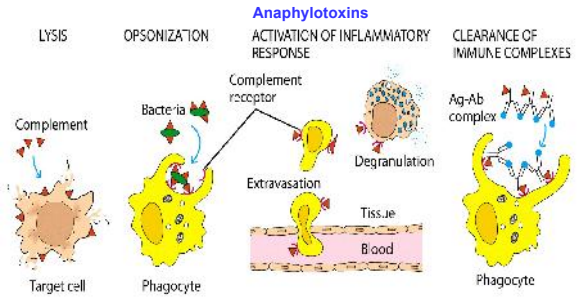
Chapter 7

Complement

History

- Jules Border in 1890's discovered complement
- Paul Ehrlich coined the term "complement"
- "The activity of blood serum that **completes** the action of antibody"
- **Now**: "Set of serum proteins that act in a cascade fashion to increase the immune response"

The Functions of Complement



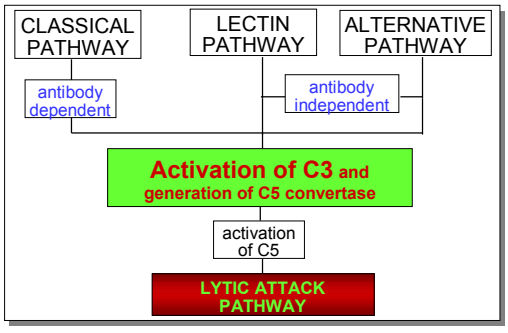
Complement Components

- Over 20 serum proteins
- Cascade fashion
- Components designated: C1 – C9
- Proteolysis results in: Large fragments "b" and smaller fragments "a" → C3a, C5b
- The **exception** is C2, where C2a is the large fragment and C2b is the smaller fragment and diffuses away

Complement Pathways

- 1) **Classical Pathway** – activated by antigen-antibody interaction. Best Ab for complement activation: IgM, IgG1, IgG2
- 2) **Alternative pathway** – activated by C3b binding to microbial cell surfaces
- 3) **Lectin pathway** – binding of the mannose-binding lectin (MBL) to the surface of pathogens.

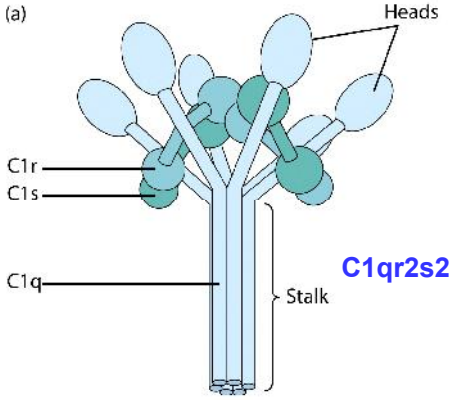
Pathways of complement activation



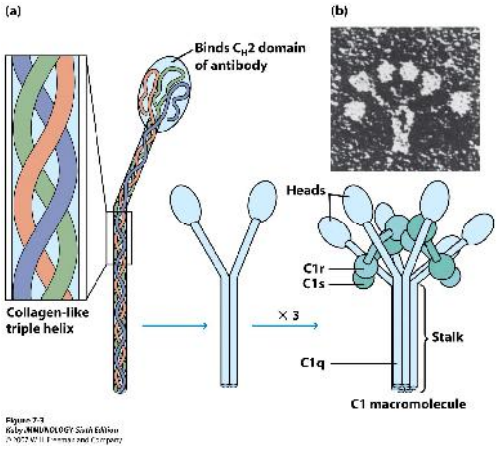
1. Classical Pathway

- Activated by Ag-Ab complexes
- Complement bind to Fc region
- Antibodies: IgM, IgG (IgG3>IgG1>IgG2)
- Divided into two steps:
  - A) Pre-C3 (C1 to C4)
  - B) Post-C3 (C5 to C9)

Structure of C1

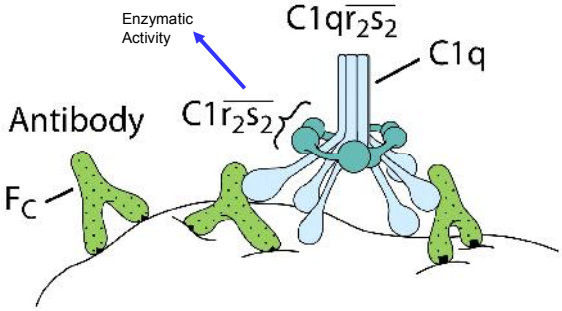


C1qr2s2

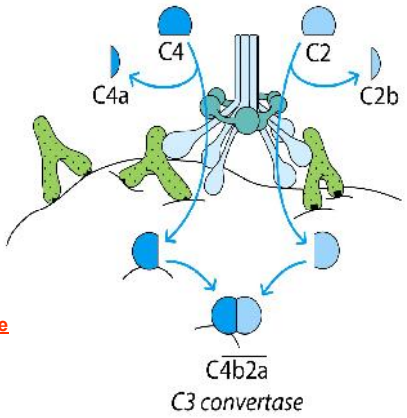


- **C1q**
- **2 molecules of C1r**
- **2 molecules of C1s**

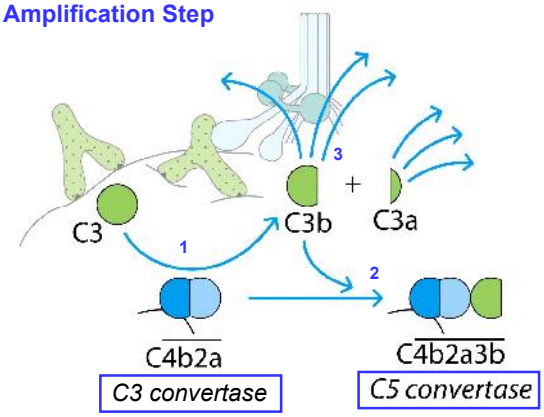
- **Binding of C1 complex to Ab leads to activation of C1r and C1s**
- **Substrate for C1s is C4 and C2**

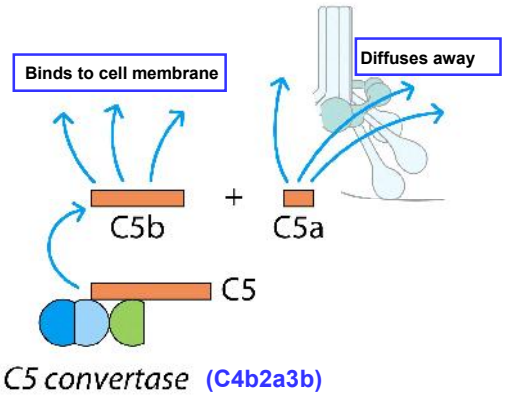


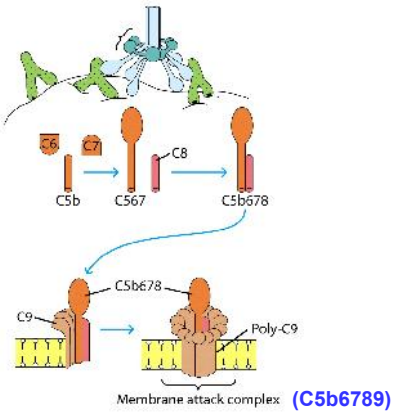
- **C1complex (C1s) hydrolysis C4, resulting in C4a (small) and C4b. C4b binds to the cell surface**
- **C1 complex (C1s) hydrolysis C2 resulting in C2b (small) and C2a which binds to C4b to form the C3 convertase**



Amplification Step



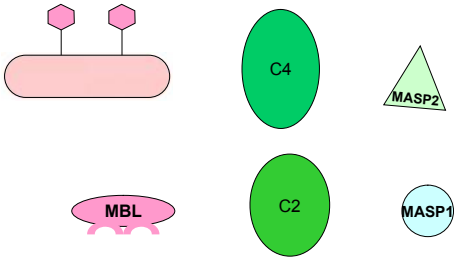




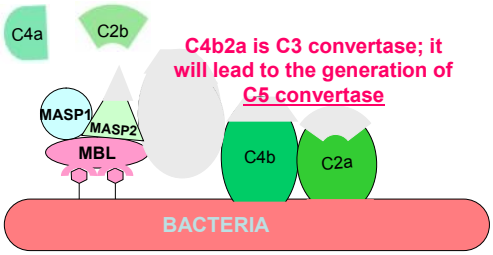
2. Lectin Pathway

- **Lectins** are carbohydrate-binding proteins
- Does not require antibody
- Recognizes mannose residues on glycoproteins
- The mannose-binding lectin **(MBL)** is an acute phase protein that increases during inflammation
- Plays a similar role to that of C1q
- After binding to mannose-residues on the cell surfaces, associates with MBL-associated serine proteases (**MASP-1** and **MASP-2**).
- This complex activates C4 and C2 just as in the classical pathway
- MASP-1 and MASP-2 very similar to **C1r** and **C1s**

Components of mannose-binding lectin pathway



Mannose-binding lectin pathway



3. Alternative Pathway

- Activated by microbial cell wall and cell wall components

TABLE 7-1 Initiators of the alternative pathway of complement activation

PATHOGENS AND PARTICLES OF MICROBIAL ORIGIN
Many strains of gram-negative bacteria
Lipopolysaccharides from gram-negative bacteria
Many strains of gram-positive bacteria
Teichoic acid from gram-positive cell walls
Fungal and yeast cell walls (zymosan)
Some viruses and virus-infected cells
Some tumor cells (Raji
Parasites [trypanosomes]
NON PATHOGENS
Human IgG, IgA, and IgE in complexes
Rabbit and guinea pig IgG in complexes
Cobra venom factor
Heterologous erythrocytes (rabbit, mouse, chicken)
Anionic polymers (dextran sulfate)
Pure carbohydrates (agarose, inulin)

SOURCE: Adapted from M. K. Pangburn, 1986, in Immunobiology of the Complement System, G. Ross, ed., Academic Press, Orlando.

Table 7-1
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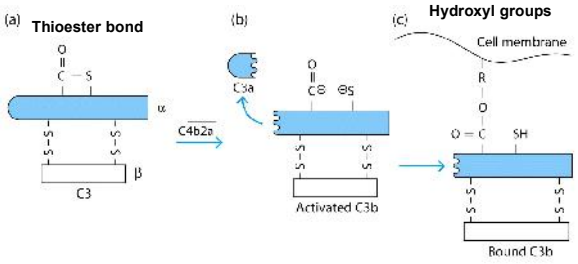
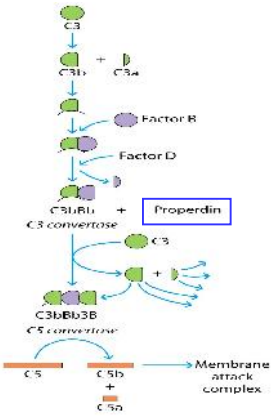
- C3 in serum undergoes spontaneous hydrolysis → C3a, C3b
- The half life of these products is very short, **except**…if C3b can bind to host and bacterial cell surfaces!!!!
- In mammals there is **high** levels of **sialic acid** → **inactivation of C3b**
- This is not the case in bacteria and fungi → **longer half life**



- **C3b bound to cell surfaces is stabilized by Factor B**
- **Factor D is recruited and acts on Factor B to generate a large (Bb) and small fragment (Ba)**
- **C3bBb is the C3 convertase**
- **Properdin (small protein) stabilizes the C3 convertase**



Summary

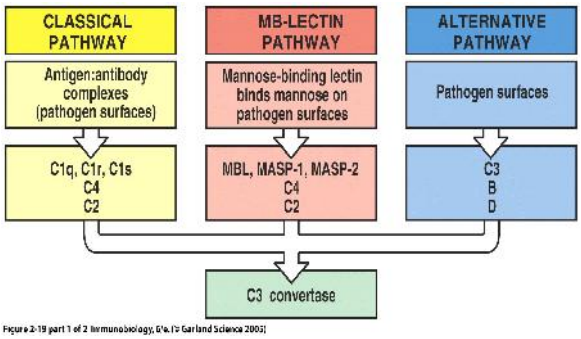
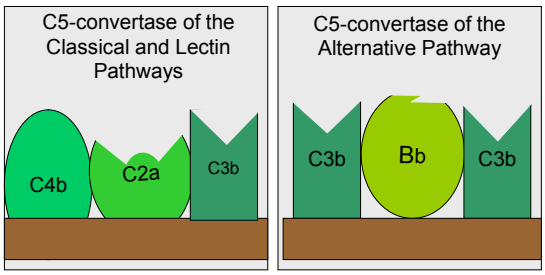


Figure 2-19 part 1 of 2 Immunobiology, 6/e, © Garland Science 2005

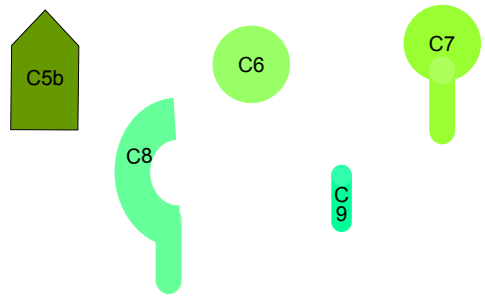
C5-convertase of the two pathways



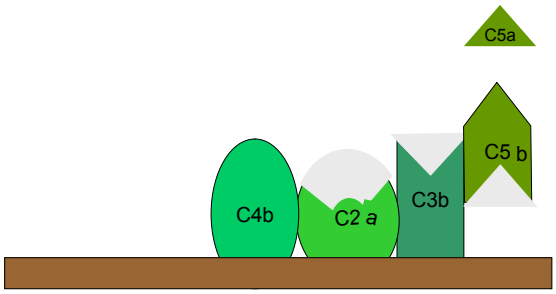
Generation of C5 convertase leads to the activation of the

Lytic pathway

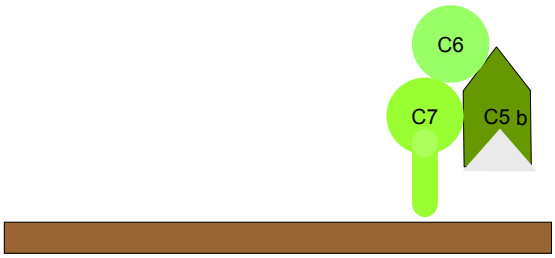
Components of the lytic pathway



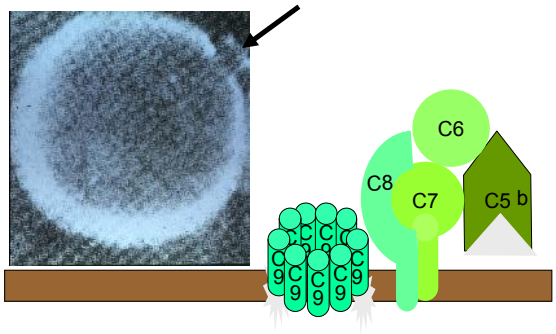
Lytic pathway
C5-activation

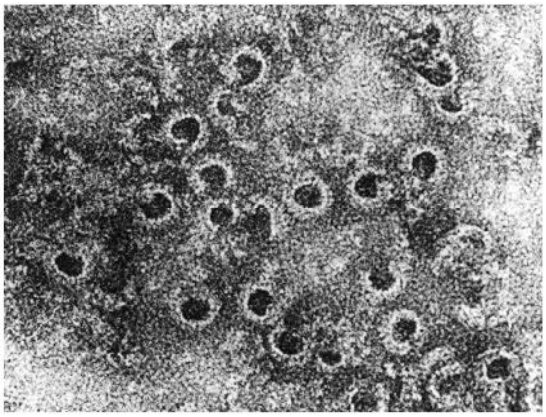


Lytic pathway
assembly of the lytic complex



Lytic pathway:
insertion of lytic complex into cell membrane



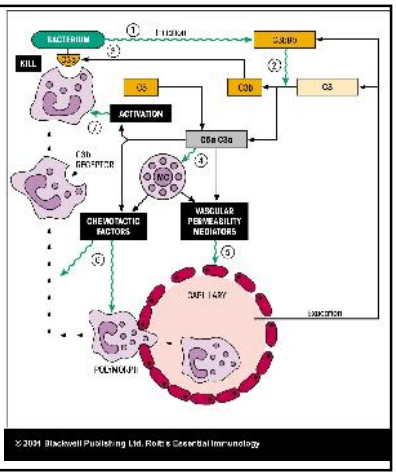


Biological Effects of Complement Components

- 1) Cell Lysis ---------- C5b-C9
- 2) Inflammation
  - Degranulation of mast cells/basophils --- C3a, C5a (C4a)
  - Chemotactic for leukocytes ---------- C3a, C5a
- 3) Opsonization ---------- C3b, **iC3b**
- 4) Solubilization and clearance of Immune complexes ---------- C3b

Overall Picture!!!!

1) Alternative Pathway
2) Amplification Step
3) Opsonization
4) Anaphylactic Factors
5) Vascular Effect
6) Cell Recruitment
7) Neutrophil Recruitment
8) Cell Activation



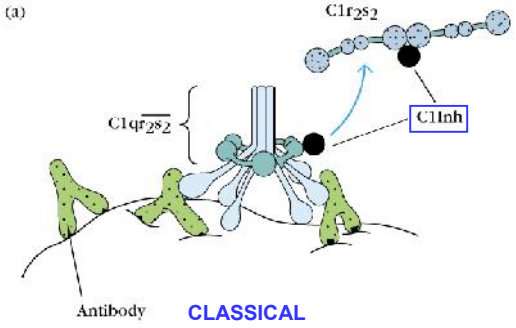
Regulation of Complement

TABLE 13-2 Proteins that regulate the complement system

Protein	Type of protein	Pathway affected	Immunologic function
C1 inhibitor (C1Inh)	Soluble	Classical	Serine protease inhibitor; causes C1r$_2$s$_2$ to dissociate from C1q
C4b-binding protein (C4bBP)*	Soluble	Classical and lectin	Blocks formation of C3 convertase by binding C4b; cofactor for cleavage of C4b by factor I
Factor H*	Soluble	Alternative	Blocks formation of C3 convertase by binding C3b; cofactor for cleavage of C3b by factor I
Complement-receptor type 1 (CR1)* Membrane-cofactor protein (MCP)*	Membrane bound	Classical, alternative, and lectin	Block formation of C3 convertase by binding C4b or C3b; cofactor for factor I–catalyzed cleavage of C4b or C3b; C3bBb
Decay-accelerating factor (DAF or CD55)*	Membrane bound	Classical, alternative, and lectin	Accelerates dissociation of C4b2a and C3bBb (classical and alternative C3 convertases)

*An RCA (regulator of complement activation) protein. In humans, all RCA proteins are encoded on chromosome 1 and contain short consensus repeats.

C1inh (C1 inhibitor) – removes C1r and C1s from the active C1 complex



CLASSICAL

-- Complement Regulatory Protein (CR1), Factor H, Membrane Cofactor Protein (MCP) and Decay-accelerating factor (DAF) → Target C3 convertase (C2b)

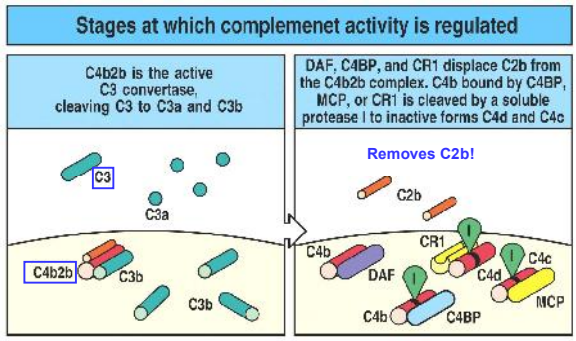


Figure 2-37 part 2 of 4 Immunobiology, 6/e, (c) Garland Science 2005]

CD59 and S protein – MAC complex

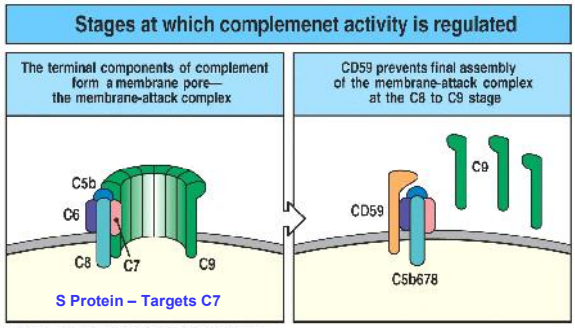


Figure 2-37 part 4 of 4 Immunobiology, 6/e, (c) Garland Science 2005]

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Factor H*	Soluble	Alternative	Blocks formation of C3 convertase by binding C3b; cofactor for cleavage of C3b by factor I
Complement receptor type 1 (CR1 or CD35)* Membrane-cofactor protein (MCP or CD46)*	Membrane bound	Classical, alternative, and lectin	Block formation of C3 convertase by binding C4b or C3b; cofactor for factor I–catalyzed cleavage of C4b or C3b
Decay-accelerating factor (DAF or CD55)*	Membrane bound	Classical, alternative, and lectin	Accelerates dissociation of C4b2a and C3bBb (classical and alternative C3 convertases)
Factor I	Soluble	Classical, alternative, and lectin	Serine protease; cleaves C4b or C3b using C4BP, CR1, factor H, DAF, or MCP as cofactor
S protein	Soluble	Terminal	Binds soluble C5b67 and prevents its insertion into cell membrane
Homologous restriction factor (HRF); also called membrane inhibitor of reactive lysis (MIRL or CD59)*	Membrane bound	Terminal	Bind to C5b678 on autologous cells, blocking binding of C9
Anaphylatoxin inactivator	Soluble	Effector	Inactivates anaphylatoxin activity of C3a, C4a, and C5a by carboxypeptidase N–catalyzed removal of C-terminal Arg

*An RCA (regulator of complement activation) protein. In humans, all RCA proteins are encoded on chromosome 1 and contain short consensus repeats.

Table 7-2
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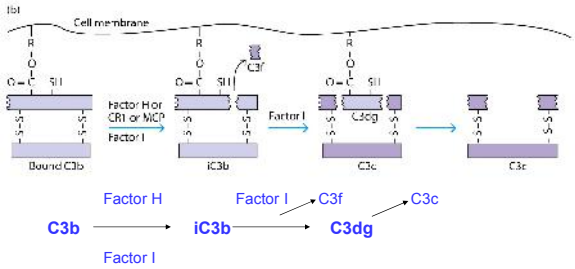
THE GOOD SIDE OF THE STORY: Factor H and Factor I → actively neutralize C3b generating smaller products (**iC3b**). However, if C3b binds to a surface this mechanism is less effective. The chances of C3b to bind to a surface are higher if hydrolysis occur near a cell surface (**Classical and Lectin Pathways**)



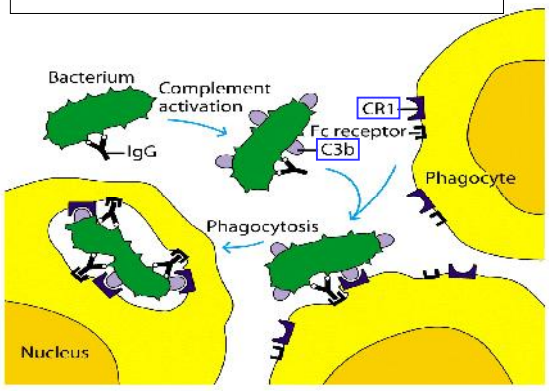
THE ALSO GOOD SIDE OF THE STORY: CR1 and Factor I

- C3b ---- Binds to CR1
- CR1 recruits Factor I (C3b protease)
- CR1 - C3b → iC3b → C3d (Factor I)
- **iC3b** – Opsonin; **C3d** – Antibody production

What is the point of all this?? …3 major functions of complement activation:

1. **Phagocytic cells have receptors for C3b (CR1) and iC3b (CR3, CR4). Phagocytosis of cells coated with C3b is enhanced. (i.e. C3b is an opsonin)**

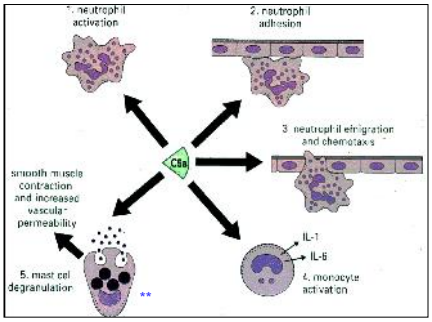
Opsonization and phagocytosis

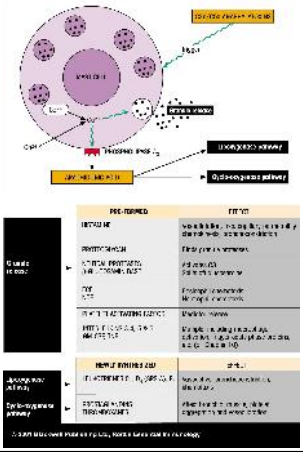


What is the point of all this?? …3 major functions of complement activation:

2. **C3a (and C5a) are anaphylatoxins. They bind to mast cells and basophils through specific receptors. They also act on macrophages, neutrophils, basophils and mast cells to promote chemotaxis of these cells (particularly neutrophils) to the site, degranulation and the respiratory burst. This creates a local inflammatory response that damages any pathogens in the vicinity (and also host tissue). (Cell degranulation)**

Biological effects of C5a





When activated, mast cells and basophils can release a large number of inflammatory mediators, and also produce leukotrienes, prostaglandins and thromboxanes.

These compounds contribute to the characteristic features of inflammatory responses:

Vasodilation – results in redness at the site

Increased capillary permeability results in swelling at the site.

Cytokines – IL-6, TNF-α

What is the point of all this?? …3 major functions of complement activation:

1. Phagocytic cells have receptors for C3b and iC3b. Phagocytosis of cells coated with C3b is enhanced. (I.e. C3b is an opsonin)
2. C3a (and C5a) are anaphylatoxins I.e. they act on macrophages, neutrophils, basophils and mast cells to promote chemotaxis of these cells (particularly neutrophils) to the site, degranulation and the respiratory burst. This creates a local inflammatory response that damages any pathogens in the vicinity (and also host tissue).
3. **Further enzyme reactions produce a complex (the membrane attack complex, MAC) that creates pores in the microbial cell membrane, resulting in lysis and death of the cell.**

TABLE 7-3 Summary of biological effects mediated by complement products

Effect	Complement product mediating*
Cell lysis	C5b-9, the membrane-attack complex (MAC)
Inflammatory response	
Degranulation of mast cells and basophils†	C3a, C4a, and C5a (anaphylatoxins)
Degranulation of eosinophils	C3a, C5a
Extravasation and chemotaxis of leukocytes at inflammatory site	C3a, C5a, C5b67
Aggregation of platelets	C3a, C5a
Inhibition of monocyte/macrophage migration and induction of their spreading	Bb
Release of neutrophils from bone marrow	C3c
Release of hydrolytic enzymes from neutrophils	C5a
Increased expression of complement receptors type 1 and 3 (CR1 and CR3) on neutrophils	C5a
Opsonization of particulate antigens, increasing their phagocytosis	C3b, C4b, iC3b
Viral neutralization	C3b, C5b–9 (MAC)
Solubilization and clearance of immune complexes	C3b

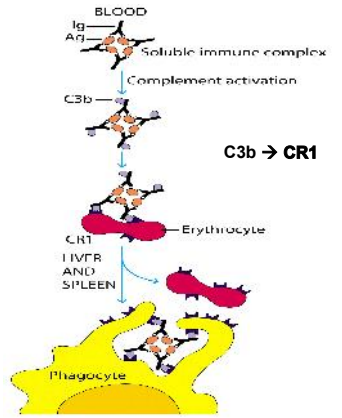
*Boldfaced component is most important in mediating indicated effect.
†Degranulation leads to release of histamine and other mediators that induce contraction of smooth muscle and increased permeability of vessels.

Table 7-1

What is the point of all this?? …3 major functions of complement activation:

3. Further enzyme reactions produce a complex (the membrane attack complex, MAC) that creates pores in the microbial cell membrane, resulting in lysis and death of the cell.

4. **Removal of inmmune complexes in the spleen or liver**
   Presence of CR1 on RBC



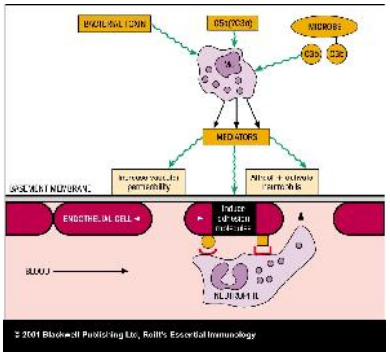


Figure 1.17

Complement Receptors

Receptors	Ligand	Cells
CR1 (CD35)	C3b, C4b	RBC – Phagocytes
CR2* (CD21)	C3d, iC3b	B cells*
• CR3	iC3b	Phagocytes, NK cells
• CR4	iC3b	Phagocytes, NK cells
• C3aR/C4aR	C3a, C4a	Mast cells, Basophils
• C5aR	C5a	Mast cells, Basophils, Phagocytes

TABLE 7-4 Complement-binding receptors

Receptor	Major ligands	Activity	Cellular distribution
CR1 (CD35)	C3b, C4b	Blocks formation of C3 convertase; binds immune complexes to cells	Erythrocytes, neutrophils, monocytes, macrophages, eosinophils, follicular dendritic cells, B cells, some T cells
CR2 (CD21)	C3d, C3dg,* iC3b	Part of B cell coreceptor; binds Epstein-Barr virus	B cells, follicular dendritic cells, some T cells
CR3 (CD11b/18) CR4 (CD11c/18)	iC3b	Bind cell adhesion molecules on neutrophils, facilitating their extravasation; bind immune complexes, enhancing their phagocytosis	Monocytes, macrophages, neutrophils, natural killer cells, some T cells
C3a/C4a receptor	C3a, C4a	Induces degranulation of mast cells and basophils	Mast cells, basophils, granulocytes
C5a receptor	C5a	Induces degranulation of mast cells and basophils	Mast cells, basophils, granulocytes, monocytes, macrophages, platelets, endothelial cells

*Cleavage of C3dg by serum proteases generates C3d and C3g.

Table 7-4
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Microbial Evasion

- **Gram negative bacteria**:
  - Long LPS
  - Outer membrane
  - Elastase (C3a and C5a are inactivated)
- **Gram positive bacteria**:
  - Peptidoglycan in cell wall
  - Capsule
- **Viruses, Bacteria, Parasites**
  - Incorporation or microbe production of regulatory components of the complement cascade

TABLE 7-5 Microbial evasion of complement-mediated damage

Microbial component	Mechanism of evasion	Examples
	GRAM NEGATIVE BACTERIA	
Long polysaccharide chains in cell wall LPS*	Side chains prevent insertion of MAC into bacterial membrane*	Resistant strains of *E. coli* and *Salmonella*
Outer membrane protein	MAC interacts with membrane protein and fails to insert into bacterial membrane	Resistant strains of *Neisseria gonorrhoeae*
Elastase	Anaphylatoxins C3a and C5a are inactivated by microbial elastase	*Pseudomonas aeruginosa*
	GRAM POSITIVE BACTERIA	
Peptidoglycan layer of cell wall	Insertion of MAC into bacterial membrane is prevented by thick layer of peptidoglycan	*Streptococcus*
Bacterial capsule	Capsule provides physical barrier between C3b deposited on bacterial membrane and CR1 on phagocytic cells*	*Streptococcus pneumoniae*
	OTHER MICROBES	
Proteins that mimic complement regulatory proteins	Protein present in various bacteria, viruses, fungi, and protozoans inhibit the complement cascade	Vaccinia virus, herpes simplex, Epstein-Barr virus, *Trypanosoma cruzi*, *Candida albicans*

*LPS = lipopolysaccharide; MAC = membrane-attack complex; CR1 = complement receptor type 1.

Table 7-5
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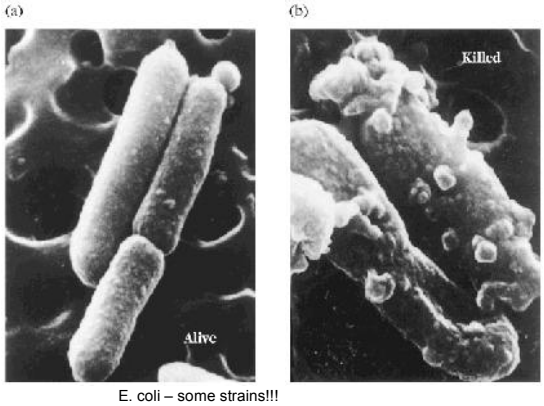
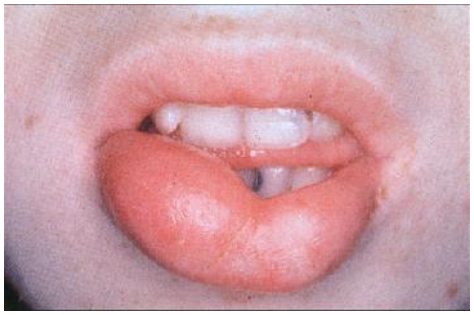


E. coli – some strains!!!

Kuby Figure 13-11

C1-inhibitor deficiency:
angioedema

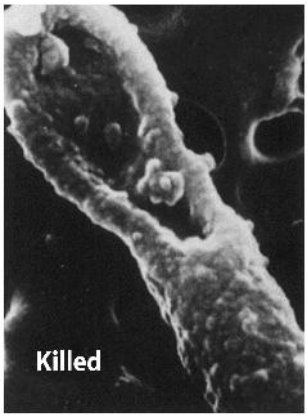


Deficiencies:

- **Systemic lupus erythomatosus (SLE)** is an autoimmune disease that results in tissue damage due to complement activation by Ag-Ab complexes
- C1, C2, C4 and CR1 predispose to SLE
- Lack of C1q or C4 results in 90% of SLE
- Deficiencies in C1q, C1r, C1s, C2 or C4 results in low levels of C3b required for clearance of Ag-Ab complexes (glomerulonephritis, vasculitis)

Deficiencies:

- Deficiency in C3 → Severe bacterial infections with *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*.
- Deficiency in C5, C6, C7, C8 or C9 results in high risk for bacterial meningitis caused by *Neisseria meningitidis*,



The End