus report

Investigations

**Serology**

All patients: C3, C4, CFH, (CFP)
Paraprotein screen
C3NeF

Selected: CFB, C3 activation, C5 cativation
Autoantibodies to CFB and CFH

**Genetic screening**

All patients: CFHR5

Selected: C activating and regulatory factors
CNV CFH-CTHR locus
Spectrum of C3 nephropathy

From Zipfel et al. Molec Immunol 2015
Revised Chapel Hill Consensus Conference nomenclature for vasculitis – 2012

- Immune Complex Small Vessel Vasculitis
  - Cryoglobulinemic Vasculitis
  - IgA Vasculitis (Henoch-Schönlein)
  - Hypocomplementemic Urticarial Vasculitis
    - (Anti-C1q Vasculitis)

- Medium Vessel Vasculitis
  - Polyarteritis Nodosa
  - Kawasaki Disease
  - Anti-GBM Disease

- ANCA-Associated Small Vessel Vasculitis
  - Microscopic Polyangiitis
  - Granulomatosis with Polyangiitis
    - (Wegener’s)
  - Eosinophilic Granulomatosis with Polyangiitis
    - (Churg-Strauss)

- Large Vessel Vasculitis
  - Takayasu Arteritis
  - Tiel Arteritis
Anti-Neutrophil Cytoplasmic Antibodies

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>Fluorescence alone</td>
<td>80 - 85 %</td>
<td>75%</td>
</tr>
<tr>
<td>Fluorescence plus ELISA</td>
<td>90%</td>
<td>90%</td>
</tr>
</tbody>
</table>

- Anti-PR3
- Anti-MPO
- Anti-Neutrophil Cytoplasmic Antibodies
- Anti-PR3
- Anti-MPO
- Fluorescence alone
- Fluorescence plus ELISA
ANCA-associated vasculitis: the standard model
Pauci-immune FNGN and ANCA-associated vasculitis
Pauci-immune FNGN and ANCA-associated vasculitis

C dependent injury caused by passive anti-MPO nephritis
Antibodies to MPO and PR3 activate neutrophils to release C5a
C5a Receptor Mediates Neutrophil Activation and ANCA-Induced Glomerulonephritis

Adrian Schreiber,* Hong Xiao,† J. Charles Jennette, † Wolfgang Schneider,* Friedrich C. Luft,* and Ralph Kettritz*

[Image of graph showing percentage of glomeruli involved in crescents and necrosis in wild-type and C5aR-/- mice.]

[Images of tissue samples labeled μ and θ.]
Complement and AAV

Adapted from Kallenberg & Heeringa Kidney Int 2012
Alternative Complement Pathway Activation Products in Urine and Kidneys of Patients with ANCA-Associated GN

Shen-Ju Gou, Jun Yuan, Chen Wang, Ming-Hui Zhao, and Min Chen
Association of Serum C3 Concentration and Histologic Signs of Thrombotic Microangiopathy with Outcomes among Patients with ANCA-Associated Renal Vasculitis

Lucio Manenti,* Augusto Vaglio,* Elisa Gnappi,* Umberto Maggiore,* Landino Allegri,* Marco Allinovi,* Maria L. Urban,* Marco Delsante,* Maricla Galetti,* Maria Nicastro,* Francesco P. Pilato,† and Carlo Buzio*

![Graph showing plasma levels comparison between vasculitis and healthy groups.](image)

Plasma Bb levels (µg/ml)

Vasculitis

Healthy

P=0.02

Plasma Sc5b9 levels (ng/ml)

Vasculitis

Healthy

P<0.001
Association of Serum C3 Concentration and Histologic Signs of Thrombotic Microangiopathy with Outcomes among Patients with ANCA-Associated Renal Vasculitis

Lucio Manenti,* Augusto Vaglio,* Elisa Gnappi,* Umberto Maggiore,* Landino Allegrì,* Marco Allinovi,* Maria L. Urban,* Marco Delsante,* Maricla Galetti,* Maria Nicastro,* Francesco P. Pilato,† and Carlo Buzio*
CCX168 is an orally active C5aR inhibitor from Chemocentrix

Phase II double blind RCT of CCX168 in AAV recently completed – shows benefit
Phase II clinical trial of the oral C5a receptor inhibitor CCX168 in AAV

First Morning Urine Albumin:Creatinine Ratio
Mean % change from baseline

BVAS Total Score
%Change from Baseline

David Jayne – ACR 2014
Complement in active AAV

Raised circulating concentrations of Bb and sC5-9 are proof of active alternative pathway and terminal component activity.

Glomerular deposition Bb and C5-9 together with raised urinary concentrations are proof of local activation.

Initial results of C5aR blockade suggest complement makes an important contribution to injury.