The evolution of complement and the alternative pathway

Prof. Peter Lachmann - University of Cambridge, UK

The Evolution of Complement and the Alternative Pathway

Prof. Peter Lachmann
Emeritus Sheila Joan Smith Professor of Immunology
University of Cambridge

Two ways to look at complement evolution

- History of Discovery
  Starts from classical pathway

- Evolutionary History
  Starts from alternative pathway

Leon Rosenberg, 1965
Ann-Rev-Microbial 19, 285-300

"I believe... that no physically comprehensible meaning can be given to the "one hit theory"
It is instructive to compare the timidity of the physical chemist with the temerity of the student of complement in formulating reaction rate functions for complex sequential chemical events

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The historical evolution of complement

- 1885-1889: Pfeiffer & Issaeff show lysis of Vibrio Cholerae in peritoneum of immune guinea pigs & normal guinea pigs when antibody is given IP.

The historical evolution of complement

- 1894: Bordet & Ehrlich & Morgenroth show that erythrocytes are lysed by antibody and complement.

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The historical evolution of complement

Bordet & Gengou describe complement fixation – showing complement is a substance & not an activity

Nobel Laureate in Medicine, 1919

Jules Bordet
1870 – 1961

Complement

“Heat labile activity” in serum
(1880-1890s Buchner, Bordet, Erhlich)

“Material” absorbed by Ag/Ab
(1901 Bordet and Gengou)

Two components
Globulin/midpiece (C1) and Albumin/endpiece (C2)
(1907 Ferrata, Brand)

Cobra venom (1912 Ritz) and yeast (1934 Coca)
Destroy C but not C1 and C2
Defines “C3”

NH₃ destroys C
(1926 Gordon et al.)
Defines “C4”

Multiple components
20+ proteins make up C system

Alternative Pathways

Covalent binding site
* Internal thioester bond

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The kinetic analysis of complement action and the “one hit” theory of complement lysis

- 1948 - 1961 Developed by Mayer, Rapp & Borsos
- Believed originally to measure molecules of complement & thereby underestimated concentrations by about 1000-fold for C3
- Delayed purification of complement by a decade & caused complement to be viewed as an arcane & obscure mystery for much longer
- 1961 Adapted to detecting “functional molecules” after identification of C3 as β1c-globulin

Leon Rosenberg, 1965
Ann-Rev-Microbiol 19, 285-300

“I believe... that no physically comprehensible meaning can be given to the “one hit theory”...
It is instructive to compare the timidity of the physical chemist with the temerity of the student of complement in formulating reaction rate functions for complex sequential chemical events

Herbert J Rapp & Tibor Borsos, 1970
“Molecular Basis of Complement Action”
Meredith Corp, NY

This book is dedicated to the “one-hit” theory of immune haemolysis...

Evidence for this theory has been growing for the last 20 years and it now appears to be completely accepted...

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The identification of C3 as β1c globulin

- Paradigm shift in study of Complement
- Showed that at least one component was present in mg/ml range and initiated studies of complement immunochemistry
- [C3] is c. 1.2 mg/ml serum and half life is 48 hours
- Body makes as much C3/day as it does IgG – c. 5-6 Gm/day
- C3 is also major acute phase protein in terms of the amount extra synthesised

Hans Muller-Eberhard
(1927 – 1998)

When activated to C3b, C3 labels invading organism by covalent reaction through a thioester

Structure of C3 – a multi-domain protein at the heart of the complement system

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The mechanism of complement fixation
the internal thioester bond and covalent binding

- Described covalent binding of C3 to receptive surfaces and explain this by transesterification – or by transamidation - from an internal thioester bond
- C4 and (alpha2 macroglobulin) have similar properties
- C4A has preference for amide bonds and C4B for ester bonds

Covalent binding finally explains complement fixation!

The thioester reactions

Native C3

- O-O
- S
- CH₃ NH₂
- O-C-NH(CH₃)
- SH

Activated

- O-O
- S
- CH₃ OH
- O-C-OH
- O-C-OR
- SH

- H₂O
- H₂O
- Surface

Amide binding – to protein

C3 to

Ester binding – to CHB

C3 coating bacterial flagella

Feinstein A, Munn EA 1966 (Babraham Institute)

First demonstration that fixation of C3 extends far beyond complement fixation site and will coat whole organism/s organelles

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Archeo – complement system

The complement system in insects

Structure of Mosquito TEP1r compared with Human C3

“Primitive” feedback

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Novel features of vertebrate complement to cope with pumped circulation and plasma full of protease inhibitors

- Need a control mechanism to stop any activating stimulus going to exhaustion – hence the C3b breakdown cycle
- Can no longer rely on microbial proteases for cleavage – hence two specialised induced fit enzymes:
  - FD (adipsin) for feedback cycle to cleave Fb to Ba and Bb
  - Fi for breakdown cycle to cleave C3b to iC3b and further to C3dg
- Both are secreted as inactive proenzymes that need processing
  - Pro-FD is converted to FD predominantly by MASP3
  - Pro-Fi is converted to Fi by furin

Novel features of vertebrate complement to cope with pumped circulation and plasma full of protease inhibitors

- Both are enzymatically active – and susceptible to inhibition – only when substrate is complexed with a further protein cofactor
  - Cofactor for FD is C3b
  - Fi has multiple cofactors - FH, MCP & CR1 (& C4bp for C4 cleavage)
- Concentration of both is highly regulated – which is very unusual for triggered enzyme cascades

C3b feedback and breakdown
The alternative pathway of vertebrate complement

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**The properdin system**

**the first discovery of the alternative pathway**

- Pillemer Laboratory claimed that a new protein – properdin could activate complement without antibody
  - *Science* 120 279
- 14 papers followed in 1955 -1961 claiming that properdin was important for immunity to bacteria, viruses and cancer cells...
  - Great public interest
- Claims of non-specificity rejected and bitter controversy results

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**Second coming of the Alternative Pathway**

- Ritz (1912) & Coca (1914) had shown that Cobra venom and yeast respectively consumed C₃ but not mid-piece or end piece
- In 1950s Pillemer had described properdin as initiating a new C₃ pathway, debunked by Nelson
  - Recently revived by Hourcade & others, debunked again by (inter-alia) Harboe et al. –
    - “Old fictions never die – and they don’t even always fade away”
- In 1960s C₃ activators that did not consume C₁, 4 or 2 and/or worked in C₄ or C₂ deficient serum described in several labs:
  - Gewurz, Reid, Muller-Eberhard, Frank...
    - These included LPS, Fab'2 and IgA precipitates, some subclasses of IgG as well as CVF, Yeast – and Nef

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**Second coming of the Alternative Pathway**

- Shown to need additional novel factors eventually called FB & FD as well as properdin, nephritic factors played a part
- How it worked was however obscure
  - Activation of FD by properdin or “Initiating factor”, suggested as homologue of C₁ activation, could not be confirmed
    - (Recently FD activation by MASP1 has been suggested – but is not a plausible mechanism for AP activation)

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The alternative pathway mechanism
A reaction entirely governed by the rates of two opposing reaction cycles

- Claims on initiating factor and direct activation of properdin and Fd not sustained
- Explanation arose from study of a patient with uncontrolled activation of AP = deficiency of FI
  Abramson, Alper, Lachmann, Rosen & Jandl, 1971
- Similar activation follows in-vitro FI depletion, gave rise to "tickover" explanation
  Nicol & Lachmann, 1973
- Molecular basis of tickover ascribed to hydrolysis of C3 internal thiocarbonate
  Schreiber & Muller-Eberhard, 1981
- However it is likely that tickover has multiple mechanisms – C4b2a, MASP2, elastase, thrombin, plasmin etc.

C3b feedback and breakdown
The alternative pathway of vertebrate complement

What produces the initial activated C3?

The Cy-Tickover
Initiation of alternative complement pathway

Tick-over maintained by spontaneous hydrolysis of C3 internal thioester bond to ‘C3b-like’ molecule and by cleavage to C3b

Tick-over “fired” by –

1. Mechanisms that increase C3b production
   a) ‘Exogenous’ C3 splitting enzymes e.g. C4b2a, Plasmin, Leucocyte proteases – elastase, Bacterial proteases
   b) Stabilisation of C3b, C1s, P, Nef, sp also CVF, Bp

2. Mechanisms that reduce C3b destruction
   a) Fixation on “protected surface”
   b) Deficiency of FH and FI
   c) Local sequestration of FH

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How to inhibit C3b Breakdown

- FH – absence or [low] or loss of function mutants
- FI – absence or [low]
- MCP – [low] or loss of function mutants
- Protected Surface for bound C3
- C3F – C3F binds FH with less affinity than does C3S and is cleaved more slowly by FI

(Heinrich et al. 2011 PNAS 108: 8762-66)

Predisposes to immunopathology associated with over activity of the alternative pathway

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C3b bound on a protected surface binds FB better than it binds FH

### B and H binding

<table>
<thead>
<tr>
<th></th>
<th>C3b</th>
<th>B (K x 10^5)</th>
<th>H (K x 10^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep E</td>
<td>30,000</td>
<td>34,500 (2.3)</td>
<td>25,900 (10)</td>
</tr>
<tr>
<td>Sheep E (Salivary)</td>
<td>8,000</td>
<td>7,790 (3.8)</td>
<td>1,620 (31)</td>
</tr>
<tr>
<td>Sheep E (NaI)</td>
<td>10,000</td>
<td>10,790 (3.2)</td>
<td>2,390 (9)</td>
</tr>
<tr>
<td>Zymosan</td>
<td>2,800</td>
<td>4,000 (2.4)</td>
<td>650 (57)</td>
</tr>
</tbody>
</table>

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C3b bound on a protected surface binds FB better than it binds FH

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</thead>
<tbody>
<tr>
<td>Sheep E</td>
<td>30,000</td>
<td>34,500 (2.1)</td>
</tr>
<tr>
<td>Sheep E (laxidase)</td>
<td>8,000</td>
<td>7,790 (3.9)</td>
</tr>
<tr>
<td>Sheep E (NaI/O3)</td>
<td>10,000</td>
<td>10,790 (3.2)</td>
</tr>
<tr>
<td>Zymosan</td>
<td>2,800</td>
<td>4,000 (1.4)</td>
</tr>
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</table>

What does complement do?

- Enhances inflammation by neutrophil (and monocyte) activation, opsonisation, chemotaxis, vasoactivity...
- Causes membrane damage and cell death (or activation) by MAC insertion
- Plays a necessary role in immune complex metabolism
- Plays an enhancing role in antibody formation
- It is an important component of innate immunity
- It is a principal effector for antibody mediated immunity

How to amplify feedback

- **Increase C3b input**
  - Other C3 convertases (CP and Lectin pathways)
  - Other C3 splitting enzymes (Elastase, plasmin etc.)

- **Accelerate feedback reactions**
  - Gain of function mutants of FB
  - Increase [FD] and or [Mg]

- **Stabilise AP C3 convertase**
  - Properdin
  - Nef
  - (Cobra Venom Factor)

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Nephritic factors (Nef)
Autoantibodies that stabilise the AP C3-convertase & convert C3 when added to NHS

Spitzer et al. find Nef ~ MCGN

Thompson shows Nef to be ~ IgG(3)

Valliota et al. deny Ig nature of Nef

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Nef and Disease

- Nef can be ~ MCGN/DD and/or with partial lipodystrophy or neither (Sisson et al. 1976)
- Suggests that Nef is a risk factor for these diseases and not their invariable cause. PLD is often precipitated by a virus infection
- Carriers of C3F allele have relative risk of 2.1 for making Nef (Finn & Mathieson, 1993)
- Regions of fat prone to PLD make more FD (adipsin); (Mathieson & Peters, 1997) Lysis by AP susceptible to raised [FD]
- But we still have no idea of what provokes its formation

Partial Lipodystrophy

- PLD occurs at sites where fat cells make high levels of FD
- This allows AP to become lytic

The hyperinflammatory complement phenotype

- This is produced by any one or more of the genetic alleles that increases amplification
- Protects against infection particularly in early life before IgG antibodies are formed against the relevant microbes
- In later life predisposes to inflammatory disease. May include accelerated atherosclerosis and, possibly, Alzheimers Disease as well as DDD, HUS and AMD
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**C3b feedback and breakdown**
The alternative pathway of vertebrate complement

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**iC3b**
- iC3b reaction with CR3 on PMN is principal phlogistic event after complement activation
  (Extensive work by Gordon Ross and colleagues)
- Synergistic with C5a activation of PMN
- iC3b generation is **absolutely necessary** for development of MCGN in mice (Pickering et al. 2007)

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**Inflammation**
- Inflammation is the response of living tissue to injury
- It is mediated by (at least) three immunological pathways (which often act together):
  - **Allergic**
    - Mediated by IgE – primary effector cells: mast cells, basophils, eosinophils (Coombs & Gell type 1)
  - **Humoral**
    - Mediated by antibody and complement – primary effector cell: neutrophil (Coombs & Gell types 2 & 3)
  - **Cellular**
    - Mediated by cytokines – effector cells: T-lymphocytes and macrophages (Coombs & Gell Type 4) (A inflammation activation)
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Neutrophils are essential for humoral inflammation

- Depletion of neutrophils with nitrogen mustard or anti-neutrophil antiserum inhibit:

- Deficiency of CD18 – the common light chain of CR3 (CD11b), CR4 (CD11c) and LFA (CD11a) – [Ross et al. 1985 Blood 66: 882-890]
  - Causes failure to generate an inflammatory response to bacterial infection (no pus is formed) and these children die of “silent” infections

- On the other hand C3 deficiency facilitates bacterial infection only in childhood before IgG Abs to bacteria are made, then Fc receptors recruit neutrophils (Neisseria are an exception)

Complement R CR3

- Glycoprotein member of the integrin family (CD11b/CD18)
  - Noncovalently linked dimer 185kDa α chain (CD11b)
  - 95kDa β chain (CD18)
  - B chain same as in LFA-1 (CD11a)
  - p150 (CD11c)

- Whole molecule termed the CD11/CD18 complex
- Resides in 2 pools in neutrophils as does CR1

Structure and domains of CR3 (CD11b/CD18 or α₅β₂ – Integrin)

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**iC3b reaction with CR3/CR4 is necessary for most complement mediated inflammation**

- Fi deficiency
  - Can generate no iC3b
  - Suffer from bacterial infection
  - Can make pus
  - Normal wound healing
  - No "C3 glomerulopathy"
  - Can live normal life after childhood but cave Neisseria

- CD 18 Deficiency
  - Lacks ligand for iC3b (inter alia)
  - Suffer from bacterial infection
  - Make no pus
  - Paper thin scars
  - ?? "C3 glomerulopathy"
  - Die of silent infections & now given BM transplants soon after birth

NB Isolated CD11b (ITGAM) deficiency can make renal disease worse - ?macrophage CR3 may be anti-inflammatory in some situations

(Barbour et al. 2016 Kidney International 89: p823-832)

THE END

AND THANK YOU

From "Bill The Mindful"

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