LECTURE: 29

Title COMPLEMENT PROTEINS AND THEIR RECEPTORS

LEARNING OBJECTIVES:

The student should be able to:

- **Determine the locations of the complement receptors.**
- **Enumerate the importance of the complement receptors.**
- **Enumerate the multiple physiological activities of the complement receptor 1 (CR1).**
- **State the other names for CR1.**
- **Enumerate the four physiological activities of the CR1.**
- **Indicate the importance of complement receptor 2 (CR2) in the activation of B-lymphocytes.**
- **Indicate the main pathophysiological activity of CR2.**
- **Identify the family of the complement 3, and 4 (C3, and C4).**
- **Identify the specific location of the CR3.**
- **Determine the importance of the CR3 (CD18/11b).**
- **Give examples for some pathogens that bind directly to CR3.**
- **Enumerate the ligands for CR3.**
- **Identify the specific locations of the CR4.**
- **Identify the specific locations of the CR5a**
- **Explain the mechanism of regulation and limitation of C5a activity.**
- **Enumerate some receptors that bind to collectins.**
- **Enumerate some common diseases caused due to deficiency in C1 (q, r, or s), C4, C2, C3, C5, C6, C7, C9, C1INH, Factor H, and Factor I.**
- **Describe the methods of quantitation of complement components, and changes in complement components in various diseases states.**
- **Should know the principal & application of complement fixation test.**

LECTURE REFRENCE:

1. TEXTBOOK: ROITT, BROSTOFF, MALE IMMUNOLOGY. 6th edition. Chapter 3. pg. 22, 27, 29, 54, 55, 57, 135, 155, 156, 176, 251.

2. TEXTBOOK: ABUL K. ABBAS. ANDREW H. LICHTMAN. CELLULAR AND MOLECULAR IMMUNOLOGY. 5TH EDITION. Chapter 14. pg 333, 335-336, 335t.

THE COMPLEMENT PROTEINS AND THEIR RECEPTORS

INTRODUCTION

In **1894 Pfeiffer** discovered that cholera bacilli (Vibrio cholerae) were dissolved or lysed in vitro by addition of guinea pig anti-cholera serum. Heating the serum at 56C for 30 minutes abolished this activity, but did not abolished the activity of antibodies against the bacilli, since the heated serum could still transfer immunity passively from one guinea pig to another. Pfeiffer discovered that the addition of normal, fresh serum to the heat-labile component present in immune as well as normal serum, were necessary for the lysis of V. cholera in vitro.

A few years later, **Bordet** confirmed that bacteriolysis by immune serum required a heat-labile component that he termed "**Alexine**". The term **complement**, applied some years later by **Ehrlich**, displaced "Alexine" and is used to donate the heat-labile components in normal serum which, together with antigen-bound antibodies, exhibit a variety of biological properties, one of which is the ability to lyse cells or micro-organisms.

Complement consists of a group of serum proteins that act in concert and in orderly sequence to exert their effect. These proteins are not immunoglobulins, and their concentrations in serum do not increase after immunization. Bordet discovered that the action of complement, in the presence of appropriate antiserum, results in the lysis of red blood cells. Based on this observation, Bordet developed the <u>complement fixation test.</u>

Now we know that the complement system plays a **major** role in host defense and the inflammatory process (part of the innate immune system) in addition to lysis, the activation of complement results in the generation of many powerful biologically active substances. Activation of the complement cascade has widespread physiologic and pathophysiologic effects.

Metabolism

Synthesis

A. **Liver** is the major site of synthesis of complement proteins, although **macrophages** and **fibroblasts** can synthesize some.
B. Complement proteins first appear in the fetus during the second month of pregnancy
C. The concentration of most components **at birth** is about 50% of adult levels.
D. Inflammation increases the synthesis of complement components, presumably through the action of interleukin-1 and gamma interferon.

Catabolism the catabolic rate of complement proteins is high, 1% to 3% pre hour.

The MAJOR biological activities of the complement system

Activation of complement system generates a wide range of biological activities, these can be grouped into three major function:

1. **Opsonic function (opsonization).** Opsonization occurs as activated complement coat pathogenic organisms or immune complexes, facilitating the process of phagocytosis.
2. **Activation of leukocytes.** Polymorphs and macrophages have specific receptors for small complement fragments that are generated on the target by the complement cascade. These fragments diffuse away from the target, and stimulate directed cellular movement (Chemotaxis) and activation when bound by effector cells. Also activation of the complement system results in the release of histamine from mast cells and basophils and in stimulation of the inflammatory response.
3. **Lysis of the target cells (cytotoxic function).** In the final stage of the complement cascade the membrane of the target cell is inserted by a **hydrophobic 'plug'** into the target cell lipid membrane bilayer. This causes osmotic disruption and lysis of the target (e.g., bacteria and tumor cells are attacked, leading to cell death) (figure-1).

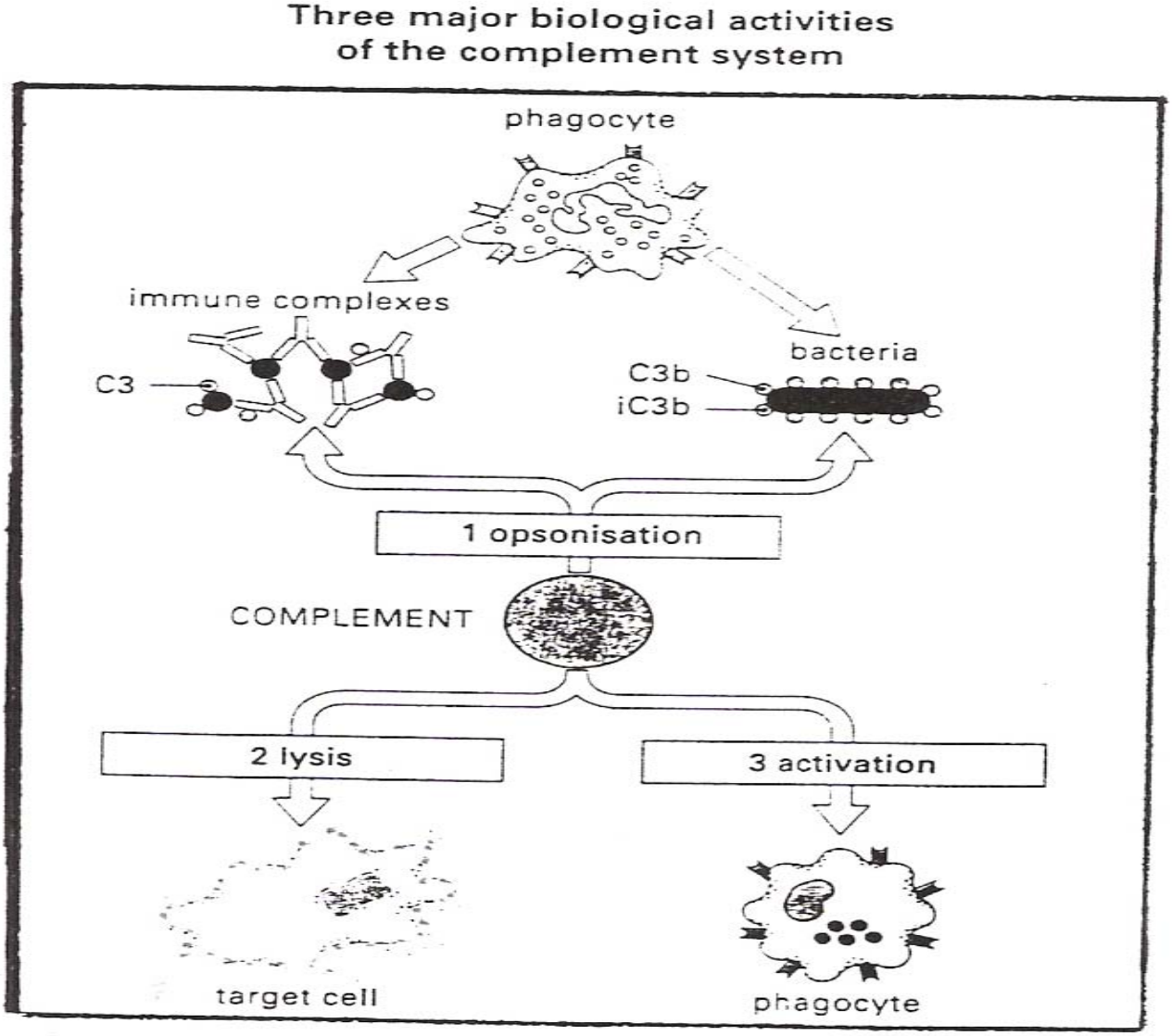


Figure-1 Three major biological activities of the complement system

Complement control proteins (CCPs) also known as Regulators of complement activation (RCA).

The complement action and activation is controlled by several proteins some are found in the plasma and some are cell membrane-attached. These proteins are:

1. **Complement receptor type 1 (CR1, CD35) and complement receptor type 2 (CR2, CD21).**
2. **Decay accelerating factor (DAF, CD55), a membrane protein attached by unusual glycophospholipid 'foot'.**
3. **Factor H: a plasma globulin with an elongated configuration.**
4. **C4-binding protein (C4-bp): a heptameric plasma protein with a "spider-like" configuration.**
5. **Membrane cofactor protein (MCP, CD46)**

Some of these proteins such as Factor H, C4pbp, DAF, MCP and CR1 share a number of functions for example; all inhibit stable formation of the classical and alternative pathway of C3 convertase enzymes, respectively C4b2a and C3bBb. All CCPs contain a domain of approximately 60 amino acids, called the **short consensus repeat (SCR).** SCRs provide the structural scaffold of each molecule, and may also provide the binding specificity of the protein.

Complement can distinguish self from non-self

Complement has evolved many mechanisms for self non-self discrimination. For example, the rapid and widespread binding of C3b to non-self, such as microorganisms or immune complexes, while the individual's own cell surfaces are protected by **surface molecules** that very effectively **limit C3b deposition.**

ACTIVATION OF COMPLEMETN

The complement proteins are **activated** in two ways, both lead to the formation of a convertase that cleaves **C3** to **C3a** and **C3b,** this is a **central** step in the complement activation. One cascade is called the **classical pathway** and the other is known as the **alternative pathway (figure-2).**

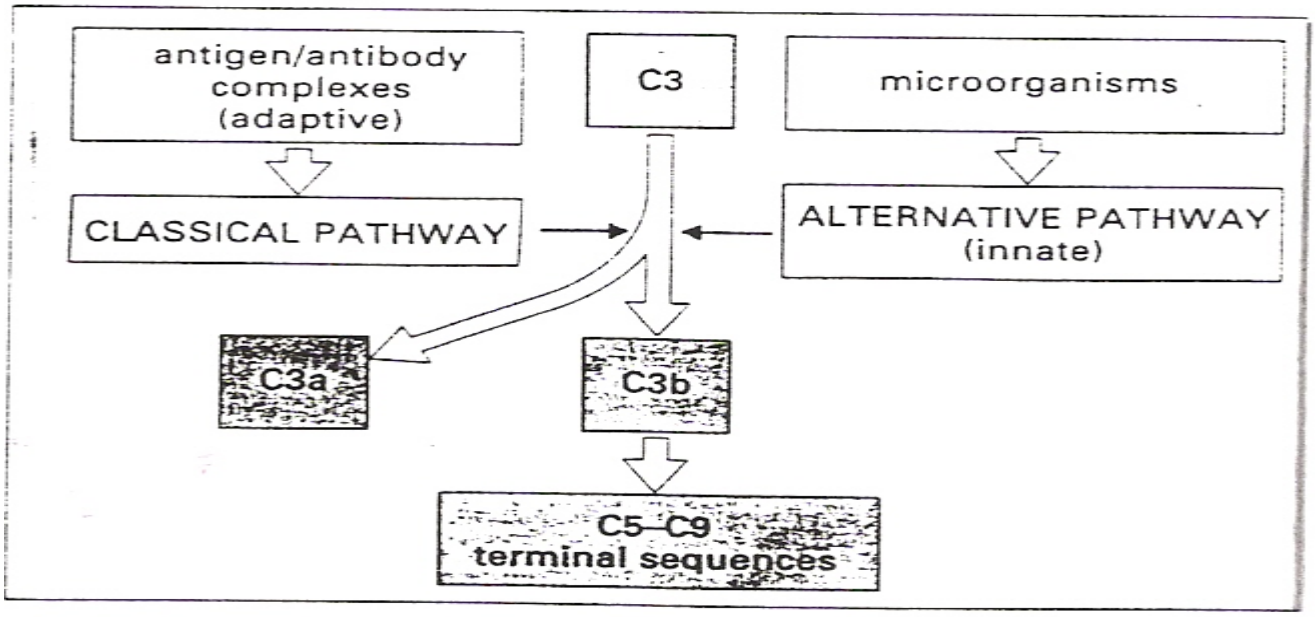


Figure-2 Activation of complement

Plasma proteins of the complement system

A. Complement plasma proteins involved in the classical pathway are:

1. C1

a. C1q
b. C1r
c. C1q

2. C4
3. C2
4. C3

B. Complement plasma proteins involved in the alternative pathway are:

1. Factor B
2. Factor D
3. Properdin

C. Complement plasma proteins involved in the membrane attack are:

1. C5
2. C6
3. C7
4. C8
5. C9

D. Regulatory proteins are:

1. C1 inhibitor (C1 INH)
2. Factor I
3. C4-bp
4. Decay accelerating factor (DAF)
5. Membrane factor protein (MAC)
6. Complement receptors 1, and 2 (CR1, and CR2)

Convertase enzymes involved in the classical and the alternative pathways

1. The convertase which cleaves the C3 into C3a and C3b in the classical pathway is the combination of two complement proteins C4 and C2 which lead to C4b2a complex (the classical pathway C3 convertase).
2. The alternative pathway convertase is a combination of C3 with FB, which lead in turn to C3bBb.

The C3b generated by these two enzymes binds to the target membrane and becomes a focus for further C3b production, this part of the cascade is called **amplification loop.** Both types of C3 convertase can be converted into a **C5 convertase** by the addition of a further molecule of **C3b**; **C5 convertase** catalyses the first step in a cascade that lead to the production of membrane attack complexes.

The nomenclature of the complement system is complex

The proteins of the classical pathway and membrane attack system are each assigned a **number** and react in the order; C**1**q, C**1**r, C**1**s, **C4**, C**2**, C**3**, C**5**, C**6**, C**7**, C**8**, and C**9**. Many of the proteins are zymogens (pro-enzymes requiring proteolytic cleavage to become active). The activated form is distinguished from its precursor by a **bar** drawn above its notation, e.g. C1r. The cleavage products of complement proteins are distinguished from the parent molecules by suffix letters; conventionally the **small** initial cleavage fragment is designated the "**a**" fragment and the **larger** portion, the "**b**" fragment, e.g., C3**a** and C3**b**. **(An exception of this rule is seen for C2, so that the small fragment is designated C2b and the larger fragment, C2a).**

The proteins of the alternative pathways are known by the term **"Factors"** and are identified by **single letters.** Conventionally, 'Factors' can be abbreviated to '**F**' or even omitted altogether, so that '**Factor B**' may be represented as **'FB'** or quite simply as **'B'.**

Complement receptors are named either according to their ligand (e.g., C5a receptor) or using the Cluster of Differentiation (CD) system. There also numbering system for receptors of the major fragments of C3, complement receptor 1 to 4 (CR1 to CR4). This has the unfortunate consequence that some receptors have three names in current usage: the receptor for C3b is variously referred to as C3b receptor, CR1 and CD 35.

The classical pathway

1. Activation

a. The classical pathway is activated mainly by immune complexes, or by the aggregated immunoglobulins.

(1) **IgG** mainly (**IgG1** and **IgG3**) and **IgM** are most efficient in reacting with complement.

(a) Only one molecule of pentameric IgM is required, whereas at least two adjacent molecules of the monomer IgG are needed.
(b) IgG4, IgA, IgD, and IgE do not activate the classic component cascade at all in their native configuration; IgG2 binds complement weakly.

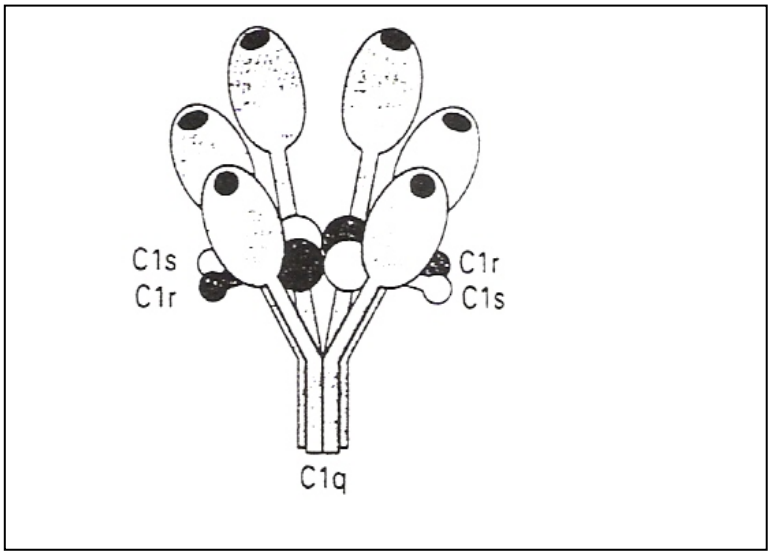
(2) Classically, an **antigen-antibody complex** activates the classical complement proteins in a orderly sequence to form macromolecular complex **ag-ab C142356789.**

b. Activation follows the binding of complement component C1 to a site on the **Fc** fragment of the immunoglobulin. The site is the **C_H2 domain** on **IgG** or **C_H3** on **IgM.**

2. Components and steps

a. C1 (the recognition unit). C1 is a pent molecular $Ca2^{+}$ dependent complex consisting of a single **C1q molecule, two C1r, and two C1s** held together by calcium ions. With the removal of the calcium, C1 breaks down into its three sub units (figure-3).

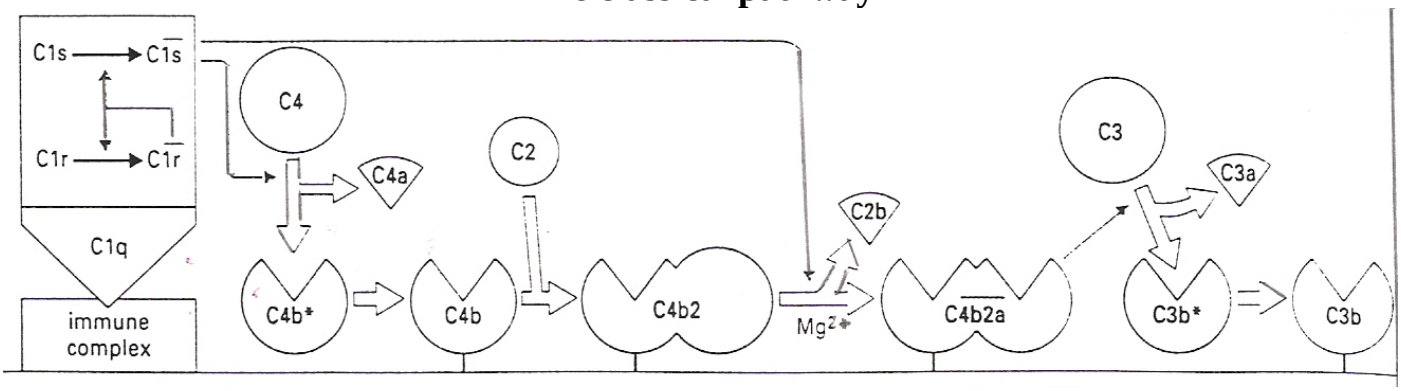
Intact C1



(1) The first step is the binding of antibody to two or more of the six globular domains of C1q. C1q binds with high avidity to the C_H2 domain of the aggregated IgG molecules in an immune complex or C_H3 of the IgM antibody. This binding lead to conformational changes in the C1 complex. This makes one of the C1r to activate itself (autocatalysis) and then the other C1r, to yield two active C1r enzymes. These two enzymes then cleave the two C1s molecules to give active C1s serine esterases.

The classical pathway can also be activated in an antibody-independent fashion. Mannan-binding protein (MBP) is a member of calcium dependent **lectins** known as collectins. This protein can bind to the terminal mannose groups on the surface of bacteria and then be able to react with the serine proteinase known as MASP (mannan-binding protein-associated serine proteinase). MASP is hologous to C1r and C1s; the interaction of MBP to MASP is analogous to the interaction with the C1q with C1r and C1s, and lead to antibody-independent activation of the classical pathway. C1q can bind directly to certain microorganisms, including mycoplasmas and some retroviruses, in an antibody-independent process.

The classical pathway



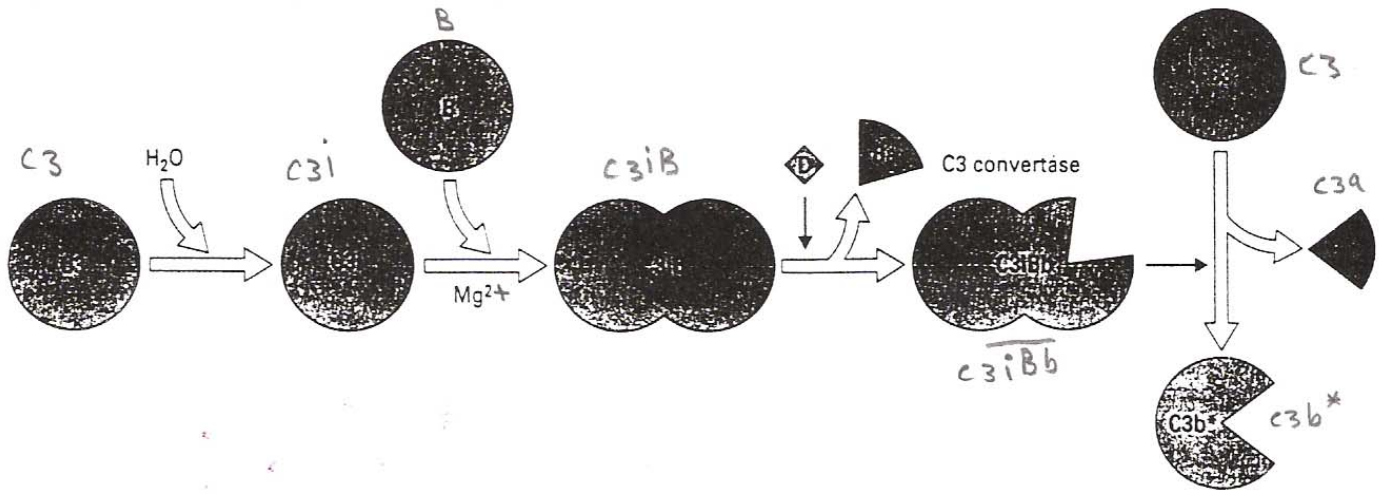
b. C1 cleaves C4 and generates activated C4b. The complement protein C4 contains an internal thioester bond in a sequence that is closely homologous to a thioester containing sequence in C3. When C1s cleaves C4 two fragments are generated C4a (which has weak anaphylaxis activity) and a larger unstable intermediate C4b* (the* indicate unstable state) within a few milliseconds this is attacked by nucleophilic groups (electron donors).

The majority of C4b* is **hydrolyzed by water to form inactivated iC4b. However, C4b* can also form covalent bonds with amine or hydroxyl groups on cell surface molecules, to form surface bound C4b. Surface bound C4b acts as a binding site for C2 in the presence of Mg^{2+}, leading to the formation of classical C3 convertase (C4b2a).** C3b generated by C3 convertase can bind covalently to cell surface molecules. Since C3 convertase (C4b2a) are usually generated on non-self surfaces or on immune complexes, this indicated the C3b as well is deposited at non-self membranes. The bound C3b becomes as a focus for further complement activation via the **alternative pathway amplification loop.**

THE ALTERNATIVE PATHWAY

The alternative pathway of complement activates spontaneously. The Tick-over activation continuously generates low levels of C3b* in serum (figure). The internal thioester bond in native C3 is susceptible to spontaneous hydrolysis by water, generating an activated form of C3 known as C3i**. <u>This steady low level spontaneous C3 activation in plasma is known as tick-over activation.</u>** C3i.then acts as a binding site for Factor B to produce C3iB. (This is analogous to the binding of C2 to C4b). The Factor B bound to C3i. is cleaved by Factor D to release Ba. The remaining complex, C3iBb, is a fluid phase, alternative pathway C3 convertase. The alternative pathway can be triggered immunologically (e.g., by aggregated IgA, or IgG4, or IgE) or nonimmunologically (e.g., by certain microbial cell products) less efficient than the classical pathway.

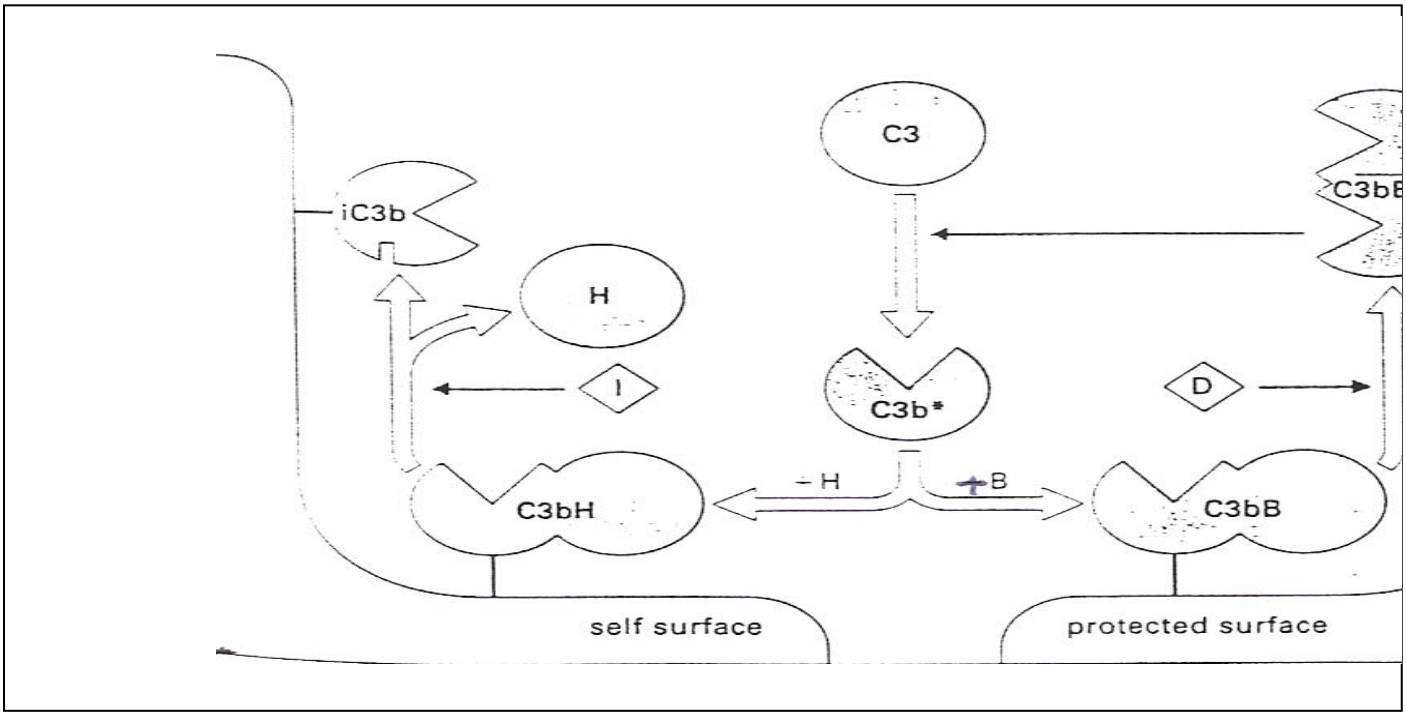
C3 tick-over



Because the convertase is operating in the fluid phase, most of the C3b* generated by C3iBb is hydrolysed and inactivated by water. However, **if it should happen to come into contact with a non-self surface, such as a bacterial cell membrane, it will covalently bind and initiate the amplification loop of the alternative pathway. C3b that bind to autologous cell surface is prevented form initiating the amplification loop by the complement control proteins.**

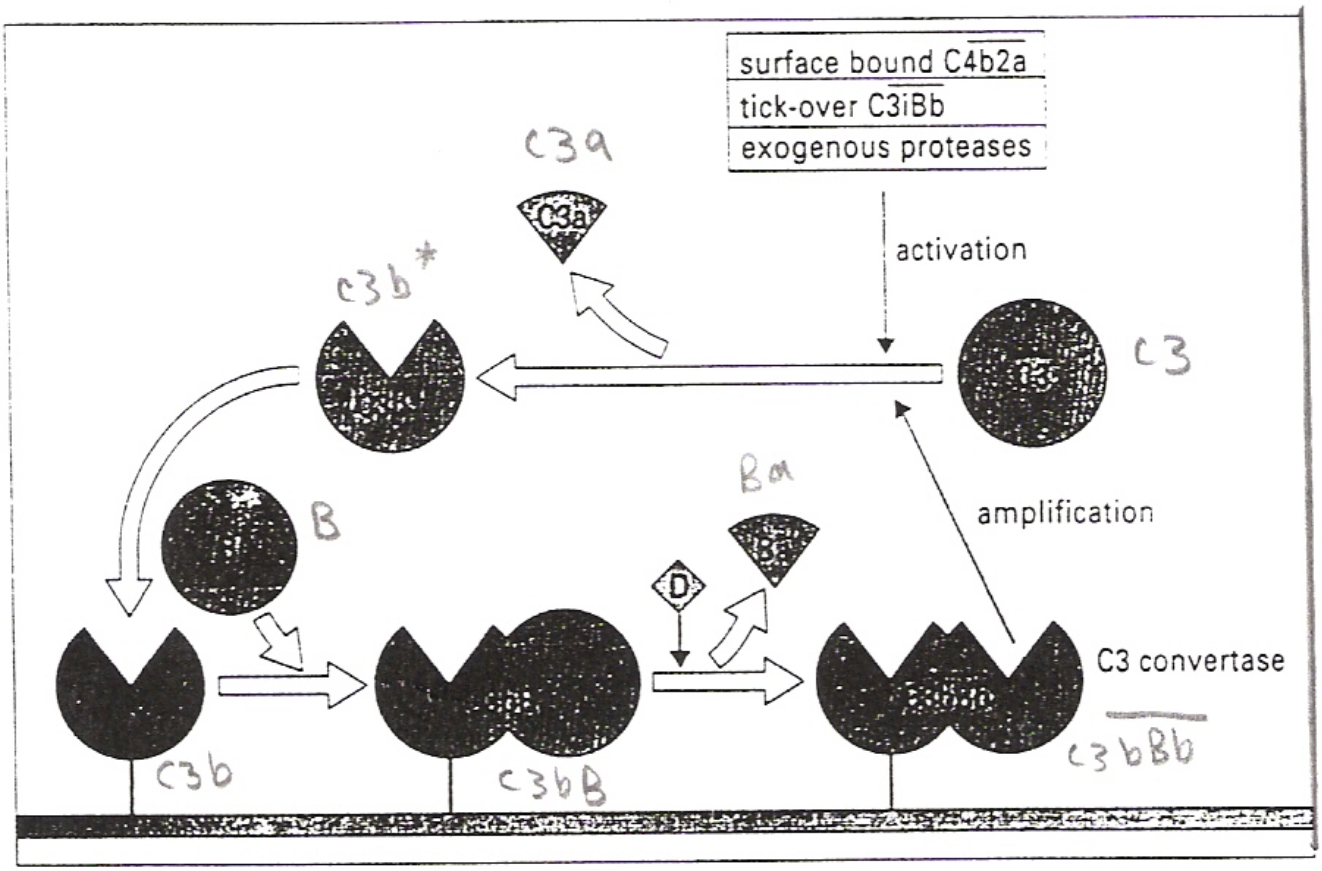
Surfaces that are good activators of complement are known as "protected" surfaces (non-self surfaces). Protected means that bound C3b is protected from proteolytic degradation. Bacterial cell wall are protected for C3b because C3b has a higher affinity for Factor B than Factor H at these sites (for self surface Factor H is favored and C3b becomes inactivated by Factor I), and is therefore more likely to form a stable convertase (C3bBb) which derives the amplification loop of the alternative pathway (figure). Non-self surfaces also lack the host regulatory proteins that inhibit complement activation.

Regulation of the amplification loop



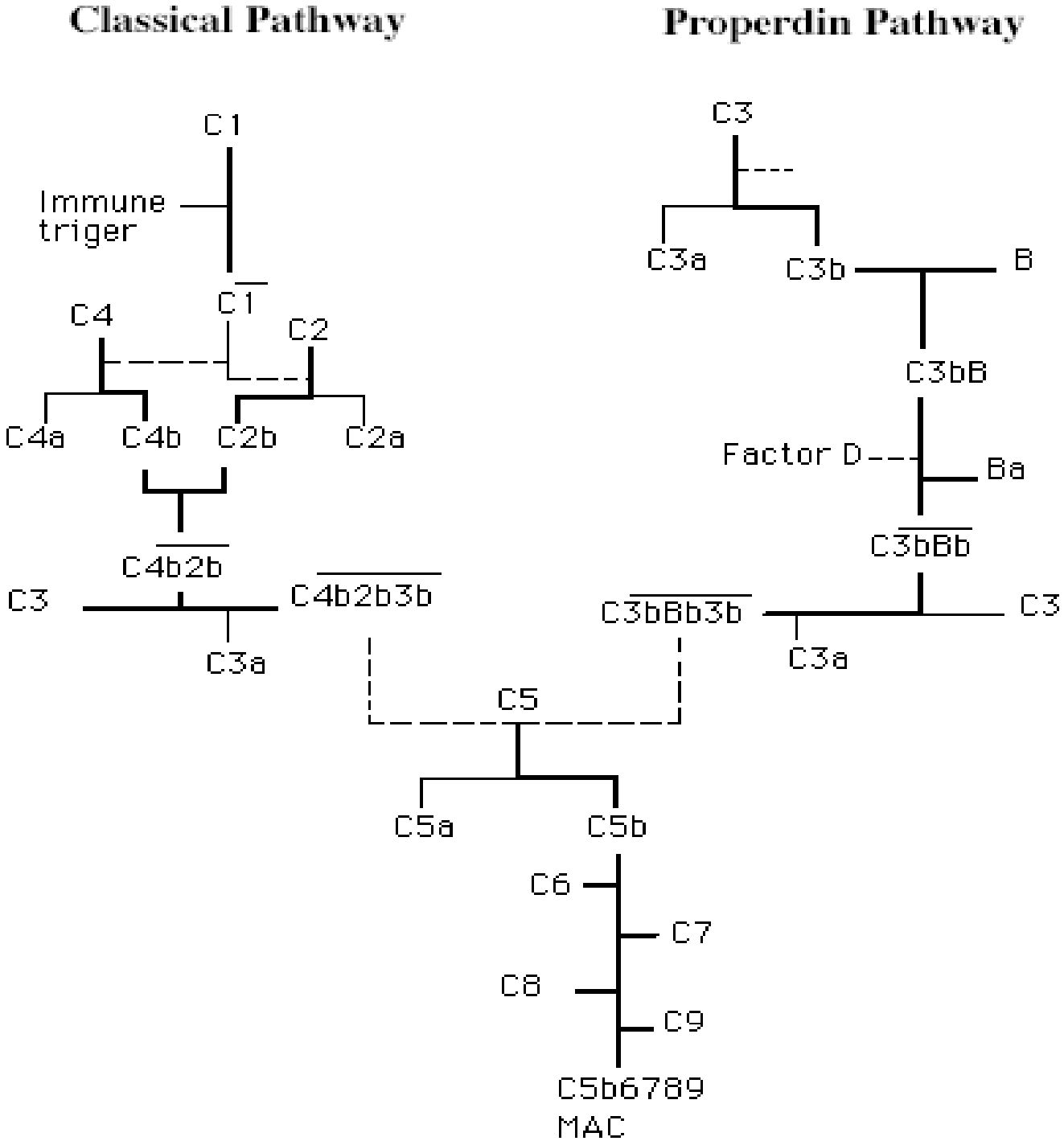
The alternative pathway amplification loop uses a positive feedback mechanism. Surface-bound C3b binds Factor B to give C3bB. This becomes substrates for Factor D, a serine esterase that cleaves Factor B to release a small fragment, Ba, leaving C3bBb bound to the surface. The C3bBb dissociates fairly rapidly unless it is stabilized by the binding of properdin (P), forming the complex, C3bBbP. This constitutes the surface-bound C3 convertase enzyme of the alternative pathway. C3bBbP cleaves many more C3 molecules; the location of the convertase means that C3b* thus generated will tend to bind to the nearby protected surface, rather than elsewhere (figure). The amplification loop also works for C3b deposited as a result of (antibody-dependent) classical pathway activation.

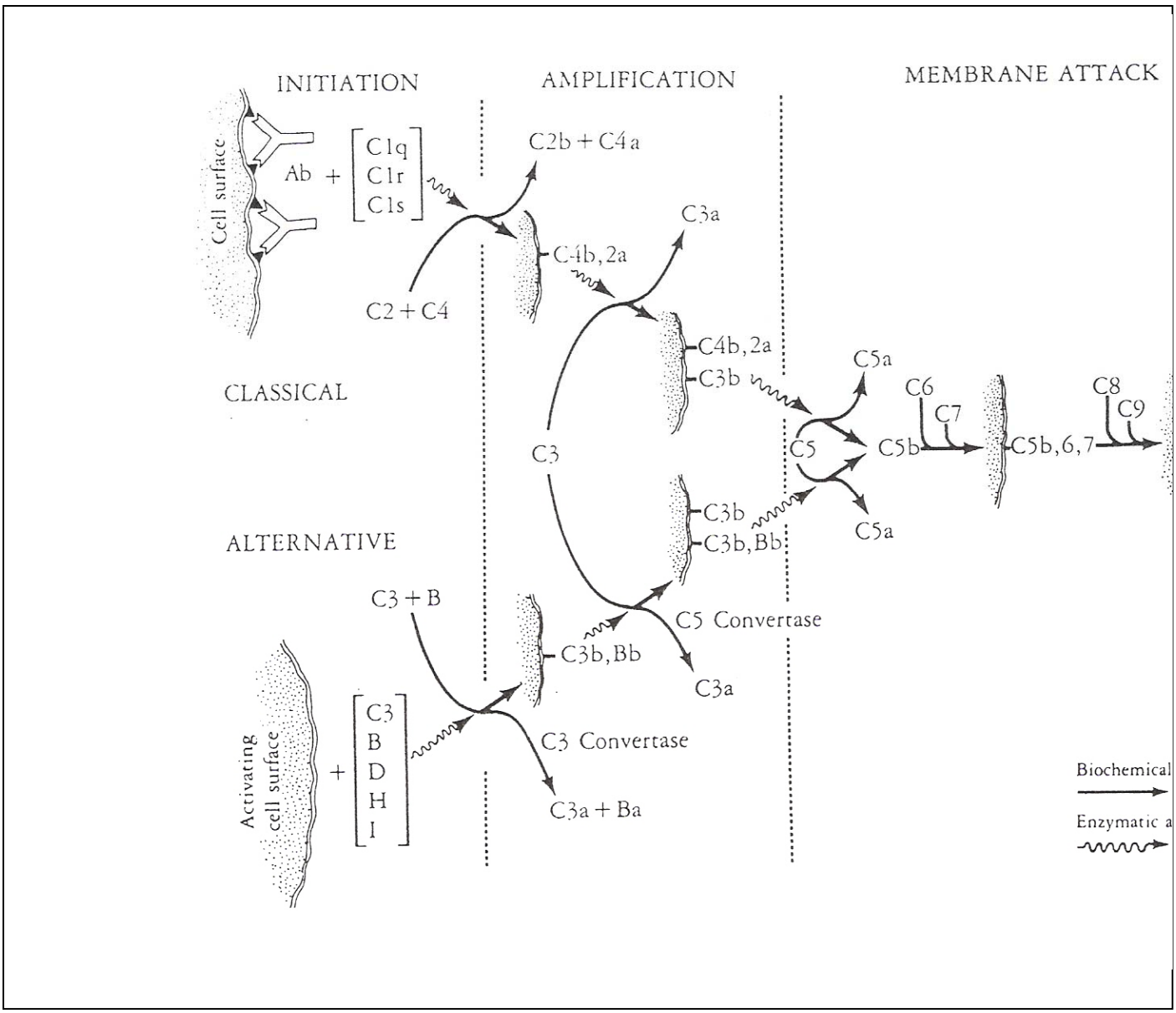
The amplification loop of C3 activation



The analogous action of the classical and alternative pathways

Both pathways generate a C3 convertase: C4b2a (classical pathway) and C3bBb (alternative pathway). In the classical sequence, C1s is activated by complexed antibody and splits both C4 and C2. The small fragments, C4a and C2b, are lost and the major components from C4b2a. In the alternative pathway, surface-bound C3b (generated by tick-over activation) binds Factor B, which is split releasing a small fragment, Ba. The major fragment, Bb, remains bound to form C3bBb. This converts more C3, so creating positive feedback. Activator surfaces, on microorganisms for example, stabilize C3b by facilitating its combination with Factor B. This activity promotes alternative pathway activation. The C3 convertase of both pathways may bind further C3b to yield C5 convertase enzymes. These activate the next component of the complement system, C5. The classical pathway C5 convertase is C4b2a3b, and the alternative pathway convertase is C3bBb3b.





Activation of the classical pathway is efficiently regulated

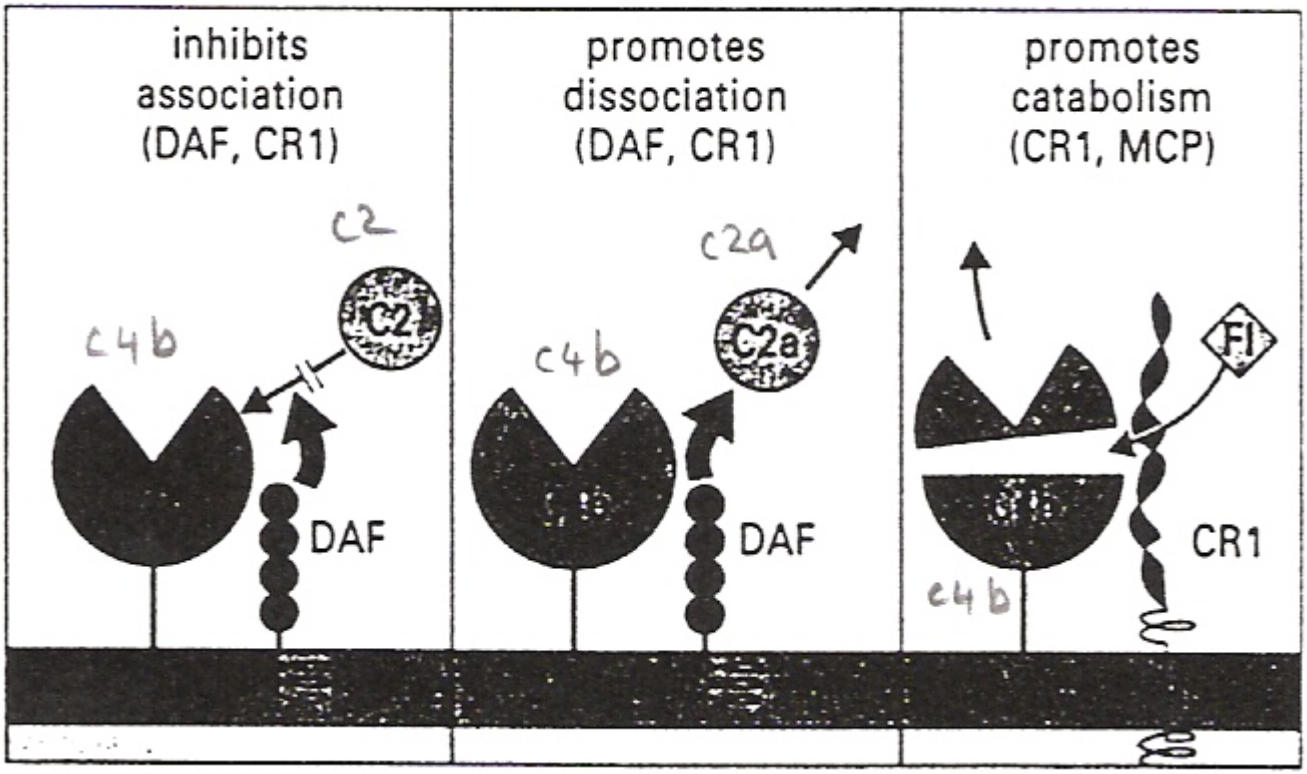
Classical pathway activation is regulated in the **fluid phase** by two mechanisms. The first is C1 inhibitor, a serine proteinase inhibitor (serpin) that binds and inactivates C1r and C1s.

The second mechanism blocks the formation of the classical pathway C3 convertase enzyme, C4b2a. The formation of C4b2a is inefficient in the fluid phase, due to the presence of Factor I and C4 binding protein (C4-bp), which together catabolize C4b. C4-bp also promotes the dissociation of C2a from C4b2a.

Classical pathway activation is also regulated by inhibiting complement binding to host cell surfaces. This is achieved by the complement control proteins (CCPs), including decay accelerating factor (DAF), complement receptor 1 (CR1), and membrane cofactor protein (MAC). These proteins operate in the following ways (figure):

a. They inhibit the binding of C2 to C4b (DAF or CR1)
b. They promote 'decay acceleration', the dissociation of C2a from C4b (DAF or CR1)
c. They act as cofactor to promote the catabolism of C4b by Factor I (MCP or CR1).

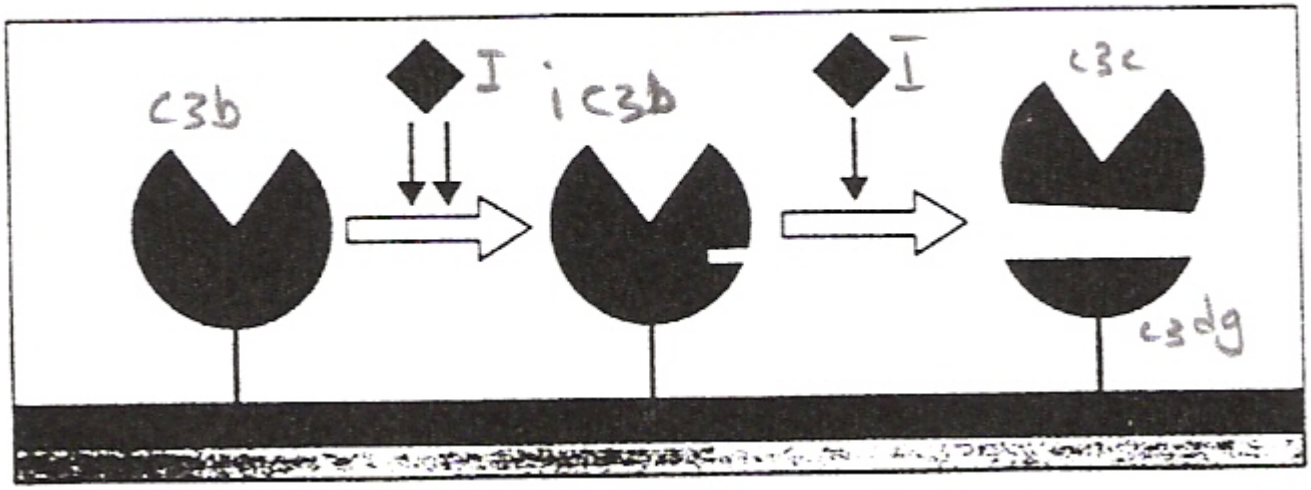
Regulation of C3 convertases



Alternative pathway and amplification loop activation are regulated

Activation of the alternative pathway in the fluid phase, where C3b does not bind to surfaces, is regulated by Factor H which is homologous to C4-bp, promotes the dissociation of Bb from both C3i. and C3b. Factor H also functions as a cofactor to Factor I for the catabolism of C3i. and C3b (figure).

Breakdown of C3b



Regulation of the amplification mechanism is important for host: this is a positive feedback system, and it will cycle until all C3 is completely cleaved unless it is regulated. On Autologous cell membranes, both DAF and CR1 accelerate the dissociation of C3bBb, promoting the release of the C3b from the complex. CR1 and MAC both act as cofactors for the cleavage of C3b by Factor I. This reaction is exactly analogous to the activities of DAF, MCP and CR1 in controlling the activity of classical pathway C4b2a when it is bound to cell membranes. For summarize the regulation of the bound C3b, there are two ways:

1. Amplification, C3b acts as a binding site for Factor B, forms a convertase enzyme, and focuses the deposition of more C3b to the same surface.
2. Inhibition, C3b is catabolized by Factor I using one of three cofactors, Factor H (from plasma), and CR1 or MCP (surface-bound).

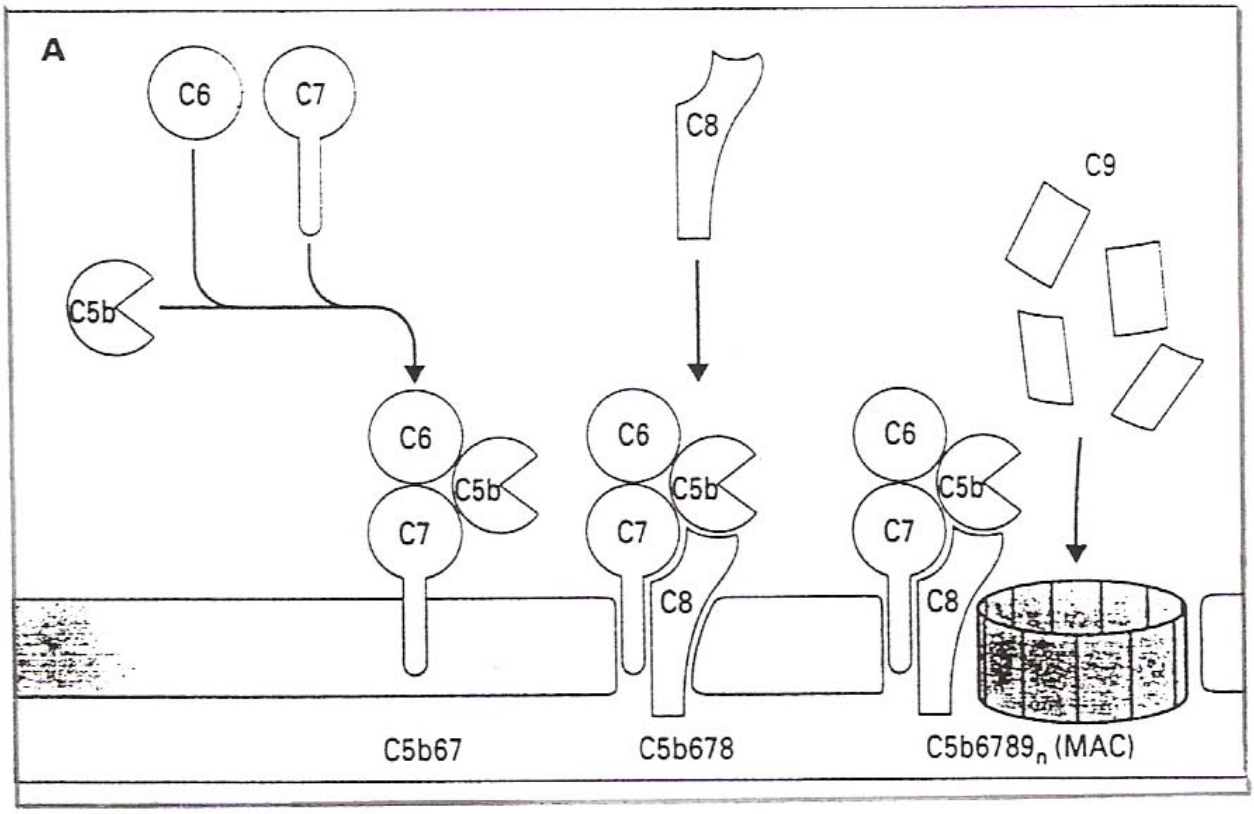
The nature of the surface to which the C3b is bound regulates which of these two ways is mostly likely. It is the presence of intrinsic molecules such as DAF, CR1, and MCP, on self surfaces, particularly the cell membranes that effectively limits the formation of C3 convertase enzymes. On the other hand, non-self surfaces, for example bacterial cell membranes, act as a protected site for C3b since factor B has a higher affinity for C3b than Factor H at these sites. Thus the deposition of few molecules of C3b on to non-self surfaces is followed by the formation of the relatively stable alternative pathway C3 convertase enzyme, C3bBbP, which focuses more C3b deposition in the near vicinity.

MEMBRANE ATTACK COMPLEX

The formation of the membrane attack complex (MAC) is the final phase in the activation of the complement cascade **by** the **enzymatic cleavage of the C5.** C5 is homologous to C3 and C4 but lacking the internal thioester bond. C5 must be bound to the C3b before it can be cleaved by C5 convertase enzyme. The classical pathway C5 convertase is trimolecular complex **C4b2a3b** in which the C3b is covalently bound to C4b, than for C3b which is bound to other cell surface molecules. The alternative pathway C5 convertase is also trimolecular **C3bBb3b** in which one C3b is covalently bound to the other. Cleavage of C5 release the small peptide fragment **C5a**, which is a **potent anaphylatoxin**.

The **reminder of the formation of the MAC is non-enzymatic**. C5b binds C6, forming C5b6, and this then binds C7 to form a C5b67 complex (figure). The binding to the C7 marks the transition of the complex from hydrophilic to a hydrophobic state that preferentially inserts into lipid bilayer. C8 then binds to this complex, followed by a stepwise addition of up to 14 C9 monomers, resulting in the formation of a lytic 'plug' or pore-forming molecule. Although a small amount of lysis occurs when C8 binds to C5b67, it is the polymerized C9 that causes the majority of lysis.

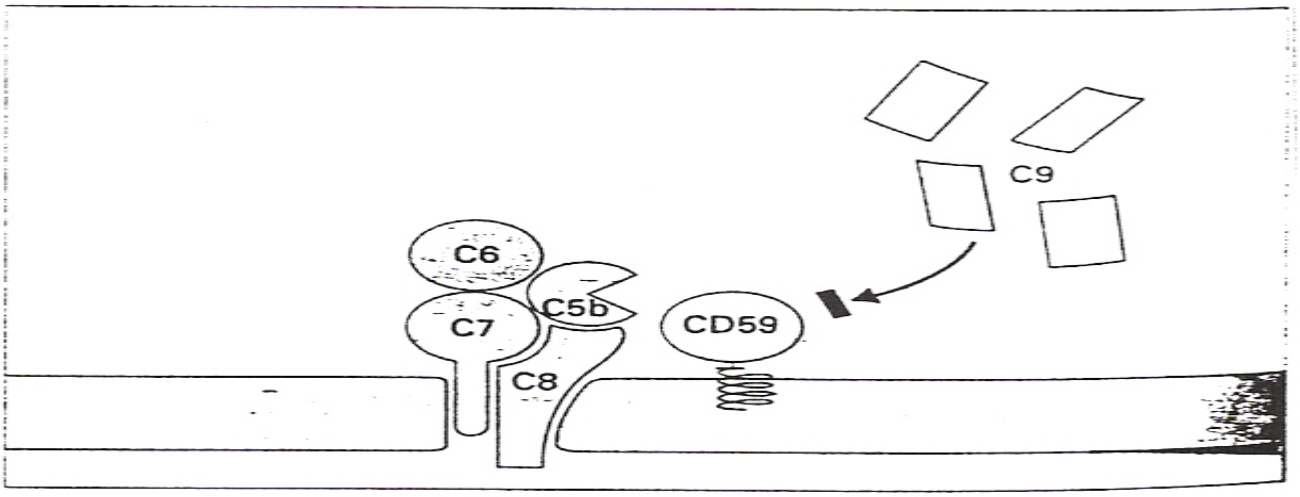
The lytic pathway



Once the hydrophobic C5b67 complex has formed it can insert itself into other cell membranes close to the primary surface on which complement activation is focused. This process of 'reactive lysis' could, if unregulated, have damaging consequences to self/host tissues. Thus the regulation of the formation of the MAC must be regulated to reduce the 'reactive lysis'. This regulation occurs through the binding of the **S proteins** (vitronectin) present in plasma which bind to the C5b67 in the **fluid phase** and form inactive SC5b67 complex which is unable to insert into lipid bilayers. If **C8** binds to the C5b67 in the **fluid phase** this also form a complex incapable of membrane insertion.

Two species restricted proteins are found **on the membrane** of the host cells which mediate the process of the host protection from the reactive lysis. These are the CD59 which is a protein anchored by a glycophospholipid foot. It is widely distributed in the cell membranes, binds to C8 in C5b-8 complexes, and inhibits the insertion and unfolding of C9 into cell membranes (figure).

CD59 inhibits C9 binding to C5b-8



COMPLEMENT RECEPTORS

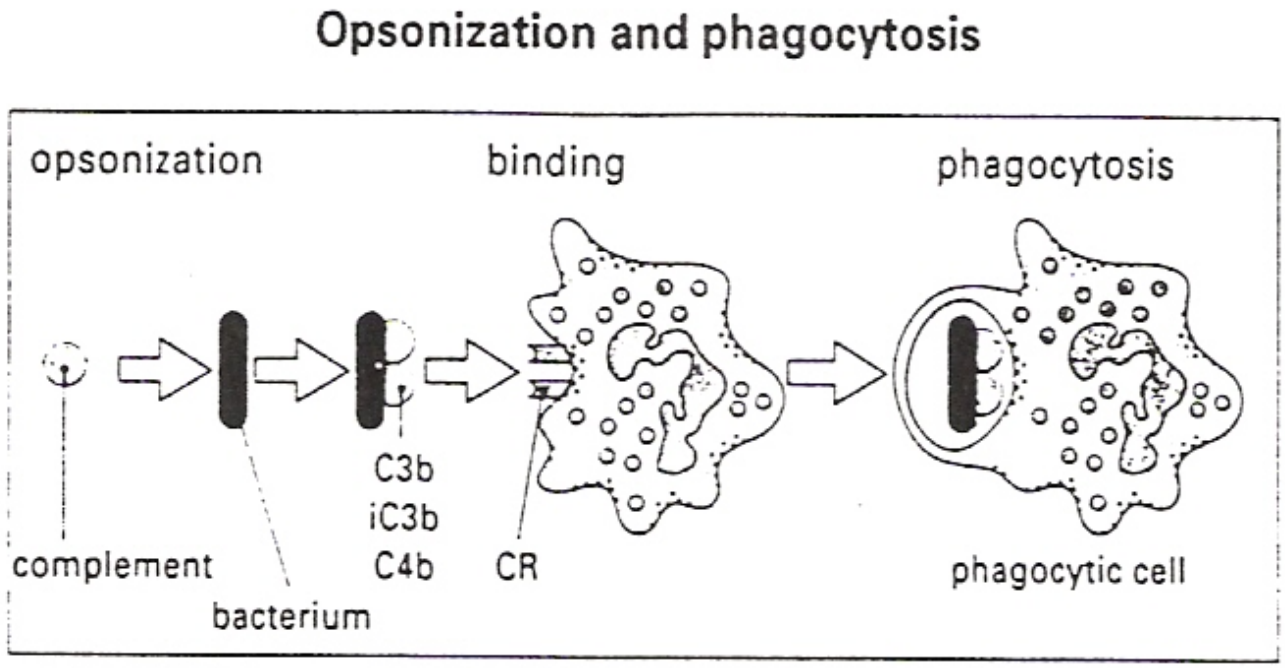
Many of the fragments of complement proteins produced during activation bind to specific receptors on the surface of immune cells. This is an important mechanism for the mediation of the physiological effects of complement, including uptake of particles opsonized by complement, and activation of the cell bearing the receptor.

There are **four** receptors for the three fragments of the complement component **C3.** These fragments are **C3b, iC3b and C3dg.** These fragments bind to the membranes of the target cells. The four different receptors for these opsonic fragments are named complement receptors 1 to 4 (CR1, CR2, CR3, and CR4). The ligands and the types of cells bear CR1 is illustrated in the table below.

Complement receptors for fragments of C3

receptor	ligands	cellular distribution
CR1 (CD35)	C3b>iC3b, C4b	B cells, neutrophils, monocytes, macrophages, erythrocytes, follicular dendritic cells, glomerular epithelial cells
CR2 (CD21)	iC3b, C3dg Epstein–Barr virus interferon-α	B cells, follicular dendritic cells, epithelial cells of cervix and nasopharynx
CR3 (CD18/CD11b)	iC3b zymosan certain bacteria fibrinogen factor X ICAM-1	monocytes, macrophages, neutrophils, NK cells, follicular dendritic cells
CR4 (p150-95) (CD18/CD11c)	iC3b fibrinogen	neutrophils, monocytes, tissue macrophages

CR1 (CD35) It is a receptor for C4b, C3b, and iC3b CR1 functions as an opsonin (figure) (cofactor in factor I cleavage of C3b to C3dg, and also clear the antigen-antibody complexes. On B cells CR1 and CR2 mediate the lymphocyte activation. Where on red cells or platelets CR1 picks up the opsonized immune complexes or bacteria and transport them to mononuclear phagocytic cells.



CR2 (CD21) It binds iC3b, C3dg, Epstein-Barr virus, and alpha interferon CR2 involved in the activation of B lymphocytes. CR2 on B lymphocytes function as an accessory receptor to antibody in activating specific immune responses. Immune complexes, containing cross-linking antigen and complement are more effective at activating B cells than antigen alone.

CR3 and CR4 Both are calcium dependent, and belong to the leukocyte (Beta-2) integrin family of adhesion molecules. Both bind to fibrinogen and iC3b. CR3 binds to zymogene, certain bacteria, and ICAM-1 (inter cellular adhesion molecule). CR4 is found on both myeloid and lymphoid lineages, where it may be important receptor for particles opsonized with iC3b.

The C5a Complement Receptor

Two small fragments of complement proteins **C3a and C5a** can trigger the degranulation of mast cell, and are known as **anaphylatoxins.** The effects of the anaphylatoxins C3a and C5a are mediated by binding to specific receptors. Receptors for C5a are found on all cells derived from **myeloid lineage (neutrophils, eosinophils, basophils and mast cells, monocytes and macrophages).** The C5a receptor is homologous in structure to several receptors mediating **chemotactic signals,** including the f-met-leu-phe receptor (binding bacterial peptides) and receptors for the chemokinesis, IL-8.

Following receptor binding, C5a is internalized and degraded to inactive peptide fragments; this is an important mechanism for regulating and limiting C5a activity.

BIOLOGICAL EFFECTS OF COMPLEMENT

The biological effects of the complement are either harmful or beneficial to the host:

A. The major beneficial activities are:

1. Promotion of the killing of the microorganisms
2. The efficient clearing of immune complexes
3. The induction and enhancement of antibody responses.

B. Harmful effects are caused by complement activation under certain circumstances these are:

1. If activated systemically on a large scale; e.g., in Gram-negative septicemia
2. If activated by tissue necrosis, e.g., during myocardial infraction.
3. If activated by an autoimmune response to host tissues.

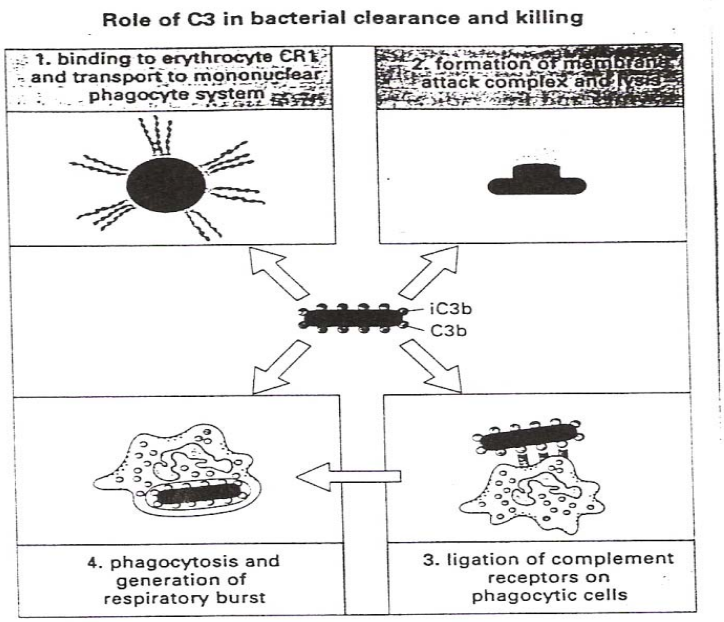
Complement promotes the killing of microorganisms

The enhancement of killing of microorganisms by complement is achieved by several ways:

1. By generation of anaphylatoxins, which increase vascular permeability and therefore recruit other components of the inflammatory response to the site of the infection
2. By opsonizing the microorganisms to enhance phagocytosis
3. By insertion of the membrane attack complex into the cell membranes of microorganisms.

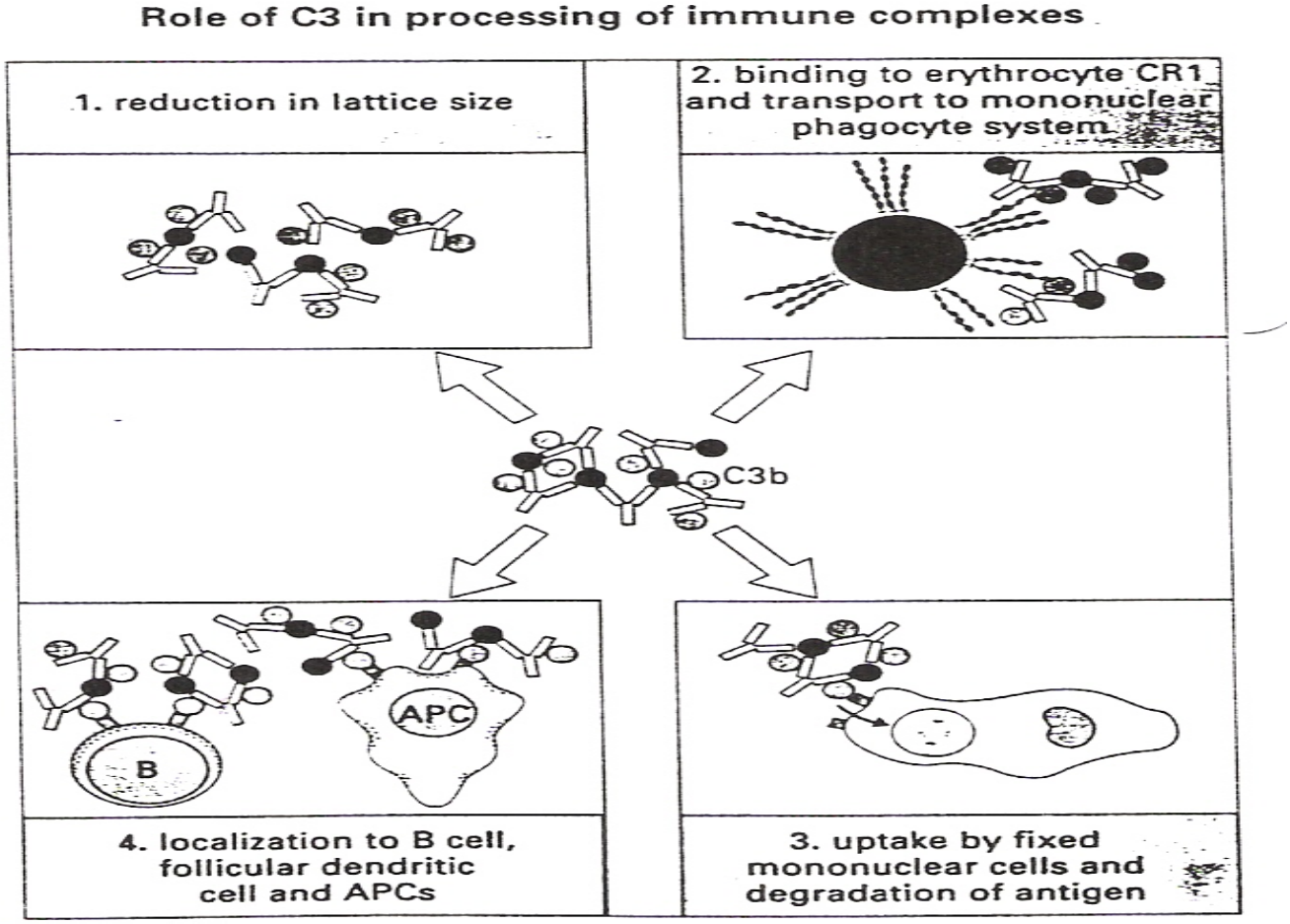
Role of C3 in bacterial clearance and killing

C3 bound to bacteria as C3b or iC3b **(figure)** (1) binds to CR1 on erythrocytes, which transport bacteria through the circulation, (2) acts as a focus for the deposition of lytic membrane attack complex on the bacterial cell membrane, (3) ligates complement receptors on phagocytic cells, which in turn (4) activates the phagocytic cell leading to bacterial phagocytosis, respiratory burst generation and bacterial killing.



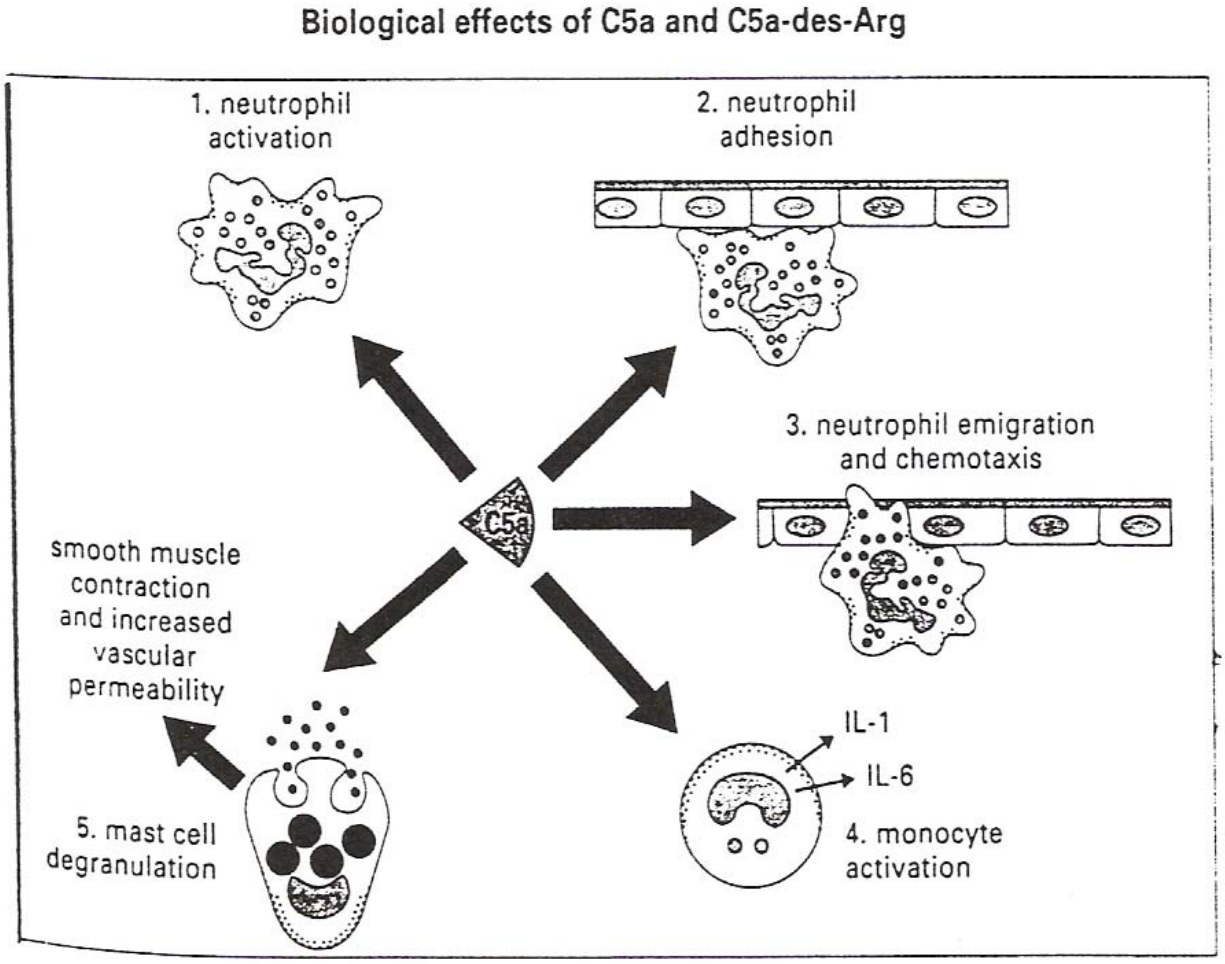
Role of C3 in processing of immune complexes

C3 binds to the immune complexes (figure) and (1) reduces the lattice size of the complex; (2) promotes the binding of immune complexes in the circulation to CR1 on erythrocytes which transport immune complexes through the circulation; (3) promotes the uptake of immune complexes by fixed mononuclear phagocytic cells leading to the generation of antigen; and (4) promotes the localization of antigen in the form of immune complexes to B lymphocytes and to antigen presenting cells, including the specialized follicular dendritic cells of lymph nodes.



Anaphylatoxins are potent inducers of inflammation

Activation of complement results in the production of the anaphylatoxins C5a and C3a, their functions are important in recruiting the inflammatory cells to sites of inflammation and activate their effector functions. Systemic administration of C5a or profound intravascular activation of complement may associate with development of cardiovascular collapse, and bronchospasm. C5a is a potent activator of all types of cells of the myeloid lineage (figure). Neutrophils respond to C5a by chemokinesis and Chemotaxis. They degranulate and the respiratory burst is occurred, leading to the production of oxygen free radicals. Membrane arachidonic acid is mobilized with production of prostaglandins. Surface expression of adhesion molecules also increases, which promotes adhesion to vascular endothelium. C3a is much less active than C5a, and its receptor is not yet characterized. It induces a weak neutrophil aggregation and activation of the respiratory burst. C3a is not a strong and significant chemotactic as C5a. **The anapylatoxins (C3a, and C5a) are not only produced as a result of the complement activation, but also from some enzymes systems such as lysosomal enzymes (neutrophil elastse), kallikrein, bacterial protases such as gingipain-1, and plasmin which all <u>directly</u> cleaves C3, C4, and C5.**



Complement deficiency is associated with an increase in the number of infections

The role of the complement as an opsonin is illustrated by two different hereditary deficiency states in human. Deficiency of either the components of the classical pathway and C3, or of the CR3, CR4 produces several infections caused by pyogenic bacteria. Complement plays an important role in the removal of these bacteria by phagocytosis and intracellular killing. Complement is important in the pathogenesis of some diseases:

1. **Systemic complement activation causes the production of large amount of anaphylatoxins (C3a, and C5a).** A large amount of these anapylatoxins are produced by the endotoxins of the Gram-negative bacteria cause activation and degranulation of neutrophils, basophils, and mast cells which lead to the state of **shock.**
2. **Tissue necrosis activates complement**. Ischemic (in adequate blood flow to a part of an organ) necrosis cause complement activation and abundant deposition of MAC **(e.g., myocardial infraction).**
3. **Complement activation may cause tissue injury following the formation of immune complexes in vivo.** An example of the immune complexes formation is the production of autoantibodies to the glomerular basement membrane so that the immune complexes form in the glomeruli; in **myasthenia gravis** where the autoantibodies are produced against the acetylcholine receptors. In bacterial endocarditis, an infected heart valve is the source of immune complexes that deposit in the kidney and other micro vascular beds.

<u>Complement mediates inflammation in these diseases in two major ways:</u>

1. Activate leukocytes are attracted to sites of immune-complex deposition by locally produced anaphylatoxins, and then bind to the C3b and C4b fixed to the immune complexes. This is the mechanism of **damage** in **Goodpasture's syndrome;** and inflammation which can be inhibited either by complement depletion or neutrophil depletion.
2. MAC causes cell membrane injury and thus **stimulates** prostaglandin **synthesis from arachidonic acid.**

ASSAYS FOR COMPLEMENT

Complement components are assayed for their total individual concentration and their functional levels. Total levels of individual complement proteins are usually measured by **RIA** or by **ELISA** using antibody specific for the protein under investigation. Functional levels are measured in assays tailored to detect each individual component protein by providing a **cocktail of sensitized red cells plus all** the components required for lysis**, except** the one under investigation.

Complement Fixation Test

1. General considerations

a. Complement, a protein constituent of normal serum, is **consumed (fixed)** during the interaction of antigens and antibodies.

(1). This interaction between complement and antigen-antibody complexes is called **complement fixation** when it occurs in vitro.

(2). When the same interaction occurs in vivo it is known as **complement activation** .

b. This phenomenon forms the basis for the **complement fixation test,** a sensitive in vitro procedure that is **widely used to <u>detect</u> or <u>quantitate</u> antigens or antibodies.**

2. The primary reacting ingredients are antigen, antibody, and complement.

a. Normal guinea pig serum is often used as a source of complement because the animal has high levels of complement with efficient lytic properties.

b. Different sources of complement are used in other tests; rabbit complement, for example, is used in cytotoxic tests performed for transplantation antigen detection.

3. Complement fixation is often used to determine whether a patient's serum contains antibody to a particular antigen. Both a **test system** and an **indicator system** are required, because the reaction can not be seen without an indicator system.

a. Test system

(1) The serum to be tested is heated to **56C** to **inactivate native complement**, and measured amounts of the antigen and complement (guinea pig serum) are then added.

(2) If antibody specific for that antigen is present in the serum, antigen-antibody complexes will be formed, that **will fix all the complements.**

b. Indicator system

(1). Sheep red blood cells (SRBCs) plus **hemolysin,** an antibody specific for SRBCs, are added to test for the presence of free (active) complement.

(3) Interpretation of the test is based on the presence of **hemolysis.**

(1) If all the complement has been fixed, none will be free to lyse the SRBCs; this constitutes a **POSITIVE** complement fixation test.

(2) If no antibody is present in the patient's serum, then the complement is not fixed and is free to interact in the indicator system, lysing the SRBCs; this constitutes a **NEGATIVE** complement fixation test.

4. Complement fixation tests require appropriate controls to ensure that **no anticomplementary (AC) factors** are present to affect the results adversely.

a. The antigen or the serum itself may have anticomplementary properties (e.g., they may contain denatured or aggregated immunoglobulin, heparin, or microbial contaminants).

b. The AC factors may fix all the complement in the system.

c. They may remove calcium or magnesium ions, both of which are essential for complement-mediated lysis.

COMPLEMENT DEFICIENCY

Impaired complement activity is usually **secondary** to diseases which consume of complement via the classical or alternative pathways. A common example is the **systemic lupus erythmatosus (SLE)** in which consumption of the early classical pathway complement components **C1, C4, and C2** impair the ability of complement to solubilize immune complexes, the degree of impairment correlating with disease activity.

In humans, **inherited deficiencies of complement components** are associated with characteristic clinical syndromes. Many patients with **C1, C4 or C2 deficiency** have presented with lupus-like syndrome of:

1. **malar flush**
2. **arthralgia**
3. **glomerulonephritis**
4. **Fever or chronic vasculitis and rarely with recurrent pyogenic infections.**
   Deficiency of any of these early classical pathway components probably compromises the ability of the host to eliminate immune complexes.

Patients with C3 deficiency have an **increased susceptibility to recurrent bacterial infections (e.g., pneumonia, septicemia, and meningitis**).

Deficiencies of **membrane attack complex** (**MAC**) components are associated with **increased susceptibility to infections** by ***Neisseria meningitidis*** Complement seem to be less important in host defense against viral infections, since T-cells play a more important role.

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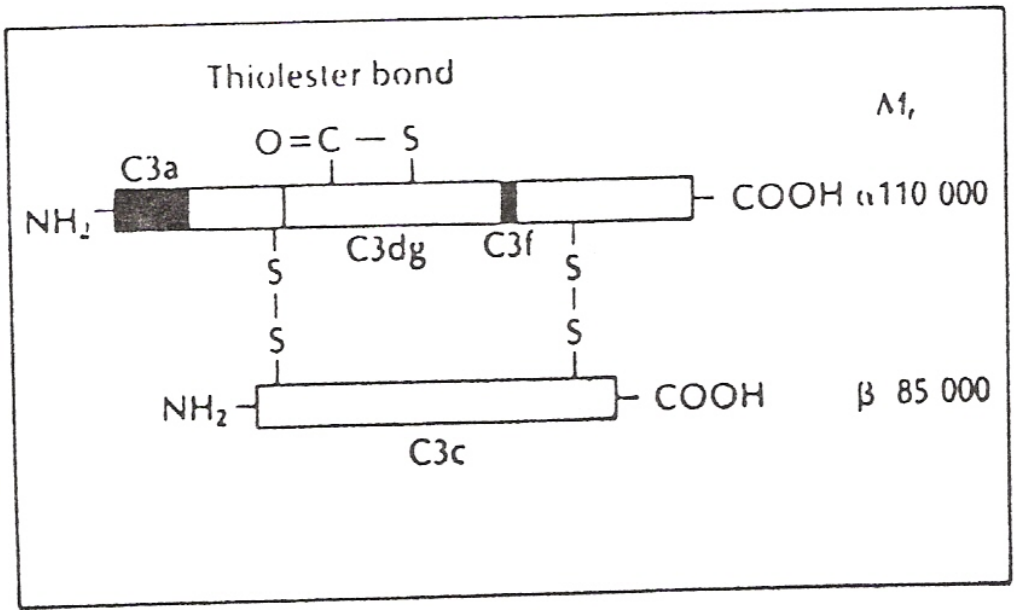
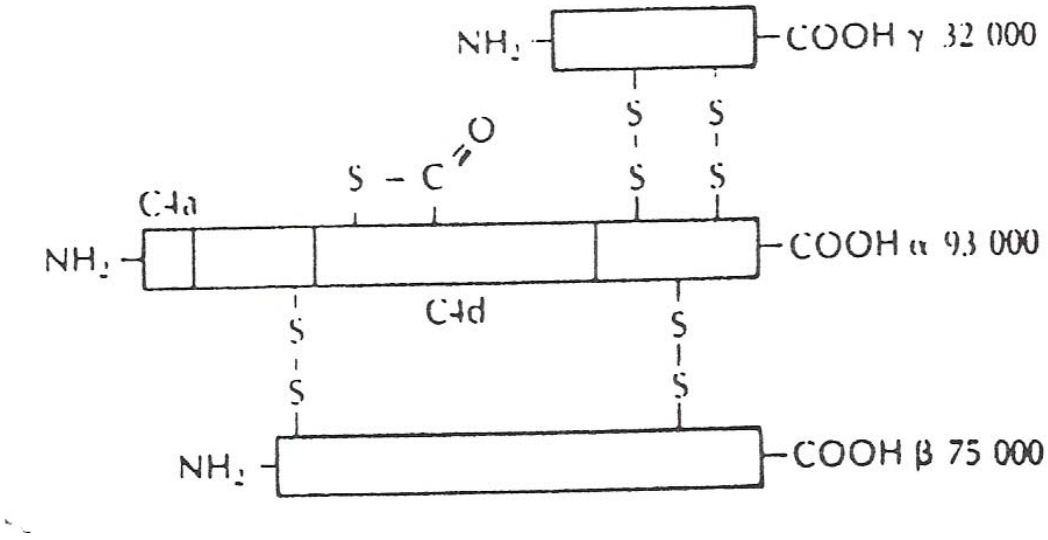
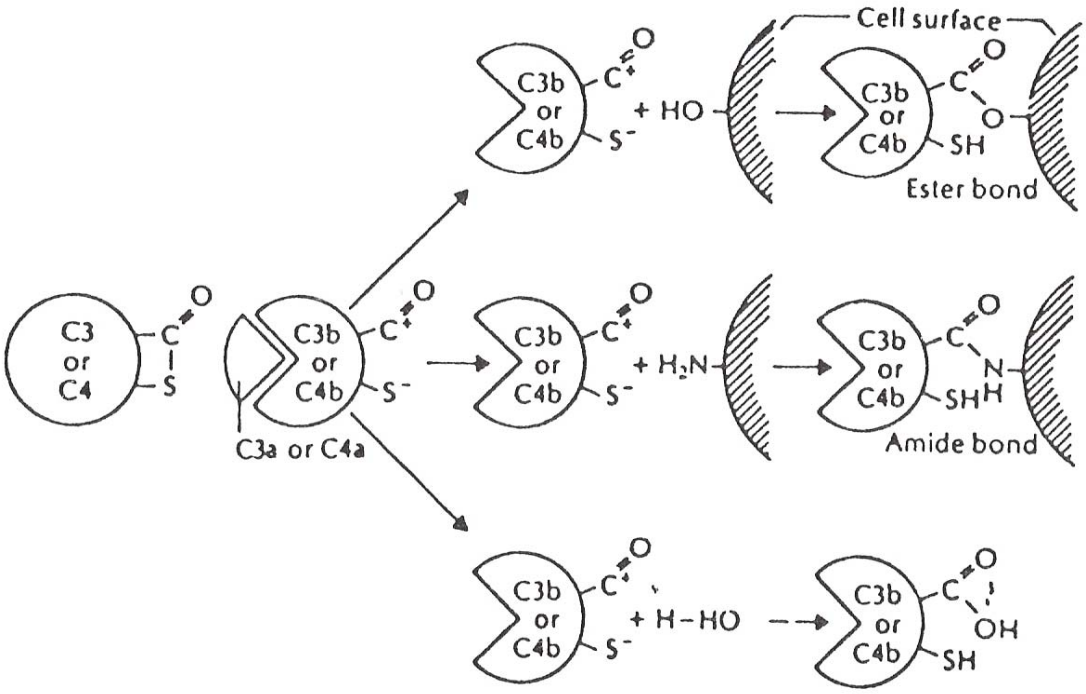


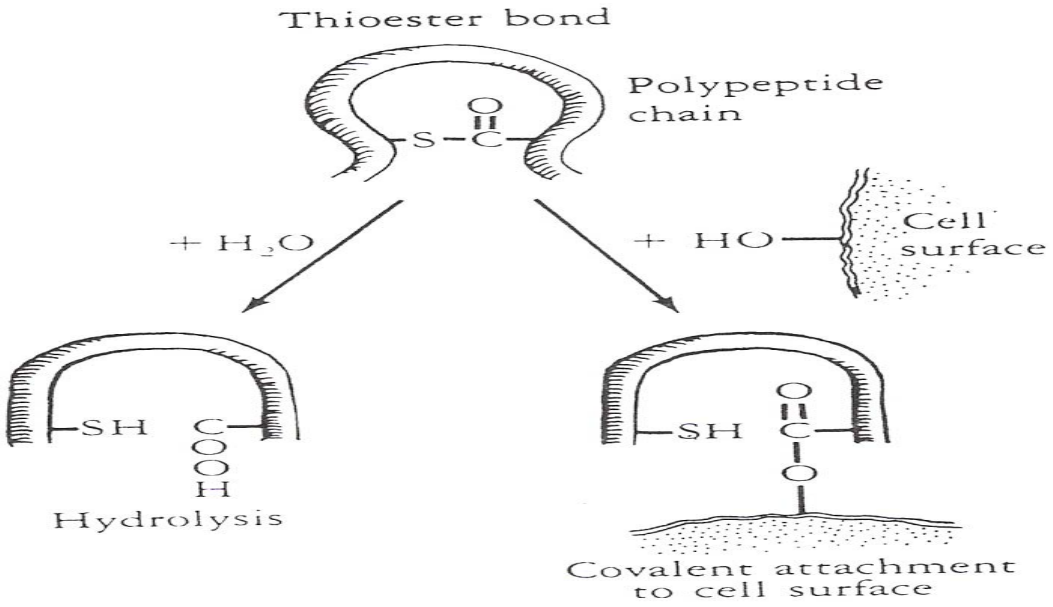
The structure of C3 complements protein



The structure of C4 complements protein



Thioester bond



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