THE COMPLEMENT SYSTEM

components and functions of the complement system

- complements (C) are heat labile proteins found in mammalian blood and make up the complement system.
- This complex, multi-component system is composed of about 26 proteins.
- "Complement cascade" is non-specific but it must be activated in order to function.

The functions of complements include:

- making bacteria more susceptible to phagocytosis
- directly lysing some bacteria and foreign cells
- producing chemotactic substances
- increasing vascular permeability
- causing smooth muscle contraction promoting mast cell degranulation

Activation of the complement system

- Two distinct pathways; the *classical pathway* and the *alternate pathway*.
- Once initiated, a cascade of events (the "complement cascade") ensues, providing the functions listed above.
- Some complement components are numbered (e.g. C1, C2, C3, etc.) while others are referred to as "Factors".
- Some complement components must be enzymatically cleaved to activate their function; others simply combine to form complexes that are active.

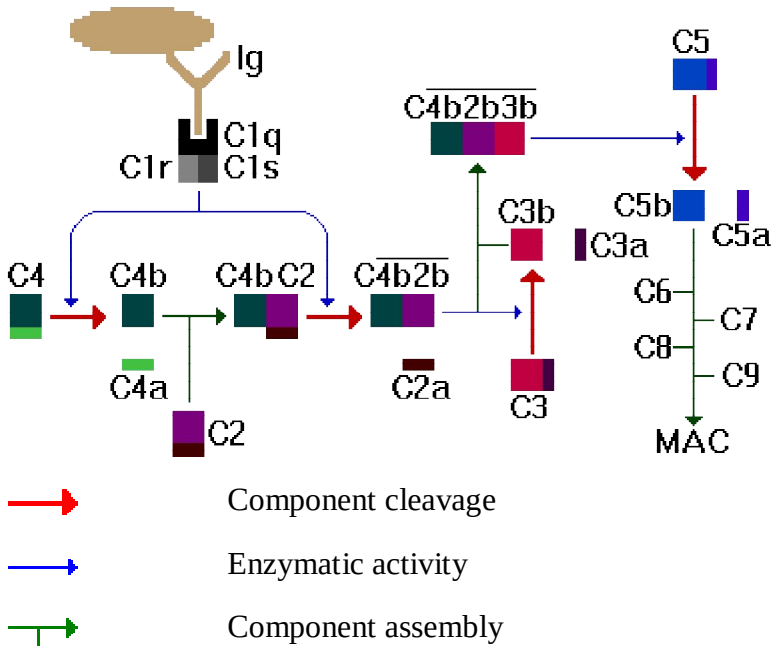
ACTIVATION OF THE COMPLEMENT CASCADE

Classical Pathway

- Starts with C1; C1 binds to immunoglobulin Fc (primarily IgM and IgG);
- C1 is composed 3 subunits; C1q, C1r, C1s.
- C1q (glycoprotein) is the actual recognition portion.
- C1q is made up of hydroxyproline and hydroxylysine that looks like a tulip flower.

- Upon binding via C1q, C1r is activated to become a protease that cleaves C1s to a form that activates (cleaves) both C2 and C4 to C2a/b and C4a/b.
- C2b and C4b combine to produce C3 convertase (C3 activating enzyme). C4a has anaphylactic activity (inflammatory response) and flows away.
- C3 is central to both the classical and alternative pathways.
- In classical, C4b2b (C3convertase) cleaves C3 into C3a/b. C3a is a potent anaphylatoxin.
- C3b combines with C4b2b to form C4b2b3b complex that is a C5 convertase. C3b can also bind directly to cells making them susceptible to phagocytosis.
- C5 is converted by C5 convertase (i.e. C4b2b3b) to C5a/b. C5a has potent anaphylatoxic and chemotaxic activities. C5b functions as an anchor on the target cell surface to which the lytic membrane-attack complex (MAC) forms.
- MAC is formed by C5b, C6, C7, C8 and C9. Once C9 polymerizes to form a hole in the cell wall, lysis ensues.

Classical Pathway



Components of the Classical Pathway

Native component	Active component(s)	Function(s)
C1(q,r,s)	C1q	Binds to antibody that has bound antigen, activates C1r.
	C1r	Cleaves C1s to activate protease function.
	C1s	Cleaves C2 and C4.
C2	C2a	Unknown.
	C2b	Active enzyme of classical pathway; cleaves C3 and C5.
C3	C3a	Mediates inflammation; anaphylatoxin.
	C3b	Binds C5 for cleavage by C2b. Binds cell surfaces for opsonization and activation of alternate pathway.
C4	C4a	Mediates inflammation.
	C4b	Binds C2 for cleavage by C1s. Binds cell surfaces for opsonization.
Components of the Alternate Pathway		
Native component	**Active component(s)**	**Function(s)**
C3	C3a	Mediates inflammation; anaphylatoxin.
	C3b	Binds cell surfaces for opsonization and activation of alternate pathway.
Factor B	B	Binds membrane bound C3b. Cleaved by Factor D.
	Ba	Unknown.
	Bb	Cleaved form stabilized by P produces C3 convertase.
Factor D	D	Cleaves Factor B when bound to C3b.
Properdin	P	Binds and stabilizes membrane bound C3bBb.

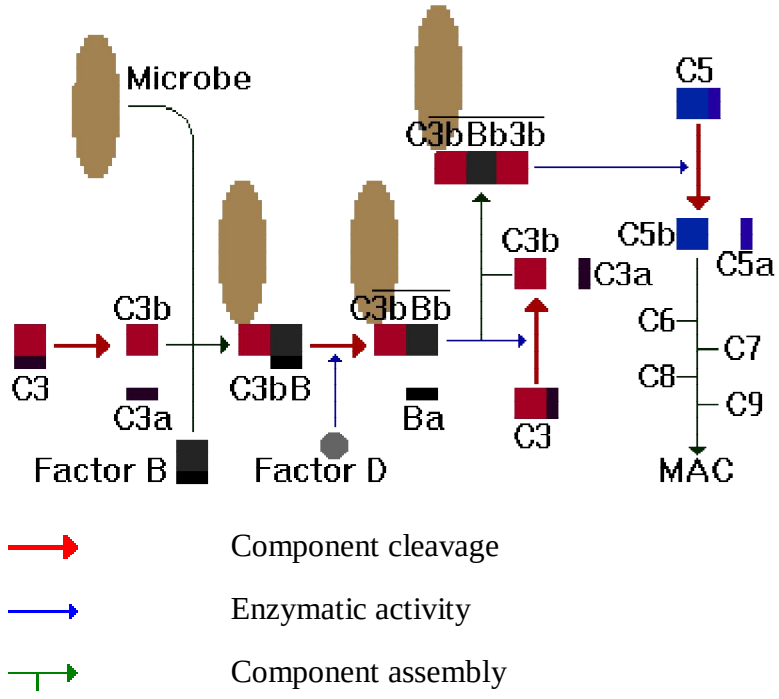
Components of the Membrane-Attack Complex		
Native component	**Active component(s)**	**Function(s)**
C5	**C5a**	**Mediates inflammation; anaphylatoxin, chemotaxin.**
	C5b	**Initiates assembly of the membrane-attack complex (MAC).**
C6	**C6**	**Binds C5b, forms acceptor for C7.**
C7	**C7**	**Binds C5b6, inserts into membrane, forms acceptor for C8.**
C8	**C8**	**Binds C5b67, initiates C9 polymerization.**
C9	**C9n**	**Polymerizes around C5b678 to form channel that causes cell lysis.**

Alternate Pathway

- Initiated by immunologic (e.g. IgA or IgE) or non-immunologic (e.g. LPS) means.
- Cascade begins with C3.
- As a result of spontaneous cleavage of C3, small amount of C3b is always found in circulating in the blood but concentration is always in check by some disintegrating factors in the blood.
- When C3b binds covalently to sugars on a cell surface (Microbes), it can become protected.
- Factor B binds to C3b on the cell surface.
- In the presence of Factor D, bound Factor B is cleaved to Ba and Bb; Bb contains the active site for a C3 convertase.

- Next, properdin binds to C3bBb to stabilize the C3bBb convertase on cell surface leading to cleavage of C3. Finally, a C3bBb3b complex forms and this is a C5 convertase, cleaving C5 to C5a/b. Once formed, C5b initiates formation of the membrane attack complex as described above.
- Only Gram-negative cells can be directly lysed by combination of antibody and complement;
- Gram-positive cells are mostly resistant to the above combination. However, phagocytosis is greatly enhanced by C3b binding (phagocytes have C3b receptors on their surface) and antibody is not always required. In addition, complement can neutralize virus particles either by direct lysis or by preventing viral penetration of host cells.

Alternate Pathway



REGULATION OF THE COMPLEMENT CASCADE

- Complement activation is mediated via 3 proteins and affects the complement component C3b due to it central role in both pathways of complement activation.

1. **C1 Inhibitor** inhibits the production of C3b by combining with and inactivating C1r and C1s. This prevents formation of the C3 convertase, C4b2b.

2. **Protein H** inhibits the production of C3b by inhibiting the binding of Factor B to membrane-bound C3b, thereby preventing cleavage of B to Bb and production of the C3 convertase, C3bBb.

3. **Factor I** inhibits the production of C3b by cleaving C3b into C3c and C3d, which are inactive. Factor I only works on cell membrane bound C3b, mostly on red blood cells (i.e. non-activator surfaces).

HYPERSENSITIVITY

- This occurs due to inappropriate response of the immune system to antigen.
- There are four different types of hypersensitivities that result from different alterations of the immune system. These types are classified as:

- Type I: Immediate Hypersensitivity
- Type II: Cytotoxic Hypersensitivity
- Type III: Immune Complex Hypersensitivity
- Type IV: Delayed Hypersensitivity

TYPE I HYPERSENSITIVITY

Type I or Immediate Hypersensitivity can be illustrated by considering the following experiment:

1. First, a guinea pig is injected intravenously with an antigen. For this example, bovine serum albumin (BSA, a protein) will be used. After two weeks, the same antigen will be re-injected

into the same animal. Within a few minutes, the animal begins to suffocate and dies by a process called *anaphylactic shock*.

2. Instead of reinjecting the immunized guinea pig, serum is transferred from this pig to a "naive" (unimmunized) pig. When this second guinea pig is now injected with BSA, it also dies of anaphylactic shock. However, if the second pig is injected with a different antigen (e.g. egg white albumin), the pig shows no reaction.
3. If immune cells (T-cells and macrophages instead of serum) are transfered from the immunized pig to a second pig, the result is very different; injection of the second pig with BSA has no effect.

These results tell us that:

- The reaction elicited by antigen occurs very rapidly (hence the name "immediate hypersensitivity").
- The hypersensitivity is mediated via serum-derived components (i.e. antibody).
- The hypersensitivity is antigen-specific (as one might expect for an antibody-mediated reaction).

The details of this reaction can be summarized as follows:

1. Initial introduction of antigen produces an antibody response. More specifically, the type of antigen and the way in which it is administered induce the synthesis of IgE antibody in particular.
2. Immunoglobulin IgE binds very specifically to receptors on the surface of mast cells, which remain circulating.
3. Reintroduced antigen interacts with IgE on mast cells causing the cells to degranulate and release large amounts of histamine, lipid mediators and chemotactic factors that cause smooth muscle contraction, vasodilation, increased vascular permeability, bronco-constriction and oedema. These reactions occur very suddenly, causing death.

Examples of Type I hypersensitivities include allergies to penicillin, insect bites, molds, etc. A person's sensitivity to these allergens can be tested by a cutaneous reaction. If the specific antigen in question is injected intradermally and the patient is sensitive, a specific reaction known as *wheal and flare* can be observed within 15 minutes. Individuals who are hypersensitive to such allergens must avoid contact with large inocula to prevent anaphylactic shock.

TYPE II HYPERSENSITIVITY

- Type II or Cytotoxic Hypersensitivity also involves antibody-mediated reactions. However, the immunoglobulin class (isotype) is generally IgG.
- In addition, this process involves K-cells rather than mast cells. K-cells are, of course, involved in antibody-dependent cell-mediated cytotoxicity (ADCC).
- Type II hypersensitivity may also involve complement that binds to cell-bound antibody. The difference here is that the antibodies are specific for (or able to cross-react with) "self" antigens. When these circulating antibodies react with a host cell surface, tissue damage may result.

Examples of Type II hypersensitivity include:

- **Pemphigus:** IgG antibodies that react with the intracellular substance found between epidermal cells.
- **Autoimmune hemolytic anemia (AHA):** This disease is generally inspired by a drug such as penicillin that becomes attached to the surface of red blood cells (RBC) and acts as hapten for the production of antibody which then binds the RBC surface leading to lysis of RBCs.
- **Goodpasture's syndrome:** Generally manifested as a glomerulonephritis, IgG antibodies that react against glomerular basement membrane surfaces can lead to kidney destruction.

TYPE III HYPERSENSITIVITY

- Type III or Immune Complex hypersensitivity involves circulating antibody that reacts with free antigen. These circulating complexes can then become deposited on tissues. Tissue deposition may lead to reaction with complement, causing tissue damage. this type of hypersensitivity develops as a result of systematic exposure to an antigen and is dependent on i) the type of antigen and antibody and ii) the size of the resulting complex.

More specifically, complexes that are too small remain in circulation; complexes too large are removed by the glomerulus;

- intermediate complexes may become lodged in the glomerulus leading to kidney damage.

Example of a Type III hypersensitivity is **serum sickness**, a condition that may develop when a patient is injected with a large amount of e.g. antitoxin that was produced in an animal. After about 10 days, anti-antitoxin antibodies react with the antitoxin forming immune complexes that deposit in tissues. Type III hypersensitivities can be ascertained by intradermal injection of the antigen, followed by the observance of an "Arthus" reaction (swelling and redness at site of injection) after a few hours.

TYPE IV HYPERSENSITIVITY

Type IV or Delayed Hypersensitivity can be illustrated by considering the following experiment:

1. First, a guinea pig is injected with a sub-lethal dose of *Mycobacterium tuberculosis* (MT). Following recovery of the animal, injection of a lethal dose of MT under the skin produces only erythema (redness) and induration (hard spot) at the site of injection 1-2 days later.
2. Instead of reinjecting the immunized guinea pig, serum is transfered from this pig to a "naive" (unimmunized) pig. When this second guinea pig is now injected with MT, it dies of the infection.
3. If immune cells (T-cells and macrophages instead of serum) are transfered from the immunized pig to a second pig, the result is very different; injection of the second pig with MT causes only erythema and induration at the site of injection 1-2 days later.
4. In a separate experiment, if the immunized guinea pig is injected with a lethal dose of *Listeria monocytogenes* (LM) instead of MT, it dies of the infection. However, if the pig is simultaenously injected with both LM and MT, it survives.

These results tell us that:

- The reaction elicited by antigen occurs relatively slowly (hence the name "delayed hypersensitivity").
- The hypersensitivity is mediated via T-cells and macrophages.
- The hypersensitivity illustrates both antigen-specific (T-cell) and antigen non-specific (macrophage) characteristics.

1. Initial introduction of antigen produces a cell-mediated response. *Mycobacterium tuberculosis* is an intracellular pathogen and recovery requires induction of specific T-cell clones with subsequent activation of macrophages.
2. Memory T-cells respond upon secondary injection of the specific (i.e. MT) antigen, but not the non-specific (i.e. LM) antigen.
3. Induction of the memory T-cells causes activation of macrophages and destruction of both specific (MT) and non-specific (LM) microorganisms.