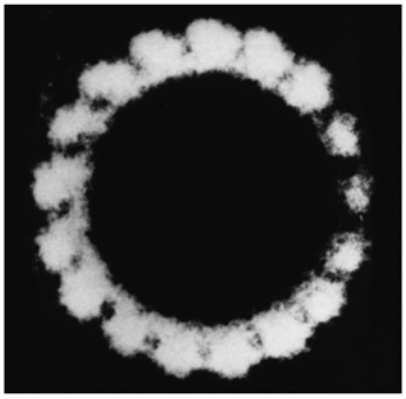
Chapter 13

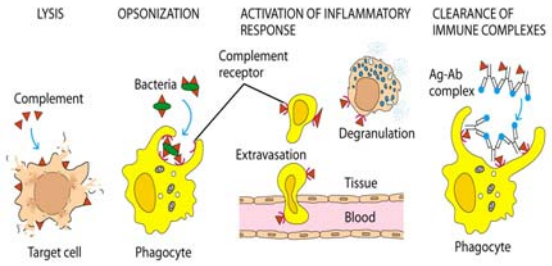
Complement

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

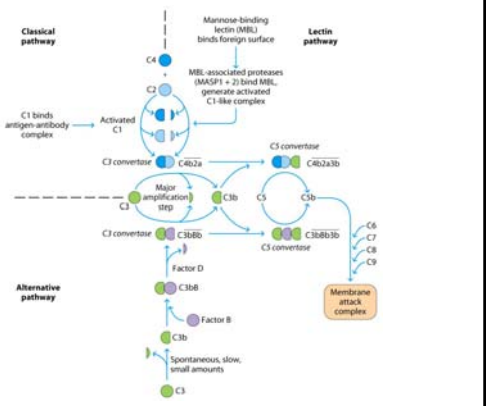


Complement Components

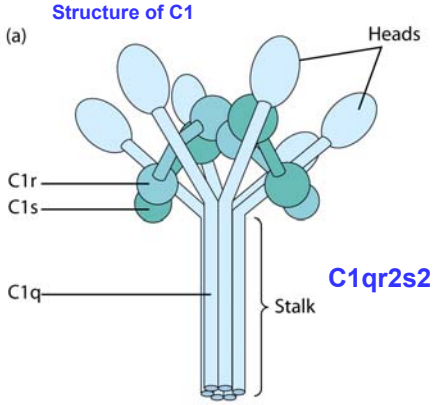
- Over 20 serum proteins
- Cascade fashion
- Components designated: C1 – C9
- Proteolysis results in: Large fragments "b" and smaller fragments "a" → C3a, C5b
- The **only exception** is C2, where C2a is the large fragment and C2b is the smaller fragment and diffuses away

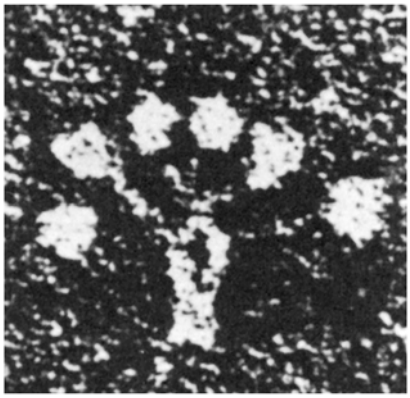
Complement Pathways

- 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.



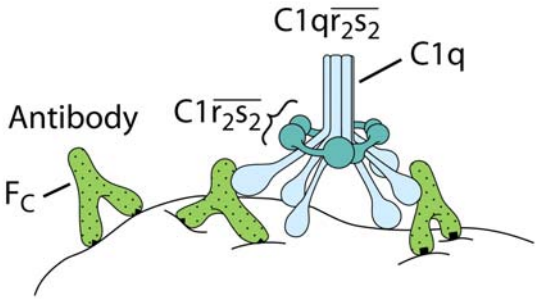
Structure of C1





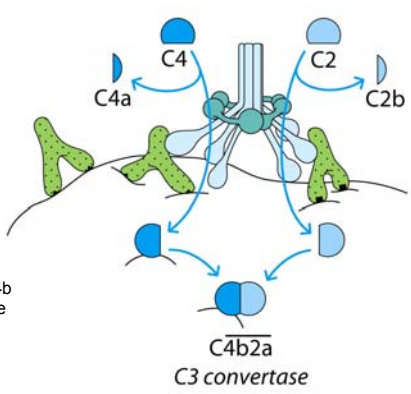
- C1q
- 2 molecules of C1r
- 2 molecules of C1s

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

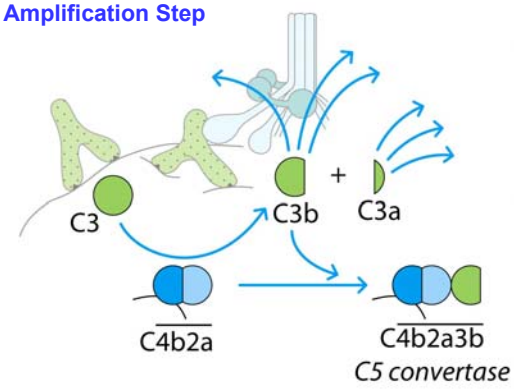


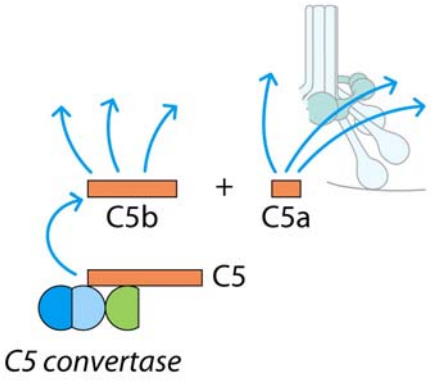
C1complex (C1s) hydrolysis C4, resulting in C4a and C4b. C4b binds to the cell surface

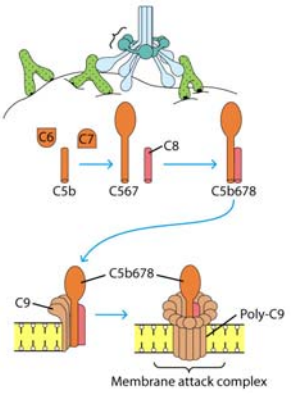
C1 complex (c1s) hydrolysis C2 resulting in C2b (small) and C2a which binds to C4b on the cell surface to form the **C3 convertase (C4b2a)**



Amplification Step







- **ALTERNATIVE PATHWAY**

- **C3 in serum undergoes spontaneous hydrolysis → C3a, C3b**
- **The half life of these products is very short, except…**
- **C3b can bind to host and bacterial cell surfaces**
- **Mammals have high levels of sialic acid → inactivation of C3b**
- **This is not the case in bacteria, longer half life**

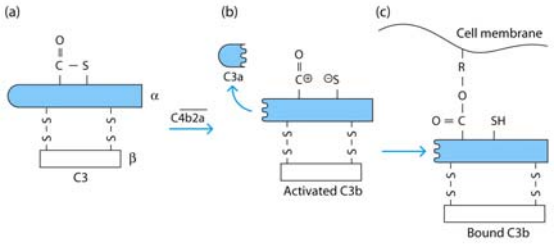


TABLE 13-1 Initiators of the alternative pathway of complement activation

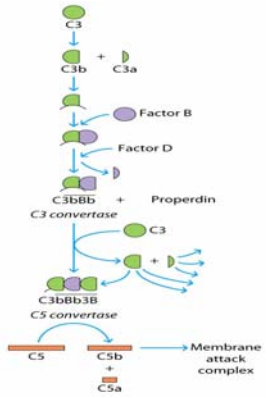
PATHOGENS AND PARTICLES OF MICROBIAL ORIGIN
Many strains of gram-negative bacteria
Lipopolysaccharides from gram-negative bacteria
Many strains of gram-positive bacteria
Teichoic acid from gram-positive cell walls
Fungal and yeast cell walls (zymosan)
Some viruses and virus-infected cells
Some tumor cells (Raji)
Parasites (trypanosomes)

TABLE 13-1 Initiators of the alternative pathway of complement activation

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)

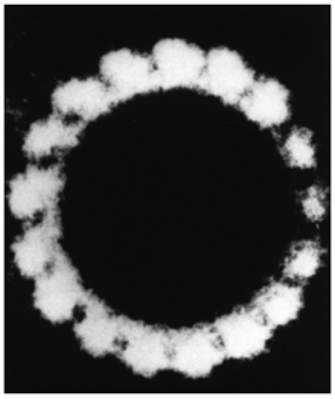
SOURCE: Adapted from M. K. Pangburn, 1986, in *Immunobiology of the Complement System*, Academic Press.

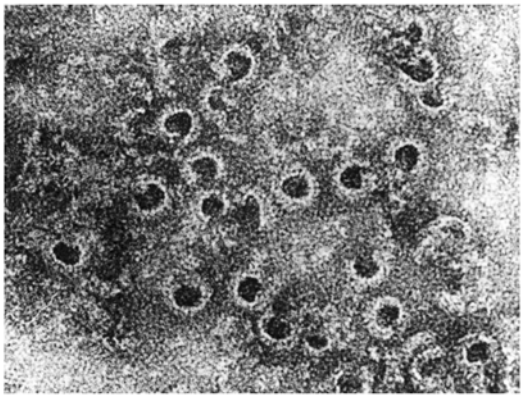
- C3b bound to cell surfaces is stabilized by Factor B
- Factor D cleaves C3bB and generates large fragment (Bb) and small fragment (Ba)
- C3bBb is the C3 convertase



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 of C1q to 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 very similar to C1r and C1s





Biological Effects of Complement Components

- 1) Cell Lysis ---------- C5-C9
- 2) Inflammation
  - Degranulation of mast cells/basophils ----C3a, C4a, C5a
  - Chemotactic for leukocytes ----------C3a, C5a
- 3) Opsonization ---------- C3b, iC3b
- 4) Solubilization and clearance of Immune complexes ---------- C3b

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 $C1r_2s_2$ 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 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
Membrane-cofactor protein (MCP)*			
Decay-accelerating factor (DAE 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.

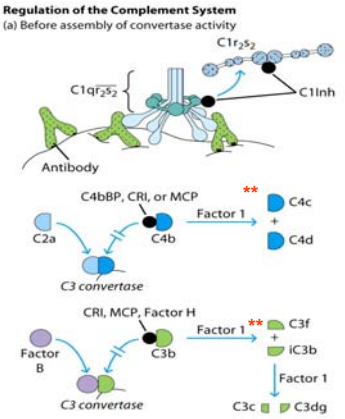
TABLE 13-2 Proteins that regulate the complement system

Protein	Type of protein	Pathway affected	Immunologic function
Factor-I	Soluble	Classical, alternative, and lectin	Serine protease: cleaves C4b or C3b using C4bBP, CR1, factor H, DAE, or MCP as cofactor
S protein	Soluble	Terminal	Binds soluble C5b67 and prevents its insertion into cell membrane
Homologous restriction factor (HRF) 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 removal of C-terminal Arg

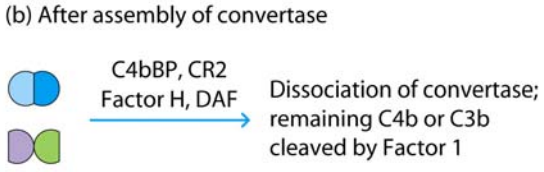
*An RCA (regulator of complement activation) protein. In humans, all RCA proteins are encoded on chromosome 1 and contain short consensus repeats.

I. EARLY

1. **C1inh → Classical → Dissociation of C1r2S2**
2. **C4BP → Classical & Lectin → X C3 Convertase (C4 to C2)**
3. **MCP (Membrane-cofactor protein) → Classical, Lectin & Alternative → X C3 Convertase by binding to C4b or C3b**

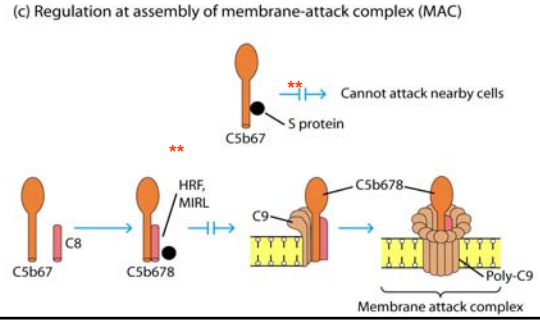


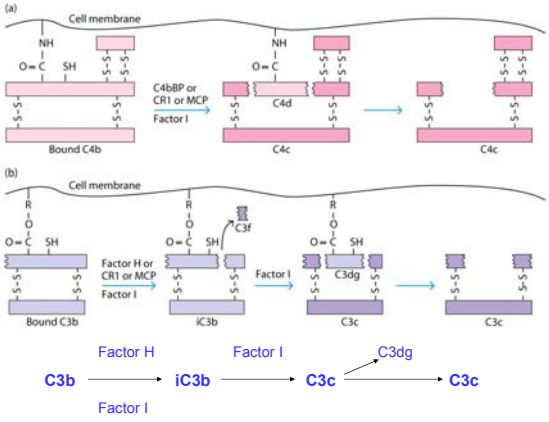
II. DAF (Decay Accelerating Factor) → Classical, Alternative & Lectin → Accelerates dissociation of C3 convertase (C4b2a, C3bBb)



III. LATE: S protein → binds to C5b67 and prevents insertion into cell membrane

HRF (Homologous restriction factor) → binds to C5b678 and block binding of C9





Biological Effects of Complement Components

- 1) Cell Lysis ---------- C5-C9
- 2) Inflammation
  - Degranulation of mast cells/basophils ----C3a, C4a, C5a
  - Chemotactic for leukocytes ----------C3a, C5a
- 3) Opsonization ---------- C3b, iC3b
- 4) Solubilization and clearance of Immune complexes ---------- C3b

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)**

1. **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).**

2. **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 13-3 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.

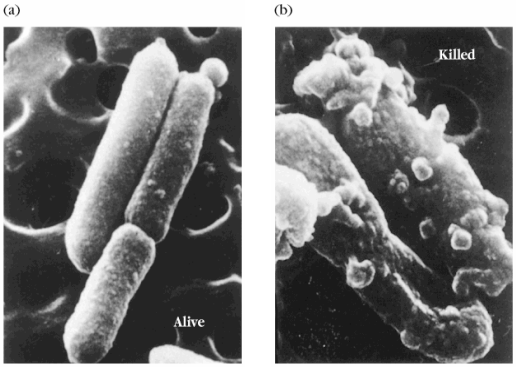
Complement Receptors

Receptors	Ligand	Cells
• CR1	C3b	RBC – Phagocytes
• CR2*	C3d, iC3b	B cells*
• CR3	iC3b	Phagocytes, NK cells
• CR4	iC3b	Phagocytes, NK cells
• C3a/C4a	C3a, C4a	Mast cells, Basophils
• C5a	C5a	Mast cells, Basophils, Phagocytes

TABLE 13-4 Complement-binding receptors

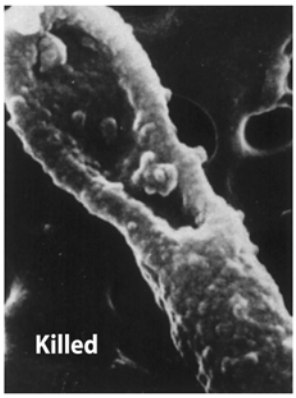
Receptor	Major ligands	Activity	Cellular distribution
CR1 (CD35)	C3b, C4b	Blocks formation of C3 convertase; binds immune complexes to cells	Erythrocytes, neutrophils, monocytes, macrophages, eosinophils, follicular dendritic cells, B cells, some T cells
CR2 (CD21)	C3d, C3dg,* iC3b	Part of B-cell coreceptor; 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.



E. coli

Kuby Figure 13-11



Microbial Evasion

- Gram negative bacteria:
  - Long LPS
  - Outer membrane
  - Elastase (C3a and C5a are inactivated)
- Gram positive bacteria:
  - Peptidoglycan in cell wall
  - Capsule

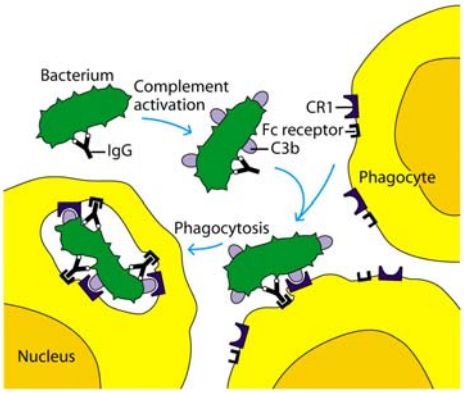
TABLE 13-5 Microbial evasion of complement-mediated damage

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 (C5b – 9); CR1 = type 1 complement receptor.

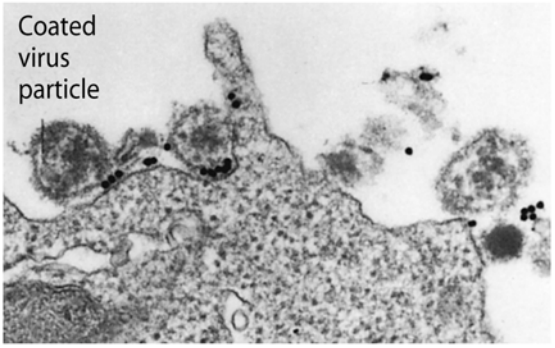
Deficiencies:

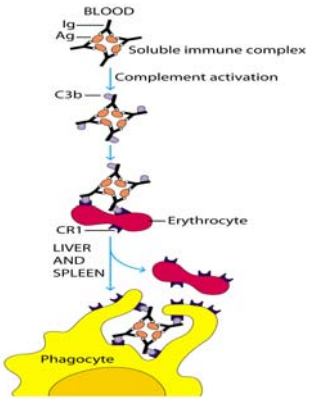
- **Systemic lupus erythomatosus (SLE)** is an autoimmune disease that results in tissue damage due to complement activation by Ag-Ab complexes
- C1, C2, C4 and CR1 predispose to SLE
- Lack of C4 results in 90% of SLE
- Deficiencies in C1, C2 and C4 results in low levels of C3b required for clearance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



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.**





C3bBb is usually rapidly inactivated, but it is stabilized if it comes into contact with microbial surfaces.

C3bBb bound to microbial surfaces has enzyme activity – it is a C3 convertase:

$$C3 \xrightarrow[C3bBb]{\text{(faster rate)}} C3b + C3a$$

The additional C3b formed by this enzyme binds to the microbial surface, where it is progressively broken into smaller fragments:

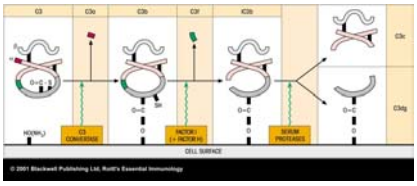
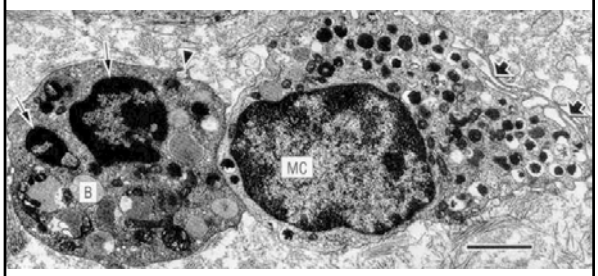


Figure 1.11

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

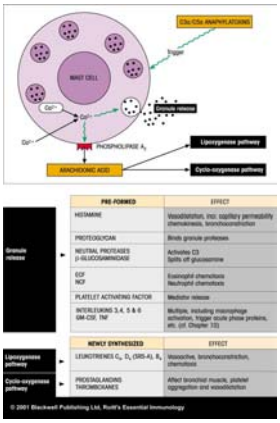
1. **Phagocytic cells have receptors for C3b (CR1) and iC3b (CR3, CR4). Phagocytosis of cells coated with C3b is enhanced. (i.e. C3b is an opsonin)**

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).**



A mast cell (MC) and basophil (B)

from Costa, JJ et al (1997) JAMA 278:1815-1822



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

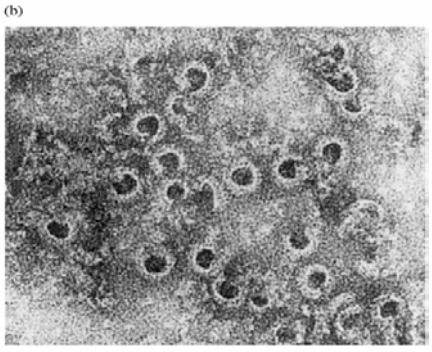
Increased capillary permeability results in swelling at the site.

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)**

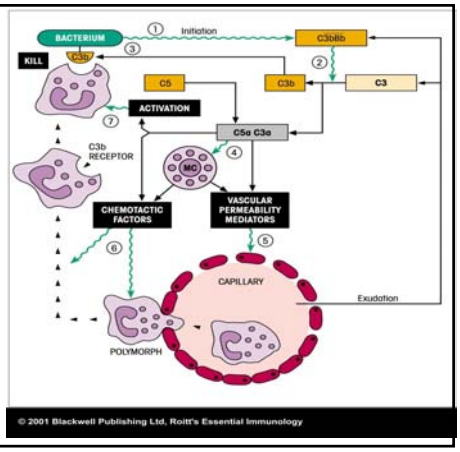
1. **C3a (and C5a) are anaphylatoxins I.e. they act on macrophages, neutrophils, basophils and mast cells to promote chemotaxis of these cells (particularly neutrophils) to the site, degranulation and the respiratory burst. This creates a local inflammatory response that damages any pathogens in the vicinity (and also host tissue).**

2. **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.**



Lesions on erythrocyte membrane

Kuby Figure 13-8b



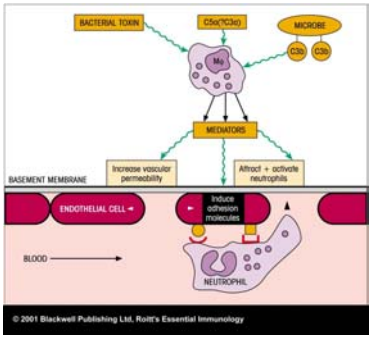


Figure 1.17