Lecture 2: Introduction to Complement

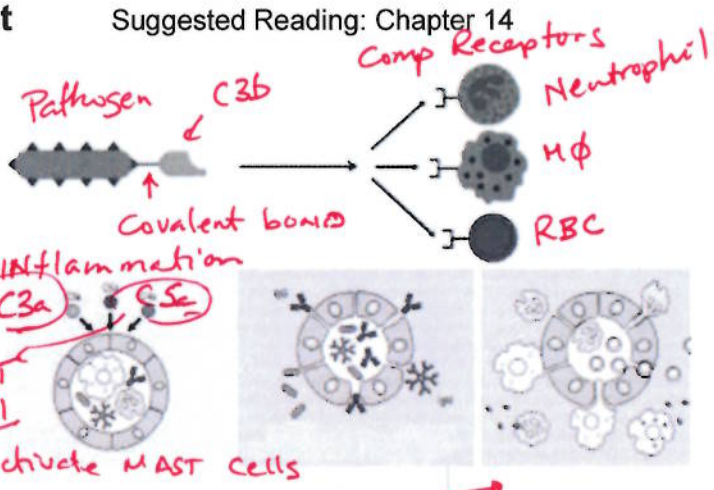
Suggested Reading: Chapter 14

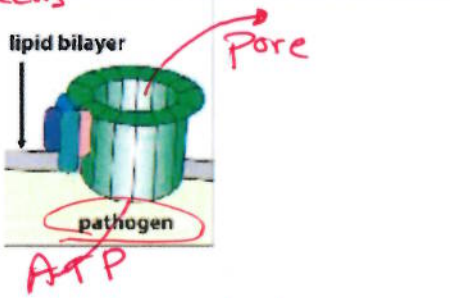
Key part of the innate immune response and important effector arm of humoral (acquired) immunity.

- >30 proteins from liver and inflammatory cells; short half-lives; tightly regulated enzymatic cascades.
- Regulation is finely tuned and very tight to prevent host damage.
- Up-regulated in the acute phase response during substantial inflammation.

Major roles:

1. Opsonization of pathogens with **C3b**. (Opsonins are proteins that become attached to pathogens, facilitating receptor mediate endocytosis)
2. Activation of Mast cells via **C3a, C5a** → inflammation.
3. Formation of membrane attack complex, leading to leakage of cell contents.





Membrane attack complex (MAC)

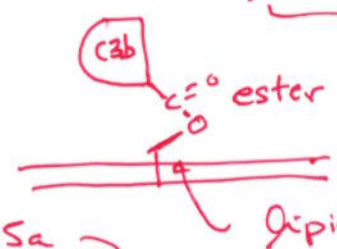
Some Definitions:

i) Convertases & Split products:

C3 convertase = (C3b)(Bb) — catalytic activity

$C3 \xrightarrow{\text{(protease)}} C3a + C3b$ — split products

ii) Complement Fixation:



lipids, sugars, proteins (Tyr, Ser, Thr)

iii) Anaphylatoxin: C3a, C5a (*phylaxis=prevention against infection Anaphylaxis =opposite of prevention*) → activate MAST cells → anaphylactic shock → prompt inflammatory response.

iv) PAMP – Pathogen associated molecular pattern. (e.g. mannose)

Overview:

INNATE

1 Alternative Pathway
First pathway to become involved in pathogen destruction. **Spontaneous** activation, **amplification** occurs if foreign substances (e.g. pathogens) are present.

(C3bBb)

C3b

2 Lectin Pathway
Converges with classical pathway at C3 convertase. Triggered by terminal mannose residues on bacteria. A high level of mannose binding protein complex produced during inflammatory response.

C3 + C3b → C3 convertase → C5 → C5 convertase → MAC

3 Classical Pathway
Activated during inflammatory response via C-reactive protein (from liver).

Also triggered by Ag-Ab complexes on microbial surfaces. ← Antibody

(C4bC2a) → (C4bC2aC3b) = C3 convertase

Acq. System

Alternative Pathway:
Phase I - Generation of C3 Convertase & C3b opsonization.

A. Generation of Soluble C3 convertase:

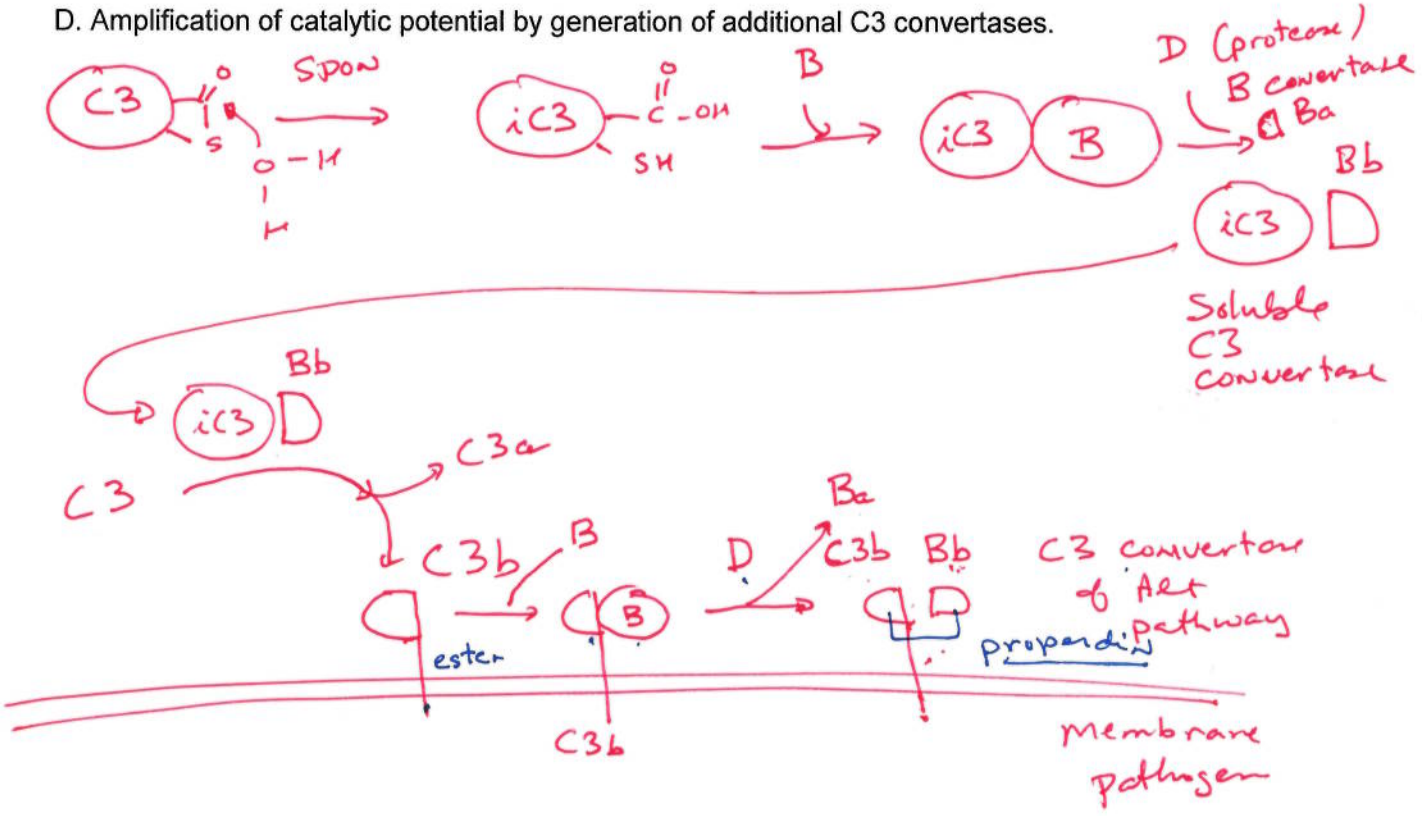
1. **C3** (soluble) spontaneous hydrolysis of thio-ester, producing **iC3**.
2. iC3 (soluble) binds **factor B**, producing iC3-B complex
3. **Factor D** cleaves B to give Ba + Bb, **iC3Bb** complex, a soluble C3 convertase.
4. iC3Bb converts soluble C3 to C3b and C3a.

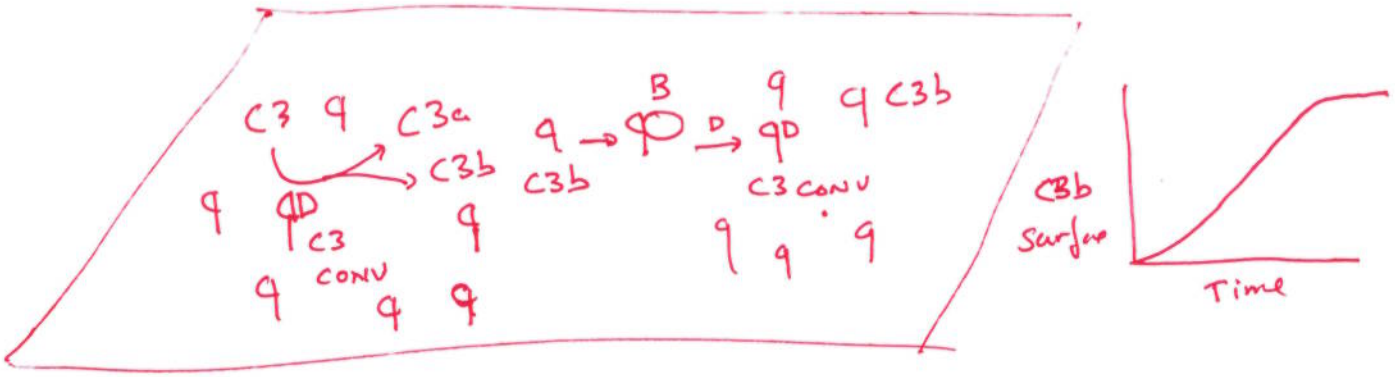
B. Generation of fixed C3 Convertase

5. C3b fixed by covalent ester bond on pathogen surface.
6. Factor B binds to *immobilized* C3b, giving C3bB
7. Factor D cleaves B into Bb and Ba, giving *immobilized* **C3bBb** (**C3 convertase of the alt pathway**).
8. C3bBb complex stabilized by **properdin.**

C. Production of large amounts of C3 split products, fixation of C3b to membrane in vicinity of active C3 convertase.

D. Amplification of catalytic potential by generation of additional C3 convertases.





Phase II – Formation of C5 Convertase & the Membrane Attack Complex.

1. Fixed C3 convertase (C3bBb) binds soluble C3b, forming C5 convertase = **$(C3b)_2Bb$.**
2. C5 converted to C5a + C5b (soluble), C5b binds to membrane.
3. C5b binds to soluble C6 + C7, forming the initial membrane complex.
4. C5b-C6-C7 complex binds C8.
5. C5b-C6-C7-C8 complex causes polymerization of ~16 molecules of C9
6. C5b-C6-C7-C8-$C9_{16}$ is the MAC, leading to cell lysis.

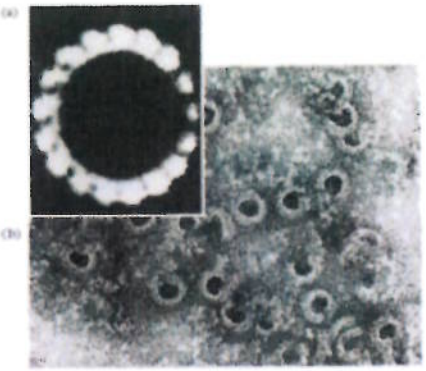


FIGURE 7-8 (a) Photomicrograph of poly-C9 complex formed by in vitro polymerization of C9 and (b) complement-induced lesions on the membrane of a red blood cell. These lesions result from formation of membrane-attack complexes. [Part a from E. R. Podack, 1986, in Immunobiology of the Complement System, C. Ross, ed., Academic Press, Orlando; part b from J. Humphrey and R. Dourmashkin, 1969, Advances in Immunology 11:75.]

C5a

C5b + C6 + C7 + C8 + 20x C9 ⇒ MAC

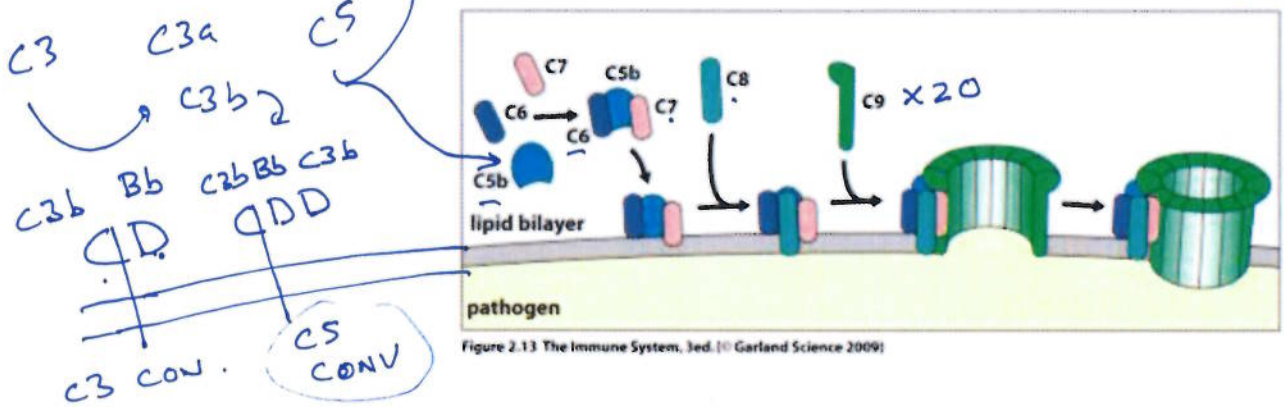


Figure 2.13 The Immune System, 3ed. [© Garland Science 2009]

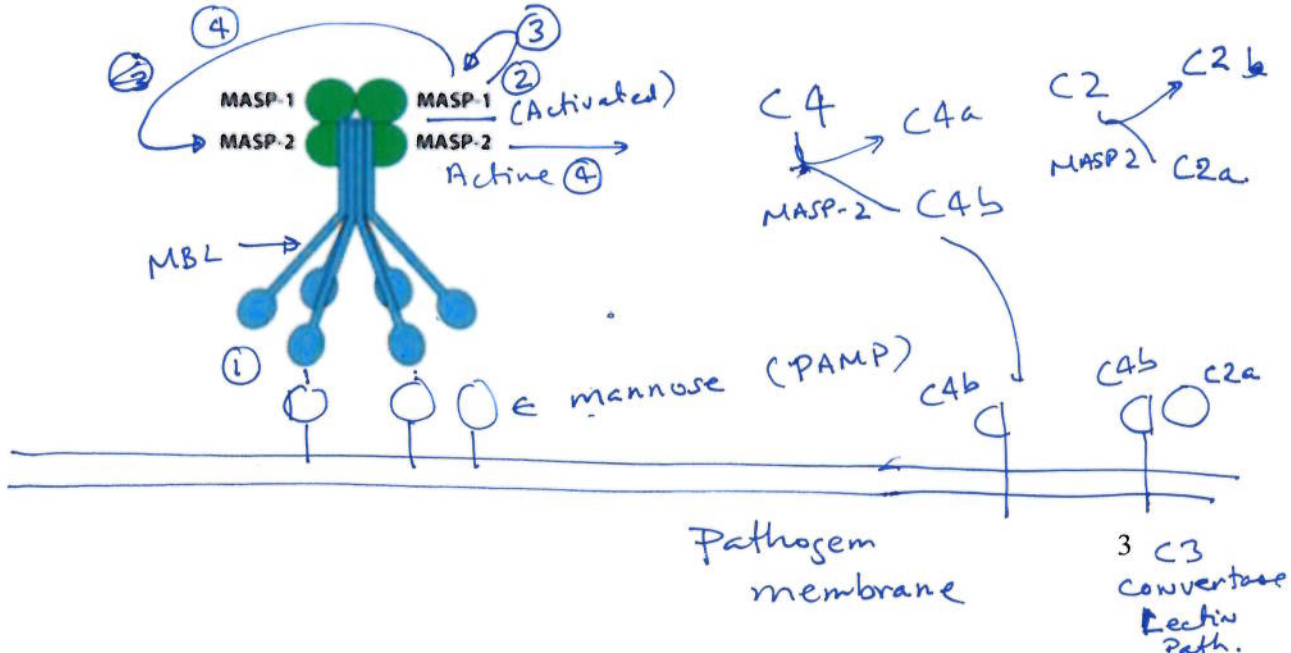
Activation of other Branches of the Complement Pathway:

Lectin pathway:

1. Recognition of mannose residues by mannose binding lectin (MBL). This is an example of recognition of a PAMP.

PAMP = Pathogen Associated Molecular Pattern.

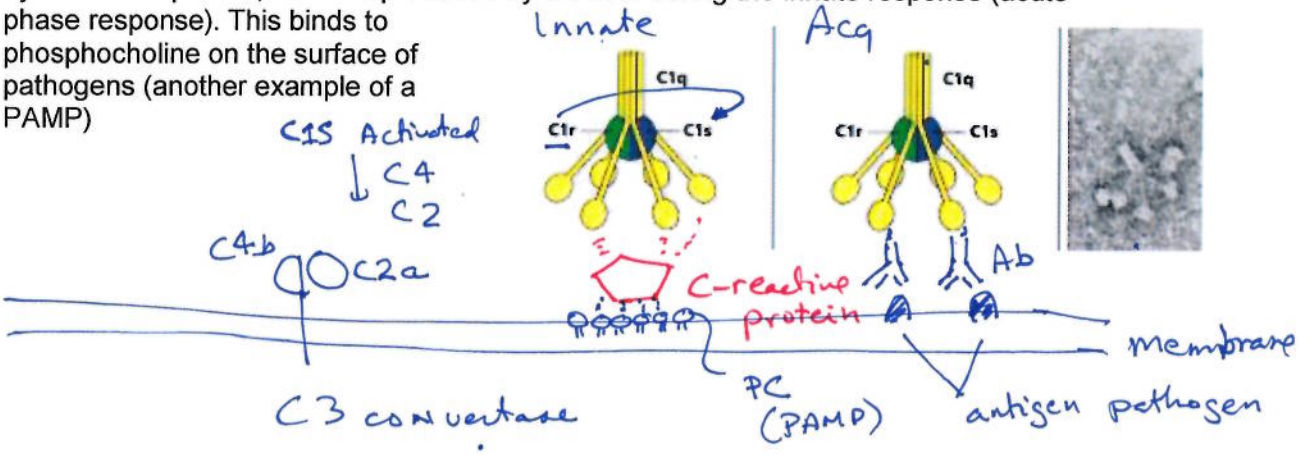
2. MASP-1 (MBL associated serine protease) is activated.
3. MASP-1 activated, cleaves itself and other MASP-1 (auto-activation)
4. MASP-2 activated by cleavage by MASP-1
5. MASP-2 cleaves C4, C4b is fixed on surface
6. MASP-2 cleaves C2, forming C2a & C2b
7. C2a binds to C4b, generating C3 convertase.



Classical Pathway:

Triggered:

- by antigen-antibody (Ab) complexes on microbial surfaces
- by c-reactive protein, which is produced by the liver during the innate response (acute phase response). This binds to phosphocholine on the surface of pathogens (another example of a PAMP)



C1q: *Recognizes IgM or c-reactive protein*
C1r: *Activated by C1q, auto-cleavage and cleavage of other C1r.*
C1s: *Cleaved by activated C1r, producing active protease.*
C4 cleaved, and C4b fixed
C2 cleaved, and C2a binds to C4b = C2aC4b = C3 convertase

Homology between Lectin & Classical Pathways

MBL	C1q	— recognition
Masp-1	C1r	— activation of 2nd protease
Masp-2	C1s	— cleavage C4, C2

Biological Consequences of Complement Activation

1. Opsonization: C3b

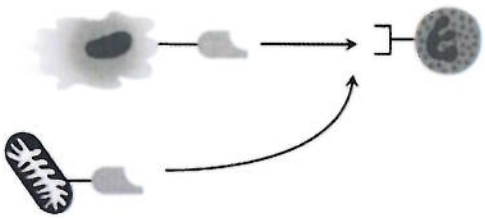
Receptor	Cells bearing receptor	Ligands	Consequences of ligand binding
CR1	RBC	C3b/iC3b	clearance of pathogen spleen
CR2	B-cell	C3b/iC3b/C3d	Activation of B-cell
CR3	Neutrophil Macrophage	iC3b	enhance receptor mediated endocytosis
CR4			

(iC3b is an inactive form of C3b, C3d is yet another split product of C3.)

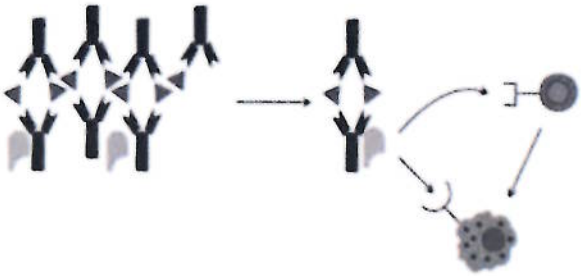
2. Inflammation: C3a, C5a

3. Direct killing: formation of functional MAC on cell surfaces causes cell lysis

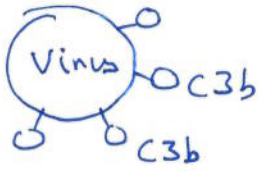
4. Removal of necrotic and apoptotic cells: C3b deposits on dying cells and released organelles, signaling rapid clearance by phagocytic cells



5. Clearance of immune complexes: C3b deposits on antibody-antigen complexes and causes their dissociation and clearance.

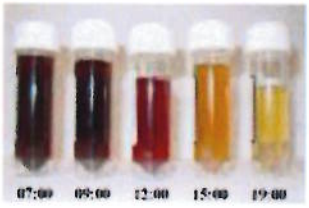


6. Neutralization of viral infection: Complement on antibody-antigen complexes forms a thick coat and blocks viral entry into cells.



7. Enhances activation of B-cells during an infection (via CR2, see above table).

Discussion: In the genetic disease *paroxysmal noctural hemoglobinuria*, the urine contains high levels of hemoglobin at various times of the day. Can you suggest what might be causing this disease?



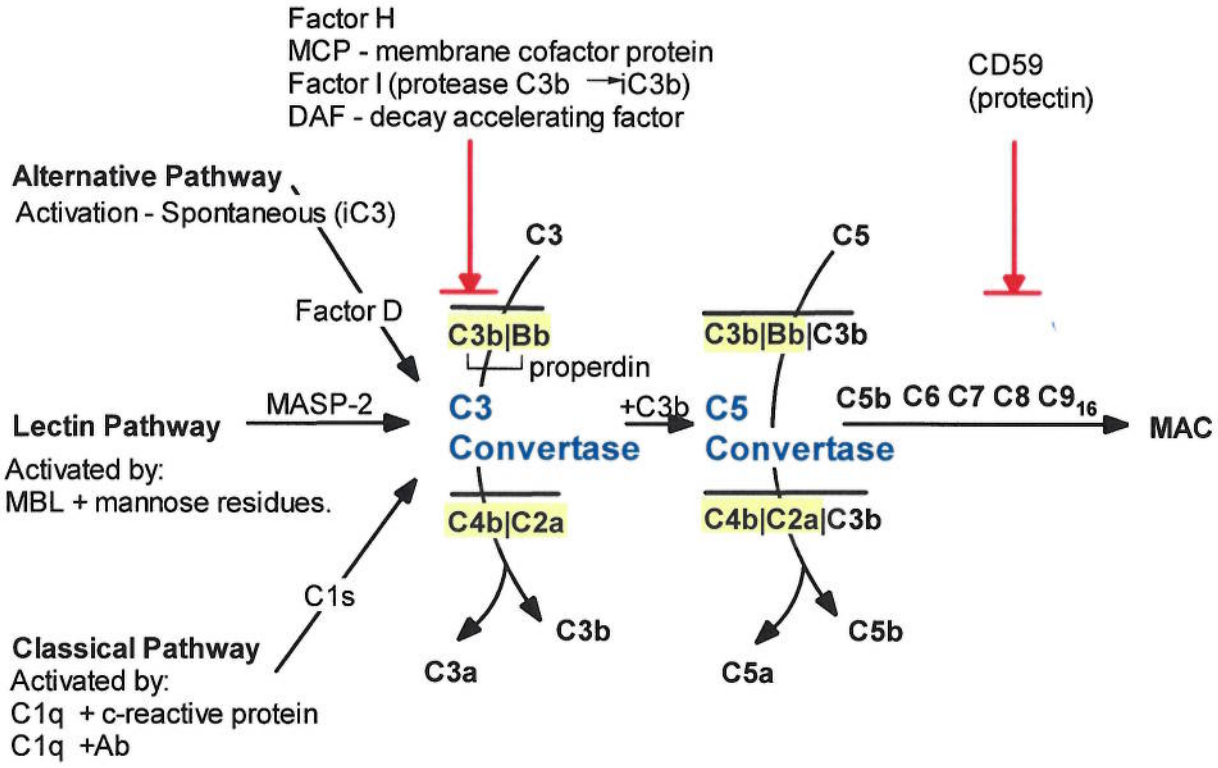
Regulation of Complement Activity (Alternative pathway)

- Factor H and MCP (membrane co-factor) prevent B binding to fixed C3b.
- Factor I (protease) converts fixed C3b to iC3b, an inactive form. Cleavage enhanced by H and MCP.
- DAF (decay accelerating factor) disrupts C3 convertase by causing loss of Bb.
- CD59 (protectin) inhibits formation of the MAC.

Summary/Learning Goals

- Distinguish between the activation of each pathway (innate versus acquired)
- Describe the role of C3b.
- Describe the role of C3a, C5a.
- State the composition of the C3 and C5 convertase in each pathway.
- Describe the formation of the MAC complex.
- Provide examples of regulation of complement.
- Describe the outcome of C3b binding to complement receptors & biological functions of complement (1-7 above).
- Two examples of PAMP receptors – MBL and C1q, recognizing mannose and phosphorylcholine.

Overview of Complement Pathways and Regulation



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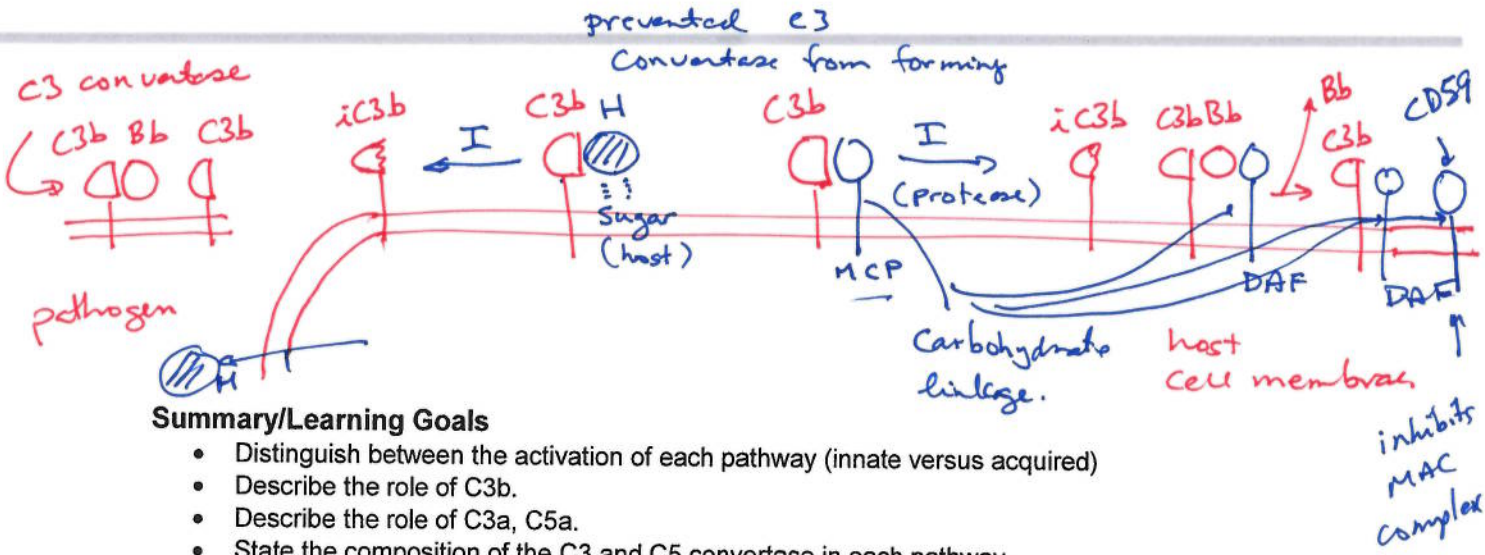

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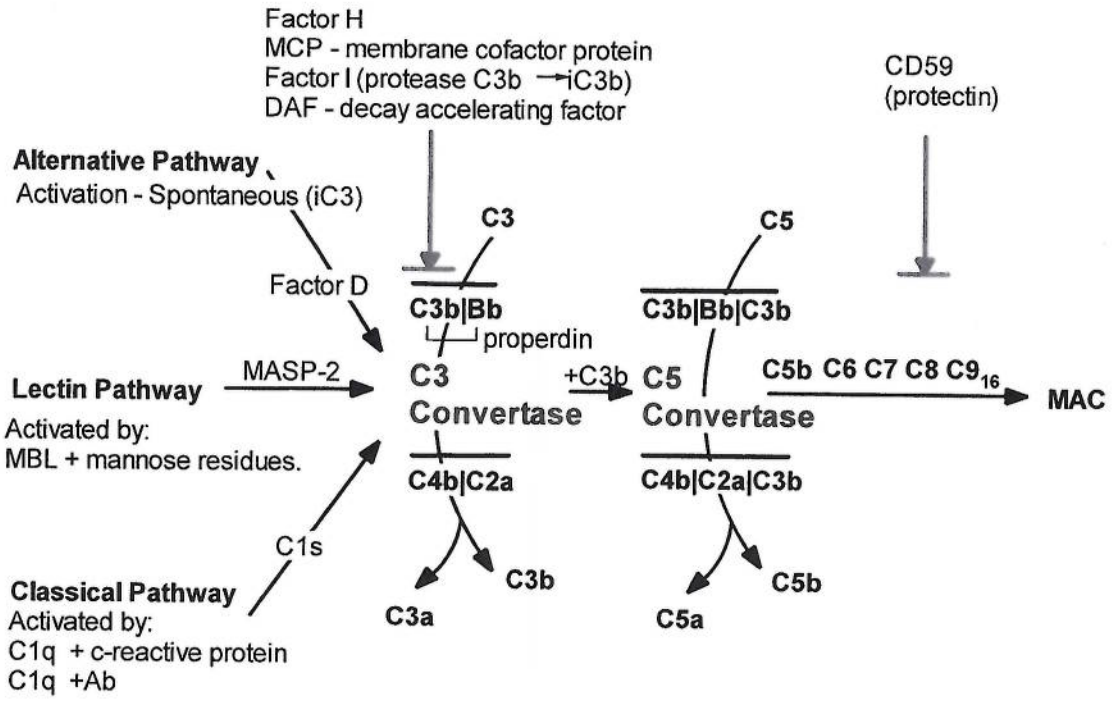


03-390 Immunology Bonus Quiz 1 – August 29, 2016 Name:_____

1. Which of the three complement pathways are associated with the innate immune system? Which are associated with the acquired immune system?

a) alternative I

b) lectin I

c) classical I + Acquired

2. State (but do not describe the process) how the classical pathway activated.

C-reactive protein (innate), Ab on surface (Acquired)

3. State (but do not describe the process in detail) how the alternative pathway activated.

C3 → iC3 → Soluble C3 convertase → fixed C3 convertase

↑ Spontaneous

4. What is the C3 convertase of the alternative pathway?

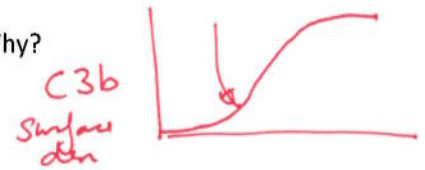
C3bBb.

5. What is the major opsonin that is produced by the complement pathways?

C3b.

i) catalytic activity
ii) forming new C3 conv

6. What is the rate of opsonization, ~~linear~~? Or ~~something else~~? Why?



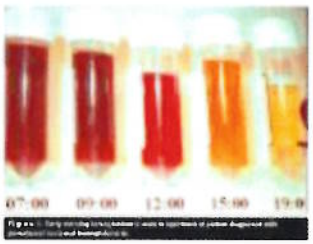
7. What are the two major anaphylatoxins produced by the complement pathway?

C3a, C5a

8. What is the MAC complex, and how does it kill pathogens? ← ion leakage

C5b + C6 + C7 + C8 + C9 (15-20) ⇒ MAC

9. Speculate what might be causing the disease Paroxysmal nocturnal hemoglobinuria?



- MAC complexes on RBC
→ leakage of Hb
- lack of regulation.