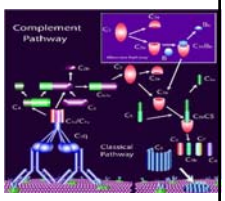
Foundations of Public Health Immunology

Complement



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Objectives

- Identify functions of complement
- Recognize the similarities and differences of the three complement pathways
- Identify important complement components & their functions
- Identify deleterious effects of complement activation and deficiency

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Complement (C')

- Series of approximately 30 heat-labile proteins
- Normally inactive in the serum
  - Inactive complement proteins known as zymogens
- Can be **sequentially activated** in a controlled sequence
  - Amplification of the reaction occurs at each step
- Production of **biologically active** fragments for lysis or killing of the target

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Complement Proteins

- Synthesized in the liver and by several cells types (splenic macrophages)
- Plays an **essential role in inflammation** and in facilitating antibody effectiveness
- Severe infections or autoimmune diseases can result from complement deficiencies (rare in the population)

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Functions of Complement

- Essential role in inflammation
- Assists antibodies in **effector functions** (Antibody Dependent Cell-mediated Cytotoxicity- ADCC)
- Assist in clearing immune complexes
- **Opsonization and facilitation of phagocytosis**
- *No antigen specificity*

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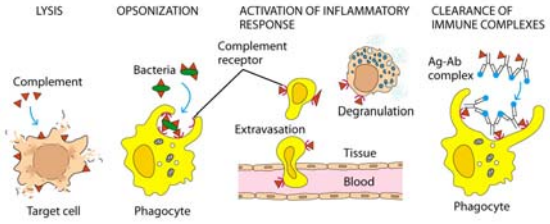
Functions of Complement



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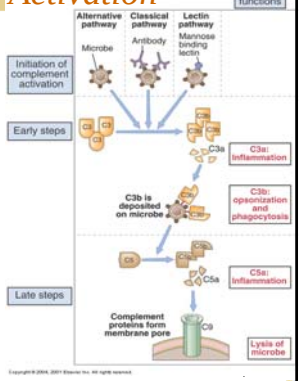
3 Pathways of Activation

- **Classical**
  - Triggered when IgM or certain IgG subclasses bind antigens
- **Alternative (Properdin)**
  - Triggered by the deposition of complement protein, C3b, onto microbial surfaces
  - **No antibodies required for activation**
- **Lectin**
  - Triggered by the attachment of plasma mannose-binding lectin (MBL) to microbes
  - **No antibodies required for activation**



Complement Activators

Activators of complement

	immunoglobulins	microorganisms: viruses	microorganisms: bacteria	microorganisms: other	other
classic pathway	complexes containing IgM, IgG1, IgG2 or IgG3	murine retroviruses, vesicular stomatitis virus	–	*Mycoplasma*	polyanions, esp. when bound to cations, PO_4^{2-} (DNA, lipid A, cardiolipin), SO_4^{2-} (dextran sulphate, heparin, chrondroitin sulphate
lectin pathway			many Gram-positive and Gram-negative organisms		arrays of terminal mannose gro
alternative pathway	complexes containing IgG, IgA or IgE (less efficient than the classic pathway)	some virus-infected cells (e.g. EBV)	many strains of Gram-positive and Gram-negative organisms	trypanosomes, *Leishmania*, many fungi	dextran sulphate, heterologous erythrocytes, carbohydrates (e.g. agarose)

Fig. 4.3 This table summarizes the activators of the classical lectin and alternative pathways.

Activators start the domino effect…

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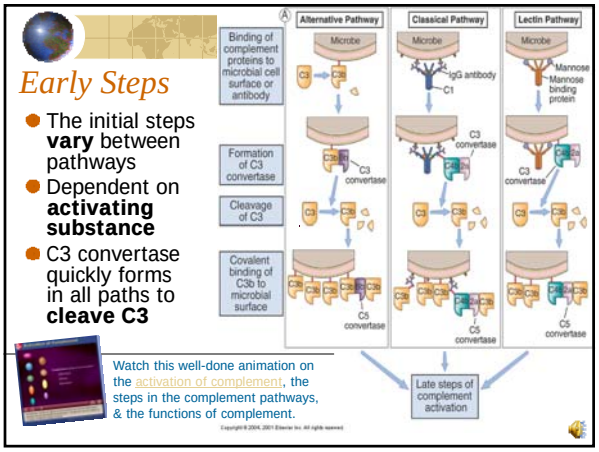
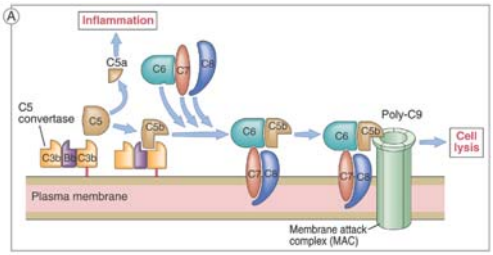
Early Steps

- The initial steps **vary** between pathways
- Dependent on **activating substance**
- C3 convertase quickly forms in all paths to **cleave C3**



Watch this well-done animation on the activation of complement, the steps in the complement pathways, & the functions of complement.

Late Steps



- Late steps (after C5 convertase) are **same in all pathways**
- Lead to formation of MAC

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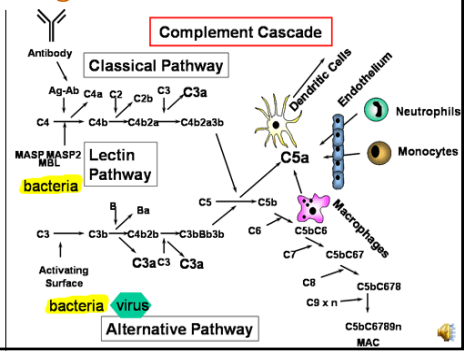
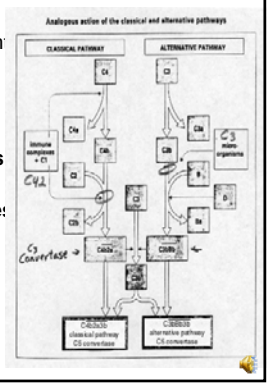
All Step Together…

Watch this professional animation on the Classical Complement Pathway which reviews all of the steps in the pathway.



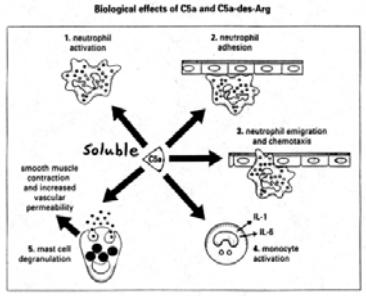
Important Complement Proteins

- C3
  - **Most abundant** complement protein
  - **Common to all three pathways**
  - a & b fragments have **important biologic effects**
- C3a
  - Anaphylatoxin that **promotes inflammation**
- C3b
  - Binds the microbial surface thus **acting as an opsonin**
  - C3b also a component of the C3 & C5 convertases



Important Complement Proteins

- C5a



anaphylatoxin
promote inflammation
C_{3a} - weaker

Fig. 4.18 C5a causes (1) neutrophil activation, (2) increased expression of adhesion molecules, (3) emigration of neutrophils and chemotaxis, (4) monocyte activation and (5) mast cell degranulation, which in turn causes smooth muscle contraction and increased vascular permeability.

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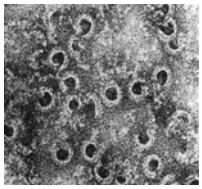
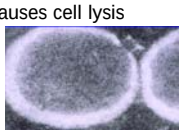
Important Complement Proteins

- C5b
  - **Initiates the late steps** of complement activation (Common Pathway)
  - C6, C7, C8, and C9 sequentially bind C5 in the Common Pathway
  - **C9 polymerizes to form the Membrane Attack Complex** (MAC) which forms a pore in the target cell and causes cell lysis



Complement membrane attack complexes (above) punch holes in the membranes of microbial invaders.



Another view of the membrane attack complex creating a pore in the cell membrane.

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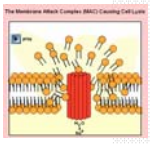
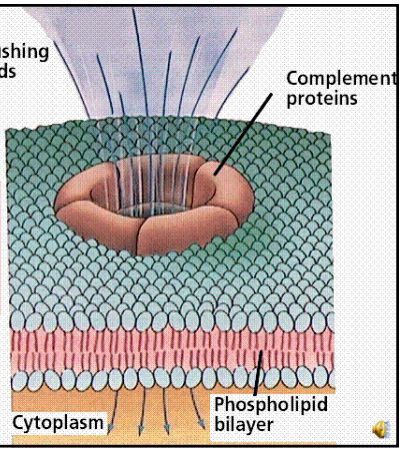
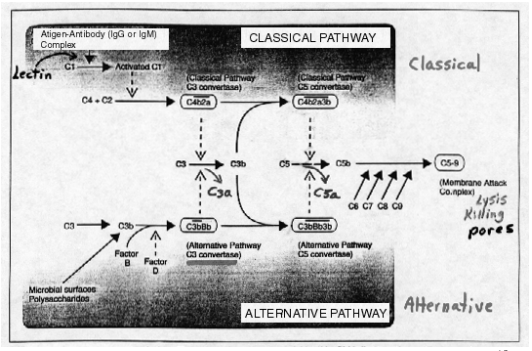
Membrane Attack Complex



Watch this short animation of the formation of the MAC & subsequent cell lysis.

Source: Gary Kaiser.





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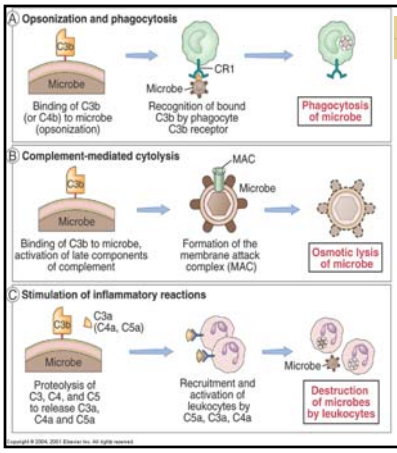

Functional Proteins

- Overview of how complement effects innate immunity & inflammation

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Complement Effects

- Lysis (destruction of target cell)
  - Membrane Attack Complex
  - **Antibody makes complement more efficient and guides complement deposition**
- Opsonization (enhanced phagocytosis)
  - Due to the formation of C3b and C5b (larger fragments than "a" fragments)
  - **C3b and C5b bind substrate**
  - **Attract phagocytes** to microbes coated with complement

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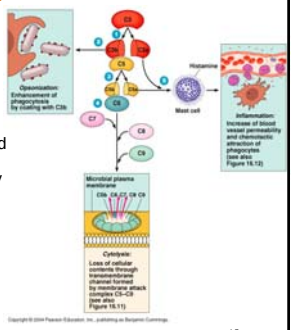
Complement Effects

- **Activation of the inflammatory response**
  - Anaphylatoxins C3a and C5a promote inflammation
  - C3a and C5a are freely soluble in solution
  - Increased cellular attraction and activation
  - Increased vascular permeability
- **Induction and enhancement of antibody response**
  - Complement receptors on APC and B lymphocytes enhance antigen presentation
  - Accessory role in antibody response



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Complement Effects

- Viral neutralization
- Clearance of immune complexes
  - Efficient removal from tissue by phagocytic cells
  - Solubilize immune complexes

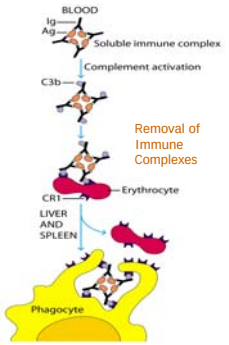

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TABLE 13-3 Summary of biological effects mediated by complement products

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.

Especially note the Complement Mediated Inflammatory Responses!!

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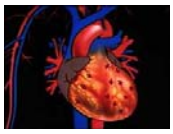
Deleterious Effects of Complement

- Systemic Activation
  - Triggered by Gram Negative organisms
  - Leads to septicemia, anaphylatoxins and shock
- Activation by unrelated tissue necrosis
  - Ischemia (myocardial infarction)



Myocardial ischemia is reduction of blood flow to the heart

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Regulation of Complement

- Tight regulation of complement system **necessary to prevent autoimmunity**
- Opsonization by binding to complement receptors on cells
- Recognition of "non-self" by **C3b** which doesn't bind to self or is **limited in formation**
- **C3 convertase enzyme also produces inhibition** of complement activity (feedback loop)

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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-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 $C4b\overline{2}a$ and $C3b\overline{Bb}$ (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 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.

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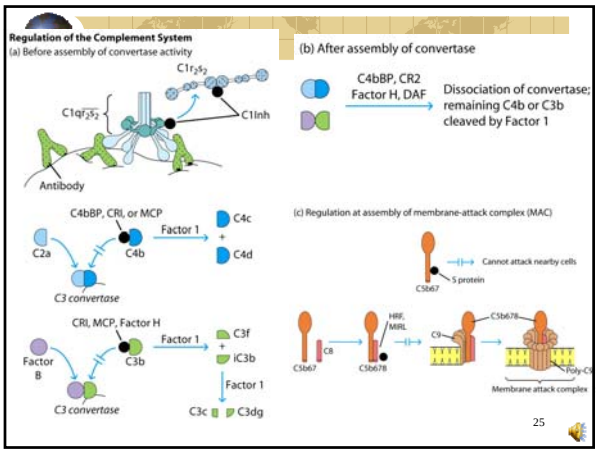
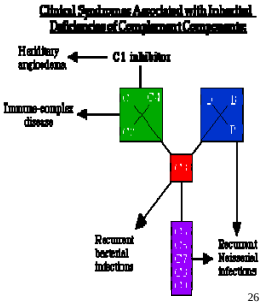
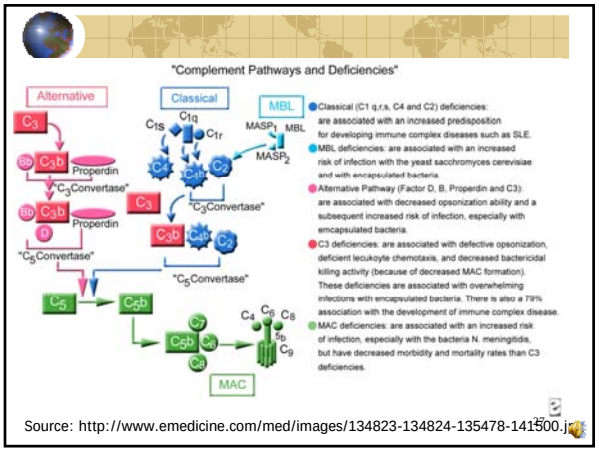


Complement Deficiencies

- Clinical symptoms are determined by the Complement Pathway affected
- **Can be acquired or inherited**
- No specific treatments
  - Antibiotics and immunizations used to reduce risk of disease





Source: http://www.emedicine.com/med/images/134823-134824-135478-141500.j

C2 & C4 Deficiencies

- C2 deficiency is most widely reported deficiency of all components in complement pathways
- Immune complex disorders are the main problem with a deficiency of C2
- Complete C4 deficiency is rare
- Almost all the patients with complete C4 deficiency have discoid or systemic lupus erythematosus (with or without associated glomerulonephritis)

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C3 Deficiencies

- C3 is central to all three complement pathways!!
- Usually rare and leads to an inability to form the membrane attack complex (MAC)
- Predisposes person to frequent bouts of pyogenic **bacterial infection**
  - Especially Gram-negative bacteria such as meningococci and pneumococci

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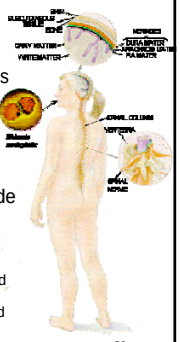
Meningococcal disease

- *Neisseria meningitidis* is the most frequently isolated pathogen from patients with bacterial meningitis
- Only humans can harbor *N. meningitidis*
- Susceptibility to meningococcal disease is highest in children aged 3-24 months
- Meningococcal meningitis occurs worldwide
  - Prevalent serotypes vary according to the geographic region
  - 'African Meningitis Belt' in sub-Saharan Africa
    - In 1996, Africa experienced the largest recorded outbreak of epidemic meningitis in history, with over 250,000 cases and 25,000 deaths recorded



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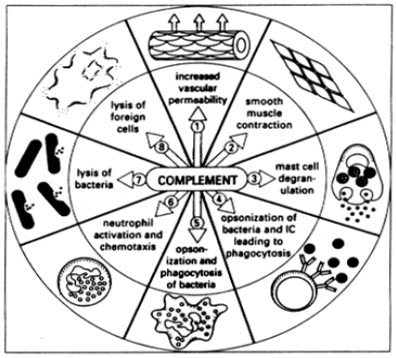
Summary of the actions of complement and its role in the acute inflammatory reaction



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In Summary

- Identify the similarities and differences of the 3 complement pathways
- Identify the functions (effects) of complement
- Identify deleterious effects of complement activation
- Identify deleterious effects of complement deficiency

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Self-Test Questions

- What is complement?
- What are the 3 pathways of activation?
- Which pathways are not activated by antibodies?
- Which complement components stimulate inflammation?
- Name 2 effects of complement.
- What disease or infection may a person deficient in C3 be pre-disposed to?

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