Complement: Classical Pathway

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The classical pathway of complement is a major recognition system in innate immunity that is found in mammals and other animal species. It triggers various mechanisms via two multimolecular proteases – C1 and the MBL/MASP complex – to eliminate pathogenic microorganisms and other antigens from blood and tissues.

Secondary article

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Introduction

The classical pathway of complement is a major system of innate (nonantibody-dependent) immunity. It is triggered through activation of two different multimolecular proteases: C1 and the mannan-binding lectin/MBL-associated serine protease (MBL/MASP) complex. These convert an initial recognition signal into proteolytic activity, thereby initiating the 'classical' and 'lectin' routes of complement activation. Both routes then lead to the formation of a further complex protease (C3 convertase) and the generation of protein fragments triggering diverse biological activities, such as opsonization, endocytosis and inflammation.

Activators of the Pathway

Classical pathway activators

A wide variety of pathogenic microorganisms, including a number of bacteria and viruses, as well as many infected cells, efficiently activate the classical pathway after their recognition by antibodies. Activation is triggered upon interaction of the serum C1 complex with antigen–antibody complexes or immune aggregates containing immunoglobulin G (IgG) or IgM. The most reactive of the IgG subclasses is IgG3, followed by IgG1 and IgG2, whereas IgG4 exhibits very little reactivity. The C1-binding site in Ig has been located within the $C_\gamma 2$ and $C_\mu 3$ domains of the Fc regions of IgG and IgM, respectively. C1 binding to immunoglobulins is mediated by its C1q subunit and involves the heterotrimeric C-terminal globular 'heads' of the protein (**Figure 1**). Because of the hexameric structure of C1q, the C1 binding affinity to Ig-containing activators increases markedly with their state of aggregation: soluble monomeric IgG binds C1 about 10 000-fold less efficiently than do IgG-containing immune complexes.

Although the classical pathway is often described as an antibody-dependent defence mechanism, it should be remembered that its initial component C1 is also activated directly by a remarkably diverse range of substances other than antibody (**Table 1**; Cooper, 1985). These include some Gram-positive and Gram-negative bacteria, certain viruses (including human immunodeficiency virus, HIV), cellular and subcellular structures from damaged cells, as well as several proteins, carbohydrates, lipids, polyanions, and other compounds such as sodium urate crystals. The acute-phase C-reactive protein, which recognizes phosphocholine groups on pneumococci and other pathogens, activates the classical pathway and thereby triggers elimination of the pathogens by phagocytic mechanisms. In contrast, C1 activation by fibrils of the β-amyloid peptide may contribute, through the development of uncontrolled inflammation, to the progression of Alzheimer disease pathogenesis. There are no obvious structural features shared by the diverse nonimmune C1 activators, some of which activate C1 more efficiently than immunoglobulins. In most cases, C1 binding to these activators occurs through the C1q subunit, and has been reported to involve either its globular 'heads', or its collagen-like regions (**Figure 1**), or both.

Lectin pathway activators

In addition to the well-characterized C1-mediated initiation of the classical pathway, a second activation route, the 'lectin pathway', was discovered in the early 1980s. It is triggered by a C1-like complex protease in which the recognition function is mediated by mannan-binding lectin (MBL), a member of the collectin family (Turner, 1996). Through its multiple C-terminal C-type lectin modules (**Figure 1**), MBL recognizes arrays of oligosaccharides at the surface of a wide spectrum of microorganisms, including yeasts (*Candida albicans*), viruses (HIV, influenza A), and a number of bacteria (*Salmonella montevideo*, *Listeria monocytogenes*, *Haemophilus influenzae* and *Neisseria meningitidis*). There is increasing evidence that the lectin pathway of complement activation plays an important role

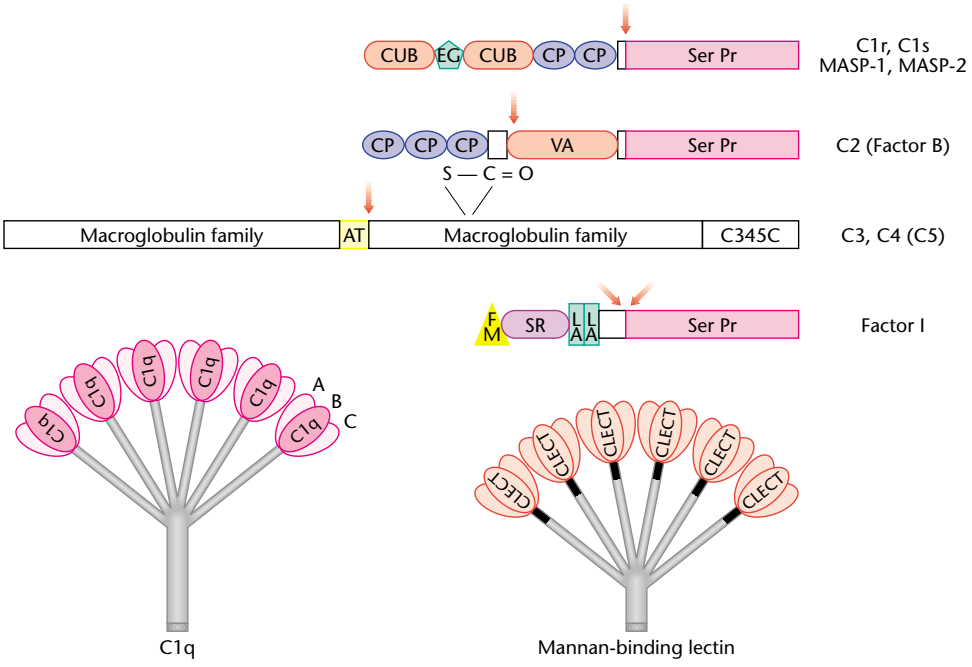

Figure 1 Modular structures of the complement classical pathway proteins. The nomenclature and symbols used for protein modules are those defined by Bork and Bairoch (1995). AT, anaphylatoxin; CP, complement control protein (CCP) module; CUB, module found in complement C1r/C1s, Uegf, and bone morphogenetic protein; C345C, complement proteins C3/C4/C5 C-terminal module; EG, epidermal growth factor (EGF)-like module; FM, factor I/membrane attack complex proteins C6/C7 (FIMAC) module; LA, low-density lipoprotein receptor class A (LDLRA) module; SR, scavenger receptor cysteine-rich (SRCR) module; Ser Pr, serine protease domain. VA, von Willebrand factor type A (VWFA) module. Heterotrimeric associations of 'C1q' modules (A, B, C), and homotrimeric associations of C-type lectin (CLECT) modules are found at the C-terminal end of the collagen 'arms' of C1q and mannan-binding lectin, respectively. Unlabelled portions of the molecules represent connecting segments or sequence areas with no known homology to other proteins. Areas of collagen-like structure are grey, and coiled-coil structures are shown in black. C1q only occurs as a hexamer, whereas mannan-binding lectin is present in serum under multiple oligomeric forms. Arrows indicate peptide bonds cleaved on activation of proteolytic enzymes. In the case of C3 and C4, the only cleavage shown is that mediated by C3 convertase and C1s or MASP-2, respectively. The location of the internal thioester group of C3 and C4 is indicated. The relative sizes of the proteins are approximate.

Table 1 Activators of the classical pathway of complement

Immune activators
Antigen–antibody complexes containing IgM or IgG
Nonimmune activators
Various bacterial strains (*E. coli*, *Salmonella*, *Klebsiella*)
Mycoplasma: *Mycoplasma pneumoniae*
Various viruses (Sindbis, Newcastle disease, Epstein–Barr virus) and retroviruses (HIV)
Parasites: *Schistosoma mansoni*, *Trypanosoma brucei*
Cellular and subcellular membranes
Proteins: C-reactive protein, myelin, β-amyloid peptide, serum amyloid P component
Oligosaccharides and polysaccharides
Lipids (lipid A, cardiolipin)
Polyanions (heparin, DNA)

Modified from Cooper (1985).

in immune defence, particularly during the phase of primary contact with microorganisms.

Components

A characteristic feature shared by most complement components is the occurrence in their structure of contiguous protein motifs or modules (**Figure 1**; **Table 2**). These belong to different types which may be recognized at the sequence level by specific consensus sequences and have probably evolved from a common ancestor by gene duplication. Protein modules fulfil specific functions, often the ability to mediate protein–protein interactions.

The recognition proteins C1q and MBL are both oligomers of trimeric subunits, comprising collagen-like regions prolonged by C-terminal globular domains, with overall structures resembling a bunch of tulips (**Figure 1**). C1q is a hexamer of three homologous but distinct A, B and C chains. Because of repeating Gly-Xaa-Yaa triplets in

Table 2 Components of the classical and lectin activation pathways of human complement

Protein	Molecular mass (Da)		Carbohydrates	Serum concentration	Biological function
	Molecule	Chains	(content, w/w; type)	(mg L^{-1})	
C1q	459 300	A: 27 550 B: 25 200 C: 23 800	8.3% glucosylgalactosyl and *N*-linked	80	Recognition unit of the C1 complex
MBL	457 000[a]	25 400	5.5% *O*-linked	1.5	Recognition unit of the MBL/MASP complex
C1r	173 000[b]	A: 55 300 B: 31 200	9.5% *N*-linked	34	Activation unit of the C1 complex
C1s	79 800	A: 52 150 B: 27 650	6.1% *N*-linked	31	Active component of C1; cleaves C4 and C2
MASP-1	86 000	A: 57 850 B: 28 150	10.3% *N*-linked	6	Activation unit of the MBL/MASP complex
MASP-2	74 150	A: 47 650 B: 26 500	none	ND[c]	Active component of the MBL/MASP complex
C4	205 000	α: 97 000 β: 75 000 γ: 33 000	7.0% *N*-linked	600	C1 substrate; yields active fragments C4a and C4b
C2	102 000	C2a: 70 000 C2b: 30 000	15.9% *N*-linked	20	C1 substrate; yields active fragments C2a and C2b
C3	185 000	α: 110 000 β: 75 000	5.9% *N*-linked	1300	Substrate of the C3-convertase; yields active fragments C3a and C3b

[a] Molecular weight of the hexameric form of MBL
[b] Molecular weight of the C1r–C1r dimer
[c] Not determined

their N-terminal region, these form six collagen triple helices which associate as a 'stalk' and then, due to interruptions in the Gly-Xaa-Yaa motif, diverge to form six 'arms'. Each arm ultimately merges into a C-terminal globular 'head' region formed from the heterotrimeric association of protein modules of the C1q type. MBL differs from C1q in that it occurs in serum as a mixture of oligomers ranging from dimers to hexamers, made up of a single type of chain. A further difference is that the collagen triple helices of MBL merge into carbohydrate-recognition domains formed from the homotrimeric association of calcium-dependent (C-type) lectin modules held together by a coiled-coil of α helices called the 'neck' region.

The proteases associated with C1q and MBL – C1r and C1s in the classical pathway and MASP-1 and MASP-2 in the lectin pathway – share 39–45% sequence identity and exhibit the same type of modular organization (**Figure 1**). Each comprises, from the N-terminus, two CUB modules surrounding a single epidermal growth factor (EGF)-like module, a pair of contiguous complement control protein (CCP) modules, and a serine protease domain. All four proteases are single-chain proteins in their proenzyme form and are split upon activation, through cleavage of a single Arg–Ile bond, into disulfide-linked polypeptide chains A and B (the serine protease domain). The latter all belong to the chymotrypsin-like family and have an Asp residue in their substrate-binding pocket, indicative of trypsin-like specificity. However, the serine protease domains of C1r, C1s and MASP-2 lack the 'histidine loop', a disulfide bridge present in all known mammalian serine proteases including MASP-1. The regions of C1r and C1s responsible for catalytic activity comprise both the serine protease domain and the preceding CCP modules, and the latter have been shown to participate in the recognition by C1s of its substrate C4. The N-terminal CUB-EGF module pairs of C1r and C1s each contain one high-affinity Ca^{2+}-binding site. These regions mediate the Ca^{2+}-dependent C1r–C1s interactions involved in the assembly of the C1s–C1r–C1r–C1s catalytic subunit of C1 and also take part in C1 assembly.

C2 exhibits the same type of structural organization as factor B, its functional homologue in the alternative complement pathway, consisting of, from the N-terminus, three CCP modules, a single von Willebrand factor type A (VWFA) module, and a serine protease domain (**Figure 1**). Activation by C1s occurs through cleavage of a single Arg–Lys bond in the N-terminal region of the VWFA module, which splits the molecule into the N-terminal fragment C2b and the C-terminal catalytic fragment C2a. The serine protease domain of C2 lacks the highly conserved

hydrophobic N-terminal sequence generated during activation of other zymogens including C1r, C1s and the MASPs, indicating that assumption of the active conformation in fragment C2a is achieved through a particular mechanism. Another unique feature is that, despite the known trypsin-like specificity of C2a, a Ser residue is substituted for the Asp residue normally present in the binding pocket. The VWFA module of C2 contains a free thiol group and the MIDAS (metal ion-dependent adhesion site) motif described in integrins, which likely mediates binding of a Mg^{2+} ion. Both sites appear to be involved in the assembly of the C3 convertase C4bC2a.

C3 and C4, as well as component C5 of the lytic pathway, are synthesized as single-chain precursors with similar overall structures (**Figure 1**). The major part of the C3 and C4 precursor molecules is accounted for by polypeptide sequences showing homology with α_2-macroglobulin. Sequence homology between these three proteins is particularly high in the region containing an internal thioester bond, which forms between the side chains of a cysteine and a glutamine residue in the sequence ...Cys-Gly-Glu-Gln... (**Figure 1**). C3 and C4 also have in common a short anaphylatoxin (AT) motif in their central part and a complement C3/4/5 C-terminal (C345C) module. Proteolytic processing of C3 and C4 involves intracellular excision of a basic tetrapeptide at the N-terminal end of the AT module, and a further, two-step removal of a 26-residue sequence in the case of C4. The circulating mature proteins comprise two chains (α, β) in the case of C3 and three chains (α, β, γ) in the case of C4, all linked by disulfide bridges. Activation of C3 by C3 convertase, and of C4 by C1 involves cleavage of a single arginyl bond at the C-terminus of the AT module, thereby leading to the release of the corresponding fragments C3a and C4a (**Figure 2**).

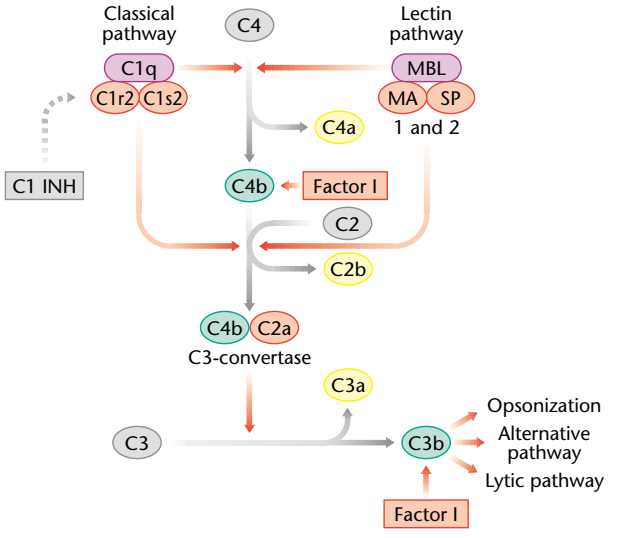

Figure 2 Classical and lectin pathways of complement. Activation of the classical pathway is triggered by direct or antibody-dependent recognition of a microorganism by C1q, whereas the lectin pathway is initiated by interaction of the mannan-binding lectin (MBL) with arrays of carbohydrates at the surface of a microorganism. Proteins expressing proteolytic activity are shown in red and proteolytic cleavages are indicated by red arrows. Fragments C4b and C3b exhibiting a reactive thioester group are green, whereas small fragments generating inflammatory reactions are yellow. C1 inhibitor (C1 INH), a member of the serine protease inhibitor (serpin) family, controls both C1 activation and C1 proteolytic activity. Its reactivity towards MASP-1 and MASP-2 is not fully established. Factor I cleaves C4b and C3b, preventing formation of the convertases and generating C3b fragments endowed with important biological activities.

Functioning of the Pathway

Triggering of the classical pathway may occur through activation of two different multimolecular proteases, C1 and the MBL/MASP complex. These complex enzymes comprise distinct but homologous subunits, and appear to share similar structural and functional features, including the ability to cleave the same protein substrates C4 and C2, thereby allowing formation of a further complex protease (C3 convertase) and generating fragments endowed with biological activities (**Figure 2**).

C1 and the MBL/MASP complex

C1 is a Ca^{2+}-dependent complex of molecular mass about 790 000 Da containing three subcomponents (C1q, C1r, C1s) in a 1:2:2 molar ratio. It is assembled from two subunits, C1q (the recognition subunit) and the Ca^{2+}-dependent tetramer C1s–C1r–C1r–C1s (the catalytic subunit). The isolated C1s–C1r–C1r–C1s complex is an elongated, reversed 'S'-shaped molecule comprising a linear array of domains, with an average width of 3–4 nm and a contour length of 51–59 nm. The C1r–C1r dimer forms the core of the tetramer, with its catalytic regions in the centre, each of its interaction regions being connected in a Ca^{2+}-dependent manner to the corresponding region of a C1s molecule. In this extended conformation, the catalytic regions of C1s are located at both ends of the tetramer.

Although early studies have indicated that C1q and C1s–C1r–C1r–C1s interact weakly under physiological conditions, it was shown more recently that the dissociation constant of human C1 at room temperature is in the picomolar range, indicating that virtually all of the C1q, C1r and C1s in serum is associated in C1. The strength of the association increases markedly when the temperature is raised, and decreases upon activation of the C1s–C1r–C1r–C1s subunit. Several lines of evidence indicate that the sites of C1q responsible for the interaction with C1s–C1r–C1r–C1s are located within the collagen-like region of the protein, and electron microscopy of the chemically cross-linked C1 complex reveals that the tetramer forms a compact mass centred on the C1q 'arms', between the

'stalk' and the globular 'heads' (Strang *et al.*, 1982). Solution scattering studies provide further support to the hypothesis that the elongated C1s–C1r–C1r–C1s tetramer folds into a more compact conformation upon interaction with C1q. The binding of the tetramer to C1q appears to be a complex process involving ionic bonds and multiple sites contributed by both C1r and C1s and mainly located in their N-terminal CUB-EGF interaction regions, which therefore represent key elements of the architecture of macromolecular C1.

Several of the low-resolution C1 models proposed so far are based on the assumption that the C1s–C1r–C1r–C1s tetramer adopts a compact, '8'-shaped conformation in C1 (**Figure 3**). This conformation brings the catalytic regions of C1r and C1s into the same vicinity and thereby allows the two-step C1 activation to proceed, namely (1) autoactivation of the C1r–C1r dimer through mutual cleavage of each monomer by its neighbour, and (2) C1s activation by active C1r. Although the molecular mechanisms involved in C1 activation and control are not fully understood, current knowledge supports an overall scheme involving: (1) the ability of C1 to activate spontaneously, reflecting the intrinsic property of C1r to autoactivate; (2) the occurrence in serum of a permanent negative control of C1 activation exerted by C1 inhibitor; and (3) the release of this control upon interaction of C1 with strong activators. The nature of the signal that triggers C1 activation remains, however, an intriguing question. Once C1 activation has occurred, the C1s–C1r–C1r–C1s tetramer may adopt a more relaxed, open 'S'-shaped conformation in which both C1s catalytic regions protrude outside the C1q molecule and thereby gain access to C4 and C2, the substrates of active C1.

Little information is available on the structure and function of the MBL/MASP complex that triggers the lectin pathway of complement activation. Although MBL was initially found associated in human serum with a single MASP species (MASP-1), more recent studies indicate that the catalytic component of the MBL/MASP complex contains two distinct serine proteases, probably functionally equivalent to C1r (MASP-1) and C1s (MASP-2). However, further analyses of the stoichiometry of the MBL/MASP-1/MASP-2 complex and of the structure and enzymatic properties of MASP-1 and MASP-2 are required to get a clear picture of its assembly, function and control.

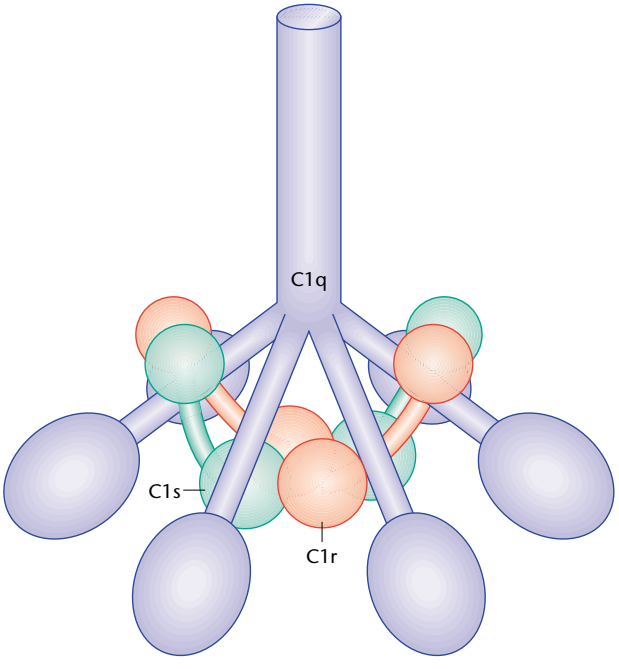

Figure 3 C1 complex of complement. C1q, C1r, and C1s are blue, red and green, respectively. Upon assembly of the complex, the elongated C1s–C1r–C1r–C1s tetramer is thought to bend around two opposite collagen 'arms' of C1q, resulting in a compact, distorted '8'-shaped conformation. The catalytic regions of C1r and C1s responsible for C1 activation and proteolytic activity towards C4 and C2 are located inside the cone defined by the C1q arms, whereas the interaction regions involved in both the assembly of the C1s–C1r–C1r–C1s tetramer and its interaction with C1q lie outside the collagen 'arms' of C1q. Modified from Arlaud *et al.* (1987).

C3 convertase

The thioester bond and the covalent binding of C4b and C3b

Proteolytic cleavage of C4 by C1 or the MBL/MASP complex, and of C3 by C3 convertase leads to concomitant exposure of their internal thioester bond. The acyl group of the thioester in C4 becomes highly reactive with amino and hydroxyl groups, with differences due to C4 polymorphism. The two isoforms of human C4, C4A and C4B, mainly differ by the nature of the amino acid at position 1106 (about 100 residues C-terminal of the thioester), which is an Asp in C4A and a His in C4B. Binding of the C4A isotype involves a single-step reaction between the thioester and amino-nucleophiles, whereas the C4B isotype reacts with hydroxyl-nucleophiles in a two-step mechanism in which the His contributes to an acyl-imidazole intermediate. Native C4b is thus able to form amide or ester bonds or reacts readily with water. The reactivity of activated C4 is extremely short-lived: the half-life of activated C4A is about 10 s and that of C4B less than 1 s (Dodds *et al.*, 1996).

The above characteristics are also valid for C3, but this protein only exhibits reactivity of the C4B type. Overall, this B type reactivity appears to be the most frequent, leading to formation of ester links between C3b or C4b and acceptor sites. Although binding of C3b or C4b is indiscriminate, the occurrence of regulatory proteins on normal host cells prevents complement deposition and amplification on their surfaces, providing self/nonself discrimination.

Assembly of C3 convertase

When activated C1 cleaves C4, the resulting C4b fragment is able to bind covalently to $-NH_2$ or –OH groups on proteins or polysaccharides in the immediate vicinity of C1 at the surface of the activator. Bound C4b then binds C2 in the presence of magnesium, which renders this protein available for limited proteolysis by C1. The catalytic fragment C2a remains bound to C4b, resulting in the formation of a bimolecular complex C4bC2a, the classical pathway C3 convertase, with a serine active site in C2a and exhibiting specific proteolytic activity towards C3. This complex protease is central to the complement system as it generates fragments C3a and C3b, both responsible for major biological activities.

Incidentally, native C3b, like C4b, is able to bind covalently to the activator surface in the vicinity of the convertase, as well as to the C3b moiety of the C3 convertase itself, leading to a three-protein complex (C4bC2aC3b), the classical pathway C5 convertase, in which C2a acquires specificity for the cleavage of C5.

Biological effects of the pathway (Table 3)

Anaphylatoxins C3a and C4a

C4a and C3a are 77 amino acid polypeptides released from the α chains of C4 and C3 respectively upon proteolysis by C1 or the MBL/MASP complex (C4) and C3 convertase (C3). Binding of these peptides to receptors on the surface of mast cells and basophils initiates a release of vasoactive amines from intracellular granules. Released histamine and serotonin act on smooth muscle to cause contraction or on small blood vessels to increase vascular permeability. Binding to receptors on neutrophils stimulates a release of lysosomal enzymes. C3a is by far the most efficient of the two anaphylatoxins, both of which contribute to inflammation. Control of anaphylatoxin activity is achieved efficiently *in vivo* by a plasma carboxypeptidase N (anaphylatoxin inactivator) which removes the C-terminal arginine residue from each peptide.

Table 3 Biological activities of components of the classical complement pathway

C2b and C2k	Vascular permeability and contraction of smooth muscles
C3a, C4a	Vascular permeability Contraction of smooth muscles
C3b (C4b)	Immune adherence and opsonization Elimination of immune complexes Antigen localization in lymphoid tissues Phagocytosis of immune complexes Antigen presentation
iC3b	Phagocytosis Antibody-dependent cytotoxicity

Kinin-like activity of C2b

The C2b fragment itself or a smaller proteolytic fragment C2k (about 1500 Da) from the C-terminal end of C2b has been implicated as the causative factor for hereditary angioedema. Uncontrolled activity of C1 towards C4bC2 leads to overproduction of C2k and angioedema stroke. In contrast with C3a and C4a activities, C2k is not inhibited by antihistaminic drugs.

Biological activities of C3b and C4b

Opsonization and endocytosis

Native C4b and C3b are able to bind covalently to nucleophilic groups on any antigen or pathogen. The bound fragments then bind noncovalently to membrane receptors on many phagocytes, leading to endocytosis of soluble antigens or to phagocytosis of diverse pathogens. Binding and cellular uptake are mediated by complement receptor CR1. The binding of C4b or C3b to CR1 is transient, due to proteolysis of these fragments by factor I into C4c and C4d and into iC3b and C3f, respectively. In the case of iC3b the affinity for CR1 is decreased, whereas a capacity to bind with high efficiency to complement receptor CR3 appears. Subsequent proteolysis of iC3b leads to fragment C3dg, able to bind complement receptor CR2. Receptor CR3 appears from the life length of iC3b as the most efficient in phagocytosis. This activity of C3 and C4 fragments is one of the first defence mechanisms of the human body against foreign pathogens.

Clearance of immune complexes

C3b and C4b are able to bind covalently to immune complexes which activate the classical complement pathway. C3b- and C4b-associated immune complexes are loaded on erythrocytes through CR1 receptors and transported to the liver where proteolysis of C3b or C4b leads to the release of the complexes from the erythrocytes, with subsequent binding of the immune complexes to Fc receptors of Kupffer cells, and their uptake by these cells.

Roles in the immune response

C3b- or C4b-associated antigens are more efficiently processed and presented by antigen-presenting cells to T lymphocytes than antigens alone. In the case of presenting B lymphocytes, improved uptake and processing of C3b/C4b-bound antigens has been observed. Also, signalling by

C3b/C4b-bound antigens has been shown to modulate antigen presentation through interactions involving receptors CR1, CR2, CD19 and the immunoglobulinic antigen receptor.

Regulation of the Pathway

Regulatory proteins (Table 4)

These proteins have different targets, at the level of C1 (C1 inhibitor), and at the level of C3 convertase: C4b- and/or C3b-binding proteins such as decay-accelerating factor (DAF), C4-binding protein (C4BP), factor H, membrane cofactor protein (MCP) and complement receptor 1 (CR1). Whereas DAF only acts as a binding protein, the other four proteins act, in addition, as cofactors for factor I, the proteolytic regulator of C4b and C3b. Intrinsic instability characterizes the classical pathway C3 convertase, contributing by itself to the control of this enzyme. DAF, C4BP, factor H, MCP and CR1 are all encoded by genes clustered on chromosome 1 in human and all contain repeating CCP modules, which provide the binding sites for C4b and/or C3b.

Regulation of C1

C1 inhibitor (C1 INH) is a heavily glycosylated serine protease inhibitor (serpin) that is the only known serum inhibitor of C1, but also controls several serine proteases of the kinin and coagulation pathways. Upon reaction with activated C1s and C1r in C1, C1 INH is cleaved into two fragments, the major one forming a covalent complex with the active site of both proteases which thus become irreversibly inhibited. Reaction of C1 INH with the C1 proteases is followed by a disruption of the C1 complex which leaves C1q bound to the activating surface. It has been suggested that C1 INH also prevents C1 activation in the absence of activator, through noncovalent binding to C1.

Table 4 Regulators of the classical pathway of complement

Soluble proteins

Protein	Molecular mass (Da)	Carbohydrates (content, w/w; type)	Serum concentration (mg L^{-1})	Specificity	Role
C1 INH	110 000	35% N-linked and O-linked	200	C1r, C1s	Inhibitor of C1 protease
C4-binding protein (C4BP)	560 000	12%	250	C4b	Decay of C4b2a, cofactor of C4b cleavage by factor I
Factor H	150 000	ND[a]	500	C3b	Cofactor of C3b cleavage by factor I
Factor I	88 000	27% N-linked	35	C4b, C3b	Proteolysis of C4b and C3b with cofactors C4BP, H, CR1, or MCP

Membrane-associated proteins

Protein	Molecular mass (Da)	Carbohydrates (content, w/w; type)	Positive cells[b]	Specificity	Role
Complement receptor type 1 (CR1) (four structural allotypes)	type D: 250 000 type B: 220 000 type A: 190 000 type C: 160 000	ND[a]	E, B, G, M	C3b, C4b	C3b breakdown
Membrane cofactor protein (MCP)	45 000–70 000	*N*-linked and O-linked	B, T, N, M	C3b, C4b	Regulation of C3b breakdown
Decay accelerating factor (DAF)	70 000	*N*-linked and O-linked	E, L, P	C4b2a, C3bBb	Decay of C3/C5 convertases

[a] Not determined.
[b] Human cell types: B, B lymphocytes; E, erythrocytes; G, granulocytes; L, leucocytes; M, monocytes/macrophages; N, neutrophils; P, platelets; T, T lymphocytes.

Regulation of C3 convertase

Binding of DAF, a membrane-bound protein, to C4b in C3 convertase leads to increased decay of the convertase, an activity facilitated by the glycolipid anchor of DAF, which confers to this protein a large area of control on the cell membrane. Destabilization of C3 convertase by cofactors of factor I leads to the formation of bimolecular associations between C3b or C4b and one of the cofactors, which are soluble proteins (C4BP for the cleavage of C4b, factor H for the cleavage of C3b) or membrane-associated proteins (MCP and receptor CR1). Under their associated forms, C3b and C4b become available as substrates for proteolysis by factor I, suppressing the activity of the convertase. C4b ligands like C4BP and DAF are also able to bind C4b alone and thereby interfere with C3 convertase assembly.

Factor I

The mature protein in blood consists of two disulfide-linked 50-kDa and 38-kDa polypeptide chains, the latter being a serine protease domain. Maturation results from intracellular removal of four basic residues linking the two chains in profactor I. Its high carbohydrate content corresponds to a probable glycosylation of the six potential sites in the sequence. Factor I exhibits a unique modular structure, with a factor I/membrane attack complex proteins C6/7 (FIMAC) module at the N-terminal end, followed by a scavenger receptor cysteine-rich (SRCR) module, two low-density lipoprotein receptor class A (LDLRA) modules, and a serine protease domain (**Figure 1**).

Protein Biosynthesis and Deficiencies

The major site of biosynthesis for most of the circulating complement components is the hepatocyte, and more than 90% of serum complement proteins is produced in the liver. However, C1 subcomponents are synthesized primarily in the gut epithelium, and a growing list of other cells (monocytes/macrophages, fibroblasts, endothelial cells, leukocytes, etc.) produce complement proteins. Evidence is also emerging that locally produced complement proteins might be an important factor in triggering inflammation in different peripheral tissues, especially those shielded from plasma components by blood–tissue barriers. Thus, the cells of the central nervous system biosynthesize a complete, functional complement system and express complement regulators and receptors. Complement biosynthesis in the brain is thought to be important in immune defence but probably also has implications in inflammatory and degenerative diseases.

Cases of hereditary deficiencies have been described for each of the components C1q, C1r, C1s, C4, C2, and C3 of the classical activation pathway, and these deficiencies are generally associated with an increased susceptibility to infection by pyogenic microorganisms (*Staphylococcus*, *Streptococcus*) and to immune complex disease such as systemic lupus erythematosus (Morgan and Walport, 1991). Deficiency of C1 inhibitor is associated with hereditary angioedema, which is probably due to the kinin-like activity of a fragment released from C2b, and may cause fatal obstruction of the upper airways. Inherited deficiency of the membrane regulatory component DAF is characterized by paroxysmal nocturnal haemoglobinuria, whereas deficiency of either factor I or factor H results in a state of acquired C3 deficiency, due to the lack of C3b catabolism leading to uncontrolled C3 cleavage by the alternative pathway C3 convertase.

The major site of MBL biosynthesis is the hepatocyte. Levels of MBL in serum show a wide range (0–5 mg L^{-1}), and low serum concentrations are found associated with increased susceptibility to recurrent bacterial and viral infections. There are three common structural alleles of MBL, each characterized by a single point mutation in the collagen-like region, and these are the most important determinants of the MBL level.

Phylogeny

Opsonic complement activities have been found in all vertebrate species tested, from the mammals back to the cartilaginous fish (sharks and rays). More recent studies have led to identification of MASP-like proteins in the urochordate *Halocynthia roretzi*, suggesting occurrence of a lectin pathway in this species which is intermediate between vertebrates and invertebrates. Current phylogenetic knowledge provides support to the hypothesis that complement arose as an innate defence system, possibly based on lectin recognition, before the emergence of antibodies. Contrary to the old view that complement 'complements' the adaptive immune system, it may be considered that antibodies arose to 'complement' complement by increasing its versatility in recognition.

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