IMMUNOLOGY AND MEDICAL MICROBIOLOGY

Complement System

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Introduction

The complement (C) was discovered by Jules Bordet and others during 1894-‘96 as a non-specific factor that was required along with specific antibodies in the serum to cause lysis of the bacterial cells (immunological bacteriolysis). This non-specific factor was found to be heat-labile (destroyed at 56°C in 30 minutes), unlike specific antibodies in the antiserum. In subsequent years, it came to be known that C was not a single factor but a complex system of several different serum proteins.

At present, we know that the complement (C) system is made up of about 35 soluble and cell-surface proteins and glycoproteins that are involved in innate immunity, effector arm of the adaptive immunity, inflammation, leukocyte migration, anaphylactic response, immunoregulation, etc. Some soluble C components are proenzymes/ zymogens that have to be activated to participate in a sequence or ‘cascade’ of enzymatic reactions. Other soluble C components are involved in protein-protein interactions so as to make bigger functional complexes. Most cell-surface associated C components are regulatory proteins or mediate effector functions of the C system.

Several different kinds of substances (bacteria, viruses, other pathogens, immune complexes, complex polysaccharides, etc.) can activate C system in three different pathways: classical, alternative and the recently discovered lectin pathway. Activation of the classical pathway is antigen (Ag) -dependent in the sense that it is predominantly initiated by IgM or IgG antibodies (Abs) after binding specifically to the Ag. Thus, the classical pathway plays an important role in the effector arm of adaptive immunity. The alternative pathway is triggered in an Ag-independent manner by non-specific substances including those from bacteria, viruses and other pathogens, and thus plays important role in innate immunity. The lectin pathway is also Ag-independent and is initiated by bacterial carbohydrate (mannans or terminal mannose- or N-acetyl glucosamine containing) ligands, and is therefore a part of the innate immune system. The three pathways converge in the cascade to a common terminal pathway that leads to the formation of a ‘pore-forming unit’ or ‘membrane attack complex’ (MAC) that lyses or disrupts the membranes of the foreign cells and other membrane-bound complex Ags.

Certain products of the enzymatic proteolysis of C components at various steps in the activation pathways are released into the fluid-phase (plasma or tissue fluids) and are therefore not the part of the cascade-associated protein complexes. These activation products of the C system have various patho-physiological effects in the body, such as inflammation, chemotaxis, anaphylactic response, etc.

Complement receptors (CRs) are present on various leukocytes and other cells that bind C components and are responsible for immune adherence and opsonization, Ag presentation, immunoregulation, etc. Functions of the C system are well regulated by a number of soluble and cell surface-associated molecules in order to protect the self and destroy the foreign (non-self) invaders in the body. Deficiencies of C components lead to reduced capacity of the affected host to fight against infections, particularly pyogenic bacterial infections, and are associated with autoimmune disorders and other diseases. Major historical events in the study of complement system are presented in Table 1.

Table 1: Major historical events in the study of complement system		
Year/ period	**Discovery/ event**	**Most prominent figure(s)**
1894-1896	Discovery of complement (‘alexine’) as an accessory factor in normal serum required for lysis of antibody-coated bacteria	Buchner, Pfeiffer, Bordet
1900	Development of complement fixation test as a serodiagnostic test	Bordet & Gengou
1940s	Functions of the first 4 components (C1, C2, C3 & C4) described	Pillemer
1953	Immune adherence receptor of erythrocytes & neutrophils (CR1) discovered	Nelson
1954	Discovery of alternate pathway of C activation; role of properdin defined	Pillemer
1966	All 9 C components in the hemolytic form of guinea pig C published	Nelson
1969-‘72	Congenital C3 deficiency discovered; central role of C3 in C activation and host defence confirmed	Alper
1971	Factor B discovered	Gotze & Muller-Eberhard
1972	Membrane attack complex ‘doughnut model’ for lesions in the cell membrane presented	Mayer
1980s-onwards	Application of rec DNA methods in C research; biosynthesis, structure, functions & genetics of C systems	Several investigators
1992	Discovery of the lectin pathway	Matsushita & Fujita

Components of the complement system

1. The C components in the cascade

The C components are designated by the capital letter C followed by an Arabic numeral. Some components have multiple proteins and therefore designated by suffixing a small letter to the main component. The components of the classical pathway in a sequence are as follows: C1q, C1r, C1s, C4, C2, C3, C5, C6, C7, C8 and C9. Out of these, C1r, C1s, C4 and C2 are zymogen or proenzyme serine proteases that need to be activated by their cleavage. The cleavage products of C4, C2, C3 and C5 are designated as C4a & C4b, C2a & C2b, C3a &C3b, C5a & C5b. Activated enzyme forms of these components that participate in the cascade reaction are larger than the non-participant ones and are written as $\overline{C1}$r, $\overline{C1}$s, $\overline{C4}$b, $\overline{C2}$a, $\overline{C3}$b and $\overline{C5}$b, all having a bar on the numeral and the small letter suffix.

Certain components of the alternative pathway are called ‘factors’. Different factors are B, D and properdin, designated respectively as factor B (or FB or simply B), FD, and P. Activated form of

B is written as Bb with a bar over it. The components of the 'lectin pathway' are mannan or mannose binding lectin (MBL), ficolins and MBL-associated serine proteinases (MASP-1 and MASP-2). C1q and MBL belong to the family of 'collectins'. MASP-1 and MASP-2 are the homologues of C1r and C1s respectively. Activated forms of MASP-1 and MASP-2 are designated by putting a bar on their abbreviated names.

The terminal 'lytic' pathway components are C5b, C6, C7, C8 and C9. C5b, the ultimate product of the cascade reaction, is the first component of the lytic pathway. $\overline{C4b2a}$ and $\overline{C3bBb}$ are called 'C3 convertases' of the classical and the alternative pathway respectively. C5b6789 is called the 'membrane attack complex' (MAC). Each MAC can have several C9 components that arrange themselves to make a pore or a channel in the plasma membrane of the foreign invader cell in the host. Table 2 presents all the components of the C system.

Table 2: The complement components in different activation pathways

Activation pathway	The C component designations	The activated C component designation	C regulators	The triggering/ initiator C component
Classical	C1q, C1r, C1s, C2, C3, C4, C5, (C6, C7, C8, C9)	$\overline{C1}$r, $\overline{C1}$s, $\overline{C2}$a, $\overline{C3}$b, $\overline{C4}$b, $\overline{C5}$b	C1INH, C4-bp, FI, CR1, DAF, MCP (CD46)	C1q
Alternative	$C3_{H2O}$, FB, FD, P, C5, (C6, C7, C8, C9)	$\overline{C3}$b, $\overline{Bb}$	FI, FH, CR1, DAF, MCP(CD46)	$C3_{H2O}$
Lectin	MBL, Ficolins, MASP-1, MASP-2, C4, C2, C5, (C6, C7, C8, C9)	$\overline{MASP}$-1, $\overline{MASP}$-2, $\overline{C4}$b, $\overline{C2}$a, $\overline{C5}$b	C1INH, C4-bp, FI, FH, CR1, DAF, MCP (CD46)	MBL/ ficolins
The common terminal 'lytic'	C5b, C6, C7, C8, C9	$\overline{C5}$b	S protein (vitronectin), clusterin, protectin (CD59), HRF	C5b

Abbreviations: C1INH: C1 inhibitor; C4-bp: C4-binding protein; MBL: Mannose/ mannan binding lectin; MASP: MBL-associated serine protease; DAF: Decay accelerating factor; MCP: Membrane cofactor protein; HRF: Homologous restriction factor; CR1: Complement receptor 1

2. The C receptors

Five different C receptors, viz., CR1, CR2, CR3, CR4 and CR5 are expressed on surface of various types of cells including leukocytes, endothelial cells and erythrocytes. Different CRs bind various C components, mostly their cleavage products obtained from the cascade reaction in the activation pathways. Receptors for C1q, anaphylatoxins (C3a, C4a, and C5a) and factor H (FH) also exist on various leukocytes and other cells.

3. Regulatory C components or C control proteins

Several proteins, known as regulators of complement activation (RCA), are involved in the regulation of the C system at different steps in different pathways (table 2). These include FI, decay accelerating factor (DAF) or CD55, C1 inhibitor (C1INH), CD59, clusterin, S protein, etc. FI is a serine protease that requires different co-factors (FH, C4-bp, MCP, and CR1) for regulation of C3b. C1INH, a serine protease inhibitor (*serpin*), inactivates C1r and C1s and their respective homologues, MASP-1 and MASP-2. DAF, CR1, FH and MCP are the regulators of the 'amplification loop' of the alternative pathway. CD59, clusterin, S protein and homologous restriction factor (HRF) regulate "MAC' to avoid or reduce 'reactive lysis' of the host cells as a result of C activation against foreign invaders.

Activators of the complement system

1. Activators of the classical pathway

Immune complexes of IgM and IgG antibodies with antigens are the best known activators of the C system in the classical pathway. C1q binding on at least two Fc regions simultaneously initiates the classical pathway. Pentameric IgM is many-fold more efficient than IgG for activating C by classical pathway. IgG subclasses vary in their C activating efficiency. Efficiency of binding C1q by human IgG subclasses is in the order: IgG3> IgG1>>IgG2. IgG4 does not bind C1q. Other classes of Abs, viz., IgA, IgE and IgD do not bind C1q and therefore are unable to activate the classical pathway. Some other substances can however activate C by binding to C1q, which are C-reactive protein, nucleic acids, LPS lipid A, polyanions, β-amyloid fibrils, mitochondria, apoptotic cells, etc. (Table 3).

Table 3: Activators of the three different complement activation pathways

Activation pathways		
Classical	**Alternative**	**Lectin**
• Immune complexes of IgM and IgG classes • pentraxins • nucleic acids • mitochondria • LPS lipid A • bacterial capsular polysaccharide • C-reactive protein bound to pneumococcal C-type polysaccharide • other microbial components • apoptotic cells • serum β-amyloid protein	• Bacteria • yeast & fungi • some viruses • parasites • some tumor cells • heterologous erythrocytes • LPS • teichoic acid • zymosan • cobra venom factor • anionic polymers • agarose • immune complexes of human IgG, IgA and IgE	• Mannose- and N-acetyl glucosamine -containing polysaccharides on yeast, bacteria, and viruses • glycosylation variants of IgG

2. Activators of the alternative pathway

Several different Gram + and Gram – bacteria, fungi, protozoa and other parasites are able to activate the alternative pathway. In addition, erythrocytes from different sources, dextran sulphate and carbohydrates can also activate this pathway. IgA, IgE or IgG immune complexes are also able to activate this pathway, but less efficiently than the classical pathway (table 3).

3. Activators of the lectin pathway

Gram + and Gram − bacteria having terminal mannose-/ N-acetyl glucosamine-containing poly-saccharides activate this pathway as these serve as ligands for mannose-binding lectin (MBL) and ficolins in the host (Table 3).

Structure and functions of the complement proteins

Some biochemical features of C components are presented in Table 4. The structure and functions of different categories of C components are discussed in brief below:

1. Components involved in the classical pathway cascade

i) C1q

C1q is a hexamer of >410 kDa size, each subunit being made of 3 dissimilar polypeptide chains (A, B and C) of approximately equal size (23 kDa). C1q has N-terminal common 'stalk', six different 'stems' and six C-terminal globular heads, making shape of a 'bouquet of tulips'. Polypeptide chains A, B and C in each subunit towards N-terminal region arrange to make heterotrimeric collagen-like triple-helical fibers and the fibers of the six subunits arrange themselves to form a common 'stalk', but each fiber then bends to make its own 'stem' due to differences in collagen repeat sequence and a C-terminal heterotrimeric globular 'head'. The globular heads are involved in recognition function of C1, i.e, they have binding sites for Cμ3 domain of IgM or Cγ2 domain of IgG that are already complexed with the multivalent antigens, and for other complement activators. The stem has sites for interaction with C1r and C1s enzymes, and C1q receptors. C1q structure provides a scaffold for proper folding of the catalytic subunit C1s-C1r-C1r-C1s. In the globular head the B lies outside the A and C chains. A chain of the head is N-glycosylated. Lys59 of A chain and Lys61 of B chain in the stem interact with C1r and C1s molecules.

C1q has serum concentration of 70 μg/ ml, is thermolabile (56°C, 30 min) and has γ2 electrophoretic mobility. It is not a zymogen, but its binding to Cμ3 or Cγ2 domains produces allosteric change necessary as activation signal for the associated zymogen C1r. It belongs to 'collectin' protein family, as also is MBL of the lectin pathway. Each of the six heads can bind with a Cμ3 domain of IgM or Cγ2 domain of IgG, but a minimum of two heads are required to produce allosteric change in its stem-stalk structure so as to transmit activation signal to the associated C1r zymogen or to its cellular receptors, C1qRs.

ii) C1r

C1r is a zymogen serine protease, two copies of which are associated with C1q. It is thermolabile (56°C, 30 min).

iii) C1s

C1s is a zymogen serine protease homologous to C1r, two copies of which are associated with C1q. Like C1r, it is thermolabile (56°C, 30 min).

C1 complex is a 790 kDa multimolecular protease formed by the association of a recognition protein C1q with the catalytic component, C1s-C1r-C1r-C1s tetramer. The tetramer is stabilized by 8 Ca^{2+} ions. Each globular head of C1q also binds one Ca^{2+} ion. Thus, C1 complex has a total of 14 Ca^{2+} in it. The complex is bound to immune complexes or other substances via C1q, before activation of the serine protease activity in C1r followed by that of C1s.

iv) C4

C4 is a globular protein, consisting of three polypeptide chains, namely, α, β and γ. It is not thermolabile. Activated C1s in the C1 complex cleaves C4 α chain to give a larger fragment C4b and a smaller fragment C4a. C4b can binds to the surface by making covalent bond. Several copies of C4b can be deposited in the vicinity of Ag:Ab:C1 complex on the membrane surface.

v) C2

C2 is a zymogen serine protease, having single polypeptide chain. Like C1 components, it is thermolabile. It is cleaved by activated C1s of C1 complex or activated MASP2 of lectin pathway into N-terminal short polypeptide chain, C2b, and the larger C-terminal C2a fragment. C2a is an atypical serine protease of the classical pathway. C2a after association with C4b serves as a catalytic subunit of C3- and C5- convertases. Protease activity of C2a occurs only when it is complexed with C4b in C3 convertase, and with C4b and C3b in C5 convertase of the classical pathway.

vi) C3

Human C3 is a glycoprotein, composed of disulphide-linked α and β chains. It is not thermolabile and occurs in a range of 1-2 mg/ ml in serum i.e., as the most abundant complement component.

vii) C5

C5 is a β1-globulin, having two polypeptide chains. It is cleaved by the classical as well as the alternative pathway C5 convertase into a cell surface bound C5b fragment and a fluid-phase C5a fragment. C2a of the classical pathway and Bb of the alternate pathway have the enzymatic site for cleavage of C5. Non- complement proteases also cleave C5 into biological active fragments. But these fragments may not be same as produced by C5 convertases.

Box 1: Serine proteases involved in the cascade reaction of the complement activation pathways: 1. The classical pathway

1. C1r and C1s non-covalently associated with C1q in the C1 complex are zymogen serine proteases involved in the activation of the classical pathway.
2. Auto-cleavage activates C1r, which then cleaves C1s zymogen to activate it.
3. Activated C1s serine protease of the C1 complex cleaves C4 into C4b that can bind onto the non-self surface and C4a that remains in the fluid phase.
4. Activated C1s also cleaves surface bound C4b-associated C2 into C2a that remains associated with the non-self surface and C2b that is released in the fluid phase.
5. The enzymatic activity of C1 complex is inhibited by C1INH.
6. The resulting C4b2a complex on the surface acts as 'C3 convertase' of the classical pathway
7. C2a has the serine protease activity of the 'C3 convertase' that cleaves C3 into C3b that can be associated with the C4b2a complexes on the non-self surface or can bind as isolated C3b molecules on the self as well as non-self surfaces.
8. C3b in association with C4b2a makes 'C5 convertase' on non-self surfaces.
9. C2a of the 'C5 convertase' cleaves C5 into C5b and C5a. C5b can bind independently on the non-self surface and initiate the formation of the 'membrane attack complex' (MAC).
10. MAC is a multimolecular complex of C5b:1C6:1C7:1C8:3-6C9. MAC formation does not involve serine protease.

2. Components of the alternative pathway cascade

i) $C3_{H2O}$

Under physiological conditions, the thioester bond in the native C3 undergoes hydrolysis at a very slow rate giving rise to $C3_{H2O}$. Hydrolysis of the thioester bond causes conformational changes that enable $C3_{H2O}$ to associate with FB in the presence of Mg^{2+} ions and thus to form the 'initiation' C3 convertase of the alternative pathway [$C3_{H2O}B(Mg^{2+})$].

ii) Factor B

FB is a single polypeptide glycoprotein, which is structurally and functionally similar to C2. FB is an atypical serine protease of the alternative pathway. Bb after association with C3b serves as a catalytic subunit of C3- and C5- convertases. Protease activity of Bb occurs only when it is complexed with C3b in C3 convertase, and with $(C3b)_2$ in C5 convertase of the classical pathway. FB is cleaved by FD into Ba and Bb. The active site is present on Bb rather than on Ba. Activation of FB and C2 are different from other serine proteases in certain respects.

iii) Factor D

FD is a smallest serine protease and least abundant complement protein. Most FD is present as an already activated serine protease and cleaves FB only when the latter is bound to C3b in the

presence of Mg^{2+} to form catalytically active C3bBb complex, which is called C3 convertase of the alternative pathway. After cleavage of FB bound to C3b, FD returns to its inactive form.

Box 2: Serine proteases involved in the cascade reaction of the complement activation pathways: 2. The alternative pathway

1. FB is a zymogen serine protease of the alternative pathway, equivalent to C2 of the classical pathway.
2. Surface-bound 'tick-over' $C3_{H20}$ associated FB is cleaved into Bb and Ba by a self-activated serine protease FD.
3. $C3_{H20}Bb$ is the 'initiation C3 convertase' of the alternative pathway in which Bb cleaves more C3 into C3b and fluid-phase C3a.
4. C3bBb is stabilized by P on the non-self surface and serves as the 'amplification C3 convertase' for the classical pathway also.
5. C3bBb3bP is the 'C5 convertase' of the alternative pathway and Bb cleaves C5 into C5b and C5a. At this step, the two pathways converge to produce C5b, the initiator of the 'terminal' pathway that leads to the formation of MAC.
6. Binding of C3b on the self surfaces is regulated. FI is a self-activated serine protease that cleaves self surface- bound C3b into iC3b and derivatives, such as C3dg, C3c and C3d, etc.
7. FI requires FH, CR1 and MCP as co-factors for its enzymatic activity.

iv) Properdin

Properdin is an oligomeric protein, often having non-covalently associated 3-4 identical chains. The native form is able to associate with C3bBb. However, the activated form of P is able to associate with fluid-phase C3b to help binding of B.

3. Components of the lectin pathway cascade

i) Mannose-binding lectin

MBL is a serum glycoprotein that binds to terminal mannose and N-acetyl glucosamine residues of complex polysaccharides (PS) present on a variety of microorganisms. It also interacts with two associated zymogen proteases, called MBL-associated serine protease 1 (MASP-1) and MASP-2. These two proteases are homologous to C1r and C1s respectively.

ii) MBL-associated serine protease 1

There are two serine proteases associated with MBL, designated as MASP1 and MASP2. Like C1r and C1s, the two proteases are activated by cleavage of a single Arg-Ile bond, splitting the single polypeptide into two disulphide-linked polypeptides. It exhibits only 36% and 37% sequence identity with human C1r and C1s.

iii) MBL-associated serine protease 2

MASP2 is a few amino acids shorter than MASP1 has no potential N-glycosylation sites. Only MASP2 has C4 cleavage activity, and it seems that MASP1 is equivalent to C1r and MASP2

equivalent to C1s. Both seem to be associated with MBL, like C1r and C1s with C1q in C1 complex of the classical pathway

Box 3: Serine proteases involved in the cascade reaction of the complement activation pathways: 3. The lectin pathway

1. MASP-1 and MASP-2, the homologues of C1r and C1s, are the zymogen serine proteases associated with MBL that initiates the lectin pathway by binding with mannans on bacterial surfaces.
2. Auto-cleavage activates MASP-1, which then cleaves MASP-2.
3. Activated MASP-2, like C1s of the C1 complex, cleaves C4 into C4b that can bind onto the non-self surface and C4a that remains in the fluid phase.
4. Activated MASP-2 also cleaves surface-bound C4b-associated C2 into C2a that remains associated with the non-self surface and C2b that is released in the fluid phase.
5. The enzymatic activity of MASPs is inhibited by C1INH.
6. The resulting C4b2a complex on the surface acts as 'C3 convertase'.
7. The lectin and the classical pathways thus converge at this step and all further steps are the same in both the pathways.

4. Components of the terminal pathway

i) C5b

It is produced from C5 cleaved by the classical as well as the alternate pathway C5 convertase as C5b fragment that may be bound to the surface membranes.

ii) C6

C6 is a single chain protein that binds with C5b and can anchor onto plasma membranes as C5b6 complex and then can associate with C7. It does not have protease activity.

iii) C7

C7 is a trimeric protein, structurally similar to C6. It associates non-covalently with C5b6 complex in the fluid-phase and on the membranes to form C5b67 complex which allows the binding of C8.

iv) C8

C8 is a heterotrimeric protein, having α, β and γ chains. The α and γ chains are disulphide-linked and the β chain is non-covalently associated with them. It can associate with C5b67 complex via its β chain and anchors into the membrane via its α chain. C9 binding site also resides on the C8 α chain.

Table 4: Some biochemical features of complement components				
Component	**Size (kDa)**	**Serum concentration (µg/ml)**	**Polypeptide composition**	**Structural motifs**
C1q	414	70	18 (6x3 dissimilar chains)	Collagenous sequence in the N-terminal region
C1r	85	35	2 copies	SCRs (CCP), EGF, CUB, SP, C1r/s
C1s	83	30	2 copies	SCRs, EGF, CUB, SP, C1r/s
C2	102	25	1	SCRs, VWF, SP
C3	185	1600	2 (α,β)	
C4	205	600	3 (α,β,γ)	
C5	180	85	2 (α,β)	
C6	128	60	1	SCRs, EGF, LDLR, TP, FIMAC
C7	120	55	1	SCRs, EGF, LDLR, TP, FIMAC
C8	150	15	3 (α,β,γ)	EGF, LDLR, TP
C9	79	60	1	EGF, TP
FB	93	225	1	SCRs, VWF, SP
FD	24	1	1	SP
P	220	25	4 (homo-tetramer)	TP
MBL	500	<1	18 (6x3 identical chains)	Collagenous sequence in the N-terminal region
MASP-1	85	<1	2 copies	SCRs, EGF, CUB
MASP-2	85	<1	2 copies	SCRs, EGF, CUB
FI	88	35	2 (α,β)	LDLR, FIMAC, SRCR
FH	150	500	1	SCRs
DAF	70	Membrane bound	1	SCRs
MCP(CD46)	45-70	Membrane bound	1	SCRs
C1INH	110	180	1	
C4-bp	540	250	8 (homo-octamer)	SCRs
S protein	83	600	1	
Clusterin	80	Membrane bound	2 (α,β)	
CD59	20	Membrane bound	1	
HRF	65	Membrane bound	1	
CR1	160-250	Membrane bound	4 allomorphs, with different number of repeat motifs	SCRs
CR2	140	Membrane bound	Similar to CR1	SCRs
CR3	p165,95	Membrane bound	2, non-covalently linked	VWF, MB
CR4	p150,95	Membrane bound	2, non-covalently linked	VWF, MB
CR5	95	Membrane bound	1	?

SCR: short consensus sequence; CCP: complement control protein motif; EGF: epidermal growth factor; LDLR: low-density lipoprotein receptor domain type A; TP: thrombopoeitin; VWF: von Willebrand factor repeat; SP: serine protease domain; C1r/s: C1r/C1s-specific repeat; FIMAC: Factor I/membrane attack complex protein C6/C7 repeat; MB: metal-binding domain; SRCR: the scavenger receptor Cys-rich module; CUB: C1r/C1s, the sea urchin protein UEGF, and the human Bone morphogenetic protein-1.

v) C9

C9 is a single chain protein that associates with C5b678 complex on membranes and initiates formation of a pore-forming complex into the membranes. Multiple copies (3-6) of C9 often oligomerise on the membrane. But in the presence of more copies, 12-18 C9 molecules can polymerize to make a big pore or channel in the membrane. The human complement makes pores of 10-11 nm internal diameter in erythrocytes that can be easily seen in electron micrographs.

5. Regulators of the complement activation/ complement control proteins

i) C1 INH

C1INH is a single chain heavily glycosylated protein and is a member of the serpin (*ser*ine *p*rotease *in*hibitors) family. It has role in regulation of vascular permeability, modulation of inflammation, blood pressure regulation and antifibrinolytic/ procoagulant effects. Direct interaction of Gram − LPS with C1INH protects from endotoxic shock.

C1INH inactivates C1r and C1s components of the C1 complex and is the primary regulator of the classical pathway. It binds to C1r in the C1s-C1r-C1r-C1s tetramer within the C1 complex and dissociates from C1q as two C1INH-C1r-C1s-C1INH complexes.

C1INH also inhibits the lectin pathway by making complexes with MASP1 and MASP2 in isolated forms and within the MBL complex. But the exact mechanism of inhibition is not known at present. C1INH is also able to bind to C3b resulting in inhibition of binding of FB to C3b. By binding to C3b that inhibit its association with FB, C1INH may also inhibit the activation of alternative pathway.

ii) C4- binding protein

C4-binding protein (C4-bp) is a homo-octamer of about 540 kDa size. By binding to C4b, it prevents the formation of C3 convertase and also serves as a co-factor for FI-mediated cleavage of C4b.

iii) Factor I

FI is a serine protease, composed of two –S-S- linked polypeptide chains. It prevents the formation of C3 convertase of the alternative pathway and C5 convertase of all the pathways. Therefore, FI is also called C3b/C4b INA. It cleaves C3b to yield its different derivatives, having several important biological activities. Like FD, it is not a zymogen and is regulated by reversible conformational changes.

iv) Factor H

FH downregulates C3 cleavage. Self surface, e.g., host's erythrocytes preferentially binds FH, a cofactor for FI. It can dissociate C3bBb into C3b and Bb for their decay After FH binding to C3b on self surfaces, FI cleaves the α chain of C3b to produce inactive form of C3b, i.e., iC3b.

v) Decay accelerating factor

DAF (CD55) is a surface glycoprotein expressed on all peripheral blood cells. It prevents the assembly of C3- and C5- convertases of the classical or alternative pathways on the surface of cells that express it, i.e., it acts intrinsically. It does not serve as a cofactor for FI-mediated cleavage of C3b or C4b. Its action is reversible and dissociates rapidly C2a or Bb from their binding site on C4b or C3b respectively.

vi) Membrane cofactor protein

MCP (CD46) is a heterogeneous glycoprotein on leukocytes and is structurally similar to DAF. It has wide tissue distribution and regulates C3 and C5 convertases intrinsically only. Unlike DAF, it acts as a cofactor for FI-mediated cleavage of C3b or C4b on self surfaces. It also acts as a cofactor for FI-mediated cleavage of C4b into fluid-phase C4c and inactivated cell-bound C4d. It is very effective in controlling the amplification loop of the alternative pathway. It drives human T cells to become regulatory T cells.

vii) Complement receptor 1

Complement receptor 1 [CR1 (CD35)] is a polymorphic surface glycoprotein. It is a member of the regulators of the complement activation (RCA) gene family. CR1 is expressed on erythrocytes, granulocytes, B cells, some T cells, FDCs, etc. It binds to C3b> C4b> iC3b and has a number of biological functions. Erythrocyte CR1 is involved in immune adherence for removal of immune complexes fixed with complement. It promotes opsonization of C3-bound targets by monocytes, macrophages and neutrophils. Its presence on FDCs traps the immune complexes within follicular germinal centres, and on B cells promotes B cell activation.

viii) S protein (vitronectin)

S protein or vitronectin is a single chain protein and its binding to fluid-phase C5b67 complex prevents insertion of the complex into the cell membranes.

ix) Protectin (CD59)

CD59 is a single chain polypeptide expressed on cell surface. It binds to C5b678 complex so as to prevent formation of MAC on self membranes and prevent reactive lysis of the self cells.

x) Clusterin

Clusterin is a heterodimeric protein expressed on cell surfaces. It prevents MAC formation on the cell membranes by binding to C5b678 complex.

xi) Homologous restriction fragment

HRF is a single chain protein expressed on cell membrane. Its function is similar to CD59 and prevents MAC formation on the self cells.

xii) Conglutinin

Conglutinin is a lectin that recognizes terminal N-acetylglucosamine, mannose and fucose residues. It is found in bovine and human plasma, and can bind to surface-bound iC3b in a Ca^{2+} - dependent manner. It also binds to yeast cell wall fragments, C3b, C3c and α chain of C3 *in vitro*. However, its physiological role is not clear.

6. The complement receptors

Receptors for various C components and activation products are presented in Table 5.
i) CR1 (CD35): as above

ii) CR2 (CD21)

CR2 (CD21 or C3d receptor) is a surface glycoprotein of RCA gene family. It is structurally similar to CR1 and other members of RCA gene family. It binds to C3d and iC3b> C3b and C3dg. It is also a receptor for Epstein-Barr virus (EBV) and CD23. It is expressed on B cells, FDCs, thymocytes, basophils, keratinocytes and various epithelial cells. CR2 has various biological roles, such as B cell activation, trapping of the immune complexes, production of IgE, rescue of B cells from apoptosis and activation of T cells via B cells as Ag-presenting cells.

iii) CR3 (CD11b/CD18)

CR3 is a heterodimer of non-covalently associated α and β chains and is a member of the integrins family. It is expressed on most leukocytes and erythrocytes. It binds iC3b> C3b> C3d and C3dg. It mediates opsonization of C3-bound particles by neutrophils and macrophages.

iv) CR4 (CD11c/CD18)

CR4 is a heterodimer of non-covalently associated α and β polypeptide chains sharing 87% homology to CR3. It binds to iC3b> C3b, and fibrinogen. It is expressed on most leukocytes and is functionally similar to CR3.

v) CR5

CR5 is not as well studied as other CRs. It binds to C3d of fluid-phase iC3b and C3dg dimer. It is expressed on neutrophils and platelets. Its function is not established.

vi) C1q receptors

There are three types of receptors that bind to C1q component: C1qRp, gC1qR and cC1qR. C1qRp is a glycoprotein expressed on monocytes, neutrophils, lymphocytes and platelets. By mediating the binding C1q on neutrophils and monocytes, it helps opsonize the foreign materials. gC1qR is expressed on platelets, endothelial cells and eosinophils and cC1qR, on Raji cells.

vii) FH receptor

It is expressed on B cells, monocytes and neutrophils and binds FH. Its detailed functions are not known at present.

Table 5: Receptors for complement components and their activation products			
C receptor (CR)	**Expressed on**	**Biochemical nature**	**Ligand (C component / activation product)**
CR1 (CD35)	Erythrocytes, granulocytes, B cells, some T cells, FDCs, glomerular podocytes	Polymorphic membrane glycoprotein of 160-280 kDa	C3b, C4b, iC3b
CR2 (CD21)	B cells, thymocytes, FDCs, epithelial cells	Glycoprotein of 140 kDa	iC3b, C3dg, C3d, C3b
CR3 (CD11b/ CD18)	Monocytes, neutrophils, eosinophils, lymphocytes, tissue macrophages, erythrocytes, NK cells	Heterodimer of non-covalently associated α chain (165 kDa) and β chain (95 kDa)	iC3b, C3dg, C3d
CR4 (CD11c/ CD18)	Neutrophils, monocytes, eosinophils, activated B & Tc lymphocytes, tissue macrophages, NK cells	Heterodimer of non-covalently associated α chain (150 kDa) and β chain (95 kDa)	iC3b, C3dg
CR5	Neutrophils, platelets	95 kDa protein (?)	C3d, C3dg dimer
C1qRp	Monocytes, neutrophils, lymphocytes, platelets	126 kDa glycoprotein	C1q collagen-like fragment
gC1qR	Platelets, endothelial cells, eosinophils	33 kDa glycoprotein	C1q globular head
cC1qR	Raji cells	60 kDa glycoprotein	C1q collagen tail
C3a/4aR	Basophils, mast cells, eosinophils, smooth muscle cells, B cells	57 kDa	C3a, C4a
C5aR (CD88)	Basophils, mast cells, eosinophils, neutrophils, smooth muscle cells, T cells, hepatocytes, endothelial cells	47 kDa glycoprotein oligomers	C5a, $C5a_{\text{des Arg}}$
FHR	B cells, monocytes, neutrophils	170 kDa protein	FH

viii) C3a/C4aR

C3a/4aR is expressed on basophils, eosinophils, smooth muscle cells and B cells or as a 97 kDa protein on human mast cells and mediates several biological functions following binding of C3a or C4a anaphylatoxins. Release of histamine from mast cells, chemotaxis of leukocytes, spasmogenic activity and lymphocyte stimulation are the consequences of its engagement by C3a or C4a on various cells.

ix) C5aR

C5aR is an oligomer of a glycoprotein expressed on basophils, mast cells, eosinophils, neutrophils, smooth muscle cells, T cells, hepatocytes and endothelial cells. It binds to the anaphylatoxin C5a and its inactive form C5a des Arg and therefore mediates several important biological functions, like C3a/4aR. In addition, it is involved in sepsis, liver regeneration and modulation of $CD8^+$ T cells in influenza infection.

7. Effector molecules of the complement system

i) Anaphylatoxins and pro-inflammatory molecules

a) C3a

C3a is the smaller N-terminal activation product (9 kDa) of C3 generated by C3 convertases of the classical and the alternative pathways. It is an anaphylatoxin that binds to its receptor C3a/C4aR on mast cells, basophils and various other types of cells and produce several biological effects. All the three anaphylatoxins, C3a, C4a and C5a are inactivated by carboxypeptidase N. This enzyme removes C-terminal Arg from C3a that is essential for its function. The inactive form is designated as C3a des Arg.

b) C4a

C4a is released from the C4 α chain as a 9 kDa peptide, does not participate in the cascade, and has other roles to perform by binding onto C3a/C4a receptors (C3a/C4aR) on various types of cells. But of the three, it has the least anaphylatoxin activity.

c) C5a

C5a is produced as a smaller activation product of C5 generated by C5 convertases of both the classical and the alternative pathways. It binds to various cells via C5aR and has biological roles some of which are different from those of C3a. Like C3a des Arg, C5a des Arg is anaphylactically inactive.

ii) Opsonins

a) C3b and its derivatives

C3b and its derivatives, iC3b, C3d and C3dg all can be bound to CR1, CR3 and CR4 present on phagocytes and hence act as opsonins.

b) C4b

C4b can be bound to CR1 on phagocytes and hence act as an opsonin.

c) C1q

C1q on the immune complex can be bound to C1qRp on monocytes, neutrophils, lymphocytes and platelets. Thus it acts as an opsonin.

d) MBL

Being structurally similar to C1q, MBL can bind to C1qRp and opsonize bacteria after binding on surface mannans.

iii) Cytolysin/ membrane attack complex

Molar composition of MAC (C5b-9) is: [1C5b: 1C6: 1C7: 1C8: 3-6 C9]. It behaves as an integral membrane protein on the target membranes. The components in the complex insert themselves by hydrophobic apolar interactions with the lipid bilayer. Electron microscopy of the lesions produced by the MAC in the target membranes show a 'doughnut' structure with annular rim of 15 nm in diameter and a central electron dense region of 10-11 nm diameter raised above the membrane. MAC contains a cylindrical stalk of 15-16 nm length, which is embedded into the hydrophobic core of the membrane. The annulus of the complex projects above the membrane by at least 10 nm, has extended diameter of 20-25 nm and internal diameter of 10-11 nm for human complement and 8.5-9.5 nm for guinea pig complement.

If great excess of C9 molecules are available at the site, polymerization (poly C9) occurs on the target membranes. Poly C9 formation is catalyzed by Zn^{2+} ions and low ionic strength buffer. C5b678 complex catalyzes the oligomerization of C9 into a tubular structure that is responsible for the cytolytic activity of complement. Tbe Ab- coated erythrocytes is the easiest target for the lytic action of MAC and hence has served as the best assay for MAC formation and study of lytic action of complement. Erythrocytes are however resistant to homologous complement, showing the self/ non-self discriminatory power of the complement system. However, not all MAC assembled at the target membranes are cytolytic.

Membrane channels are large enough for efflux of small ions and molecules but not macromolecular cytoplasmic constituents. Water enters through channels due to Donnan effect to cause swelling and bursting of the cells. Erythrocyte lysis by MAC is a model system for immune lysis. But MAC can make channels in all membrane lipid bilayers.

Different pathways of complement activation

1. The classical pathway

The steps-in-sequence are as follows:

i) Formation of immune complexes is most often the pre-requisite for initiation of the classical pathway

The antigen (bacteria, viruses, yeast, protozoa, other pathogens or foreign substances) binds specifically with antibody and the immune complexes are formed. But the immune complexes formed only by IgM or IgG class lead to trigger the activation of the classical pathway. Ag-bound human IgG3, IgG1 or IgG2 (but not IgG4), and mouse IgG2a, IgG2b and IgG3 (but not IgG1) initiate the classical pathway activation. IgM, is several folds more efficient than IgG in triggering classical pathway. Among human IgG subclasses, IgG3 is more efficient than IgG1 and IgG1 more than IgG2. The classical pathway can also be activated in Ab-independent manner by certain viruses, mycoplasma, lipid A, polyanions, etc. listed in Table 3.

The classical pathway is initiated with the binding of C1 component to the Ag-complexed IgM or IgG in the Fc region. Free Ab molecules are not able to initiate the classical pathway.

$$\text{Ag + Ab (IgM/ IgG)} \rightarrow \text{Ag:Ab (Immune complex)} \quad (1)$$

ii) C1q binding to Fc region of Ag-complexed IgM or IgG initiates the activation

The C1 component is a Ca^{2+} -dependent trimolecular complex composed of the recognition component C1q non-covalently associated with the catalytic components, C1s and C1s in a molar ratio of 1:2:2. The catalytic subunit of the C1 complex is arranged as C1s-C1r-C1r-C1s tetramer.

C1q of the C1 complex binds to Fc region of the Ag-complexed IgM or IgG. Being pentameric or hexameric, a single molecule of IgM allows binding of hexa-headed C1q molecule onto at least two Cμ3 domains, whereas multiple monomeric IgG molecules have to be placed close together on multivalent Ag so as to engage at least two binding site on the hexa-headed C1q. The binding site of C1q, i.e., Cμ3 domain of IgM or Cγ2 domain of IgG favors C1q binding only after engagement of these Ig classes with the Ag. Ag binding to IgM or IgG probably causes conformational change in Cμ3 or Cγ2 domains that favours binding of C1q. As mentioned earlier, C1q also directly recognizes a number of pathogens and other substances.

$$\text{Ag:Ab (IgM/ IgG)−Fc (Cμ3 or Cγ2) + C1q:(C1s-C1r-C1r-C1s)} \rightarrow \text{Ag:Ab:C1q:(C1s-C1r-C1r-C1s)} \quad (2)$$

Following binding of C1q to an activating target, its stems are displaced so as to give a mechanical activation signal. This creates a mechanical stress that is transmitted through the C1q-C1-r-C1s interface from C1q stem to the C1r catalytic region. Activation by C1q induces autocatalysis of C1r to produce two polypeptide chains of 56 kDa and 27 kDa size held together by disulphide bonds. C1r in the C1 complex then activates cleavage of zymogen C1s molecule into two polypeptide chains of 56 kDa and 27 kDa size, held together by disulphide bonds as in case of C1r. This activated C1s in the C1 complex, essentially as part of C1s-C1r-C1r-C1s tetramer has catalytic activity on C4 and C2. Activated C1s can cleave C2, but not C4 in fluid phase. For cleavage of C2, SP module and a short activation peptide of C1s are involved. But for C4 cleavage, C4 recognition sites in both CCP modules of C1s are required.

$$\text{Ag:Ab:C1q:(C1s-C1r-C1r-C1s)} \rightarrow \text{Ag:Ab:C1q:(C1s-}\overline{\text{C1}}\text{r-}\overline{\text{C1}}\text{r-C1s)} \quad (3)$$

$$\text{Ag:Ab:C1q:(C1s-}\overline{\text{C1}}\text{r-}\overline{\text{C1}}\text{r-C1s)} \rightarrow \text{Ag:Ab:C}\overline{\text{1q1r}}\text{1s} \quad (4)$$

iii) $\overline{C1q1r}_2 1s_2$ activate the zymogens C4 and C2 to produce 'C3 convertase' of the classical pathway

$C1q\overline{1r_2}1s_2$ activation initiates the cascade reaction of the classical pathway. In the cascade reaction, a proenzyme or zymogen serves as substrate for the enzyme activated in a previous step, and the resulting product of this enzymatic reaction then serves as the enzyme for the next proenzyme as substrate, and so on. In this way, proenzymes are sequentially activated by proteolytic cleavage.

Multiple C4 zymogen molecules (206 kDa) are cleaved by $\overline{C1}s$ of the $C1q\overline{1r_2}\overline{1}s_2$ complex to yield a bigger C4b (197 kDa) and a smaller C4a (9 kDa) fragment. Only the α chain (95 kDa) is cleaved so as to activate C4 into $\overline{C4}b$, a serine protease. $\overline{C4}b$ binds on the foreign surface close to the $Ag{:}Ab{:}C1q\overline{1r_2}\overline{1}s_2$ complex and further participates in the cascade. C4a is released from the C4α chain as a 9 kDa peptide, does not participate in the cascade, and has other roles to perform by binding onto C3a/C4a receptors (C3a/C4aR) on various types of cells.

$$C4\ (\alpha,\beta,\gamma) \xrightarrow{C1q\overline{1r_2}\overline{1}s_2} \overline{C4}b\ (\alpha 1,\beta,\gamma) + C4a\ (\alpha 2) \qquad (5)$$

$\overline{C4}b$ has a metastable internal thioester bond that provides an active binding site, thereby binding on the foreign surface close to the $Ag{:}Ab{:}C1q\overline{1r_2}\overline{1}s_2$ complex. $\overline{C4}b$ also has an acceptor site for reversible binding of C2 in the presence of Mg^{2+} ions.

After its association with $\overline{C4}b$, C2 zymogen is cleaved by C1s of the $\overline{C1}$ complex into a bigger C2a fragment and a smaller C2b fragment. C2a in association with $\overline{C4}b$ is a serine protease for $\overline{C3}$, also called 'C3 convertase' of the classical pathway, while C2b is released into the tissue fluid or plasma.

$$C4b \text{ on foreign surface} + C2 \rightarrow C4b.C2 \xrightarrow{C1q\overline{1r2}\overline{1}s2} \overline{C4b2}a + C2b \qquad (6)$$

These early steps in the cascade are controlled by various regulatory proteins. C1 INH inhibits the $C1q\overline{1r_2}\overline{1}s_2$ activity. Two molecules of C1 INH bind with (C1r.C1s)2 so as to inactivate the enzymatic activity and to cause their dissociation from the complex. Another regulatory protein, C4-bp, serves as a fluid-phase co-factor for FI to degrade C4b into C4d. C4bp also causes dissociation of $\overline{C4b2}a$ into C4b and C2a resulting into their decay. Surface bound C4b can be degraded by FI into C4c fragment and C4d. C4c is released and inactive C4d remains surface-bound.

$\overline{C4b2}a$ complex may be formed free in solution or on cell surface due to their binding sites. But the binding sites are labile and the complex should be deposited in short time. Once formed, this complex cleaves C3 without the help of earlier complement components.

iv) C3 – the component that has central role in all activation pathways and host defence

C3 (195 kDa) can be cleaved by $\overline{C4b2}a$ (the classical pathway C3 convertase) or $C\overline{3b\overline{B}}b$ (the alternative pathway C3 convertase) into C3a (10 kDa) and $\overline{C3}b$ (185 kDa) fragments. The α chain is cleaved by the $\overline{C2}a$ and $\overline{B}b$ component of the C3 convertases.

$$C3 \xrightarrow{\overline{C4b2}a\ (C3\ convertase)} \overline{C3}b + C3a \qquad (7)$$

In $\overline{C3}b$, the internal thioester bond is exposed in the α chain region, C3d that is involved in binding to the surface. The exposed thioester bond on $\overline{C3}b$ is metastable and can be broken by reaction with aldehydes, carboxyl groups, N nucleophiles or water molecules resulting in a new covalent bond (ester or amide) formation between $\overline{C3}b$ and electron pair- donating group on the acceptor molecules. Lability and very short half-life (60 μs) of $\overline{C3}b$ thioester bond makes available only a few $\overline{C3}b$ molecules to bind to the foreign surfaces. $\overline{C3}b$ is not able to

discriminate between self and non-self/ foreign surfaces. Its binding on self surfaces is controlled by various regulatory C proteins so as to protect the self surfaces from further cascade reaction leading to the lytic effect of the complement activation. But classical pathway C3b binds in the vicinity of the surface bound C4b2a on the foreign surface, either singly or associated with the $\overline{C4b2}$a complex to make $\overline{C4b2a}$3b, the 'C5 convertase'.

The fluid-phase C3b that can not bind on the target membrane are hydrolysed by water and can no longer bind to the target membranes. Surface-bound C3b expresses binding sites for FB, FH, FI, C5, properdin, CR1, MCP, etc. Features of the surface on which C3b is bound determines the fate of C3b. On non-self surfaces, C3b leads to amplification of C3 convertase and initiation of MAC formation. On self surfaces, control of classical and alternative pathway C3 convertase amplification is by FI-mediated inactivation of C3b into iC3b form. FI needs one of the several cofactors (FH, CR1 and MCP) to cleave α′ chain of C3b into iC3b and a smaller fragment Cf. The iC3b is surface bound bigger fragment and expresses additional binding sites of CR2, CR3 and CR4. Cf is released into the serum or tissue fluids.

iC3b is further cleaved into surface-bound C3c and fluid-phase C3dg fragments by FI, using cofactors FH, CR1 or CR2. C3dg can be further cleaved into C3d and C3g fragments by trypsin, plasmin or elastase. Conglutinin, a collagen-like lectin protein in bovine and human plasma binds to carbohydrate moiety on α chain of C3, and α′ chain of C3b, iC3b or C3c.

v) $\overline{C4b2a3b}$- the C5 convertase that activates the zymogen C5

C5 is cleaved by the classical as well as the alternative pathway C5 convertase into a cell surface bound C5b fragment and a fluid-phase C5a fragment. $\overline{C2}$a of the classical pathway and $\overline{Bb}$ of the alternate pathway have the enzymatic site for cleavage of C5. But effective cell surface binding and cleavage of C5 also requires high local concentration of $\overline{C3}$b.

$$C5 \xrightarrow{\overline{C4b2a3b}} \overline{C5}b + C5a \qquad (8)$$

Non- complement proteases, such as trypsin, plasmin, elastase, cathepsin G and proteinases from macrophages, platelets and bacteria, also cleave C5 into biological active fragments. But these fragments may not be same as produced by C5 convertases.

a) C5b formation leads to the formation of C5b-9 - the membrane attack complex

C5b forms a stable complex with C6 in serum and is able to interact with membranes. C7 rapidly associates with C5b6 complex and the fluid phase complex rapidly attaches to the acceptor surface. C5b67 binding to the bystander cell surfaces can be inhibited by S protein, high density lipoproteins, heparin, dextran sulphate and DNA. C8 limits the attachment of fluid phase C5b67 complex to the target cells, thereby serving as an internal control in the late steps of the MAC formation. Histone and protamine however enhance the lytic activity of C5b6 for the bystander erythrocytes. Under favourable conditions, C8 binds to the C5b67 complex on the surface. C5b678 allows binding of multiple molecules of C9 that oligomerises to make a pore or channel in the membrane.

$$C5b + C6 \rightarrow C5b6 + C7 \rightarrow C5b67 + C8 \rightarrow C5b678 + 3\text{-}6\ C9 \rightarrow C5b6789\ (MAC) \qquad (9)$$

b) The membrane attack complex

Molar composition of MAC is: [1C5b: 1C6: 1C7: 1C8: 3-6 C9]. MAC behaves as an integral membrane protein on the target membranes. Electron microscopy of the lesions produced by the MAC in the target membranes show a 'doughnut' structure as discussed earlier. A stable fluid phase MAC having 3C9 molecules has a molecular weight of > 1.04 MDa and bound with 3 molecules of S protein that deprives the fluid phase MAC of its lytic power. The S protein does not associate with the membrane-bound MAC, which has 3C9 in the macromolecular complex. Lipoproteins and anti-thrombin III also bind with the fluid phase MAC and serve as its inhibitors.

Prolonged incubation at 37°C *in vitro* leads to the formation of a polymeric C9 complex (poly C9) that incorporates into the phospholipids membrane. It may form in vivo, but great excess of C9 molecules must be available at the site. Poly C9 formation is catalyzed by Zn^{2+} ions and low ionic strength buffer. C5b678 complex catalyzes the oligomerization of C9 into a tubular structure that is responsible for the cytolytic activity of complement. Tbe Ab- coated erythrocytes is the easiest target for the lytic action of MAC and hence has served as the best assay for MAC formation and study of lytic action of complement. Erythrocytes are however resistant to homologous complement, showing the self/ non-self discriminatory power of the complement system. However, not all MAC assembled at the target membranes are cytolytic and sensitive bacteria have been found to mutate so as to escape lysis by MAC.

Membrane channels are large enough for efflux of small ions and molecules but not macromolecular cytoplasmic constituents. Water enters through channels due to Donnan effect to cause swelling and bursting of the cells. Erythrocyte lysis by MAC is a model system for immune lysis. But MAC can make channels in all membrane lipid bilayers. Figure 1 shows all major steps of the classical pathway leading to MAC formation.

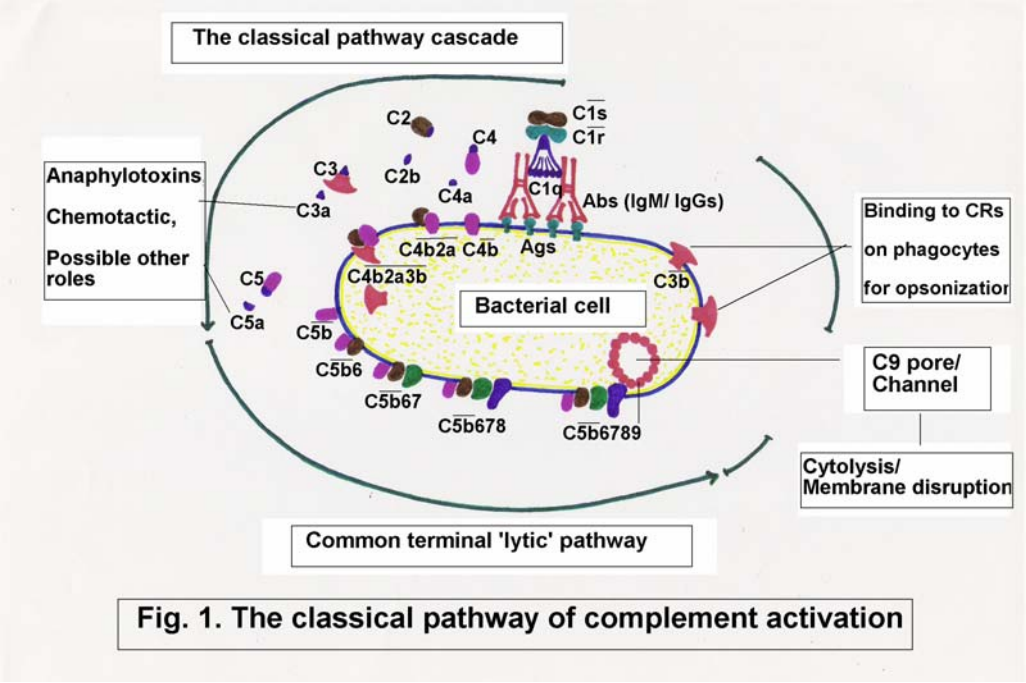

Fig. 1. The classical pathway of complement activation

Box 4: Summary of the steps in the classical pathway of C activation

Ag + Ab (IgM/ IgG) → Ag:Ab (Immune complex) (1)

Ag:Ab (IgM/ IgG)−Fc (Cμ3 or Cγ2) + C1q:(C1s-C1r-C1r-C1s) → Ag:Ab:C1q:(C1s-C1r-C1r-C1s) (2)

Ag:Ab:C1q:(C1s-C1r-C1r-C1s) → Ag:Ab:C1q:(C1s-$\overline{C1}$r-$\overline{C1}$r-C1s) (3)

Ag:Ab:C1q:(C1s-$\overline{C1}$r-$\overline{C1}$r-C1s) → Ag:Ab:C1q$\overline{1r2}\overline{1s2}$ (4)

C4 (α,β,γ) $\xrightarrow{C1\overline{q1r_2}\overline{1s_2}}$ C$\overline{4b}$ (α1,β,γ) + C4a (α2) (5)

C$\overline{4b}$ on foreign surface + C2 → $\overline{C4}$b.C2 $\xrightarrow{C1\overline{q1r2}\overline{1s2}}$ $\overline{C4b2}$a + C2b (6)

C3 $\xrightarrow{\overline{C4b2a}\text{ (C3 convertase)}}$ C$\overline{3b}$ + C3a (7)

C5 $\xrightarrow{\overline{C4b2a}3b}$ $\overline{C5}$b + C5a (8)

C5b + C6 → C5b6 +C7 → C5b67 + C8 → C5b678 + 3-6 C9 → C5b6789 (MAC) (9)

Molar composition of MAC is: [1C5b: 1C6: 1C7: 1C8: 3-6 C9]

In the excess of C9, C5b678 complex catalyzes the polymerization of C9 into a tubular (pore) structure that is responsible for the cytolytic activity of complement

2. The alternative pathway

The steps-in-sequence are as follows:

i) Non-self activating surfaces allow the binding of initial C3b generated by the 'tickover' process: (The Pangburn− Muller-Eberhard hypothesis)

C3 is the on the centre-stage of the complement activation pathways. Under physiological conditions, the thioester bond in the native C3 undergoes hydrolysis at a very slow rate giving rise to $C3_{H2O}$. Thioester bond in C3b is exposed on its surface so that it becomes metastable or extremely labile having a half-life of 60 μs and readily reacting with nucleophiles. Thus, C3b covalently binds to the surface of cells in the neighbourhood or proteins having reactive nucleophiles. The C3b thioester bond may also react with water to give fluid-phase C3b, which like $C3_{H2O}$, can form unstable fluid-phase C3 convertase. Fate of the surface bound C3b depends entirely on the nature of the surface.

Hydrolysis of the thioester bond causes conformational changes that enable $C3_{H2O}$ to associate with FB in the presence of Mg^{2+} ions and thus to form the 'initiation' C3 convertase of the

alternative pathway [$C3_{H2O}B(Mg^{2+})$]. Following this, factor D cleaves FB into two fragments i.e., Bb that remains associated as $C3_{H2O}Bb$ complex and Ba (30 kDa) that is released. This $C3_{H2O}Bb$ complex has C3 convertase activity, resulting in cleavage of C3 into C3a and C3b continuously at a slow rate. $C3_{H2O}Bb$ and C3b are rapidly inactivated by FI and FH, and C3a by C3a/C5a INA control protein.

C3−thioester bond $\xrightarrow{\text{slow hydolysis}}$ $C3_{H2O}$ (1)

$C3_{H2O}$ + FB $\xrightarrow{Mg^{2+}}$ $C3_{H2O}B(Mg^{2+})$ $\xrightarrow{FD}$ $C3_{H2O}\overline{B}b$ (2)

C3 $\xrightarrow{C3_{H2O}\overline{B}b \text{ (initiation C3 convertase}}$ $\overline{C}3b$ + C3a (3)

ii) The C3 convertase is the 'amplification' convertase

$C\overline{3}b$ bound to the non-self or activating surface (bacteria, viruses, fungi, parasites, tumor cells, bacterial LPS, polysaccharides, certain immune complexes, etc.) is preferentially bound by FB that leads to the formation of a C3 convertase. FB is a zymogen serine protease, homologous to C2 of the classical pathway in size, structure, cleavage fragments, mechanism of action, etc. and binds to fluid-phase or cell-bound C3b in the presence of Mg^{2+}. Once associated with C3b as C3bB, it is cleaved by FD into Bb and Ba. Ba has no known biological activity.

The C3 convertase on the non-self surface is also known as 'amplification' convertase, because it forms part of a positive feedback loop, forming additional C3 convertase. C3b created in the classical pathway is also used in formation of alternatve pathway C3 convertase. The activating surface- bound C3b allows binding of FB in the presence of Mg^{2+}. This C3bB complex is then cleaved by FD to give C3bBb complex. At this stage, properdin (P) binds to the complex in order to stabilize it and probably to protect it from cleavage by FI and FH at least for some time.

$\overline{C3b}$ (non-self surface bound) + FB $\xrightarrow{Mg2+}$ $\overline{C}3bB$ (4)

$\overline{C3bB}$ $\xrightarrow{FD}$ $\overline{C3bBb}$ + P ⟶ $\overline{C3bBbP}$ (C3 convertase) (5)

Certain agents have been used as modulators of the activation of the alternative pathway. Cobra venom factor (CVF) is a protein that has function analogous to C3b. It can bind with FB and promote its cleavage by FD to form an alternative pathway C3 convertase. But unlike C3b, it is not inactivated by FH and FI, and therefore can deplete C3 completely from the serum. CVF has been used to obtain C3b by completely depleting C3 from serum *in vivo* and *in vitro*. CVF from *Naja naja kaoutha*, but not *Naja haje* is also a C5 convertase that leads to consumption of all terminal components. Suramin, an anti-trypanosome drug, is an inhibitor of many serine proteases and makes C3b resistant to cleavage by FI and FH. C3 nephritic factor (C3 NeF) is an IgG autoantibody specific for a neoepitope of C3bBb and its binding stabilizes the C3bBb complex in a manner similar to properdin.

Certain agents inhibit the activity of the alternative pathway C3 convertase or directly inhibit the alternative pathway. Heparin in high doses and gold sodium thiomalate increase the affinity of

FH for C3b. Complestatin from *Streptomyces lavendulae* bind to FB preventing its binding to C3b.

C3b is unable to discriminate between self (protected) and non-self (unprotected or activating) surfaces. But this discrimination to protect the self surfaces is brought about by the regulatory proteins, i.e, FI, FH, CR1, DAF and MCP.

Self surface, e.g., host's erythrocytes preferentially binds FH, a cofactor for FI. FH can dissociate C3bBb into C3b and Bb for their decay, and also acts as a cofactor for FI under low ionic strength. After FH binding to C3b on self surfaces, FI cleaves the α chain of C3b to produce inactive form of C3b, i.e., iC3b, which is further cleaved into fluid-phase C3c and the surface-bound C3dg. CR1, DAF and MCP the membrane bound regulatory proteins, bind with C3b to prevent its binding to the self surface, and also dissociate FB from C3b. CR1 and MCP also serve as cofactors for FI. Sialic acid content of the surface interferes with the binding of FH to C3b on the surface.

iii) $\overline{C3bBb3bP}$ is the alternative pathway C5 convertase

C5 cleavage is the first step in the formation of the membrane attack complex. C5 is cleaved by the C5 convertase into the cell-bound C5b and fluid-phase C5a. Bb of the alternative pathway C5 convertase and C2a in the classical pathway convertase have enzymatic activity for C5 cleavage. Effective cell surface binding and cleavage of C5 requires a high local concentration of C3b. Only a few cell bound C5b molecules remain hemolytically active.

$$C5 \xrightarrow{\overline{C3bBb3bP}} C5b + C5a \qquad (6)$$

iv) C5b is the molecule that initiates the formation of C5b-9 (membrane attack complex) in all the three pathways

The steps for MAC formation are common among all the three pathways of C activation and have been described under the preceding classical pathway.

$$C5b + C6 \rightarrow C5b6 + C7 \rightarrow C5b67 + C8 \rightarrow C5b678 + 3\text{-}6\ C9 \rightarrow C5b6789\ (MAC) \qquad (7)$$

Figure 2 shows all major steps of the alternative pathway leading to MAC formation.

3. The lectin pathway

The steps-in-sequence are as follows:
i) Mannose-binding lectin can recognize structures on complex carbohydrates of a wide range of microorganisms

MBL and ficolins are serum glycoproteins that binds to terminal mannose and N-acetyl glucosamine residues of complex polysaccharides (PS) present on a variety of microorganisms. MBL also interacts with two associated zymogen proteases, called MBL-associated serine

protease 1 (MASP-1) and MASP-2. These two proteases are homologous to C1r and C1s respectively.

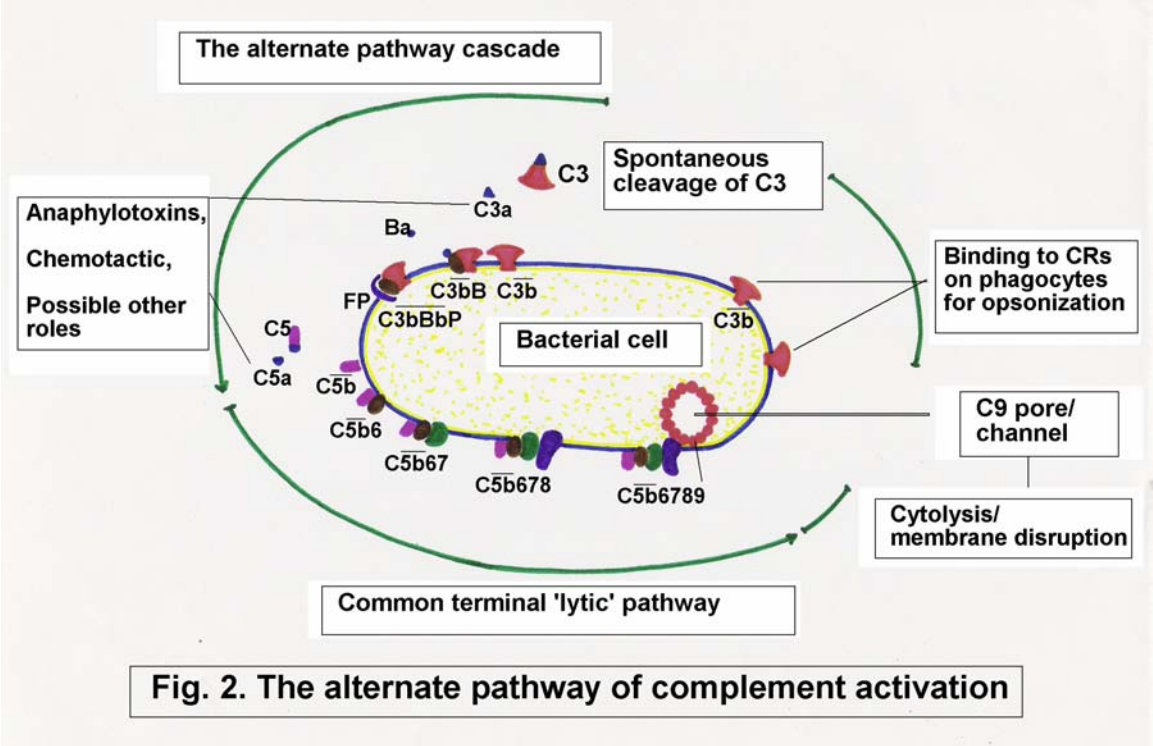

Fig. 2. The alternate pathway of complement activation

Box 5: Summary of the steps in the alternative pathway of C activation

$C3-\text{thioester bond} \xrightarrow{\text{slow hydolysis}} C3_{H2O}$ (1)

$C3_{H2O} + FB \xrightarrow{Mg^{2+}} C3_{H2O}B(Mg^{2+}) \xrightarrow{FD} C3_{H2O}\overline{Bb}$ (2)

$C3 \xrightarrow{C3_{H2O}\overline{Bb}\ \text{(initiation C3 convertase}} \overline{C3b} + C3a$ (3)

$\overline{C3b}$ (non-self surface bound) + FB $\xrightarrow{Mg2+}$ $\overline{C3}bB$ (4)

$\overline{C3bB} \xrightarrow{FD} \overline{C3bBb} + P \longrightarrow \overline{C3bBb}P$ (C3 convertase) (5)

$C5 \xrightarrow{\overline{C3bBb}3bP} C5b + C5a$ (6)

C5b + C6 → C5b6 +C7 → C5b67 + C8 → C5b678 + 3-6 C9 → C5b6789 (MAC) (7)

PS-mannose + MBL: MASP1: MASP2 ⟶ PS: MBL: MASP1: MASP2 (1)

ii) MBL complexed to PS results in activation of the associated MASPs

MASP-1 is activated by MBL after its binding to the mannose residues on foreign surfaces. The activated MASP-1 then activates MASP-2, similar to activation of C1s by the activated C1r of the classical pathway.

PS:MBL:MASP1:MASP2 → PS:MBL:$\overline{\text{MASP}}$1:MASP2 (2)

PS:MBL:$\overline{\text{MASP}}$1:MASP2 ⟶ PS:MBL:$\overline{\text{MASP}}$1:$\overline{\text{MASP}}$2 (3)

iii) Activated MASP-2 in the complex generates C3 convertase by cleavage of C4 and C2

$\overline{\text{MASP-2}}$ cleaves C4 into C4b and the fluid-phase smaller fragment C4a. Binding of C4b onto the foreign surfaces allows its association with C2, which is then cleaved by $\overline{\text{MASP-2}}$ into C4b associated larger fragment C2a and the fluid-phase smaller fragment C2b. The association of C2a with C4b on the surface leads to the synthesis of C3 convertase, i.e., C$\overline{\text{4bC2}}$a of the lectin pathway.

C4 $\xrightarrow{\text{PS:MBL:}\overline{\text{MASP1:MASP2}}}$ C$\overline{\text{4b}}$ + C4a (fluid-phase) (4)

C$\overline{\text{4b}}$ (cell-bound) + C2 → C4$\overline{\text{b2}}$ $\xrightarrow{\text{PS:MBL:}\overline{\text{MASP1:MASP2}}}$ $\overline{\text{C4b2a}}$ + $\overline{\text{C2b}}$ (5)

iv) All subsequent steps are same as of classical pathway

MASP- mediated formation of C3 convertase results in generation of C3b molecules from C3. C3b is deposited on cell-surface by covalent bond formation involving the exposed thioester bond. Some C3b is associated with C$\overline{\text{4b2}}$a on the surface so as to act as C5 convertase. Formation of C5b initiates the generation of membrane attack complex, as discussed earlier.

C3 $\xrightarrow{\text{C4b2a (C3 convertase)}}$ C$\overline{\text{3b}}$ + C3a (fluid-phase) (6)

C5 $\xrightarrow{\overline{\text{C4b2a3b}}\text{ (C5 convertase)}}$ C5b + C5a (fluid-phase) (7)

C5b + C6 → C5b6 + C7 → C5b67 + C8 → C5b678 + 3C9 → C5b−9 (MAC) (8)

Figure 3 shows all major steps of the lectin pathway leading to MAC formation. Comparison of the three C activation pathways is presented in Table 6.

C3 and its derivatives have the central role in complement activation and host defence

C3 is the most abundant and the most important complement component in serum. It can be cleaved spontaneously (tick-over conversion), and by C3 convertases of the classical and alternative pathways. C3 is converted into C3b in which the internal thioester bond is exposed and readily reacts with water, –OH and $–NH_2$ groups in various fluid-phase or membrane

macromolecules (C3 acceptor molecules). C3b and its derivatives, such as iC3b, C3dg and C3d covalently bound to C3 acceptor, can potentially interact with a number of fluid-phase and membrane-bound molecules.

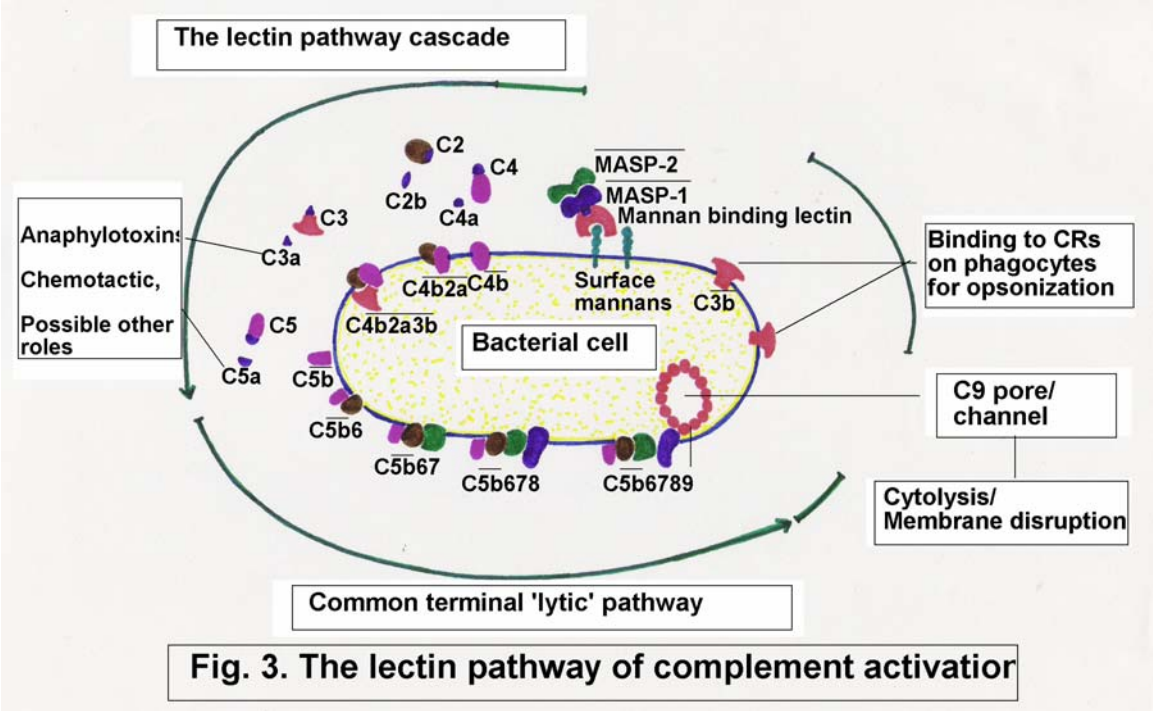

Fig. 3. The lectin pathway of complement activation

Box 6: Summary of the steps in the lectin pathway of C activation

PS-mannose + MBL: MASP1: MASP2 ⟶ PS: MBL: MASP1: MASP2 (1)

PS:MBL:MASP1:MASP2 → PS:MBL:$\overline{\text{MASP1}}$:MASP2 (2)

PS:MBL:$\overline{\text{MASP1}}$:MASP2 ⟶ PS:MBL:$\overline{\text{MASP1}}$:$\overline{\text{MASP2}}$ (3)

C4 $\xrightarrow{\text{PS:MBL:}\overline{\text{MASP1}}\text{:MASP2}}$ C4$\overline{\text{b}}$ + C4a (fluid-phase) (4)

C4$\overline{\text{b}}$ (cell-bound) + C2 → C4$\overline{\text{b2}}$ $\xrightarrow{\text{PS:MBL:}\overline{\text{MASP1:MASP2}}}$ $\overline{\text{C4b2a}}$ + $\overline{\text{C2b}}$ (5)

C3 $\xrightarrow{\overline{\text{C4b2a}}\text{ (C3 convertase)}}$ C3$\overline{\text{b}}$ + C3a (fluid-phase) (6)

C5 $\xrightarrow{\text{C4b}\overline{\text{2a3b (C5 convertase)}}}$ C5b + C5a (fluid-phase) (7)

C5b + C6 → C5b6 + C7 → C5b67 + C8 → C5b678 + 3C9 → C5b−9 (MAC) (8)

Table 6: Comparison of the classical, alternative and lectin pathway of complement activation

Feature	C activation pathway		
	Classical	**Alternative**	**Lectin**
Main activators	Ag complexed IgM & IgG molecules	Bacterial & viral structures, polyanions	Mannose- & N-acetyl glucosamine containing bacterial polysaccharides
Ag-dependence	Yes	No	No
Pathway initiation	C1q binding to IgM/ IgG complexed to Ag	Deposition of C3b deposition onto foreign (unprotected) surface	MBL binding to mannans on bacterial surface
C4 & C2 activation	C1s	Not involved	MASP-2
C3 convertase	C4b2a	C3bBbP	C4bC2a
Stabilization of C3 convertase	-	By properdin	-
C5 convertase	C4b2a3b	C3bBbP3b	C4b2a3b
Inhibitors of early stage	C1 INH, C4-bp	FI, FH, CR1, DAF, MCP	C1 INH, C4-bp
Role	Effector arm of the adaptive immunity	Innate immunity	Innate immunity
Effect of Ig deficiency	Activation prevented	Nil	Nil
Involvement in tissue injury in autoimmune disease & organ transplant	Yes, Ab-mediated	-	-
Evolution	About 400 millions years old, parallel to emergence of Igs	About 700 million years old	About ≥700 million years old
Present in	Only vertebrates, starting from cartilaginous fishes	Echinoderms	Echinoderms, sponges (?)

Surface-bound C3b and its derivatives mediate adherence to cells having receptors for these ligands leading to phagocytosis. C3b deposition on non-self surfaces also leads to the membrane attack complex formation that lyses the target cells or other substances. The pathogen-targeted destructive processes are beneficial and the self cell-targeted processes are harmful. Damage to the self cells has to be kept under control. In this manner, C3 has a central role in these processes

and therefore, its activity has to be kept under check by several control mechanisms. These regulatory mechanisms operate in a species-specific manner.

Several molecules potentially react with C3 and its derivatives, which are: i) C4b2a (the classical pathway C3 convertase), ii) C3bBbP (the alternative pathway C3 convertase), iii) FB, iv) C5, v) FH, vi) FI, vii) MCP (CD46), viii) DAF, ix) CR1, x) CR2, xi) CR3, xii) CR4, xiii) CR5, xiv) properdin, xv) conglutinin, xvi) ICAM-1, xvii) C3a/C4aR. In addition, C3 is also bound by molecules present on several pathogens (bacteria, viruses, fungi, protozoa, and helminthes).

Biological effects of complement activation

The C system is an ancient element of innate immunity present in all vertebrates and in rudimentary form in some invertebrate species. It is one of the best examples of an effective first line of host defence system. But C system needs to be activated for expression of its biological activity. A wide variety of pathogens and other substances activate C in three different pathways, namely, the classical, the alternative and the lectin, resulting in the production of various biologically active C fragments. The "Trilogy" of the C activation is shown in Figure 4.

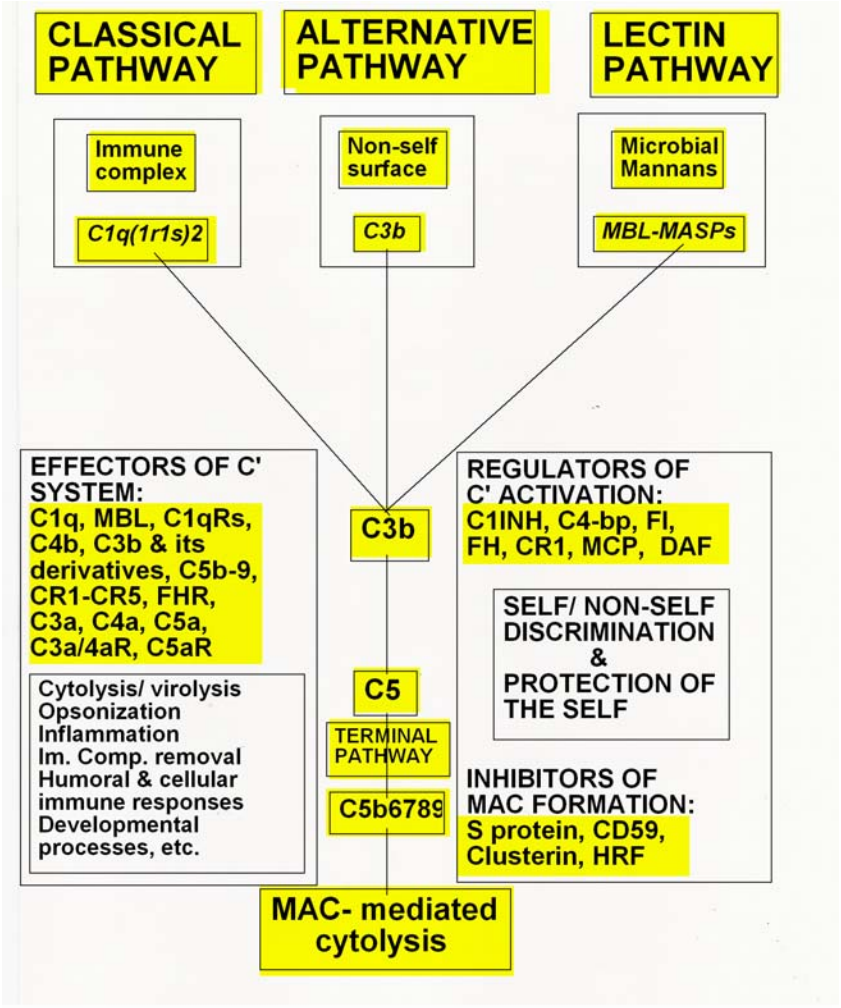

FIG.4. THE 'TRILOGY' OF COMPLEMENT ACTIVATION

As a part of the innate immunity, C system recognizes conserved repetitive structures on a variety of pathogens, and via activation eliminate the pathogens by direct lysis and opsonization. C system also provides signals to adaptive immunity for initiating immune response against the pathogens. In addition, C system also constitutes an effector arm of the humoral immunity, leading to elimination of pathogens complexed with specific Abs. C components have also been implicated for their role in developmental processes. Anaphylatoxins released during cascade reactions have several important pathophysiological effects. Important biological roles of C activation products are presented in Tble 7. Following roles are described in detail:

1. Cytolysis/ virolysis by MAC

All the three different pathways converge to generate membrane attack complex (MAC) on many different kinds of cells, such as erythrocytes, lymphocytes, platelets, bacteria, enveloped viruses, etc. But the efficiency of the target membrane lysis by MAC differs. There are also differences in MAC produced in different species, MAC in some species being more efficient than in others for lysing certain Ag:Ab combinations. MAC formation however does not always lead to lysis of the cell membranes. Resistant to lysis could be due to prevention of MAC formation on susceptible sites of the membrane, lack of binding sites on membranes for the terminal C components or the nature and structure of the cell wall or membrane itself that does not allow MAC formation. Gram – bacteria may require additional factors for their lysis by MAC. Membranes may swell as a result of changes in their ultrastructure, charge and environment after deposition of C components during activation.

Two major theories have been proposed to explain how C5b-9 complex disrupts cellular lipid bilayer membranes leading to their lysis: i) The 'doughnut' model, according to which C9 molecules arrange themselves to form channels through the membrane so that they allow passage of small molecules and initiate osmotic lysis of the cell, and ii) formation of transmembrane channels by MAC-induced reorganization of the lipids and phospholipids. More experimental evidence has been found in support of the former model.

Table 7: Important biological consequences of complement activation

Biological consequence	**Complement components involved**
Cytolysis/ virolysis	MAC (C5b-9)
Opsonization	C3b, C4b, iC3b, C3dg, C3d, C1q, MBL, ficolins
Inflammatory response	C3a, C4a, C5a
Anti-inflammatory action	C1INH
Clearance of immune complexes	C3b, C4b, C1q
Ab production to T_{dep} & T_{ind} Ags	C3, C4, C3d
B cell response	CR2 (C3dR), CR1 (C3b/C4bR) on B cells & FDCs
T cells activation	C5aR, CR3, CR4, CD46 (MCP)
Removal of apoptotic cells	C1q, MBL
Involvement in developmental processes (liver regeneration, hematopoiesis, urodel limb & lens regeneration)	C3aR, C5aR, C3, C5
Reproductive processes	CD46 (MCP)

2. Opsonization of the foreign material deposited with complement components during activation

Phagocytosis and intracellular killing are among the most important mechanism against bacteria and therefore it seems that enhancement of phagocytosis is the most important contribution of C system to host defence. Various C components and the activation products generated in different pathways act as opsonins. C1q and MBL, the initiators of the classical and the lectin pathways respectively, act as opsonins enhancing phagocytosis of their targets by binding to the C1qRp on phagocytic cells.

But the major opsonins are the activation products of C3, i.e., C3b, and its derivatives, iC3b. C3b and iC3b- coated particles are efficiently phagocytosed via CR1 (for C3b) and CR3 (for iC3b) on phagocytes. Pathogen surface- bound C4b also acts as opsonin by presenting these coated cells to CR1 (receptor for C4b) -expressing phagocytes. CR1-mediated opsonization of C3b-coated erythrocytes, however, also require a second signal in terms of IgG coating on them, so that the phagocytes bearing FcγRs effectively ingest these erythrocytes. C5a and other chemotactic factors upregulate both CR1 and CR3 both opsonization.

Removal of C3b- coated pathogens is also facilitated by the mechanism of erythrocyte-mediated immune adherence in humans. Binding of C3b-coated pathogens onto CR1 bearing human erythrocytes immobilizes the pathogens for their facilitated removal by phagocytes in spleen and liver.

3. Role in cell migration and inflammation

C system can elicit acute inflammation through its activation products C3a, C4a and C5a, and C5b-9 complex. The binding of these polypeptides to specific cellular receptors, namely C3a/4aR (for C3a and C4a) and C5aR (for C5a) leads to a multitude of cellular responses important in the initiation and maintenance of inflammatory process.

Binding C3a to C3a/4aRs on mast cells and basophils induces degranulation releasing histamine and other mediators of anaphylaxis. Therefore, C3a is also called anaphylatoxin. However, C5a is the classical anaphylatoxin generated from the complement system and about 20-fold more potent than C3a. In addition, C5a has histamine-induced effect on vascular endothelium causing vascular permeability. C4a is the least potent anaphylatoxin, about 100-fold less than C3a and 2000-fold less than C5a. All of these anaphylatoxins are rapidly destroyed in serum by carboxypeptidase N. It cleaves terminal Arg from the C5a to form C5a $_{\text{des Arg}}$ that lacks chemotactic activity *in vitro*. However, *in vivo* C5a $_{\text{des Arg}}$ retains some chemotactic activity.

C3aR engagement induces multiple effects on myeloid cells, such as chemotaxis for granulocytes and monocytes, and upregulation of β2- integrins for promoting leukocyte adhesion and migration. C5a is also a more potent chemotactic than C3a for neutrophils and monocytes. C5a also induces degranulation of granulocytes and oxidative burst.

The complement, clotting and kallikrein pathways are interrelated and are involved in initiation and control of all inflammatory processes. C1INH inhibits all the enzymes of Hageman factor-dependent pathways that include Factor XII, kallikrein, plasmin and factor XI.

4. Immune responses and immunoregulation

Role of C system is the regulation of humoral immune response has been recognized recently. In early defence against pathogens, activation of classical pathway by natural Abs (Nabs) plays an important role. C activation products may be involved in self-renewal and maintenance of B-1 cells, a distinct subset of self-renewing $CD5^{+}CD11b^{+}$ B cells, that produce Nabs.

C3 and C4 have role in induction of humoral immune response. In C3 or C4 gene knockout mice, both primary and secondary immune responses are decreased, isotype switching does not occur and germinal centres in spleen are reduced in number. Major mechanism for C-dependent regulation of B cell responses involves interaction between Ag- linked C3 activation fragments (iC3b, C3dg and C3d) and CR2 (CD21), and the fragments C3b, C4b, iC3b, C3dg and C3d with CR1 (CD35). Uptake of C3d coated Ag by specific B cell clones results in enhanced signal via BCR. Engagement of the co-receptor CD21-CD19-CD81 with BCR lowers the threshold for B cell activation and provides signal for their survival in germinal centres. Follicular dendritic cells, having high expression of CD21 and CD35, can retain C3 coated immune complexes within lymphoid follicles, providing a second mechanism for enhances B cell immune response. C3d acts an adjuvant when conjugated to Ag. CRs are also critical for localization of Ag and C3d to follicular dendritic cells for maintenance of long-term B cell memory. C deficient mice also have indicated the role of complement in negative selection of B cells.

C3a and C5a also have immunomodulatory effects. C3a suppresses and C5a enhances immune responses. Absence of C3a and C5a could influence the infiltration of $CD4^{+}$ and $CD8^{+}$ T cells. Ag presenting cells preferentially take up C3 coated viral particles for priming of $CD4^{+}$ and $CD8^{+}$ T cells via CR3 and CR4.

Complement components can affect the solubility of preformed immune complexes. Being multivalent, C1q binding increases the size of the immune complexes, thereby decreasing their solubility. C3b, generated particularly via alternative pathway can be deposited on immune complexes and reduce their size, probably by intercalating into and disrupting the immune complex lattices. These molecules help remove the immune complexes by CR-mediated clearance by phagocytes in spleen and liver.

5. Novel functions of the C system

Complement components can bind to apoptotic cells and prepare them for removal by phagocytosis. Apoptotic cells are removed directly by phagocytes or recognized by C1q and MBL and opsonized via C1qRs. C1q binding on apoptotic cells can also lead to activation of classical pathway resulting in deposition of C4b and iC3b. These molecules opsonize apoptotic cells mediated via CR1, CR3 or CR4 on phagocytes.

C1INH maintains the level of C2 and C4 which help in protection from development of autoimmune disease, particularly SLE. Direct interaction of Gram− LPS with C1INH protects

from endotoxic shock. C1INH inhibition of kallikrein plays a direct role in blood pressure regulation.

CD46 expression on spermatozoa is associated with infertility and is a target of anti-sperm antibodies. CD46 is also a receptor of many pathogens, for example, *Neisseria* spp., *Streptococcus pyogenes*, measles virus, herpesvirus 6, etc. It's binding to pathogen molecules modulate microbial pathogenesis and host immune responses in various ways. CD46 deficiency is a predisposing factor to hemolytic uremic syndrome.

Three distinct developmental processes have recently been found to involve C components: i) C3 and C5 are implicated in limb and lens regeneration in newt (a urodel amphibian) model, ii) Both C3a and C5a are growth-promoters for hepatocytes and involved in mammalian liver regeneration, and iii) C3 and C5, locally synthesized by bone marrow stroma, are implicated in hematopoiesis.

Evasion by pathogens of the destructive effects of complement

Certain bacteria, viruses and other pathogens are able to evade the destructive effects of complement system. MAC insertion into the cell walls of certain G – bacteria can be prevented by long side-chains in LPS (*E. coli and Salmonella* strains) or outer membrane protein (*Neiseria gonorrhoeae*). Multiple layers of peptidoglycan in G + bacteria can also prevent MAC formation on their membranes. *Streptococcus pneumoniae* polysaccharide capsule hampers the interaction of C3b coated bacteria with CR1 on phagocytes, thereby preventing its opsonization. Some pathogens produce proteases that degrade the C components. For example, *Pseudomonas aerugunosa* produces elastase that inactivates C3a and C5a anaphylatoxins.
Pathogens also produce certain proteins that mimick regulators of complement activation (RCA), dampen the destructive effect of C and enhance pathogenesis.
The viral complement control proteins downregulate the cellular migration to the site of inflammation, destruction of the infected cells and virus neutralization. Herpes simplex virus type-1 (HSV-1) and HSV-2 bind C3b non-covalently and promote its inactivation. EBV gp350 binds to CR2. Vaccinia virus and HIV can bind host complement control proteins onto their envelopes, resulting in resistance to complement-mediated destructive mechanisms. *Candida albicans* gp60, *Trypanosoma cruzi* and *Schistosoma mansoni* molecules bind C3d, FB and C3b respectively.

Biosynthesis of the complement components

Cell cultures have been used for studying *in vitro* biosynthesis of complement components. Genes coding for most C components have been isolated and expressed by using rDNA techniques. Liver hepatocytes seem to be the major site of C3 production. Lung, spleen, fetal intestine, hepatocytes and tissue macrophages are major sites of C5 production. Liver is the major site of C6 production. In short, liver is the principal site of biosynthesis of most complement components. But tissue macrophages and leukocytes accumulated at the site of inflammation also synthesize various C components locally. Biosynthesis of C components is mainly constitutive. But production of some C components could be induced in macrophages by proinflammatory cytokines such as IL-6, TNF and IFN-γ.

Genetics of the complement system

In humans, most genes encoding C components have been cloned, sequenced and mapped to chromosomal locations (Table 8). Interestingly, a number of closely related C components are organized in major gene clusters in the human genome, suggesting that the majority of the C components have evolved by duplication of a small number of precursor genes.

A tightly-linked gene family, called regulators of complement activation (RCA) gene cluster, is located on human chromosome 1 and includes genes for CR1, CR2, DAF, MCP, FH and C4-bp. In addition, human chromosome 1 also harbours C1q (α,β,γ) and C8 (α,β) genes. Another gene cluster, having genes for C6, C7 and C9 of the membrane attack complex is located on human chromosome 5. These three proteins, alongwith C8 α and β chains (gene on chromosome 1) share a number of structural features, suggesting that the genes of MAC may have evolved by duplication and translocation from an ancestral precursor related to perforin, a pore-forming protein of cytotoxic T cells.

Table 8: Chromosomal location and number of alleles of human C components

C component	Located on chromosome number	Approximate number of alleles
C1q	1	-
C1r	12	10
C1s	12	2
C2	6	10
C3	19	30
C4A & C4B	6	30
C5	9	2
C6	5	20
C7	5	10
C8	1 (α,β); 9 (γ)	10
C9	5	-
FB	6	20
Properdin	X	-
MBL	10	3
C1 INH	11	-
C4-bp	1	5
FI	4	5
FH	1	5
DAF	1	2
MCP	1	2
CR1	1	5
CD59	11	-
S protein	17	-

C4, FB and C2 are MHC-linked class III genes on human chromosome 6. C2 and FB are homologous and arose by duplication. FI, C5, C8γ, MBL genes are on chromosome 4, 9, 9 and 10 respectively. C1INH and CD59 are present on chromosome 11, C1r and C1s on chromosome 12, S protein on chromosome 17, C3 and C5aR on chromosome 19 and properdin on chromosome X.

In humans, C4 is encoded by two closely-linked loci, C4A and C4B, that have extensive polymorphism due to the existence of >30 alleles. There exist functional differences in the allotypes and isotypes of human C4. C2 and FB also have a large number of alleles. C3 has about 30 alleles and C6, C7, C8, C1r and C1s are also allelic. FI, FH, C4-bp and CR1 have upto 5 alleles. CR2, DAF and MCP have upto 2 alleles, C5 has 2, and MBL has 3. Chromosomal location and number of alleles of different C component is presented in table 6. Existence of distinct alleles of C3 provides a mechanism for recognizing a broader range of microorganisms.

Complement deficiencies

Complement deficiencies could be inherited or acquired and could lead to clinical conditions. Strains of guinea pig, mice and other experimental animals have been found with various hereditary C component deficiencies. C3 and FB gene-knockout mice have also been constructed for delineating the functions of these components.

In humans, inherited deficiencies have been recorded in almost all the C components of the classical and the alternative pathways, CR1, CR3, carboxypeptidase N, and regulatory C components FI, FH and C1 INH. Acquired deficiencies in CR1, DAF, CD59 and HRF have been observed. Most inherited deficiencies are autosomal codominant, except properdin (X-linked), FH (autosomal recessive). C4 and C2 genes are MHC-linked. Clinical symptoms may not be seen in certain C deficiencies because of the redundancy of the pathways and the function of the deficient C component may be compensated by another component. C deficient states may result in three types of clinical syndromes: i) recurrent and/ or persistent infections, ii) immune complex disease, and iii) defective immune response.

Deficiency of C3 or FI results in life-threatening bacterial infections. Opsonization is drastically diminished in these states. Combined deficiencies may show a defective immune response. Deficiencies of C5, C6, C7 or C8 are often associated with meningococcal and gonococcal infections, indicating the essential role of cytolytic complement in defence against these infections.

Deficiencies of C1, C2 or C4 singly or in association with other factors are associated with systemic lupus and other autoimmune diseases. It is probably due to defect in the C- dependent clearance or solubilization of immune complexes. C1INH deficiency is one of the most common abnormalities associated with hereditary angioedema. Deficiencies of C receptors are little known. In paroxymal noctural hemoglobinuria, erythrocytes are abnormally sensitive to reactive lysis due to deficiency of C8 binding factors.

C deficiencies are also associated with generation of immune responses. Absence of C3 in mice, after cobra venom factor treatment, drastically reduces Ab levels against limited amounts of T_{dep}

as well as T_{ind} Ags. Genetically C3 or C2 deficient guinea pigs and C4 deficient mice are poor responders and lack class switching to IgG.

Phylogeny and diversity of the complement system

The complement system plays its role as an effector of both the innate and adaptive immunity and also various other immunoregulatory processes in higher vertebrates. It is activated by three different pathways, i.e., classical, alternative and lectin, in higher vertebrates. In lower vertebrates, only alternative and lectin pathways are present and the complement performs the function primarily of opsonization of the foreign materials. The alternative and the lectin pathways are present in agnatha, the most primitive vertebrate species, while cartilaginous fishes are the first species in which classical pathway appears following the emergence of the immunoglobulins.

Box. 7: Phylogeny and diversity of C system

1. The C system is present in all deuterostome-derived phyla i.e., hemichordates, echinoderms, protochordates and vertebrates.
2. In higher vertebrates, three different activation pathways operate for innate and adaptive immunity, and other functions.
3. Cartilaginous fishes are the first species in which classical pathway appears following the emergence of the immunoglobulins.
4. In lower vertebrates, only lectin and alternative pathways exist to perform their role in innate immunity, primarily via opsonization of the foreign material.
5. Agnatha (jawless vertebrates, e.g., lamprey and hagfish) are the most primitive vertebrate species known to have alternative & lectin pathways.
6. The regulatory C components are present in all species beyond the protochordates (e.g., sea squirts), indicating that mechanism of C activation and regulation has developed in parallel.
7. Duplication of the primordial complement genes encoding C3/C4/C5, FB/C2, C1s/C1r/MASP-1/MASP-2, and C6/C7/C8/C9 resulted in appearance of most C components.
8. This development led to the formation of distinct pathways of C activation.
9. C-like activity has recently been detected in a variety of invertebrates.
10. In invertebrates both pathways are functionally indistinguishable and evolved for performing a limited function of opsonization of the foreign materials.
11. Tunicates (e.g., sea squirts), more ancient than echinoderms (e.g., sea urchin, star fish), have C3, MBL and MASP.
12. Sea urchin, an echinoderm, is the most ancient invertebrate known to have both C3 and FB.
13. There is no evidence of any C components in the protostomes, i.e., mollusks, platyhelminthes, annelids and arthropods. Factor C of horse-shoe crab however belongs to the family of MASP/C1r/C1s.

The C system is present in all deutrostome-derived phyla. But the regulatory C components are identified in all species beyond the protochordates. It indicates that the mechanisms of C activation and regulation have developed in parallel. The species ranging from teleosts to reptiles contain a well developed complement system similar to that of higher vertebrates. Duplication of the primordial complement genes encoding C3/C4/C5, FB/C2, C1s/C1r/MASP-1/MASP-2, and C6/C7/C8/C9 resulted in apppearance of most C components. This development led to the formation of distinct pathways of C activation. Several cold-blooded species, unlike warm-blooded ones, possess allomorphs of certain C components, such as C3 and FB, indicating their structural and functional diversity more than those of higher vertebrates.

C-like activity has recently been detected in a variety of invertebrates. Sea urchin, an echinoderm, is at present the most ancient invertebrate known to have both C3 and FB. But in tunicates (e.g., sea squirts), which are more ancient than echinoderms, C3, MBL and MASP are present, leading to the speculation that the lectin pathway could have predated the alternate pathway. However, FB being present in echinoderms is suggestive of the contrary situation. C3aR like receptor in an invertebrate urochordate has recently been reported.

Despite the possession of a conserved set of components of the innate immune system, there is no evidence of any C components in the proteostomes. Factor C of horse-shoe crab however belongs to the family of MASP/C1r/C1s.

Suggested Reading

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