Complement

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PAMB 650/720 Medical Microbiology _ Fall 2004 Lecture: 2

OBJECTIVES:

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

READING: Roitt *et al*. Immunology (6th ed.), chapter 4: pp 54-63.

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

Definitions:

C-activation: Alteration of a complement component (protein) in such a way that it can proceed to interact with the next component in the pathway (cascade).

C-fixation: Utilization of complement components by the antigen-antibody complex.

Hemolytic units: The dilution of a serum sample which can lyse a predetermined proportion of a sheep erythrocyte (SRBC) suspension coated with anti-SRBC antibody. The SRBC concentration is usually 5% and the lysis is set at 50% and the unit is recorded as CH50.

C-inactivation: Denaturation (usually by heat) of one of the early components in C-activation pathway resulting in the destruction of C-hemolytic activity.

Convertase/esterase: Activated (altered/cleaved) C-component which acts as a proteolytic enzyme specific for subsequent components.

Proteins of the Complement System

Complement system is composed of more than **25 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 via different pathways and if the activated products bind to a cellular target, their deposition leads to cell lysis. Since the complement components are activated in a cascade fashion, the absence of one of the components in the pathway can disrupt the cascade and terminate the reaction.

Table 1. Proteins of the Complement system

Classical Pathway	Lectin pathway	Alternative Pathway	Lytic Pathway
Activation Proteins: C1qrs, C2, C3, C4	Mannan binding protein (MBP), Mannan associated serine protease (MASP1, MASP2)	C3, Factors B & D*, Properdin	C5, C6, C7, C8, C9
Control Proteins: C1-INH, C4-BP Factors I* & H, DAF, CR1, *etc*.			Protein S (vitronectin)

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

Pathways of complement activation:

The complement activation can occur by three pathways, **classical**, **lectin (mannose binding protein)** and **alternative pathway**, all leading to the activation of C5 that follows the activation of the **membrane attack (lytic) pathway**.

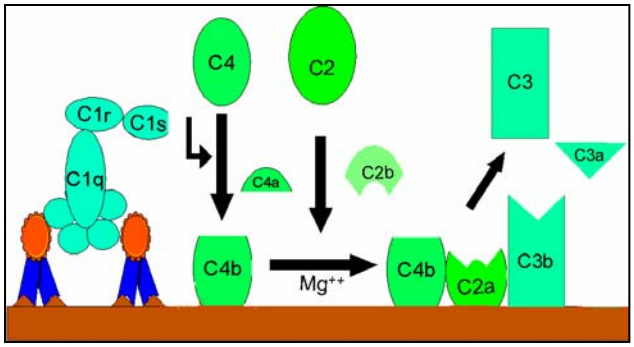

Figure 1. Activation of C3 by the classical pathway

Classical pathway

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

C1 activation: Binding of C1 (C1q, C1r & C1s: a Ca^{++} dependent complex), present in normal serum, to Ag-Ab complexes results in the autocatalysis of C1r. The altered C1r cleaves C1s and this cleaved C1s functions as **C4-C2 convertase** capable of cleaving both C4 and C2.

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 that becomes susceptible to C1s and is cleaved into C2a and C2b. C2a remains complexed with C4b whereas C2b is released in the microenvironment. 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; C3a is released in the micro-environment. 4b2a3b complex functions as **C5 convertase** that cleaves C5 into C5a and C5b. **Generation of C5 convertase marks the end of the classical pathway**.

C1qrs can also bind to a number of agents including some retroviruses, mycoplasma, poly-inosinic acid and aggregated IgG, and initiate the classical pathway.

Lectin pathway:

C4 activation can be achieved without antibody and C1 participation via the **lectin pathway** (Figure 2). This pathway is initiated by three proteins: a mannan-binding lectin (MBL), also known as mannan-binding protein (MBP) and two mannan-binding lectin-associated serine proteases (MASP-1 and MASP-2), all present in normal serum. MBL binds to certain mannose residues on many bacteria and subsequently interacts with MASP and MASP2. The MBL-MASP-1-MASP-2 complex is analogous to Ab-C1qrs comlex and leads to antibody-independent activation of C4, C2 and C3. Thus, the lectin pathway provides a means of non-specific protection against certain pathogens before any antibody response can be mounted.

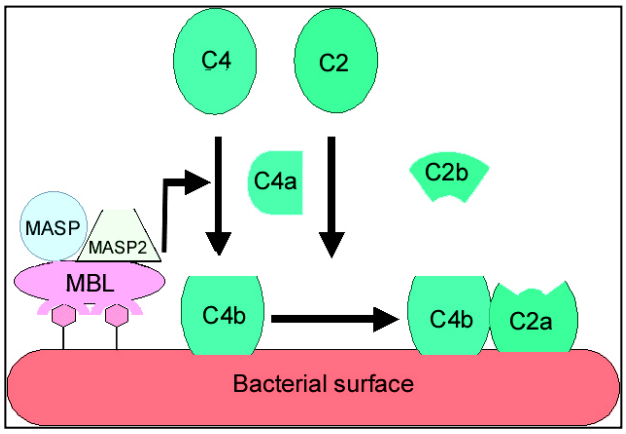

Figure 2. Lectin initiated pathway

Alternative Pathway:

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

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

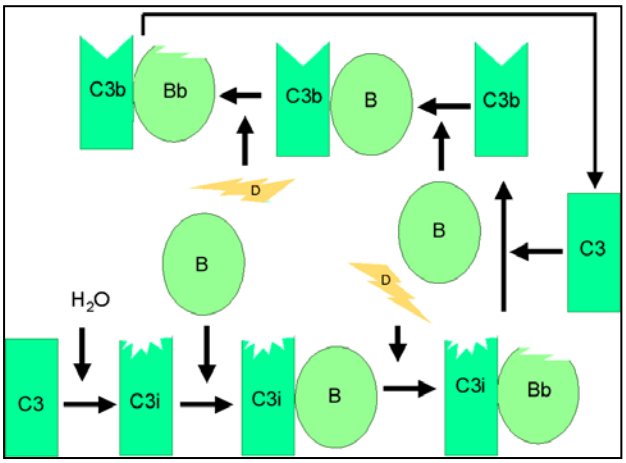

Figure 3. Spontaneous activation og C3 (C3-tickover)

Normal regulation of C3 convertase:

C3b, in fluid phase, is very short lived unless it finds a suitable stabilizing membrane or molecule (C3 activator; see later) present on many pathogens. In the absence of such a molecule, it binds quickly to autologous red cells via the C3b receptor, **CR1** at a site close to **decay accelerating factor** (**DAF**) that prevents the binding of Factor B. Binding to CR1 also makes C3b susceptible to

Factor I (Figure 4) that cleaves it into many fragments (iC3b, C3d, C3e, *etc.*: Figure 5). C4b, generated in the classical pathway, is also regulated by DAF, CR1 and Factor I. A defect in or deficiency of DAF can lead to red cell lysis and anemia, as in its absence, further activation of C will proceed and lead to the lytic pathway (see below).

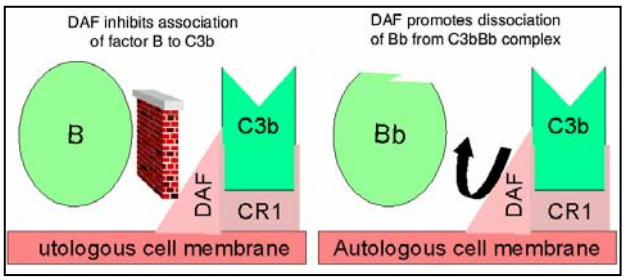

Figure 4. Regulation of activated C3 by DAF

Another serum protein, **factor H**, can displace factor B and bind to C3b. Binding of factor H also makes C3b more susceptible to factor I (see figure 5). DAF, Cr1 and Factor I also regulate, in a similar manner, C3 convertase generated by the classical pathway. The only difference is that C4b-binding protein (C4b-BP, not factor H) makes it susceptible to Factor I. A genetic deficiency of factor I (or factor H) leads to uncontrolled C3 activation and exhaustion. A genetic defect in factor I or factor H and is a significant cause of inherited C3 deficiency and increased susceptibility to certain infections.

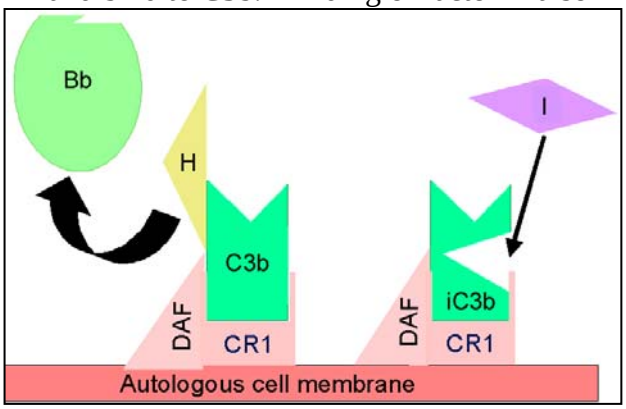

Figure 5. Regulation of activated C3 by Factor

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 becomes 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. Consequently, the alternative pathway is also referred to as the properdin pathway.

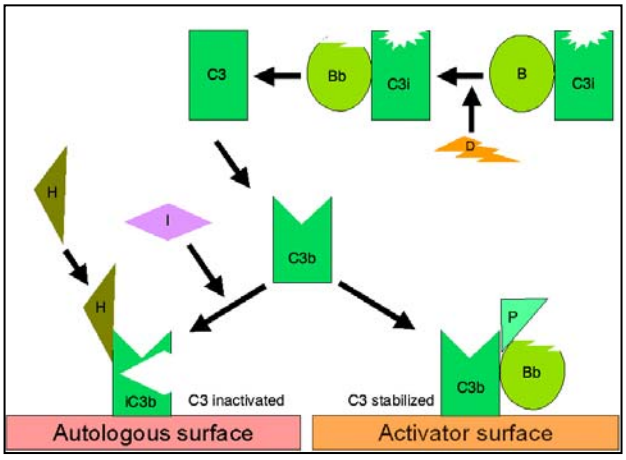

Figure 6. Stabilization of C3 convertase

Generation of C5 convertase: Stabilized C3 convertase cleaves more C3 and produces C3bBbC3b complex the **C5 convertase** of the alternative pathway (analogous to C4b2a3b of the classical pathway), which cleaves C5 into C5a and C5b. C5b initiates the membrane attack pathway that leads to cell lysis. Thus, *C3 can be activated by several pathways that are analogous to each other and they all can lead to membrane lysis*.

The alternative pathway can be activated by many Gram-negative (most significantly, ***Neisseria meningitidis* and *N. gonorrhoea***), some Gram-positive bacteria and certain viruses and parasites, that results in the lysis of these organisms. Thus, the alternative pathway of C activation provides another means of protection against certain pathogens before an antibody response is mounted. A deficiency of C3 results in an increased susceptibility to these organisms.

Lytic Pathway:

The lytic pathway involves the C5-C9 components. C5 convertase, generated by one of the pathways described above, cleaves C5 into C5a and C5b. C5b instantaneously binds C6 and subsequently C7 to yield a hydrophobic C5b67 complex that attaches quickly to plasma membrane (Figure 7). Subsequently, C8 binds to this complex and causes the insertion of several C9 molecules. The insertion of $C8(9)_n$ complex causes formation of a hole in the membrane and cell lysis. This lysis 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 their lysis. However, such an indiscriminate damage to by-standing cells is prevented by **protein S** (vitronectin) that binds to C5b67 complex and blocks its indiscriminate binding to cells other than the primary target.

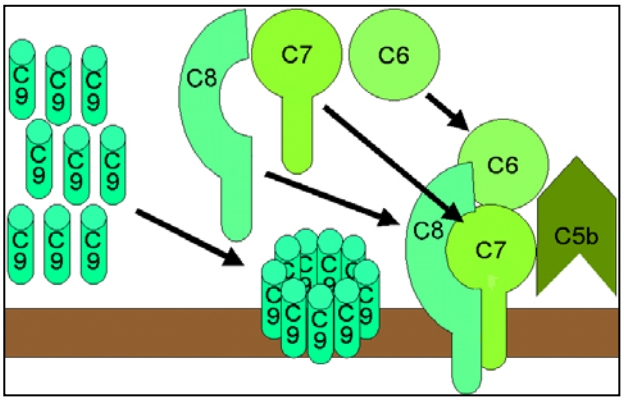

Figure 7. The lytic pathway

Biologically active products of Complement activation

Activation of complement results in the production of several biologically active molecules that contribute to nonspecific immunity and inflammation. These have been described below.

Kinin production: C2b generated during the classical pathway of C activation is a **prokinin** which becomes biologically active following enzymatic alteration by plasmin and causes vascular permeability and edema. Excess C2b production is prevented by limiting C2 activation by **C1 inhibitor** (C1-INH) also known as serpin that dismantles the activated Cq1rs 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 that promotes C1-INH production or with , ∝–amino caproic acid that decreases the plasmin activity.

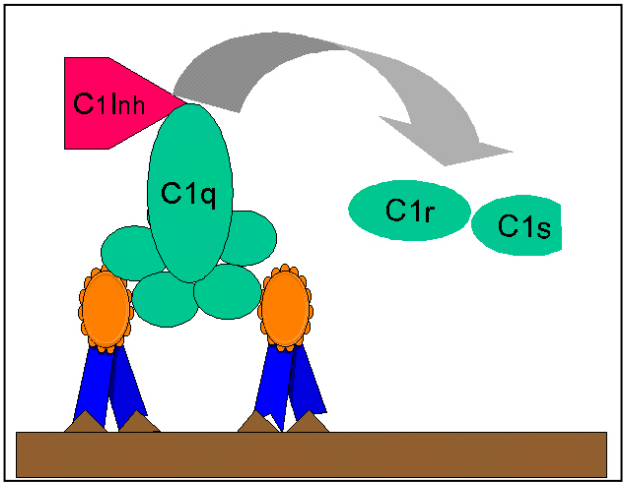

Figure 8. Regulation of C1rs (C4-2 convertase) by C1-

Anaphylotoxins: C4a, C3a and C5a are all **Anaphylotoxins** (in increasing order of activity) that cause basophil/mast cell degranulation and smooth muscle contraction. An uncontrolled production of these anaphylotoxins can lead to pathologic consequences. These anaphylotoxins are normally inactivated by **carboxypeptidase B** (C3a-INA).

Chemotactic Factors: C5a and MAC (C5b67) are both chemotactic. C5a is also a potent activator of neutrophils,and macrophages and thus amplifies nonspecific immunity. It also causes induction of adhesion molecules on vascular endothelial cells and hence promotes diapedesis.

Opsonins: C3b and C4b on 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 in is an important component of the nonspecific immune function and an adjunct to the specific immune system. It generates a number of products of biologic and pathophysiologic significance (Table 2).

There are known genetic deficiencies of most individual 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;	Anaphylaxis	Carboxy-peptidase- B (C3a-INA)
	Induction of suppressor T cells.	Immunoregulation	
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.	Anaphylaxis	C3a INA
	Chemotaxis; neutrophil aggregation; Oxidative metabolism stimulation. Stimulation of leukotriene release Induction of helper T-cells.	Inflammation Delayed anaphylaxis. Immunoregulation.	
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 among the different pathways of C3 activation.
3. Significance of the different pathways in specific and nonspecific immunity.
4. Role of different complement activation products in amplification of nonspecific and specific immunity and inflammation.