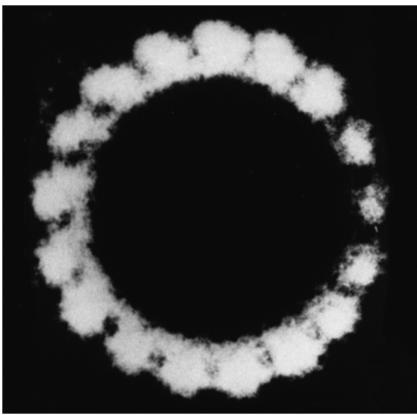


Chapter 13

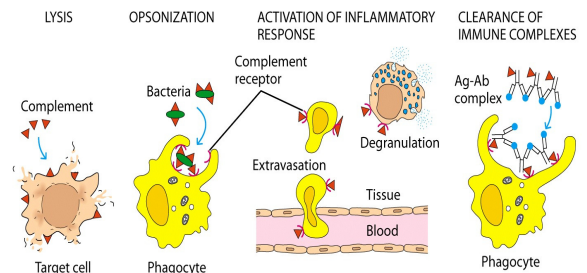
Complement

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

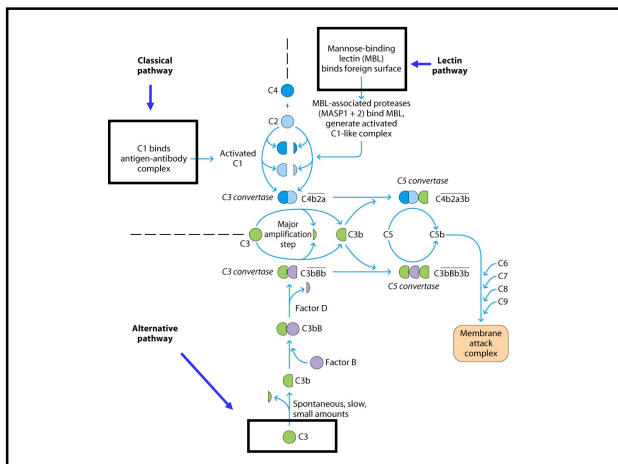
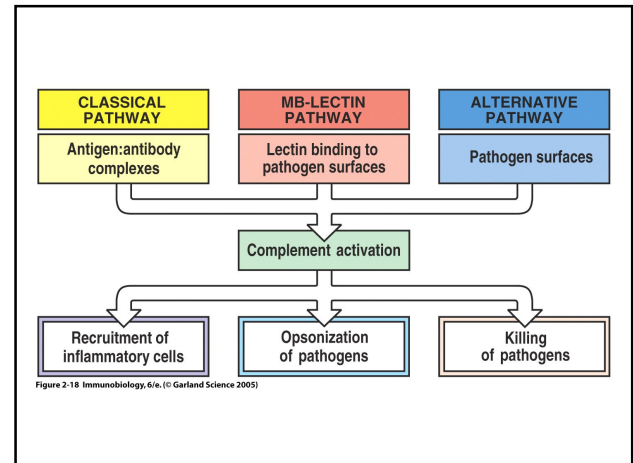
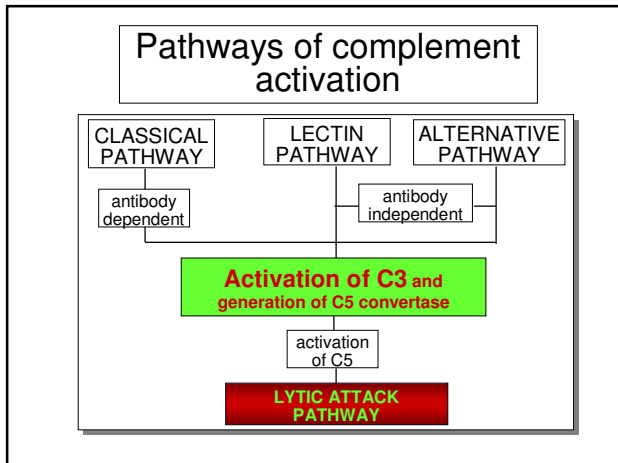


Complement Components

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

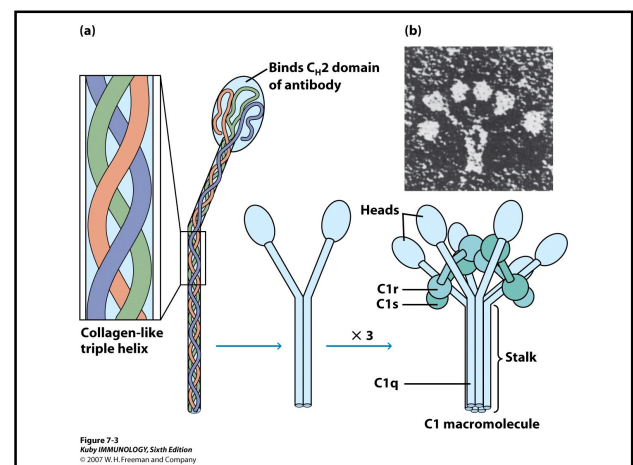
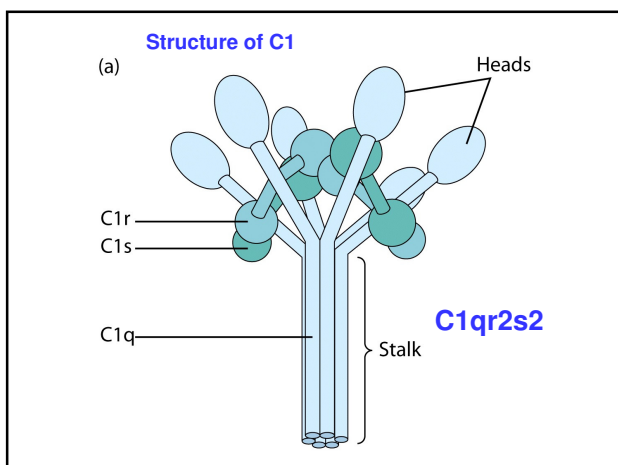
Complement Pathways

- 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 lectin mannose-binding lectin to the surface of pathogens.

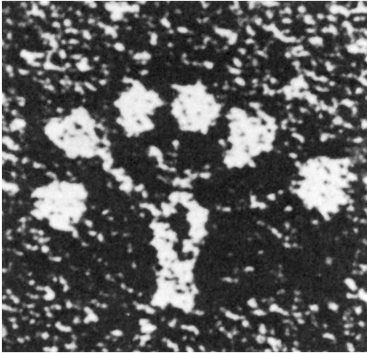


1. Classical Pathway

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

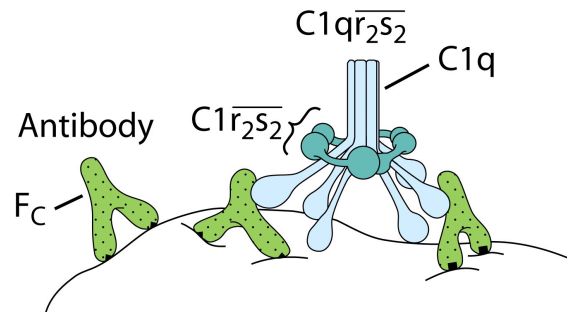


Classical Pathway



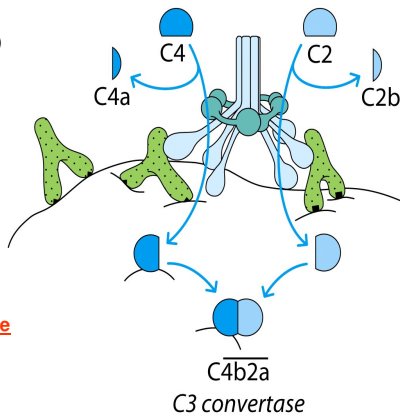
- C1q
- 2 molecules of C1r
- 2 molecules of C1s

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

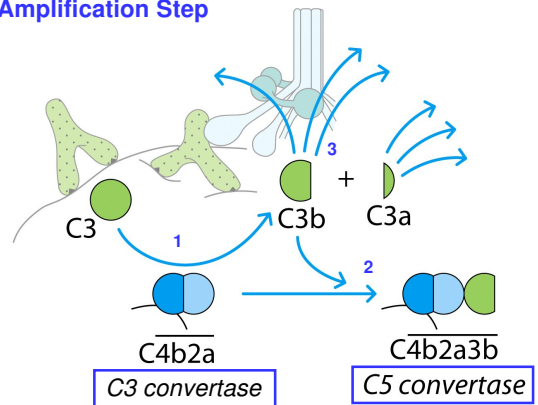


- C1 complex (C1s) hydrolysis C4, resulting in C4a and C4b. C4b binds to the cell surface

- C1 complex (C1s) hydrolysis C2 resulting in C2b (small) and C2a which binds to C4b to form the **C3 convertase**

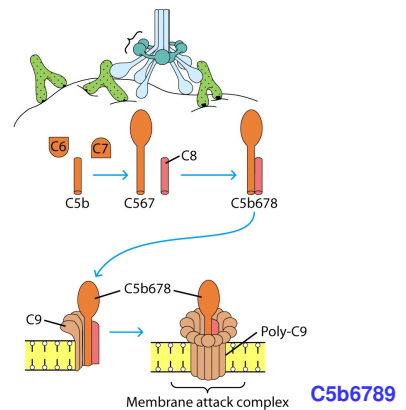
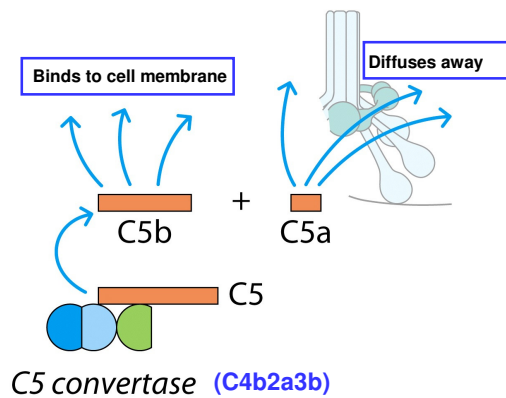


Amplification Step



Binds to cell membrane

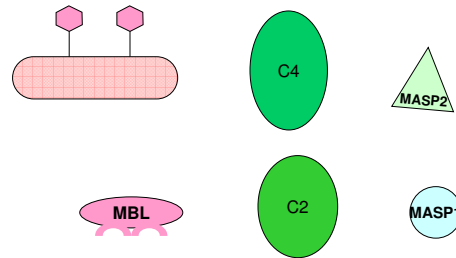
Diffuses away



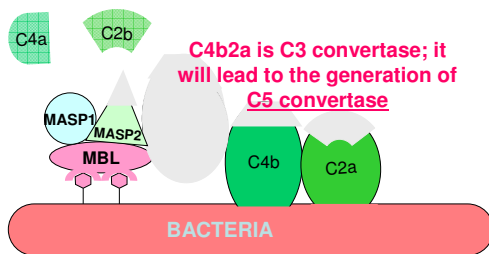
2. Lectin Pathway

- **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

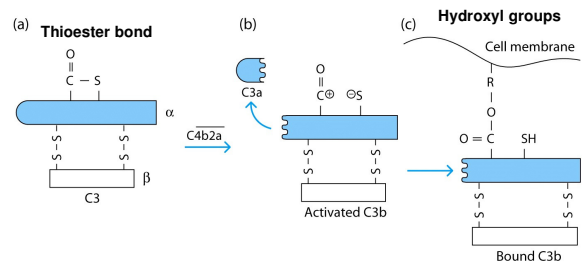
TABLE 7-1 Initiators of the alternative pathway of complement activation

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)	
NONPATHOGENS	
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
Ruby IMMUNOLOGY, Sixth Edition
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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, longer half life



- C3b has a short half life, unless it binds to a cell surface
- Mammalian cells have **high sialic acid content** – Leads to **inactivation**
- Bacteria, fungi cell – possess **low sialic acid** – C3b bound remains **active**

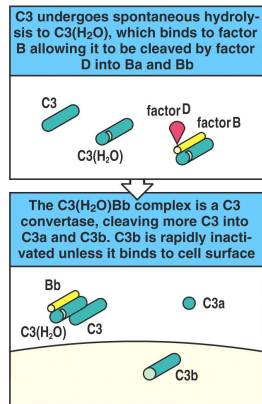
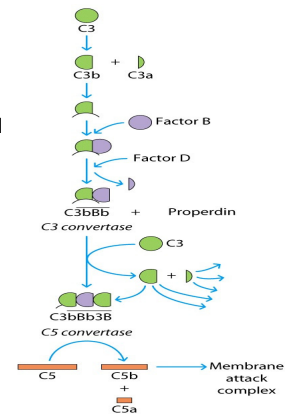


Figure 2-26 part 1 of 3 Immunobiology, 6/e, (© Garland Science 2005)

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



Summary

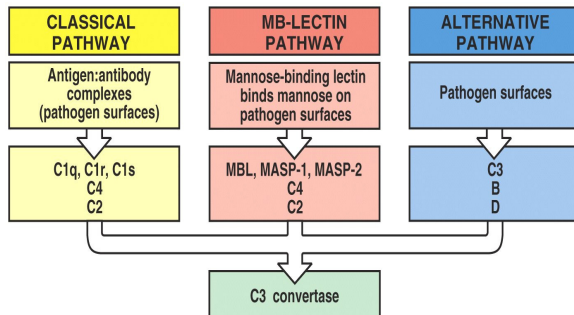
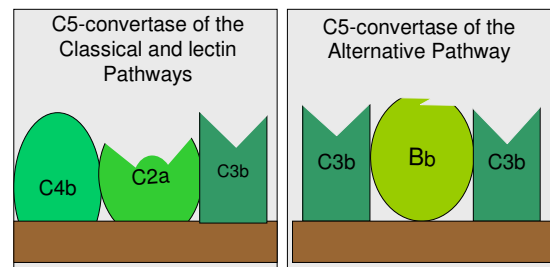


Figure 2-19 part 1 of 2 Immunobiology, 6/e, (© Garland Science 2005)

C5-convertase of the two pathways

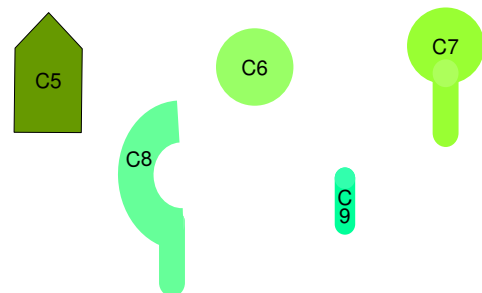


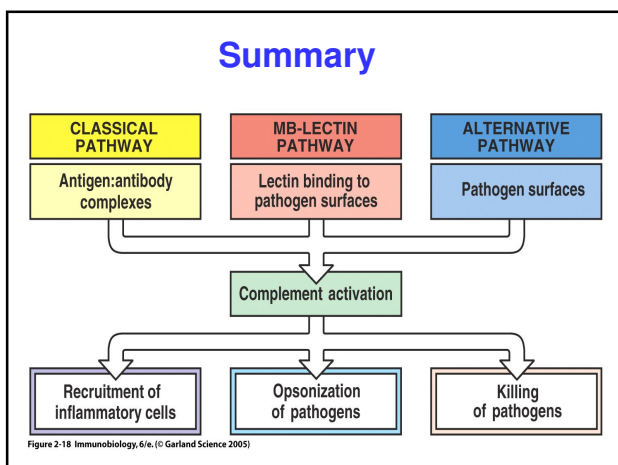
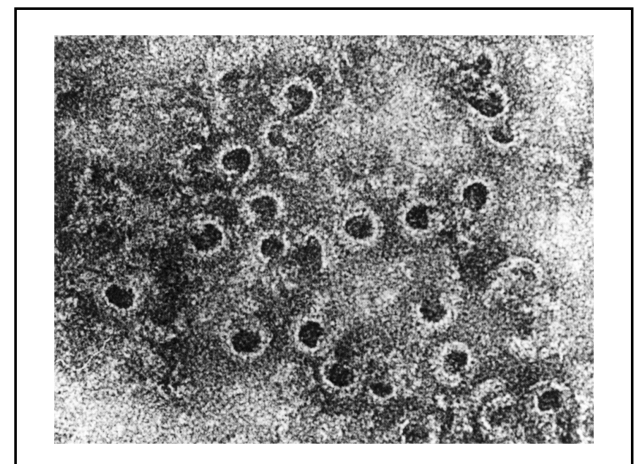
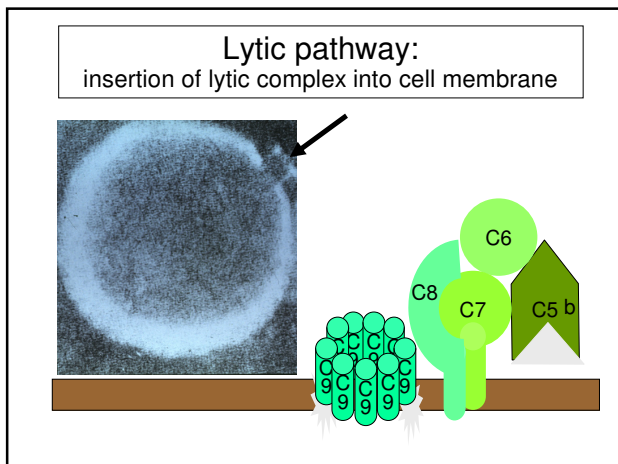
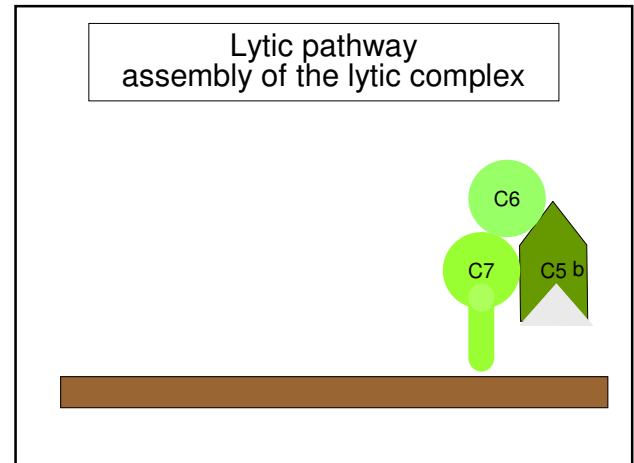
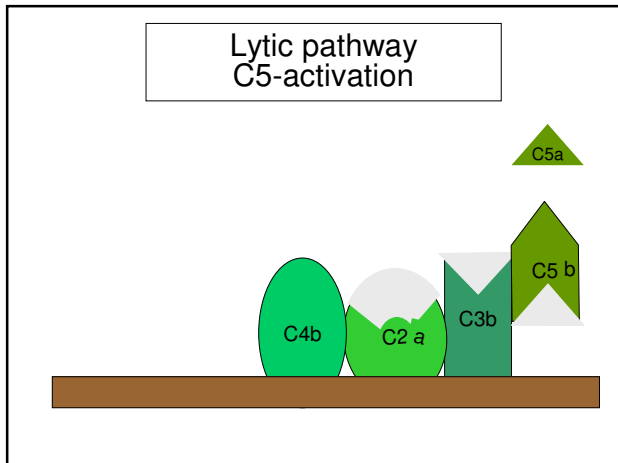
Lytic pathway

Generation of C5 convertase leads to the activation of the

Lytic pathway

Components of the lytic pathway

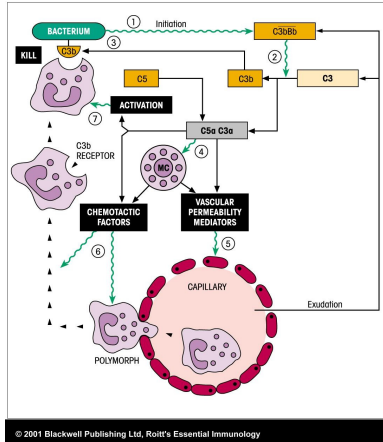




- Biological Effects of Complement Components**
- 1) Cell Lysis ----- C5b-C9
 - 2) Inflammation
 - Degranulation of mast cells/basophils --- C3a, C5a (C4a)
 - Chemotactic for leukocytes -----C3a, C5a
 - 3) Opsonization ----- C3b, iC3b
 - 4) Solubilization and clearance of Immune complexes ----- C3b

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



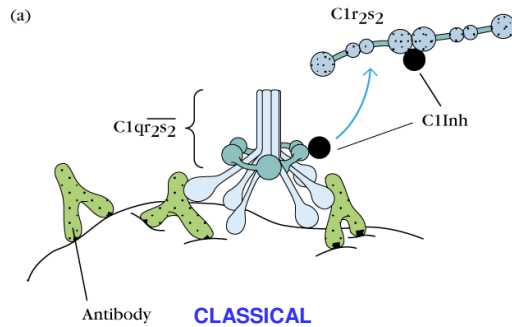
Regulation of Complement

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 (C4BP)*	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-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)

*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



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

Stages at which complement activity is regulated

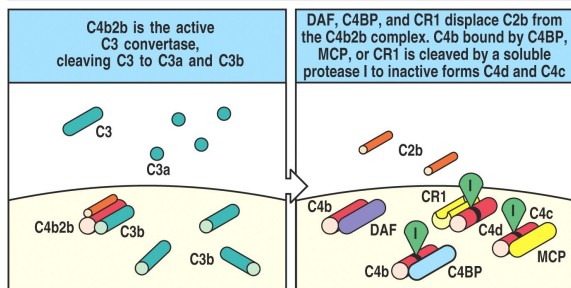
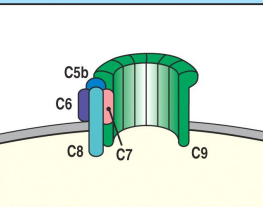


Figure 2-37 part 2 of 4 Immunobiology, 6/e. (© Garland Science 2005)

CD59 and S protein – MAC complex

Stages at which complement activity is regulated

The terminal components of complement form a membrane pore—the membrane-attack complex



CD59 prevents final assembly of the membrane-attack complex at the C8 to C9 stage

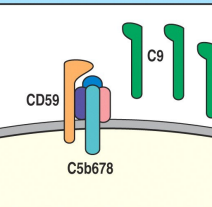


Figure 2-37 part 4 of 4 Immunobiology, 6/e. (© Garland Science 2005)

Regulatory proteins of the classical and alternative pathways

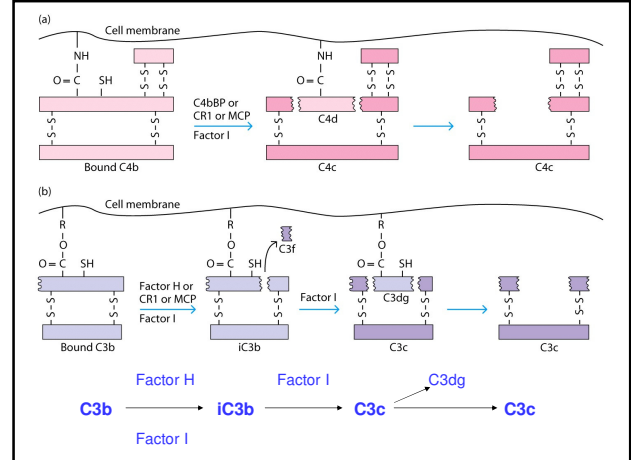
Name (symbol)	Role in the regulation of complement activation
C1 inhibitor (C1INH)	Binds to activated C1r, C1s, removing them from C1q
C4-binding protein (C4BP)	Binds C4b, displacing C2b; cofactor for C4b cleavage by I
Complement receptor 1 (CR1)	Binds C4b, displacing C2b, or C3b displacing Bb; cofactor for I
Factor H (H)	Binds C3b, displacing Bb; cofactor for I
Factor I (I)	Serine protease that cleaves C3b and C4b; aided by H, MCP, C4BP, or CR1
Decay-accelerating factor (DAF)	Membrane protein that displaces Bb from C3b and C2b from C4b
Membrane cofactor protein (MCP)	Membrane protein that promotes C3b and C4b inactivation by I
CD59 (protectin)	Prevents formation of membrane-attack complex on autologous or allogenic cells. Widely expressed on membranes

Figure 2-36 Immunobiology, 6/e. (© Garland Science 2005)

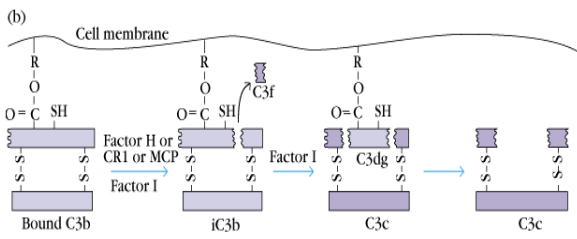
TABLE 7-2 Proteins that regulate the complement system			
Protein	Type of protein	Pathway affected	Immunologic function
C1 inhibitor (C1inh)	Soluble	Classical	Serine protease inhibitor; causes C1r ₂ s ₂ to dissociate from C1q
C4b-binding protein (C4bBP)*	Soluble	Classical and lectin	Blocks formation 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 or CD35)*	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 C4bBP, CR1, factor H, DAF, or MCP as cofactor
S protein	Soluble	Terminal	Binds soluble C5b7 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 C5b67 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 binds to a surface are higher if hydrolysis occur near a cell surface (Classical and Lectin Pathways)



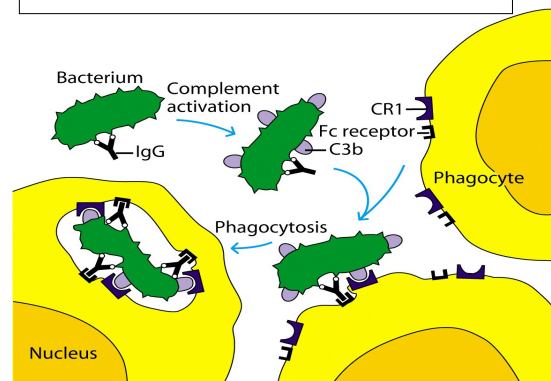
THE ALSO GOOD SIDE OF THE STORY: CR1 and Factor I

- C3b ---- Binds to CR1
- CR1 recruits Factor I (C3b protease)
- CR1 - C3b → iC3b → C3d
- 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)

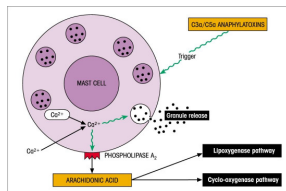
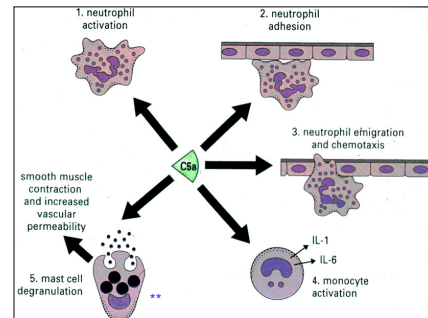
Opsonization and phagocytosis



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

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)
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).

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.

	PRE-FORMED	EFFECT
Granule release	HISTAMINE	Vasodilation, inc. capillary permeability, chemotaxis, bronchoconstriction
	TRYPTASE	Blood vessel proteases
	CHYMASE	Activates C3
	NEUTRAL PROTEASES	Activates C3
	ELASTASE	Activates C3
Cyclo-oxygenase pathway	PROSTAGLANDINS	Mediator release
	LEUKOTRIENES	Multiple, including macrophage activation, trigger acute phase proteins, etc. (cf. Chapter 10)

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.

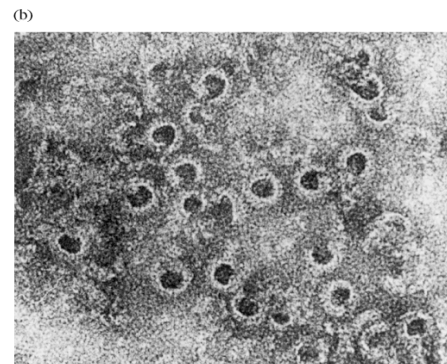
TABLE 7-3 Summary of biological effects mediated by complement products

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 7-3
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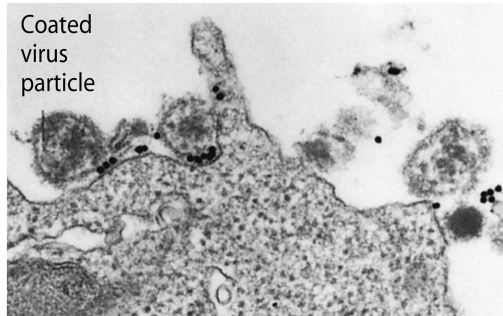


Lesions on erythrocyte membrane

Kuby Figure 13-8b

Antibody + Complement

- C3b → Viral aggregation
- Cell lysis of enveloped viruses



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 immune complexes in the spleen or liver

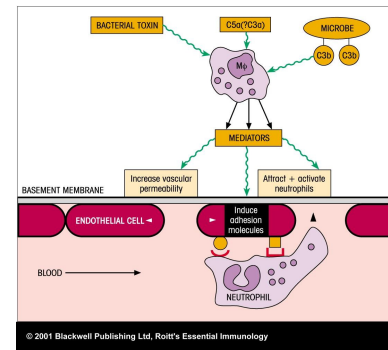
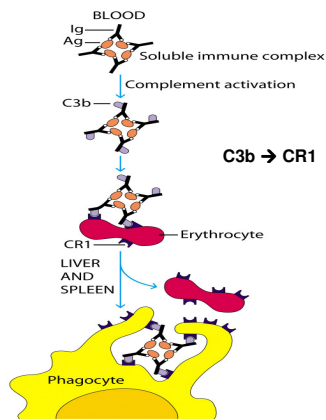


Figure 1.17

Complement Receptors

Receptors	Ligand	Cells
• CR1	C3b	RBC – Phagocytes
• CR2*	C3d, iC3b	B cells*
• CR3	iC3b	Phagocytes, NK cells
• CR4	iC3b	Phagocytes, NK cells
• C3a/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)	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
CR4 (CD11c/18)			
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 and Bacteria**
 - Incorporation or microbe production of regulatory components of the complement cascade

TABLE 7-5 Microbial evasion of complement-mediated damage

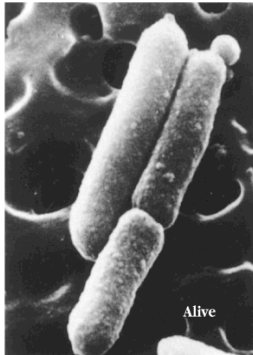
Microbial component	Mechanism of evasion	Examples
GRAM-NEGATIVE BACTERIA		
Long polysaccharide chains in cell wall LPS*	Side chains prevent insertion of MAC into bacterial membrane*	Resistant strains of <i>E. coli</i> and <i>Salmonella</i>
Outer membrane protein	MAC interacts with membrane protein and fails to insert into bacterial membrane	Resistant strains of <i>Neisseria gonorrhoeae</i>
Elastase	Anaphylatoxins C3a and C5a are inactivated by microbial elastase	<i>Pseudomonas aeruginosa</i>
GRAM-POSITIVE BACTERIA		
Peptidoglycan layer of cell wall	Insertion of MAC into bacterial membrane is prevented by thick layer of peptidoglycan	<i>Streptococcus</i>
Bacterial capsule	Capsule provides physical barrier between C3b deposited on bacterial membrane and CR1 on phagocytic cells*	<i>Streptococcus pneumoniae</i>
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, <i>Trypanosoma cruzi</i> , <i>Candida albicans</i>

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

Table 7-5

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(a)



E. coli – some strains!!!

(b)



Kuby Figure 13-11

C1-inhibitor deficiency: angioedema

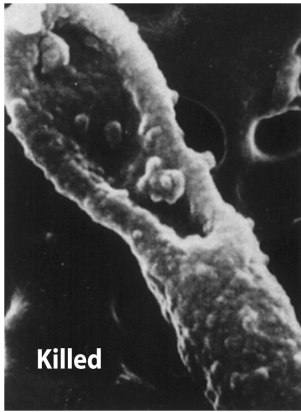


Deficiencies:

- **Systemic lupus erythematosus (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 C1 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 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