Complement System and Its Role in Immune Responses

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Introductory article

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The complement system is a network of more than 50 plasma proteins and receptors, which have the role of mediating innate and adaptive host defence mechanisms, whereas they also participate in various (patho)physiological processes. The primary functions mediated by complement proteins include phagocytosis of foreign elements (bacteria, viruses, particles etc.), cell lysis, inflammation, solubilisation of immune complexes, apoptotic cell clearance and enhancement of humoral immune responses. Dysregulation of complement activity has, therefore, been connected to various diseases, including autoimmune conditions, thrombotic pathologies and infections.

Introduction

The complement system was first recognised in late 1800s as a heat-labile component with bactericidal activity present in the serum. By 1900s, complement surface fixation and the removal of complement activity from normal human serum by antigen–antibody complexes were shown. The serum labile bactericidal activity was originally named 'alexin' by Buchner and Bordet and subsequently replaced by the term 'complement' introduced by Ehrlich and his group (reviewed by Lachmann (2006)). The name was chosen to reflect the capacity of complement system to enhance the antibacterial activity of humoral components.

The complement protein network was later shown to be an essential component of innate immunity, serving as a first-line defence against microbial invaders (for review see Refs. (Ricklin *et al*., 2010; Lambris *et al*., 2008)). As early suspected, complement also contributes to humoral immune responses by potentiating the production of antibodies and B cell memory (Carroll, 2008). Later studies suggested that complement has additional noninflammatory roles participating in several physiological processes, such as coagulation, hematopoiesis, reproduction, liver regeneration, apoptosis, homoeostasis, metabolism and central nervous system development (Ricklin *et al*., 2010).

The complement system consists of numerous plasma and membrane-bound proteins, currently numbering more than 50 components (Ricklin *et al*., 2010) (**Table 1**). Central role in the complement protein network has C3, one of the most abundant serum proteins, with concentrations generally ranging from 1.0 to 1.9 mg/mL. Most of the soluble complement proteins, including C3, are synthesised in the liver by hepatocytes, but other cell types can also be significant sources (Reis *et al*., 2006).

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Complement Activation Pathways

The complement system is comprised of three major pathways which are activated in a cascade manner (**Figure 1**; reviewed by (Ricklin *et al*., 2010; Le Friec and Kemper, 2009; Lambris *et al*., 2008; Markiewski and Lambris, 2007)): (a) the classical pathway, mainly initiated by antigen–antibody complexes; (b) the lectin pathway, triggered by carbohydrate patterns on microbial surfaces; and (c) the alternative pathway, which is based on spontaneous activation of complement component 3 (C3) by hydrolysis ($C3_{H_2O}$) or by binding of active C3 fragments, namely C3b, to properdin (Kemper *et al*., 2010; Ricklin *et al*., 2010).

Classical pathway

The classical pathway is initiated in the presence of immunoglobulins (IgM or IgG) within immune complexes

Table 1 Alternative, classical and lectin complement activation pathway components and related regulatory components

Protein	Structure	Concentration (μg/mL)	Cellular sources	Key function
Alternative pathway				
Factor B	93 kDa	210	Hepatocyte, mononuclear phagocytes, epithelial and endothelial cells, adipocytes, fibroblasts	Catalytic subunit of AP C3 convertase, forms part of the C5 convertase
Factor D	24 kDa	1–2	mononuclear phagocytes, adipocytes	Cleaves factor B that is bound to C3b or $C3_{H_2O}$
Properdin	55–220 kDa monomer to tetramer	5	Mononuclear phagocytes	Stabilises AP C3 convertase
C3 (185 kDa)	110 kDa α-chain 75 kDa β-chain	1000–1900	Hepatocyte, mononuclear phagocytes, epithelial and endothelial cells, adipocytes, fibroblasts	Activated C3 (C3b) covalently binds to activating surfaces and mediates phagocytosis and cytolysis. C3a is an inflammatory peptide It forms part of the C3 and C5 convertases. Component of both alternative and classical pathways
Factor H	150 kDa	500	Hepatocyte, mononuclear phagocytes, epithelial and endothelial cells, fibroblasts, B cells, keratinocytes, myoblasts	Accelerates the dissociation of AP C3 convertase Cofactor for factor I
Factor I	88 kDa	35	Hepatocyte, mononuclear phagocytes, myoblasts, adipocytes, fibroblasts, B cells	C4b/C3b inactivator
Classical pathway				
C1q (410 kDa)	Hexamer. Subunit contains: 26 kDa A-chain (× 6) 25 kDa B-chain (× 6) 24 kDa C-chain (× 6)	80	Hepatocyte , mononuclear phagocytes, fibroblasts, gastrointestinal epithelial cells	Binds to IgM or IgG or CRP and initiates the classical pathway
C1r	92 kDa	50	Hepatocytes, mononuclear phagocytes, fibroblasts, gastrointestinal epithelial cells	Cleaves C1s
C1s	83 kDa	50	Hepatocytes, mononuclear phagocytes fibroblasts, gastrointestinal epithelial cells	Cleaves C4 and C2
C4 (205 kDa)	97 kDa, α-chain 75 kDa, β-chain	600	Hepatocytes, mononuclear phagocytes fibroblasts, genito-urinari and alveolar	Activated C4 (C4b) covalently binds to activating surfaces Forms classical C3 convertase

	33 kDa, γ-chain		type II epithelial cells	
C2	110 kDa	20	Hepatocytes, mononuclear phagocytes, fibroblasts, genito-urinari and alveolar type II epithelial cells	Catalytic subunit of the CP C3 convertase Forms C5 convertase
C4BP	460–540 kDa 70 kDa α-chain 45 kDa β-chain	250	Hepatocytes, mononuclear phagocytes	Cofactor for factor I Accelerates the decay of CP C3 convertase
CRP	25 kDa	<10	Hepatocytes, polymorphonuclear cells	Binds microbial and apoptotic cells and activates CP via interaction with C1q
C1-inhibitor	110 kDa	200	Hepatocytes	Inhibits C1r/s and MASPs
Lectin pathway				
MBL	Dimer to hexamer subunit (192–582 kDa) contains ($\times$ 3) 32 kDa chain	1–4	Hepatocytes	Binds to carbohydrate structures of microorganisms and initiates activation of the lectin pathway
MASP1	83 kDa	11	Hepatocytes	May be involved in the direct cleavage of C3
MASP2	83 kDa	0.5	Hepatocytes	Cleaves C2 and C4
MASP3	105 kDa	5	Hepatocytes, several other tissues	Product of alternative splicing of MASP1. Possible role in the activation of AP
sMAP/MAp19	19 kDa	0.2	Hepatocytes	Unknown, truncated form of MASP2
Ficolin-1	Oligomer with 35 kDa subunits	0.06	Monocytes, neutrophils	Recognises carbohydrate patterns, initiates LP
Ficolin-2	Oligomer with 35 kDa subunits	4	Hepatocytes	Recognises carbohydrate patterns, initiates LP
Ficolin-3	Oligomer with 34 kDa subunits	7–23	Hepatocytes	Recognises carbohydrate patterns, initiates LP
Lytic pathway				
C5	110 kDa α-chain 75 kDa β-chain	75	Hepatocytes, mononuclear phagocytes, T/B lymphocyts, fibroblasts, epithelial, astrocytes	Initiates the assembly of TCC (C5b) and is involved in inflammatory processes (C5a)
C6	120 kDa	45	Hepatocytes, neutrophils, astrocytes	Participates in the formation of TCC
C7	105 kDa	55	Hepatocytes, mononuclear phagocytes, fibroblasts, astrocytes	Participates in the formation of TCC
C8	64 kDa, α-chain 64 kDa, β-chain 22 kDa, γ-chain	80	Hepatocytes, pneumocytes, astrocytes	Participates in the formation of TCC
C9	71 kDa	60	Hepatocytes, astrocytes, fibroblasts, macrophages, monocytes, platelets	Participates in the formation of TCC
Vitronectin	75 kDa	300	Hepatocytes	Binds to C5b-9 and prevents TCC formation
Clusterin	75 kDa	100	Hepatocytes	Binds to C7-C9 and prevents TCC formation

AP, alternative pathway; LP, lectin pathway; CP, classical pathway; CRP, C reactive protein; TCC, terminal complement complex.

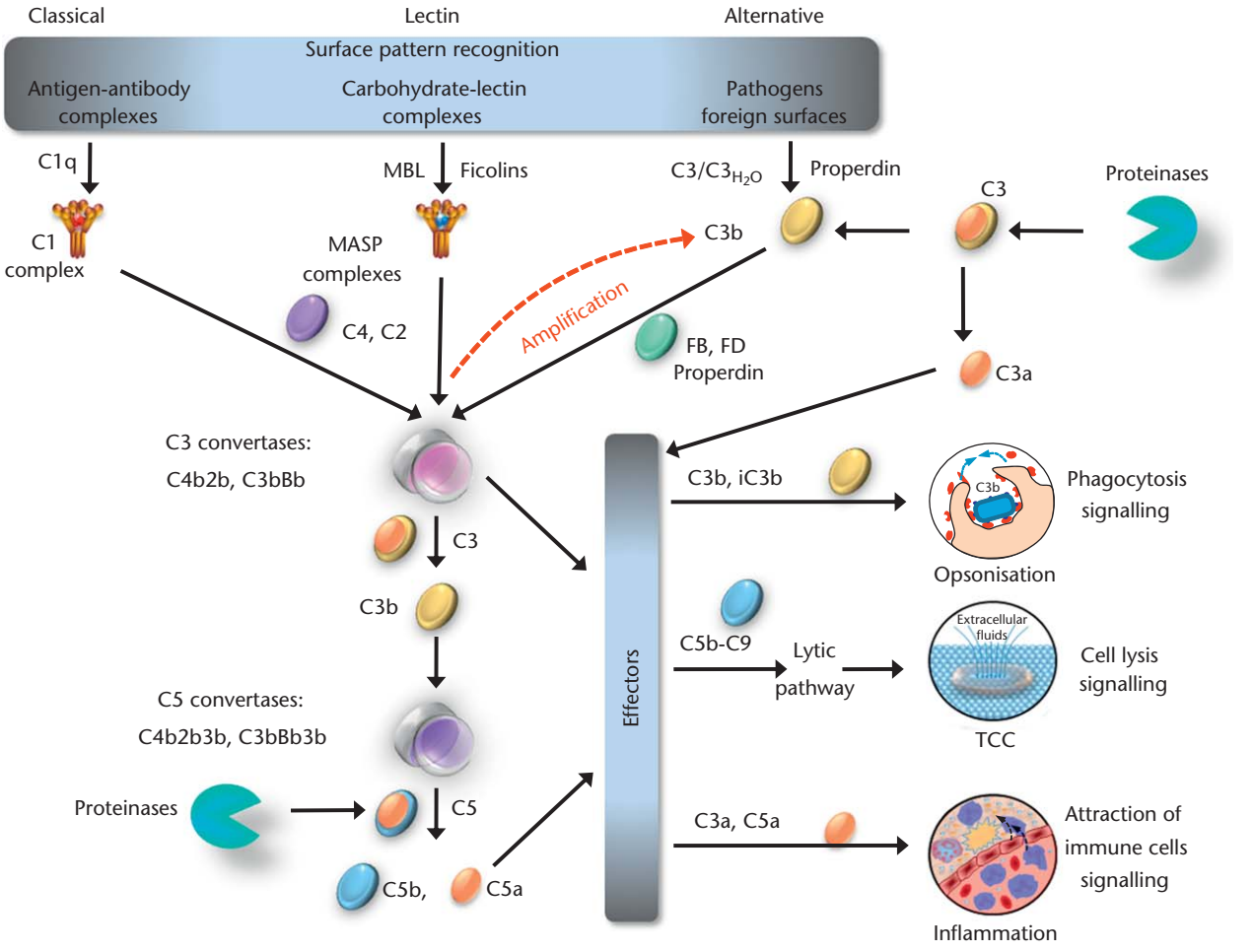

Figure 1 Complement activation pathways. The components of complement system can be organised into three major pathways: The classical pathway is mainly initiated by the binding of C1q to antigen–antibody complexes, whereas the lectin pathway is triggered by binding of mannose-binding lectin (MBL) or ficolins to glycosylated surfaces on microbial cell walls. Both pathways lead to the formation of a common C3 convertase, an enzyme complex with serine proteinase trypsin-like specificity. The alternative pathway, on the other hand, can be triggered by spontaneous hydrolysis of the internal thioester bond of C3, leading to the formation of $C3_{H_2O}$. This nonproteolytically activated form of C3 can lead to the formation of the alternative pathway C3 convertase by interacting with factors B and D. This convertase formation can be further induced and stabilised by properdin. C3 convertases generated by all pathways are able to cleave C3 into C3a and C3b, latter of which forms additional convertases, thereby rapidly amplifying complement response. C3b is vitally contributing to the clearance of pathogens by phagocytes (macrophages and neutrophils) and is a major component of the C5 convertase, which in turn cleaves C5 to C5a and C5b. The anaphylatoxins C3a and C5a mediate the inflammatory responses of complement. C5b subsequently takes the lead in formation of the terminal C5b–9 complement complex (TCC), ultimately resulting into cell lysis. Potential roles in the proteolytic activation of C3 and C5 have also been assigned to noncomplement proteinases, including enzymes of the coagulation and fibrinolysis cascades. Reproduced with permission from Springer Science+Business Media: Oikonomopoulou *et al.*, 2012; **Figure 1**.

(Ricklin *et al.*, 2010; Le Friec and Kemper, 2009). Pentraxins such as C-reactive protein, or the direct binding of viruses, bacteria and virus-infected cells can also trigger complement activation. More specifically, the C1q subunit of the Ca^{2+}-dependent C1 complex binds to immunoglobulins leading to stepwise activation of the serine proteinases, C1r and C1s, which also constitute the C1 complex (**Figure 1**). Activated C1s cleaves C4 into C4a and C4b. The thioester bond of C4b can subsequently attach to the hydroxyl or amino groups present on the microbial surfaces, ultimately leading to decoration of the surfaces by multiple C4b molecules, a process termed opsonisation. C1s proteolytically activates another complement serine proteinase, named C2, releasing C2b that together with the surface-bound C4b can form the classical pathway C3 convertase (C4b2b, also known as C4b2a).

Lectin pathway

In the lectin pathway, mannose-binding lectin (MBL) or ficolins act as the initiator of the cascade by binding to specific microbial cell surface carbohydrate groups, such as mannose and N-acetyl-glucosamine (GlcNAc) (Ricklin *et al.*, 2010; Le Friec and Kemper, 2009; Degn *et al.*, 2007). Surface binding of the MBL (ficolins) that are complexed to the MBL-associated serine proteases (MASP1, MASP2

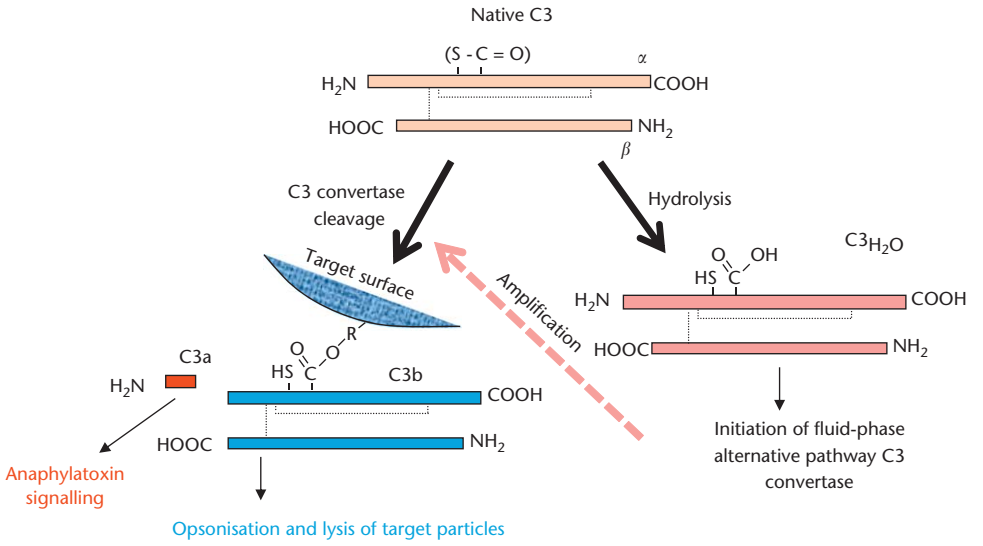

Figure 2 Proteolytic and nonproteolytic activation of C3. Native C3 is proteolytically activated by C3 convertase. Nonproteolytic activation can also occur by hydrolysis of the protected, unreactive thioester bond of native C3. The hydrolysis induces a conformational change that allows factor B to bind and form the alternative complement activation pathway C3 convertase (amplification loop is represented by the bold dotted arrow). Cleavage of native C3 by the C3 convertase results in the release of the C3a anaphylatoxin. The resulting conformational change of the remaining C3b molecule allows the thioester bond to react with target surfaces (e.g. microbes, biomaterials, apoptotic cells), leading to opsonisation or lysis of the targets. Dotted black lines within the C3 and C3-derived molecules indicate the presence of disulfide bonds.

and MASP3) allows the cleavage of C2 and C4 by the MASPs (**Figure 1**). This cascade activation pattern and the overall structure of the MBL (ficolins)/MASPs complexes mirror the ones of the C1q/C1r/C1s complex (Garred *et al.*, 2010). As with the classical pathway, the endpoint effect of the lectin pathway complex formation is the proteolytic release of C4b and C2b and the formation of the C3 convertase (C4b2b).

Alternative pathway

Native C3 contains a hidden thioester group that is exposed upon proteolytic or conformational activation. The alternative pathway is regularly kept at a low level of steady-state activation as a result of the spontaneous hydrolysis of this thioester group, which leads to the formation of $C3_{H_2O}$ (**Figure 1** and **Figure 2**) (Kemper *et al.*, 2010; Ricklin *et al.*, 2010; Le Friec and Kemper, 2009). This state of alertness ensures that the complement cascade can be quickly activated when the host comes into contact with potential pathogens, including viruses, bacteria, fungi and protozoans. The $C3_{H_2O}$ formed binds to factor B in the presence of Mg^{2+}. This interaction results in the proteolytic activation of factor B by factor D, another serine protease of the complement system, and the subsequent generation of the Bb fragment that remains bound to the hydrolysed C3. The resulting complex ($C3_{H_2O}Bb$) serves as the alternative pathway C3 convertase, which cleaves native C3 into C3a and C3b. The C3b opsonin covalently binds to nearby 'activating' surfaces (bacteria, cells, particles etc.) via its metastable thioester bond and in turn couples with factor B to generate more C3 convertase complexes (Forneris *et al.*, 2010) (C3bBb; amplification loop; **Figure 1** and **Figure 2**). The C3bBb complex can be further stabilised by properdin. The fluid-phase C3b and $C3_{H_2O}$ are mainly inactivated by the protease factor I, in the presence of cofactor molecules (e.g. complement receptor 1 (CR1), factor H, membrane cofactor protein (MCP)) that will be discussed in a later section. Therefore, the balance between available activation sites and regulatory molecules determine whether amplification (binding of factor B to C3b) or abrogation of the pathway (binding of a regulatory molecule to C3b) will occur in every setting of complement activation.

Common cascade steps

All three of the complement activation pathways converge in the formation of a C3 convertase (C3bBb or C4b2b) (Ricklin *et al.*, 2010; Le Friec and Kemper, 2009; Volanakis, 1989; Pangburn and Muller-Eberhard, 1986). The convertase can in turn trigger activation of more C3 molecules amplifying the generation of C3b and C3a (amplification loop; **Figure 1**). Downstream of C3 degradation, C3b can bind to the C3 convertases forming another multicomponent enzyme complex known as C5 convertase (C3bBb3b or C4b2b3b) (Rawal *et al.*, 2008; Pangburn and Rawal, 2002). The C5 convertases cleave C5 into the active C5a and C5b molecules (**Figure 1**). C5b is involved in the lytic pathway outlined in the relevant section below.

Lytic pathway

Downstream of the complement C3 and C5 convertases, C5b associates with C6, C7, C8 and multiple molecules of C9 to induce cell lysis (Bubeck *et al.*, 2011; Tegla *et al.*,

2011). These multicomponent interactions ultimately lead to generation of the terminal complement complex (TCC; also termed membrane attack complex or MAC) (Ricklin *et al*., 2010; Le Friec and Kemper, 2009). More specifically, the C5b–C6 complex binds to C7, causing a conformational rearrangement of the hydrophobic site of the latter molecule. This structural change drives the insertion of the C5b–C7 complex into the cell membrane and the subsequent binding of C8. The new C5b–C8 complex can act as the basis for the subsequent C9 polymerisation that leads to the formation of the TCC membrane pores.

Phylogeny of the Complement System

The complement network is one of the oldest immune mechanisms dating to more than 600 million years ago (Nonaka and Kimura, 2006; Nonaka and Yoshizaki, 2004; Sunyer and Lambris, 1998). Several complement genes and proteins have been identified in echinoderms and tunicates. In these animals, or perhaps in even more primitive species, complement may have emerged as a simple system comprising a small number of components (like C3, factor B and factor D and/or C3, MASP and MBL) with limited basic functions (such as the opsonisation of foreign materials and the C3a-mediated inflammatory responses). The classical pathway did not emerge until the antibodies first appeared in the cartilaginous fishes (e.g. sharks, rays). Also the downstream lytic pathway may have first appeared in this group of primitive vertebrates, although a C6-type molecule has been identified in the amphioxus, suggesting that at least some of the components of the lytic pathway were already present before the evolution of the fishes. The functional and structural complexity that characterises the complement system of the modern vertebrate species is possibly the result of gene duplication and exon shuffling of the genes encoding for these few components. **See also**: Complement System: Evolution

The primary sequences, structure and genomic organisations of the C3, C4 and C5 complement components are very similar (Nonaka and Kimura, 2006; Nonaka and Yoshizaki, 2004). All three molecules are comprised of individual chains (α-β in C3 and C5 and α-β-γ in C4), which are held together by single disulfide bonds. It is believed that the C3, C4 and C5 molecules are derived from a common ancestor gene, possibly the one encoding for the serum proteinase inhibitor α2-macroglobulin, which is also present in invertebrates. Similarly, the C1s, C1r and MASP components appear to have descended from a common ancestor, as they possess similar domain/module structures and functional activities. Furthermore, factor B and C2, which are both located in the major histocompatibility complex (MHC) class III region, as well as the members of the lytic pathway (C6, C7, C8 and C9) are believed to have a common ancestry. As teleost fish and chickens are known to possess a protein with roles similar to both factor B and C2, the gene duplication procedure that gave rise to the individual C2 and factor B components may be a quite recent complement evolutionary event.

Complement Effector Functions

The aforementioned steps of the complement activation pathways lead to the production of the effector molecules of the complement system. The main biological functions of complement activation can be summarised as (Dunkelberger and Song, 2010; Ricklin *et al*., 2010): (a) the opsonisation of pathogens mediated by the cleavage products of C3 (i.e. C3b, iC3b) and C4 (C4b) and the subsequent formation of the C3 convertases; (b) the recruitment and activation of inflammatory cells by the anaphylatoxins C3a and C5a, which are proteolytically released from C3 and C5; and (c) the direct elimination of pathogens by means of cell destruction as a result of the lytic pathway, initiation of which occurs with the fragmentation of C5 to C5b and C5a. Complement activation is associated with various biological processes involved in homoeostatic mechanisms or pathological pathways connected to many diseases, some of which are outlined below (Ricklin *et al*., 2010; Markiewski and Lambris, 2007).

Opsonisation and phagocytosis

All three complement activation pathways result in the generation of 'activated' C3b and C4b components, which via their thioester moiety decorate specific sites in foreign surfaces, like biomaterials, bacteria, viruses, fungi, and in apoptotic cells (Ricklin *et al*., 2010). The subsequent triggering of complement activation on these 'coated' sites results in amplification of the opsonisation process. Binding of the opsonins to their receptors (CR1 and/or CR3; **Table 2**) on the cell surface of phagocytic leucocytes results in the engulfment and destruction of the opsonised particles (outlined in the section below). C1q and MBL also have opsonising capabilities, and as such have the ability to facilitate the clearance of apoptotic cells, immune complexes and pathogens.

Solubilisation and clearance of immune complexes

In some autoimmune diseases (such as systemic lupus erythematosus, SLE) the formation and deposition of immune complexes can be massive (Cook and Botto, 2006). Therefore, clearance of immune complexes in these situations is necessary to prevent excessive complement activation that can cause damage to host tissues. In these cases, opsonisation of surfaces by C3 and C1q can result in the removal of immune complexes by the phagocytes.

Immune cell activation and signalling

A major part in translating the inflammatory role of complement effector molecules is played by cell surface

Table 2 Complement-associated cell surface components

Protein	Specificity	Structure	Cell type(s)	Key features
Cell Surface Proteins				
C1qRp (CD93)	C1q	126 kDa	Endothelial cells, platelets, neutrophils, glial cells, monocytes	Mediates phagocytosis of C1q-opsonised apoptotic cells, immune complexes and pathogens May play a role in leucocyte-endothelial cell interactions
cC1qR/CRT	C1q	46 kDa	Monocytes	Mediates phagocytosis of C1q-opsonised apoptotic cells
gC1qbp	C1q	33 kDa	Platelets, mast cells	Function mainly unknown. May play a role in C1q-mediated enhancement of P-selectin by platelets and chemotaxis of mast cells
CRIg	C3b, iC3b	44 kDa	Resting tissue macrophages	Mediates phagocytosis. Has a regulatory effect on C5 convertases
CR1	C3b, C4b	4 allotypes 160 kDa 190 220 250	Erythrocytes, eosinophils, monocytes, macrophages, neutrophils, B and some T lymphocytes, glomerular podocytes, follicular dendritic cells, mast cells, polymorphonuclear cells	Member of RCA, accelerates dissociation of CP and AP C3 convertases, cofactor for factor I, involved in phagocytosis of C3- opsonised particles
CR2	iC3b, C3dg, C3d EBV gp 350	140 kDa	B cells, some T cell subsets, follicular dendritic cells	Member of RCA, lowers threshold for B cell activation
CR3	iC3b, C3dg	170 kDa α-chain 95 kDa β-chain	Polymorphonuclear cells, monocytes, natural killer cells, some B and T lymphocytes	Involved in phagocytosis of iC3b-coated particles, adhesion of neutrophils, cytotoxicity of cells bearing activated complement components. Member of leukocyte integrins
CR4 (p150, 95)	iC3b	150 kDa α-chain 95 kDa β-chain	Monocytes, macrophages, NK and ADCC effector Lymphocytes, neutrophils	Functions in cell adhesion
DAF	C3b C4b	75 kDa	Erythrocytes, all leucocytes, platelets	Lysosomal enzyme release, leukocytosis Accelerates decay of CP and AP C3 convertases
MCP	C3b, iC3b C4b	45–70 kDa	Neutrophils, monocytes, platelets, reticulocytes, most lymphocytes, granulocytes, endothelial cells, epithelial cells, mesenchymal cells	Member of RCA, cofactor for factor I, does not accelerate decay of C3 convertases
CD59	C8, C9	18–20 kDa	widely expressed, all circulating cells, vascular endothelium, epithelial cells	Inhibits TCC formation on host cells
7-transmembrane receptors				
C3aR	C3a	54 kDa	Mast cells, neutrophils, eosinophils, basophils, monocytes/macrophages, platelets, bronchial and alveolar endothelial and epithelial cells, astrocytes and microglia	Depending on cell type, functions include chemotaxis, chemokinesis, cell aggregation and adhesion, release of lysosomal contents

(*Continued*)

Table 2 Continued

Protein	Specificity	Structure	Cell type(s)	Key features
C5aR	C5a, C5adesArg	45 kDa	Neutrophils, eosinophils, basophils, monocytes, macrophages, mast cells, liver parenchymal cells, lung vascular smooth muscle and endothelial cells, epithelial cells, astrocytes and microglia, dendritic cells, mesangial cells	Depending on cell type, functions include chemotaxis, cell adhesion and aggregation, release of granular enzymes, superoxide anions and histamine. Augments the humoral and cellular responses. Possible role in sepsis
C5L2	C5a, C5adesArg	37 kDa	Immature dendritic cells, polymorphonuclear cells, monocytes, adipocytes	Function unclear, may serve as a decoy receptor for C5a. May mediate the acylation-stimulating properties of C3adesArg, which is glucose transport and lipid metabolism. Possible role in sepsis

AP, alternative pathway; CP, classical pathway; RCA, regulators of complement activation; TCC, terminal complement complex.

receptors that recognise and bind to the complement components (Klos *et al*., 2009; Monk *et al*., 2007). The C3a and C5a anaphylatoxins, released during complement activation are rapidly converted into their desarginated derivatives, $C3a_{desArg}$ and $C5a_{desArg}$ after removal of the *C*-terminal arginine by plasma carboxypeptidases. The major role of anaphylatoxins is the recruitment of inflammatory cells, such as neutrophils, eosinophils, basophils, mast cells and macrophages, to sites of injury, a process known as chemotaxis. C3a and C5a can also increase vascular adhesion and permeability (allowing cells to reach the place of injury), trigger smooth muscle contraction, as well as result in the release of superoxide anions (respiratory burst), specific enzymes and various other pro-inflammatory mediators, such as prostaglandins and cytokines from activated cells.

The anaphylatoxins can signal to cells and tissues via two members of the G protein-coupled receptor (GPCR) family, the C3a receptor (C3aR) and C5a receptor (C5aR or CD88) (Klos *et al*., 2009). Notably, $C3a_{desArg}$ is not believed to trigger cell signalling via C3aR. A third receptor, C5a receptor-like 2 (C5L2), has been identified to bind C5a and $C5a_{desArg}$. Signalling through C5L2 for the desArg derivative of C3a, known as ASP, is still under question (Ward, 2009; Kalant *et al*., 2005). The C3aR, C5aR and C5L2 receptors are involved in a variety of processes, including chemotaxis, cell aggregation, cell adhesion, degranulation, glucose transport and lipid metabolism. Owing to their important inflammatory role, the anaphylatoxins and their receptors have been implicated in several vascular, pulmonary, autoimmune, regenerative and neurodegenerative conditions (Klos *et al*., 2009; Monk *et al*., 2007).

Cell surface receptor binding

Apart from the anaphylatoxins, several other fragments derived from complement activation have been shown to bind to cell surface partners and induce important biological processes (**Table 2**). For example, C1q, apart from its role in the initiation of the classical pathway, can also have opsonic functions that facilitate the removal of apoptotic cells, immune complexes and pathogens. It has also been implicated in additional functions, such as in chemotaxis, cytotoxicity and cytokine release. Several molecules have been hypothesised to act as C1q cell membrane receptors; however, their implication is still under investigation. **See also**: Complement Receptors

Complement receptors type 1, 2, 3 and 4 (CRs 1–4) are other cell membrane-bound proteins that are associated with complement effector functions, including regulation of B cell function, natural killer (NK) cell activity, antibody responses to T-cell-dependent and -independent antigens, as well as phagocytosis, respiratory burst and degranulation (Ricklin *et al*., 2010). Known ligands for these cell surface receptors are the C3 and C4 degradation fragments, namely C3b, iC3b, C3dg, C3d, C3c and C4b (**Table 2**). One of the most studied complement receptors is CR1 or CD35, a transmembrane glycoprotein that is present on a wide variety of cells. It functions mainly as a receptor for C3b and C4b, although it is known to bind with less affinity to iC3b and C3c. It has also been shown that CR1 binds to C1q and MBL outside of the C3b binding site. CR1 has the following functions: (a) it can act as a cofactor of the factor I-mediated cleavage of C3b and C4b; (b) it can have decay-accelerating activity, facilitating the disassembly of the C3 convertases; and (c) it can serve as a receptor for the surface-bound C3b and C4b components, promoting the binding and phagocytosis of opsonised materials, as well as the clearance of immune complexes. **See also**: Complement Receptors

Cell destruction via the lytic pathway

Formation of TTC by the complex of C5b with C6-8 and the subsequent C9 polymers leads to generation of membrane pores that can ultimately induce cell death by osmotic lysis (lytic pathway (Bubeck *et al*., 2011; Tegla *et al*., 2011)). Although polymerisation of C9 is not essential for the lysis of erythrocytes and some nucleated cells, it is believed to be important for the killing of Gram-negative bacteria, such as those of the genus *Neisseria*. Formation of sublytic concentrations of TCC, even though not functional as expected within the lytic pathway, has also been shown to play various roles in cell cycle regulation, activation, survival and signalling (Tegla *et al*., 2011).

Enhancing humoral immunity

Complement plays a fundamental role in mediating and enhancing adaptive immunity (Dunkelberger and Song, 2010; Ricklin *et al*., 2010; Carroll, 2004, 2008). C3b-opsonised particles bind to complement receptors (CR1, CR2; **Table 2**) present on antigen-presenting cells (APCs), such as dendritic cells and B lymphocytes, which process the antigens and present them complexed to MHC class II molecules to T cells. Furthermore, complement has also been found to play an important role in B cell tolerance and modulate T cell responses. The CR2-mediated localisation and retention of C3b-tagged immune complexes by germinal centres also supports the hypothesis that complement is important for the generation of memory B cells. It has also been reported that association of the CR2-complex (which includes CR2, CD19 and TAPA-1) with the B cell antigen receptor increases the potency of B cell activation by its ligands by 10- to 100-fold. For example, when an antigen is coupled to C3d, the antibody response is greatly enhanced due to the C3d–CR2 interactions. As a result of this enhancing capability, C3d is used as a natural adjuvant, bridging the innate with the acquired immune response, through CR2 (Carroll, 2008).

Regulation of T cell responses

T cell responses are greatly influenced by the activation state of antigen presenting cells (APC), in particular dendritic cells, and the local cytokine microenvironment. The

production of complement components such as Factor B, D, C3 and C5 by APCs is enhanced upon interaction with T cells. Further, the anaphylatoxins generated as a result of this activation modulate the cytokine profile produced by APCs, regulating T cell proliferation and activation. For example, the cross-talk between anaphylatoxins and toll-like receptors (TLR) signalling pathways on APCs results in the modulation of expression levels of MHC and co-stimulatory molecules, as well as in the production of cytokines such as IL-6, IL-10, IL-12 and IL-23. These APC-derived signals will in turn determine the polarisation of $CD4^+$ T cell responses towards Th1, Th2, Th17 or regulatory T cells (Klos *et al*., 2009). In addition to anaphylatoxins, activation of the complement regulator MCP on T cells drives the switch of $CD4^+$ T cells from classical IFN-γ-producing Th1 effector cells towards IL-10-producing Th1 cells with immunosuppressive properties, indicating that ligation of MCP by C3b- or C4b-opsonised microorganisms may act as a feedback mechanism to inhibit or terminate T-cell activation (Le Friec and Kemper, 2009).

Other functions

The functions of complement are now known to extend beyond the traditional idea of regulating the immune/inflammatory processes (Ricklin *et al*., 2010). Complement components have additionally been implicated in skeletal and vascular development, tissue regeneration, reproduction, hematopoiesis, CNS development, cell proliferation and apoptosis. They have also recently been linked to adipose tissue differentiation and metabolism (e.g. glucose transport, lipid removal and synthesis) (Cianflone *et al*., 2003). These novel functions link the complement branch of the innate immune system with other host cellular networks with nonimmune functions.

Regulatory Proteins of Complement Activation

Persistent complement activation can result in overwhelming inflammatory response and subsequent host cell or tissue damage. Therefore, it is imperative that not only complement activation remains under tight control but also that the host is protected from autologous 'attack' by components of the complement system. This role is played by a number of widely expressed regulatory proteins that include soluble and membrane-bound components (**Table 1**) (Morgan and Harris, 1999). Regulatory roles have also been attributed to CRs 1–4, as discussed above. Control of the complement activation pathways by their regulatory factors can occur at the following levels: (a) degradation of C3b and C4b into fragments that cannot participate in the downstream steps of the complement cascade; (b) interference with the structural integrity of the C3-convertases; (c) disruption of the TCC assembly; and (d) conversion of C3a and C5a anaphylatoxins to their inactive (or less active) desarginated forms. Many of these regulatory factors are primarily comprised of tandem short-consensus repeats (SCRs), also called complement control modules, which contain approximately 60–70 amino acids and two internal disulphide bonds. **See also**: Complement Regulatory Proteins

Soluble regulatory proteins

Factor I is a serine protease that, in the presence of the cofactor molecules factor H, MCP and/or CR1, facilitates the degradation of C3b (Nilsson *et al*., 2011; Morgan and Harris, 1999). Factor H is a single polypeptide composed of 20 SCRs. It has a cofactor role in the primary stage of factor I-mediated cleavage. It can additionally bind to surface-bound or fluid-phase C3b and enhance the decay of the C3bBb convertase. The MCP and CR1 are membrane-bound proteins. The factor I proteolytic activity results in generation of iC3b, which is further cleaved by the protease into C3c and C3dg. C3dg remains covalently associated to the surfaces due to its thioester moiety, whereas the C3c fragment, which contains the β-chain and the remainder of the C3b α-chain, detaches from the surface. C3dg is further cleaved by other proteases to produce C3d and C3g. The C3dg and C3d fragments have an important role in adaptive immune responses via its CR2 receptor. Factor I is also responsible for the cleavage of C4b in the presence of C4 binding protein (C4BP), CR1, or MCP. C4BP is composed of seven α-chains and one β-chain, which are linked by disulfide bonds to form a spider-like structure. The C4BP α- and β-chains are composed of eight and three SCRs, respectively. **See also**: Complement Regulatory Proteins

Other regulators of complement activity come from other protein families (Ricklin *et al*., 2010; Morgan and Harris, 1999). Among them, C1-inhibitor (C1-INH), a general serine protease inhibitor of the serpin superfamily, inhibits the proteolytic activity of C1r and C1 s. Other examples are the S-protein (vitronectin) and clusterin (SP-40-40), both of which acting as inhibitors of TCC formation by preventing insertion of the C5b-7 complex into cell membranes. As mentioned above, plasma carboxypeptidases are also factors that regulate anaphylatoxin activity by proteolytically removing the *C*-terminal arginyl residue of C3a and C5a.

Membrane-bound proteins

The membrane-bound regulators decay-accelerating factor (DAF or CD55) and the membrane cofactor protein (MCP or CD46) share several structural features (Ricklin *et al*., 2010; Morgan and Harris, 1999). They also have the common role to maintain complement activation in fine control in order to avoid self-attack of host tissues. More specifically, DAF contains four SCRs and remains covalently anchored to the cell membrane via a glycosylphosphatidylinositol (GPI) anchor. DAF accelerates the release of C2b or Bb from the C3 and C5 convertases

leading to their dissociation and blockage of their activity, whereas it also has a role in signal transduction. Similar to DAF, MCP contains four SCRs, but it is devoid of decay-accelerating activity. MCP rather serves as a cofactor for the factor I-mediated cleavage of C3b and C4b discussed above. Finally, CD59 is another GPI-linked membrane protein, which can bind to C8 and C9, interfering with the formation of the cell surface membrane attack complex. **See also**: Complement Regulatory Proteins

Complement Dysregulation in Clinical Settings

Poor complement activity or dysregulated tuning of the complement system functions have been reported in many settings and have been associated with diverse clinical manifestations (Oikonomopoulou *et al*., 2012; Ricklin *et al*., 2010). In line with these observations, deficiencies of specific complement components or their regulatory proteins and receptors have also been correlated with pathological settings (Skattum *et al*., 2011; Pettigrew *et al*., 2009; Reis *et al*., 2006). The complement components and their functions shown to associate with several clinical conditions are summarised below. **See also**: Complement: Deficiency Diseases

Systemic Lupus Erythematosus

Deficiency of classical pathway components such as C1q, C1r, C1s, C2 and C4 has been associated with the autoimmune disease systemic lupus erythematosus (SLE) (Skattum *et al*., 2011; Botto *et al*., 2009). Dysregulation in any of these components can affect the classical pathway activation, ultimately resulting in defective clearance of immune complexes in SLE patients. C1q deficiency has also been associated with an accumulation of apoptotic cells, which, in the presence of disturbed clearance homoeostatic mechanisms, provide another source of auto-antigens that trigger the SLE autoimmune responses.

Glomerulonephritis

Deficiency of C3 and its regulatory proteins Factor I and Factor H is commonly associated with the development of glomerulonephritis, a condition characterised by kidney damage as a result of complement activation triggered by immune complexes in renal blood vessels (Ricklin *et al*., 2010; Reis *et al*., 2006). Factor I and H deficiencies usually culminate with the secondary deficiency of the component C3, as C3 is completely consumed from plasma due to the continuous formation of fluid-phase C3 convertase.

Inflammatory thrombotic conditions

The role of uncontrolled C5a activity in inducing a thrombotic signature expression in neutrophils of patients with antiphospholipid syndrome (APS) and acute respiratory distress syndrome (ARDS) has been described (reviewed by (Oikonomopoulou *et al*., 2012; Markiewski *et al*., 2007)). Atypical hemolytic-uremic syndrome (aHUS), paroxysmal nocturnal hemoglobinuria (PNH) and hereditary angioedema (HAE) are other clinical examples of dysfunctional complement regulation that can lead to thrombotic disturbances like in platelet activation. Deficiency in factor H has been associated with aHUS, whereas dysregulation in the function of membrane regulators DAF and CD59, which results in higher C3 convertase activity and increased erythrocytes lysis, has been connected to paroxysmal nocturnal hemoglobinuria. Furthermore, C1 inhibitor deficiency has been linked to accumulation of oedema fluid in skin and mucosa in HAE patients. A role for consumptive complement activation has also been suggested in more traditional thrombotic diseases such as ischaemic stroke and ischemia-reperfusion injury. More specifically, in ischaemic stroke, an MBL deficiency has been found to be associated with a more favourable clinical outcome after acute stroke in both mice and humans.

Susceptibility to infections

C3 and MBL deficiencies have been associated with an increased susceptibility to various infectious agents (Degn *et al*., 2011; Reis *et al*., 2006). Dysregulation of properdin and factor D can result in abnormal activation of the alternative pathway, which can in principle make patients susceptible to recurrent infections. Meningococcal infections are most frequently detected in patients with deficiencies in the alternative pathway components. Factor I and H deficiencies can also render individuals more susceptible to infections by pyogenic bacteria, as a result of dysregulated C3 activation. Apart from the initiating components of complement activation pathways, dysregulation of any of the later cascade components will inhibit TCC formation. The obstructed lytic pathway ultimately signals the failure to kill foreign pathogens by means of complement-mediated lysis. The infections most frequently associated with deficiencies of late complement components are infections from meningococcal or gonococcal species. Deficiencies in the complement receptors CR3 and CR4 have also been associated with a disease characterised by dysregulated leucocyte adhesion and recurrent pyogenic infections. Finally, a link between oral infection-derived chronic inflammation, that can result in the manifestation of periodontal pathogenesis, and excessive or dysregulated complement activation has been suggested (Hajishengallis, 2010).

Systemic inflammatory response syndrome

The most widely studied clinical condition in which complement-related inflammation manifests is sepsis (Oikonomopoulou *et al*., 2012). Sepsis accounts for approximately 215 000 deaths among the 750 000 reported US cases per year, with mortality rates being higher in

advanced septic patients. The disease is characterised by a complement-mediated overwhelming inflammatory response, referred to as the systemic inflammatory response syndrome (SIRS) (Oikonomopoulou *et al*., 2012; Markiewski *et al*., 2008; Rittirsch *et al*., 2008b; Ward, 2004). In septic patients, an initial condition, such as a bacterial infection, drives immune cells to produce a variety of inflammatory mediators, including cytokines, chemokines and complement activation products. A failure of regulatory safety switches to restrain this immune hyperactivation during sepsis results in persistent inflammation, which can eventually lead to disseminated intravascular coagulation (DIC), multiple organ dysfunction syndrome (MODS) and, possibly, death. Although the exact mechanism of complement in sepsis is still under thorough investigation, it is known that complement activation products, such as C3a, C4a and C5a, are elevated in septic patients and correlated with a fatal outcome. It has also been postulated that C5a and its receptors C5aR and C5L2 are major players in the pathogenesis of disease (Rittirsch *et al*., 2008a, b; Ward, 2004).

Age-related macular degeneration

Age-related macular degeneration (AMD) is a major cause of blindness in people of European descent. Complement proteins and their activation products are detected in the drusen (deposits of cellular debris and inflammatory material) formed between the retinal pigment epithelium and Bruch's membrane, indicating local, complement-mediated inflammation in the retina of patients with AMD (Yanai *et al*., 2012; Ricklin *et al*., 2010). The Y402H polymorphism in the Factor H gene was identified as major risk factor for AMD. The Factor H H402 variant has reduced ability in binding to oxidative stress products that accumulate during pathological processes, resulting in a disruption of the balance between complement activation and regulation in the subretinal tissue and progression of AMD. Additional polymorphisms correlated with the development of AMD refer to genes coding for proteins from the alternative pathway, including C3, Factor B, Factor I, FHL-1.

Other inflammatory conditions

The association of complement activity with other inflammatory conditions such as rheumatoid arthritis, inflammatory bowel disease and several inflammatory skin conditions is only now beginning to unfold (Oikonomopoulou *et al*., 2012; Kotnik, 2011). In principle, unrestricted complement activation in these conditions can modulate the release of pro-inflammatory modulators such as C5a and TNF-α, which can in turn induce further inflammatory or coagulative responses.

Biomaterials- and organ transplantation-related complications

The past decade has seen an increasing number of evidence supporting the role of complement system in biomaterial-induced inflammation (Ekdahl *et al*., 2011; Nilsson *et al*., 2010). Medical devices and extracorporeal circuits commonly used for therapeutics, as well as vehicles used for drug delivery can potentially interfere with the activation of complement system. It has been shown that the biomaterial-induced generation of complement anaphylatoxin C5a in long-term hemodialysed patients can increase the risk for thrombotic complications (Kourtzelis *et al*., 2010); this effect was abrogated by the complement inhibitor compstatin. Similar to the biomaterial-triggered responses, complement activation may also occur in the case of organ transplantation, where regulation of complement activity can be one of the factors that may determine organ rejection or acceptance by the host (Brenner *et al*., 2010).

Complement Therapeutics

As discussed in the previous sections, unwelcomed or unrestricted complement activation can set the stage for the development of pathological conditions. In an effort to modulate the level of complement activation in human patients, inhibitory compounds for various complement components are under experimental or clinical investigation (Wagner and Frank, 2010; Qu *et al*., 2009; Ricklin and Lambris, 2007). An approach utilising peptides that can bind to factor H without disturbing its functional integrity has recently proven effective against complement actuation in a model of alternative pathway activation (Wu *et al*., 2011). Similarly, TT30, a fusion protein consisted of the C3 fragment-binding domain of CR2 and the complement-inhibitory sequence of human factor H efficiently abrogated the alternative pathway of complement activation in both *ex vivo* and *in vivo* models (Fridkis-Hareli *et al*., 2011).

In addition, eculizumab, an anti-C5-inhibitory antibody that prevents the proteolytic generation of C5a and C5b, thereby resulting in blockage of TCC assembly (Hillmen *et al*., 2007), is known to lower the risk of clinical thromboembolism in patients with PNH. Pexelizumab, an eculizumab fragment antibody, has also been suggested as an adjunctive treatment for ischaemic heart disease patients (Testa *et al*., 2008). Compounds targeting complement at the level of C5 may also find value in the treatment of septic patients, given the proposed role of C5a in sepsis (Ward and Gao, 2009; Rittirsch *et al*., 2008a, b). At the same time, restriction of complement activity at the level of C3 by compstatin, a synthetic cyclic tridecapeptide, has shown promise in several experimental disease models, including sepsis (Oikonomopoulou *et al*., 2012; Ricklin and Lambris, 2008). The clinical value of compstatin has been investigated in phase I (age-related macular degeneration (AMD)) and phase II clinical trials (Wagner and Frank, 2010; Potentia, 2007). Other preclinical studies have also pinpointed the efficacy of the compound to manage systemic thrombosis in high-risk patients, such patients undergoing haemodialyses or septic patients (Kourtzelis *et al*., 2010; Silasi-Mansat *et al*., 2010).

Another complement-related inhibitor that has already found use in the clinic is the C1-inhibitor (C1-INH), a plasma protein used in the treatment of hereditary angioedema (HAE) (Antoniu, 2011; Kaplan, 2010; Davis, 2005). C1-INH regulates complement activation by blocking the activity of C1s, C1r and MASPs but is also associated with modulation of the bradykinin–kallikrein and coagulation systems. Patients stricken with HAE show recurrent episodes of severe skin and/or mucosal oedema resulting from either decreased production of C1-INH or presence of a dysfunctional C1-INH protein. Purified or recombinant forms of C1-INH have been, therefore, successfully used for the management of HAE patients preventing the life-threatening complications.

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