Complement

Abdul Ghaffar (Phone: 733-3279; ***e-mail***: ghaffar@med.sc.edu)

MBIM 650/720 Medical Microbiology - Fall 1999 Lecture: 2

OBJECTIVES:

1. Understand different pathways of C activation.
2. Know the enzymatic and nonenzymatic mechanisms of complement activation.
3. Know the biological properties of complement activation products.
4. Know the significance of C system in host resistance, inflammation and damage to self.
5. Understand the mechanisms of regulating complement activation and it products.

READING: Roitt *et al*. Immunology (5th ed.), chapter 4.

Complement refers, historically to fresh serum capable of lysing antibody (Ab)-coated cells. This activity is destroyed (inactivated) by heating serum at 56°C for 30 minutes.

Proteins of the Complement System

Complement system is composed of more than **20 different proteins** (Table 1) produced by different tissues and cells including hepatocytes, macrophages and gut epithelial cells. These proteins are activated by a variety of agents and their activation proceeds in a cascade fashion leading to lysis. Consequently, an absence of one of the components in the cascade can terminate the reaction.

Table 1. Proteins of the Complement system

Classical Pathway	Alternative Pathway	Membrane Attack Pathway
Activation Proteins: C1qrs, C2, C3, C4	C3, Factors B & D*, Properdin	C5, C6, C7, C8, C9
Control Proteins: C1-INH, C4-BP	Factors I* & H, DAF, CR1, *etc*.	Protein S

Components underlined acquire enzymatic activity when activated.
Components marked with '*' have enzymatic activity in native form.

Pathways of complement activation:

The complement activation can be divided into three pathways: **classical pathway**, **alternative pathway** and **membrane attack pathway**. Both classical and alternative pathways lead to the activation of C5 convertase and result in the production of C5b which is essential for the activation of the membrane attack pathway.

Classical pathway

Classical pathway (Figure 1) normally requires a suitable Ab bound to antigen (Ag), complement components 1, 4, 2 and 3 and Ca^{++} and Mg^{++} cations.

C1 activation: Binding of C1qrs (a calcium dependent complex), present in normal serum, to Ag-Ab complexes results in autocatalysis of C1r. The altered C1r cleaves C1s and this cleaved C1s becomes an enzyme (**C4-C2 convertase**) capable of cleaving both C4 and C2.

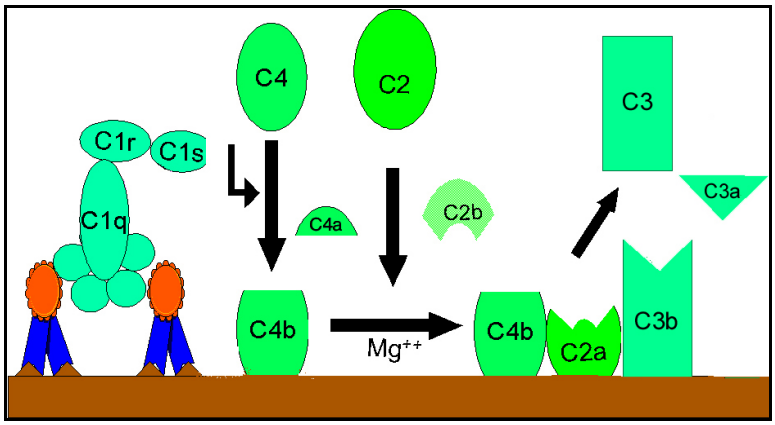

Figure 1: Activation of C and generation of C3 convertase by the classical pathway

C4 and C2 activation (generation of C3 convertase): Activated C1s enzymatically cleaves C4 into C4a and C4b. C4b binds to the Ag-bearing particle or cell membrane while C4a remains a biologically active peptide at the reaction site. C4b binds C2 which becomes susceptible to C1s and is cleaved into C2a and C2b. C2a remains complexed with C4b whereas C2b is released in the micro environment. C4b2a complex, is known as **C3 convertase** in which C2a is the enzymatic moiety.

C3 activation (generation of C5 convertase): C3 convertase, in the presence of Mg^{++}, cleaves C3 into C3a and C3b. C3b binds to the membrane to form C4b2a3b complex whereas C3a remains in the micro environment. C4b2a3b complex functions as **C5 convertase** which cleaves C5 into C5a and C5b (Figure 2). Generation of C5 convertase marks the end of the classical pathway.

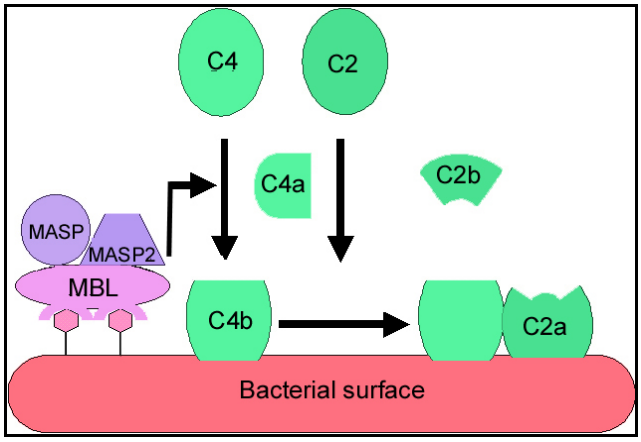

Figure 2: Lectin-initiated classical pathway

C4 activation, a crucial early event in the classical pathway can be achieved without antibodies and C1 participation by the **lectin pathway** (Figure 2). This pathway is initiated by three proteins: a mannan-binding lectin (MBL), also known as mannan-binding protein (MBP) which interacts with two mannan-binding lectin-associated serine

proteases (MASP and MADSP2), analogous to C1r and C1s. This interaction generates a complex analogous to C1qrs and leads to antibody -independent activation of the classical pathway. C1q can also bind to a number of agents including some retroviruses, mycoplasma, poly-inosinic acid and aggregated IgG, and initiate the classical pathway.

Alternative Pathway:

Alternative pathway begins with the activation of C3 and requires **Factors B** and **D** and Mg^{++} cation, all present in normal serum.

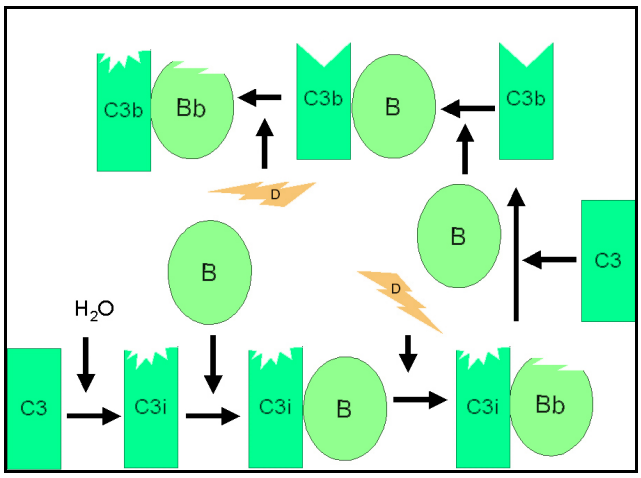

Figure 3: Spontaneous activation of C3 (tickover)

Spontaneous activation of C3: A metastable C3b-like molecule (C3i) is generated by slow hydrolysis of the native C3. C3i binds factor B which is cleaved by **Factor D** to produce C3iBb. C3iBb complex cleaves native C3 into C3a and C3b (Figure 3). C3b binds factor B, which is again cleaved by Factor D to produce C3bBb (**C3 convertase**). This C3 convertase (or the one generated by classical pathway: C4b2a), if not inactivated, will continue to act on C3 and cause its total depletion.

Normal regulation of C3 convertase:

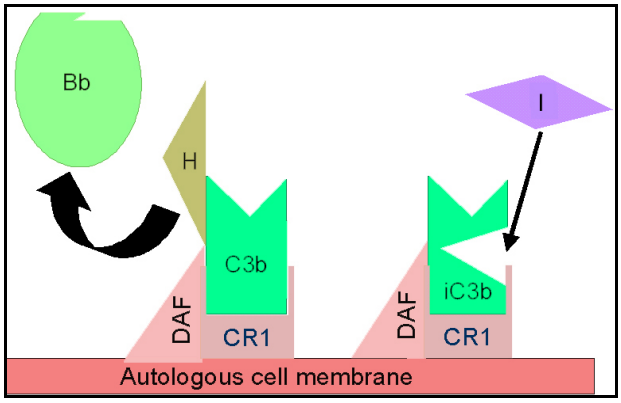

Figure 4: Regulation of C3 by Cr1

C3b, in fluid phase, is very short lived unless it finds a suitable stabilizing membrane or molecule (C3 activator; see later). It binds quickly to autologous red cells via the C3b receptor, **CR1** at a site close to **decay accelerating factor** (**DAF**) which prevents the binding of Factor B. Binding to CR1 also makes C3b susceptible to **Factor I** (Figure 4) which cleaves it into many fragments (iC3b, C3d, C3e, *etc.*). C4b, generated in the classical pathway, is also regulated by DAF, CR1 and Factor I (Figure 5). A defect in or deficiency of DAF can lead to cell lysis and anemia, as in its absence further activation of C will proceed and lead to the membrane attack pathway (see below) and cell lysis.

Another serum protein, **factor H**, can displace factor B and bind to C3b. Binding of factor H makes C3b more susceptible to factor I (see figure 4). C3 convertase generated by the classical pathway is regulated also in a similar manner by DAF, Cr1 and Factor I. The only difference is that C4b-binding protein (C4b-BP, not factor H) makes it susceptible

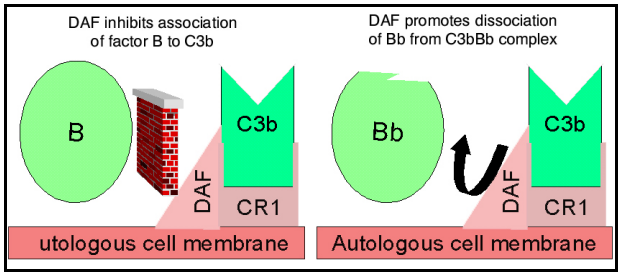

Figure 5: Regulation of C3 by DAF

to Factor I. A genetic deficiency of factor I (or factor H) leads to uncontrolled C3 activation and is a major cause of inherited C3 deficiency.

Stabilization of C3 convertase: Certain bacteria or their products (peptidoglycan, polysaccharides, etc.), provide a protected (activator) surface for C3b. Thus, C3b bound to such a surface is relatively resistant to the action of factor I (Figure 6). Even membrane bound C3bBb dissociates fairly rapidly. However, binding of another protein, **properdin**, further stabilizes this complex. It is for this reason, the alternative pathway is also called the properdin pathway.

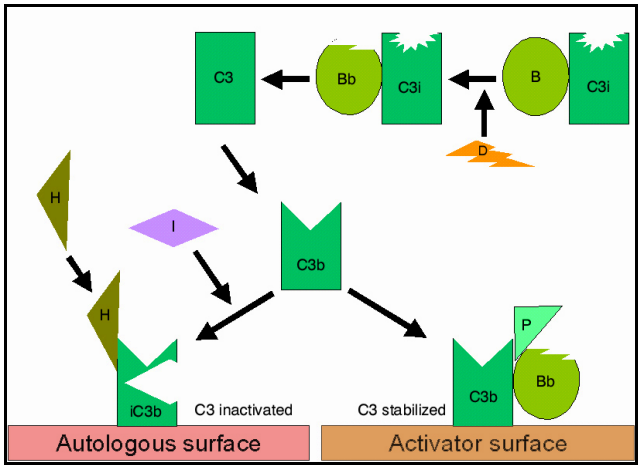

Figure 6: Stabilization of C3 convertase

Generation of C5 convertase: Stabilized C3 convertase cleaves more C3 and produces C3bBbC3b complex (analogous to C4b2b3b of the classical pathway), the **C5 convertase** which cleaves C5 into C5a and C5b (Figure 6). C5b initiates the membrane attack pathway which leads to cell lysis. *While the classical and alternative pathways are initiated by different mechanisms, they are analogous to each other and both can lead to membrane lysis*.

The alternative pathway provides a means of non-specific resistance against infection without the participation of antibodies and hence provides a first line of defense against a number of infectious agents.

Many gram negative and some gram positive bacteria, certain viruses, parasites, heterologous red cells, aggregated immunoglobulins (particularly, IgA) and some other proteins can activate the alternative pathway. One protein, **cobra venom factor (CVF)**, has been extensively studied for its ability to activate this pathway.

Membrane Attack Pathway:

Membrane attack pathway involves the C5-9 components. C5 convertase generated by the classical or alternative pathway cleaves C5 into C5a and C5b. C5b binds C6 and subsequently C7 to yield a hydrophobic C5b67 complex which attaches quickly to the plasma membrane (Figure 7). Subsequently, C8 binds to this complex and causes the insertion of several C9 molecules. bind to this complex and lead to formation of a hole in the membrane resulting in cell lysis. The lysis of target cell by C5b6789 complex is nonenzymatic and is believed to be due to a physical change in the plasma membrane. C5b67 can bind indiscriminately to any cell membrane leading to

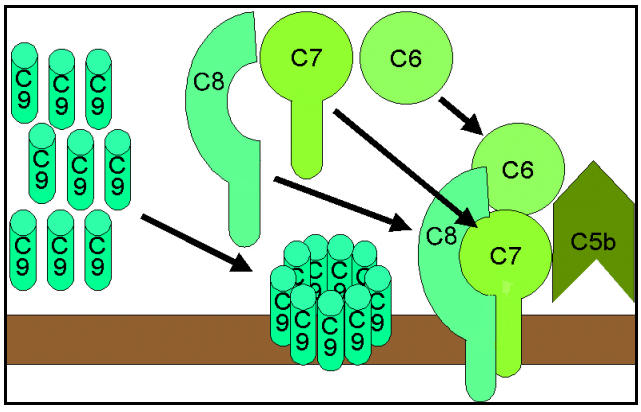

Figure 7: The lytic pathway

cell lysis. Such an indiscriminate damage to by-standing cells is prevented by **protein S** (vitronectin) which binds to C5b67 complex and blocks its indiscriminate binding to cells other than the primary target.

Biologically active products of Complement activation

Activation of complement results in the production of several biologically active molecules which contribute to resistance, anaphylaxis and inflammation.

Kinin production: C2b generated during the classical pathway of C activation is a **prokinin** which becomes biologically active following enzymatic alteration by plasmin. Excess C2b production is prevented by limiting C2 activation by **C1 inhibitor** (C1-INH) also known as serpin which displaces C1rs from the C1qrs complex (Figure 8). A genetic deficiency of C1-INH results in an overproduction of C2b and is the cause of **hereditary angioneurotic edema**. This condition can be treated with Danazol which promotes C1-INH production or with -amino caproic acid which decreases plasmin activity.

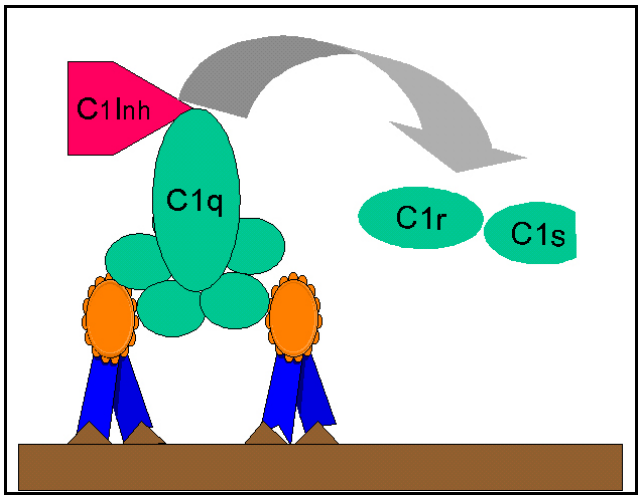

Figure 8: Regulation of C1rs by C1-INH (serpin)

Anaphylotoxins: C4a, C3a and C5a (in increasing order of activity) are all **Anaphylotoxins** which cause basophil/mast cell degranulation and smooth muscle contraction. Undesirable effects of these peptides are controlled by **carboxypeptidase B** (C3a-INA).

Chemotactic Factors: C5a and MAC (C5b67) are both chemotactic. C5a is also a potent activator of neutrophils, basophils and macrophages and causes induction of adhesion molecules on vascular endothelial cells.

Opsonins: C3b and C4b in the surface of microorganisms attach to C-receptor (CR1) on phagocytic cells and promote phagocytosis.

Other Biologically active products of C activation: Degradation products of C3 (iC3b, C3d and C3e) also bind to different cells by distinct receptors and modulate their functions.

In summary the complement system takes part in both the specific and non specific resistance and generates a number of products of biological and pathophysiological significance (Table 2).

There are known genetic deficiencies of most individual C complement components, but C3 deficiency is most serious and fatal. Complement deficiencies also occur in immune complex diseases (e.g., SLE) and acute and chronic bacterial, viral and parasitic infections.

Table 2: Biological Properties of C Activation Products and their Regulatory Molecules.

Component	Biological activity	Effect	Controls
C2b (prokinin)	Accumulation of body fluid	Edema	C1-INH
C3a (anaphylatoxin)	Basophil and mast cell degranulation; enhanced vascular permeability; smooth muscle contraction; Induction of suppressor T cells.	Anaphylaxis Immunoregulation	Carboxy-peptidase- B (C3a-INA)
C3b and its products	Opsonization; Phagocyte activation	Phagocytosis	Factors H & I
C4a (anaphylatoxin)	Basophil & mast cell activation; smooth muscle contraction; enhanced vascular permeability.	Anaphylaxis	C3a-INA
C4b	Opsonization	Phagocytosis	C4-BP, Factor I
C5a (anaphylatoxin; Chemotactic factor)	Basophil & mast cell activation; enhanced vascular permeability; smooth muscle contraction. Chemotaxis; neutrophil aggregation; Oxidative metabolism stimulation. Stimulation of leukotriene release Induction of helper T-cells.	Anaphylaxis Inflammation Delayed anaphylaxis. Immunoregulation.	C3a INA
C5b67	Chemotaxis; attachment to other cell membranes.	Inflammation; lysis of bystander cells.	Protein-S

You have learned:

1. Proteins of the complement system.
2. Differences and similarities between classical and alternative pathways.
3. Significance of the two pathways in specific and nonspecific immunity.
4. Role of different complement activation products in amplification of nonspecific and specific immunity and inflammation.