Regulation and deficiencies of complement

Regulation of the Complement System

Because many elements of the complement system are capable of attacking host cells as well as foreign cells and microorganisms, elaborate regulatory mechanisms have evolved to restrict complement activity to designated targets. A general mechanism of regulation in all complement pathways is the inclusion of highly labile components that undergo spontaneous inactivation if they are not stabilized by reaction with other components. In addition, a series of regulatory proteins can inactivate various complement components (Table). For example, the glycoprotein C1 inhibitor (C1Inh) can form a complex with $C1r_2s_2$, causing it to dissociate from C1q and preventing further activation of C4 or C2 (Figure1a (1)).

The reaction catalyzed by the C3 convertase enzymes of the classical, lectin, and alternative pathways is the major amplification step in complement activation, generating hundreds of molecules of C3b. The C3b generated by these enzymes has the potential to bind to nearby cells, mediating damage to the healthy cells by causing their opsonization by phagocytic cells bearing C3b receptors or by induction of the membrane attack complex. Damage to normal host cells is prevented because C3b undergoes spontaneous hydrolysis by the time it has diffused 40 nm away from the C4b2a or C3bBb convertase enzymes, so that it can no longer bind to its target site. The potential destruction of healthy host cells by C3b is further limited by a family of related proteins that regulate C3 convertase activity in the classical and alternative pathways. These regulatory proteins all contain repeating amino acid sequences (or motifs) of about 60 residues, termed *short consensus repeats* **(SCRs).** All these proteins are encoded at a single location on chromosome 1 in humans, known as the ***regulators of complement activation*** **(RCA)** gene cluster.

In the classical and lectin pathways, three structurally distinct RCA proteins act similarly to prevent assembly of C3 convertase (Figure 1 (2)). These regulatory proteins include soluble C4b-binding protein (C4bBP) and two membrane- bound proteins, complement receptor type 1 (CR1) and membrane cofactor protein (MCP). Each of these regulatory proteins binds to C4b and prevents its association with C2a. Once C4bBP, CR1, or MCP is bound to C4b, another

regulatory protein, factor I, cleaves the C4b into bound C4d and soluble C4c (Figure 1a 3)). A similar regulatory sequence operates to prevent assembly of the C3 convertase
C3bBb in the alternative pathway. In this case CR1,MCP, or a regulatory component called factor H binds to C3b and preventsits association with factor B (Figure 1a(4)). Once CR1, MCP, or factor H is bound to C3b, factor I cleaves the C3b into a bound iC3b fragment and a soluble C3f fragment. Further cleavage of iC3b by factor I releases C3c and leaves C3dg bound to the membrane (Figure 1 a(5)).

Several RCA proteins also act on the assembled C3 convertase, causing it to dissociate; these include the previously mentioned C4bBP, CR1, and factor H. In addition, **decay accelerating factor (DAF or CD55),**which is a glycoprotein anchored covalently to a glycophospholipid membrane protein, has the ability to dissociate C3 convertase. Each of these RCA proteins accelerates decay (dissociation) of C3 convertase by releasing the component with enzymatic activity (C2a or Bb) from the cell-bound component (C4b or C3b). Once dissociation of the C3 convertase occurs, factor I cleaves the remaining membrane-bound C4b or C3b component, irreversibly inactivating the convertase (Figure 1b). Regulatory proteins also operate at the level of **the membrane- attack complex**. The potential release of the C5b67 complex poses a threat of innocent-bystander lysis to healthy cells. A number of serum proteins counter this threat by binding to released C5b67 and preventing its insertion into the membrane of nearby cells. A serum protein called S protein can bind to C5b67, inducing a hydrophilic transition and thereby preventing insertion of C5b67 into the membrane of nearby cells (Figure 1c(1)).

Complement-mediated lysis of cells is more effective if the complement is from a species different from that of the cells being lysed. This phenomenon depends on two membrane proteins that block MAC formation. These two proteins, present on the membrane of many cell types, are *homologous restriction factor* (HRF) and *membrane inhibitor of reactive lysis* (MIRL or CD59). Both HRF and MIRL protect cells from nonspecific complement-mediated lysis by binding to C8, preventing assembly of poly-C9 and its insertion into the plasma membrane (Figure 1c (2)). However, this inhibition occurs only if the complement components are from the same species as the target cells. For this reason, MIRL and HRF are said to display homologous restriction, for which the latter was named.

Table: **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 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
Membrane-cofactor protein (MCP)*			
Decay-accelerating factor (DAE or CD55)*	Membrane bound	Classical, alternative, and lectin	Accelerates dissociation of $C\overline{4b2a}$ and $C\overline{3bBb}$ (classical and alternative C3 convertases)
Factor-I	Soluble	Classical, alternative, and lectin	Serine protease: cleaves C4b or C3b using C4bBP, CR1, factor H, DAE, or MCP as cofactor
S protein	Soluble	Terminal	Binds soluble C5b67 and prevents its insertion into cell membrane
Homologous restriction factor (HRF)	Membrane bound	Terminal	Bind to C5b678 on autologous cells, blocking binding of C9
Membrane inhibitor of reactive lysis (MIRL or CD59)*			
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.

Complement Deficiencies

Genetic deficiencies have been described for each of the complement components. Homozygous deficiencies in any of the early components of the classical pathway **(C1, C4, and C2)** exhibit similar symptoms, notably a marked increase in immune-complex diseases such as **systemic lupus erythematosus, glomerulonephritis, and vasculitis.** These deficiencies highlight the importance of the early complement reactions in generating C3b, and the critical role of C3b in solubilization and clearance of immune complexes. In addition to immune complex diseases, individuals with such complement deficiencies may suffer from recurrent infections by pyogenic (pus forming) bacteria such as **streptococci and staphylococci.** These

organisms are gram-positive and therefore resistant to the lytic effects of the MAC. Nevertheless, the early complement components ordinarily prevent recurrent infection by mediating a localized inflammatory response and opsonizing the bacteria.

Deficiencies in factor D and properdin—early components of the alternative pathway—appear to be associated with *Neisseria* infections but not with immune-complex disease.

Patients with **C3 deficiencies** have the most severe clinical manifestations, reflecting the central role of C3 in activation of C5 and formation of the MAC. The first patient identified with a C3 deficiency was a child suffering from frequent severe bacterial infections and initially diagnosed as having agammaglobulinemia (condition of absence of antibodies). After tests revealed normal immunoglobulin levels, a deficiency in C3 was discovered. This case highlights the critical function of the complement system in converting a humoral antibody response into an effective defense mechanism. **The majority of patients with C3 deficiency have recurrent bacterial infections and may have immunecomplex diseases.**

Individuals with homozygous deficiencies in the **components involved in the MAC** develop recurrent meningococcal and gonococcal infections caused by *Neisseria* species. In normal individuals, these gram-negative bacteria are generally susceptible to complement-mediated lysis or are cleared by the opsonizing activity of C3b.MAC-deficient individuals rarely have immune-complex disease, which suggests that they produce enough C3b to clear immune complexes. Interestingly, a deficiency in C9 results in no clinical symptoms, suggesting that the entire MAC is not always necessary for complement-mediated lysis.

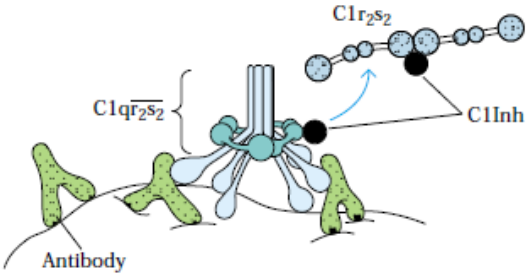
Congenital deficiencies of complement regulatory proteins have also been reported. The **C1 inhibitor (C1Inh)** regulates activation of the classical pathway by preventing excessive C4 and C2 activation by C1. Deficiency of C1Inh is an autosomal dominant condition with a frequency of 1 in 1000. The deficiency gives rise to a condition **called hereditary angioedema**, which manifests clinically as localized edema of the tissue, often following trauma, but sometimes with no known cause. The edema can be in subcutaneous tissues or within the bowel, where it causes abdominal pain, or in the upper respiratory tract, where it causes obstruction of the airway.

VISUALIZING CONCEPTS

Regulation of the Complement System

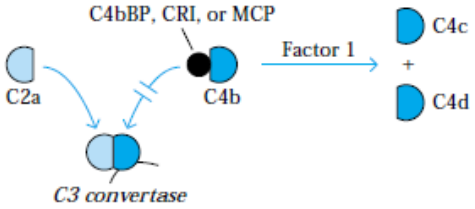
(a) Before assembly of convertase activity

1. C1 inhibitor (C1Iab) binds $C1r_2s_2$, causing dissociation from C1q



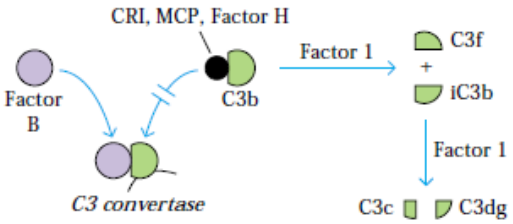
2. Association of C4b and C2a is blocked by binding C4b-binding protein (C4bBP), complement receptor type I, or membrane cofactor protein (MCP)

3. Inhibitor-bound C4b is cleaved by Factor 1



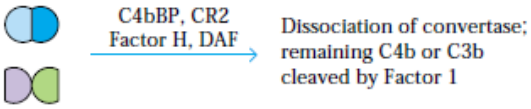
4. In alternative pathway, CR1, MCP, or Factor H prevent binding of C3b and Factor B

5. Inhibitor-bound C3b is cleaved by Factor 1



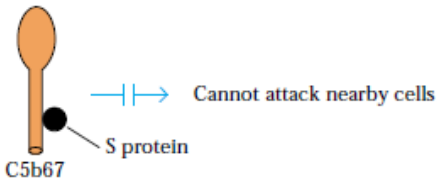
(b) After assembly of convertase

C3 convertases are dissociated by C4bBP, CR1, Factor H, and decay-accelerating Factor (DAF)



(c) Regulation at assembly of membrane-attack complex (MAC)

1. S protein prevents insertion of C5b67 MAC component into the membrane



2. Homologous restriction factor (HRF) and membrane inhibitor of reactive lysis (MIRL or CD59) bind $C8_1$, preventing assembly of poly-C9 and blocking formation of MAC

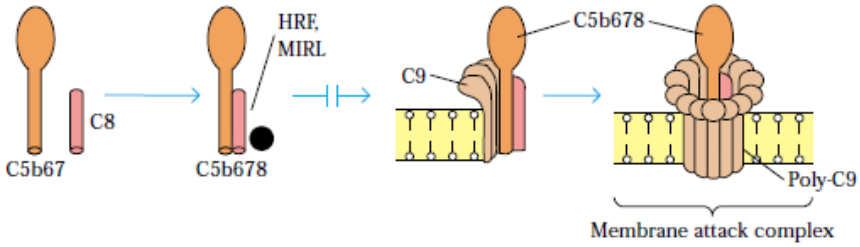


Figure 1: Regulation of the complement system by regulatory proteins (black).