Zipfel PF, Skerka CComplement regulators and inhibitory proteins. Nat Rev Immunol 9:729-740

Article *in* Nature Reviews Immunology · October 2009

Impact Factor: 34.99 · DOI: 10.1038/nri2620 · Source: PubMed

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Complement regulators and inhibitory proteins

*Peter F. Zipfel**‡ *and Christine Skerka**

Abstract | The complement system is important for cellular integrity and tissue homeostasis. Complement activation mediates the removal of microorganisms and the clearance of modified self cells, such as apoptotic cells. Complement regulators control the spontaneously activated complement cascade and any disturbances in this delicate balance can result in damage to tissues and in autoimmune disease. Therefore, insights into the mechanisms of complement regulation are crucial for understanding disease pathology and for enabling the development of diagnostic tools and therapies for complement-associated diseases.

Complement is one of the first lines of defence in innate immunity and is important for cellular integrity, tissue homeostasis and modifying the adaptive immune response[1–3]. The activated complement system recognizes and eliminates invading microorganisms and thus is beneficial for the host[4,5]. In addition, complement facilitates the elimination of dead or modified self cells, such as apoptotic particles and cellular debris[6,7]. The alternative pathway of complement forms a spontaneously and constantly activated immune surveillance system[8,9]. The individual complement reactions develop in a sequential manner, allowing regulation that modulates the intensity of the response and adjusts the effector functions for the specific immune response.

Complement was identified more than 100 years ago as a result of its 'complementary' bactericidal activity and its role in phagocytosis of cellular debris[10,11]. The activated complement system directs immune effector functions and modulates the intensity of the response in a self-controlling manner. This allows for the appropriate innate immune response, which is needed for recognition and removal of infectious agents or modified self cells[6,12]. Now, 110 years after these initial reports, the central role of this system in immune defence is much better known: many of the biological effects of complement action are understood in molecular terms and a role for complement in tissue homeostasis is emerging. Complement activation in turn activates pro-inflammatory mediators, generates anaphylactic peptides, cytolytic compounds and antimicrobial compounds, recruits effector cells and induces effector responses[13,14]. These result in a moderate and controlled outcome of complement activation, which is beneficial for the host but detrimental for the invading microorganism[15,16].

The beneficial aspects of a moderately activated complement system include immune surveillance, removal of cellular debris, organ regeneration and neuroprotection. The two anaphylatoxins complement component 3a (C3a) and C5a have a role during organ regeneration[17], in neuroprotection[18] — including in migration of neurons[19] and synapse elimination[20] — and in the release of progenitor haematopoietic stem cells[21]. In addition, crosstalk and cooperative effects between C3a receptor (C3aR), C5aR, C5a receptor-like 2 (C5L2) and Toll-like receptors have been reported[22]. These additional physiological functions highlight the role of complement in physiology and homeostasis and demonstrate that appropriate regulation and balanced or targeted activation are crucial to keep the complement system in its proper physiological state.

This Review focuses on the complement regulators that modify and control the complement system. We highlight the mechanisms of complement control on different biological surfaces, such as intact self cells, which act as non-activator surfaces, and modified self cells and microbial cell surfaces, which act as activator surfaces.

The complement cascade

Complement activation, amplification, progression and regulation. Complement activation occurs in a sequential manner and can be divided into four main steps: initiation of complement activation, C3 convertase activation and amplification, C5 convertase activation, and terminal pathway activity or the assembly of the terminal complement complex (TCC; also known as MAC) (FIG. 1). Once activated, the complement cascade generates effector compounds that are delivered to any surface in an indiscriminate manner. However, progression of the cascade and the action of the effector

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doi:10.1038/nri2620
Published online
4 September 2009

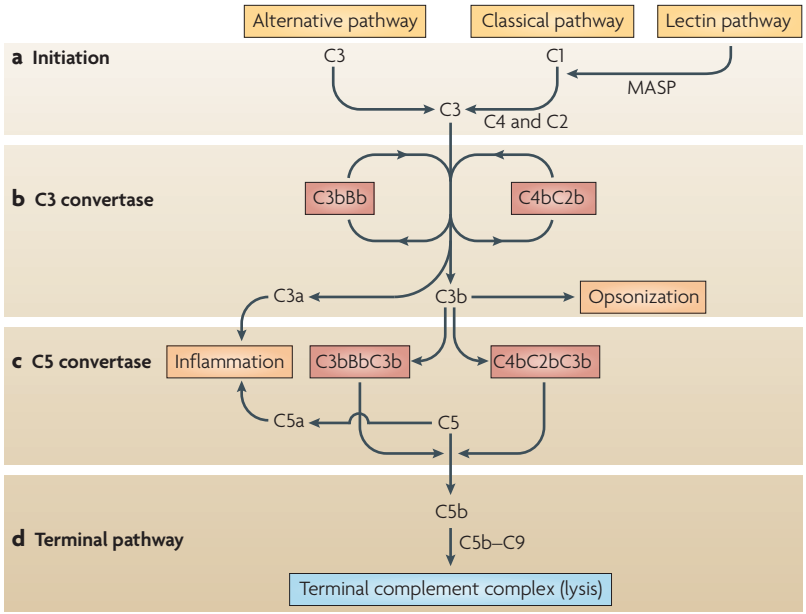

Figure 1 | **Complement: a central immunosurveillance system.** Complement is a central defence mechanism of innate immunity and is activated in a tightly regulated manner. **a** | Complement is initiated by three major pathways. The alternative pathway is spontaneously and continuously activated. The classical pathway is induced when antibodies bind to their corresponding antigen. The lectin pathway is triggered by the binding of mannan binding lectin (MBL; also known as MBP-C) to mannose residues on the surface of microorganisms. This activates the MBL-associated proteases mannan-binding lectin serine protease 1 (MASP1) and MASP2, which then cleave C4 and C2. **b** | Complement activation occurs in a sequential manner. The three pathways initiate the formation of enzymes known as complement component 3 (C3) convertases (C3bBb for the alternative pathway and C4bC2b for the classical and lectin pathways). These enzymes cleave the central complement component C3 and generate the anaphylactic and antimicrobial peptide C3a and the opsonin C3b, which can be deposited onto any nearby surface. This activation is followed by an amplification reaction that generates additional C3 convertases and deposits more C3b at the local site. Subsequently, C3b is inactivated and sequentially degraded and the various degradation products mediate important effector functions. For example, C3b deposition on a surface results in opsonization, which allows for the interaction of C3b with specific host C3 receptors expressed on the surface of immune effector cells. **c** | If activation progresses, a new enzyme, the C5 convertase (C3bBbC3b for the alternative pathway and C4bC2bC3b for the classical and lectin pathways), is generated. C5 convertase cleaves C5, releases the potent anaphylactic peptide C5a and generates C5b. **d** | C5b can initiate the terminal pathway , which recruits the components C6, C7, C8 and C9 to the surface of the target and inserts the C9 complex as a pore (termed the terminal complement complex) into the membrane. The activated complement system generates multiple effector compounds that drive and orchestrate further immune reactions.

Zymogen
An inactive pre-form of a protease that by itself lacks proteolytic activity. Upon processing or proteolytic cleavage the protein displays enzymatic activity. Using such inactive pre-forms allows targeting of protein activity to the right place at the right time. Most complement proteins, but also components of the coagulation cascade and other proteases, exist and circulate as inactive pre-forms and require modification and proteolytic processing to be converted into an active form.

molecules are strictly controlled at each level by multiple complement regulators and inhibitors. These regulators discriminate between self and non-self surfaces, such as cells — including modified self cells — tissues and infectious agents[7–9,15]. Such regulators and inhibitory circuits operate at every level of the cascade and are therefore central to understanding complement action.

The complement system consists of a set of inactive components that are linked and activated in a cascading manner. When they encounter a biological surface, the inactive zymogens are structurally modified, assemble with additional components and are converted to effector compounds or active enzymes that activate new substrates — and so the cascade progresses. The first enzymatic reactions, which activate C3 convertase, are tightly controlled to ensure that activation and generation of biologically active effector compounds occurs only at the appropriate time and site. If the activation cascade progresses, the reactions are amplified and can result in potent and powerful effector functions. The complement system has more than sixty components and activation fragments, which comprise the nine central components of the cascade (C1 to C9), multiple activation products with diverse biological activities (for example, C3a, C3b, iC3b, C3d and C3dg)[12,23], regulators and inhibitors (such as factor H, factor H-like protein 1 (FHL1), complement factor H-related protein 1 (CFHR1), CR1 (also known as CD35), C4b-binding protein (C4BP), C1q and vitronectin), proteases and newly assembled enzymes (for example, factor B, factor D, C3bBb and C4bC2b) or receptors for effector molecules (such as C3aR, C5aR, C5L2 and complement component C1q receptor (C1qR)) (TABLE 1). The number of known components of the network, particularly the regulators and receptors, is continuously increasing and new functions for these proteins are being identified.

Initiation of complement activation. Complement is activated by three major pathways. The alternative pathway is spontaneously and constantly activated on biological surfaces in plasma and in most or all other body fluids[24]. This spontaneous activation readily initiates amplification[8]. The classical pathway is triggered by an antibody bound to the target antigen[25], but this pathway can also be activated in an antibody-independent manner by viruses and Gram-negative bacteria. The lectin pathway is initiated by carbohydrates on microbial surfaces[26,27]. Activation of each of these pathways results in assembly of the first enzyme of the cascade, which is termed C3 convertase (FIG. 1). Complement is also activated by an additional bypass pathway that acts independently of C3 or bypasses the C3 convertase and is mediated by thrombin acting on the C5 convertase[28].

C3 convertase and amplification. The convertase for the alternative pathway (C3bBb) is formed by C3b and factor Bb, whereas the convertase for the classical and lectin pathways (C4bC2b) is composed of C4b and C2b. Both convertases cleave C3 to C3a and C3b. C3a aids in the recruitment and activation of innate immune effector cells and also has antimicrobial and antifungal activity[29]. If activation progresses, C3b is deposited close to the site of generation and, on formation of surface-bound convertases, amplifies the cascade[13,30]. The C3b fragment also coats microbial or apoptotic surfaces and marks the particle for phagocytosis. On the surface membrane of intact self cells, C3b deposition is prevented by regulators and further progression is blocked; by contrast, on microbial surfaces or on modified self cells activation can progress. However, pathogenic microorganisms and apoptotic cells acquire soluble regulators, which block progression of the cascade beyond the level of C3 convertase. C3b opsonizes the biological surfaces, which are then cleared by phagocytosis in a non-inflammatory manner. Interestingly, similar or related mechanisms are used for the non-inflammatory removal of modified self particles and for attack and removal of microorganisms.

Table 1 | **Complement regulators and receptors for effector proteins**

Regulator	Alternative name	Point of action	Ligand	Cell surface binding or expression	Function	Refs
Soluble regulators and effectors						
Factor H	None	Alternative pathway	C3b and C3d	Acquired to surface	Cofactor for factor I and acceleration of alternative pathway C3 convertase decay	53
FHL1	None	Alternative pathway	C3b	Acquired to surface	Cofactor for factor I and acceleration of alternative pathway C3 convertase decay	54
Properdin	None	Alternative pathway	C3	Binds to apoptotic surfaces	Stabilization of alternative pathway convertases	55,66
Carboxy-peptidase N	Anaphylatoxin inactivator	Classical pathway and lectin pathway	C3a, C4a and C5a	NA	Inactivation of anaphylatoxins C3a and C5a	56,57
C4BP	None	Classical pathway and lectin pathway	C4	Acquired to surface	Cofactor for factor I and acceleration of classical pathway C3 convertase decay	59
C1q	None	Classical pathway	IgG and IgM immune complexes	Binds to apoptotic surfaces	Activation of the classical pathway	60
C1INH	None	Classical pathway and lectin pathway	C1r, C1s and MASP2	NA	Blocks serine protease and is a suicide substrate for C1r, C1s, MASP2, coagulation factors and C3b	58
CFHR1	None	Terminal pathway	C5 convertase and TCC	Acquired to surface	Inhibition of C5 convertase and TCC assembly	62
Clusterin	SP-40,40 and apolipoprotein J	Terminal pathway	C7, C8β, C9 and TCC	NA	Transport of cholesterol, HDL, APOA1 and lipids	61,64
Vitronectin	S-protein	Terminal pathway	C5b-7 and TCC	NA	Adhesion protein, fibronectin-mediated cell attachment and Arg-Gly-Asp site coagulation in immune defence against *Streptococcus* spp.	63
Surface bound regulators and effectors						
CR1	CD35 and immune adherence receptor	C3	C3b, iC3b, C4b and C1q	Many nucleated cells and erythrocytes, B cells, leukocytes, monocytes and follicular dendritic cells	Clearance of immune complexes, enhancement of phagocytosis and regulation of C3 breakdown	68
CR2	CD21 and Epstein–Barr virus receptor	C3	C3dg, C3d and iC3b	B cells, T cells and follicular dendritic cells	Regulation of B cell function, B cell co-receptor and retention of C3d tagged immune complexes	69,70
CR3	MAC1, CD11b–CD18 and αMβ2 integrin	C3	iC3b and factor H	Monocytes, macrophages, neutrophils, natural killer cells, eosinophils, myeloid cells, follicular dendritic cells, $CD4^+$ T cells and $CD8^+$ T cells	iC3b enhances the contact of opsonized targets, resulting in phagocytosis and adhesion by CR3	76
CR4	CD11c–CD18 and αXβ2 integrin	C3	iC3b	Monocytes and macrophages	iC3b-mediated phagocytosis	77
CRIg	VSIG4	C3	C3b, iC3b and C3c	Macrophages	iC3b-mediated phagocytosis and inhibition of alternative pathway activation	74
CD46	MCP	C3	C3b and C4b	All cells except erythrocytes	C3 degradation, cofactor for factor I and factor H, and effector for T cell maturation	67,72
CD55	DAF	C3	C4b2b and C3bBb	GPI anchor expression by most cell types, including erythrocytes, epithelial cells and endothelial cells	Acceleration of C3 convertase decay	67,72
CD59	Protectin	TCC	C8 and TCC	GPI anchor expression by erythrocytes and most nucleated cells, including renal cells	Inhibition of TCC assembly and formation	73

Table 1 (cont.) | **Complement regulators and receptors for effector proteins**

Regulator	Alternative name	Point of action	Ligand	Cell surface binding or expression	Function	Refs
Receptors for complement effector proteins						
C3aR	None	C3	C3a	Neutrophils, monocytes, eosinophils, antigen-presenting cells, T cells, astrocytes, neurons and glial cells	Immune cell recruitment and inflammation	78
C5aR	CD88	C5	C5a	Myeloid cells, monocytes, neutrophils, dendritic cells, antigen-presenting cells, T cells, endothelial cells and renal tubular cells	Immune cell recruitment and inflammation	19,32, 79,82
C5L2	None	C5	C5a	Macrophages and neutrophils	Immune cell recruitment and inflammation and possibly acts as a decoy receptor	19,32, 80,82
C1qR	CD93	Classical pathway	C1q	Monocytes and B cells	Phagocytosis and cell adhesion	83,84
SIGNR1	CD209	Classical pathway	C1q	Dendritic cells and microglial cells	Signalling, inflammation and phagocytosis	85

APOA1, apolipoprotein A-1; C, complement component; C1INH, C1 inhibitor; C4BP, C4b-binding protein; C5L2, C5a receptor-like 2; CFHR1, complement factor H-related protein 1; CR, complement receptor; CRIg, complement receptor of the immunoglobulin superfamily; DAF, complement decay-accelerating factor; FHL1, factor H-like protein 1; GPI, glycosylphosphatidylinositol; HDL, high-density lipoprotein; MASP2, mannan-binding lectin serine protease 2; MCP, membrane cofactor protein; NA, not applicable; SIGNR1, a mouse homologue of DC-SIGN, SIGN-related 1; TCC, terminal complement complex.

C5 convertase. If the activation cascade is allowed to proceed beyond the cleaving of C3 into C3a and C3b, an additional C3b molecule binds to the C3 convertases. This generates C3bBbC3b, the C5 convertase for the alternative pathway, or C4bC2bC3b, the C5 convertase for the classical and lectin pathways[31]. Both enzymes cleave C5 to C5a and C5b. C5a is a powerful anaphylactic peptide and the C5b fragment can initiate the terminal pathway[32] (FIG. 1). The C5a-induced inflammation and the C5b-initiated terminal pathway seem to be separately regulated[33].

The terminal pathway. The C5b fragment bound to the active convertase initiates the terminal pathway, which results in directed, non-enzymatic assembly of the terminal pathway components C5, C6, C7, C8 and C9, which form the TCC. The assembly and conformational changes of these soluble, hydrophilic proteins generate lipophilic, membrane-inserting complexes. Ultimately, formation of a TCC leads to pore formation and cell lysis[34–36]. Additional functions for TCC components have also been reported. These include stimulating activity in T helper cell polarization, a role for membrane-bound C7 as a sink for the late complement components to control excessive soluble TCC-induced inflammation and a role for the soluble TCC in platelet-activating activity[37–39].

Complement: a double-edged sword

Complement is important for the clearance of infectious agents, but pathogens use a number of evasion strategies to modify and block complement activity. Most pathogens express surface proteins that bind to host complement regulators or recruit complement inhibitors and thus block or interfere with complement effector functions. Such surface-bound host regulators delay or even block protective host innate immune responses that would otherwise eliminate the infectious agent and keep the host organism intact. However, a persisting pathogen triggers additional immune reactions. Thus, during an infection by a pathogen, complement is induced locally and this local complement activation also attacks host bystander cells at the site of infection. In this situation, an attacked host cell requires maximal protection and uses numerous available regulators to ensure survival. So, the human complement system maintains a delicate balance between activation and inhibition to allow activation on foreign or modified self surfaces and protection of self. This explains why the regulation of complement activation is crucial for homeostasis.

Protection of self cells. The action of complement effector molecules is blocked on the surface of intact host cells and biomembranes[40,41] by a combination of integral membrane, surface-attached and fluid-phase regulators. The concerted action of regulators and inhibitors ensures cell and tissue integrity (FIG. 2).

Dysregulation of the complement cascade results in immunopathology and autoimmune disease. Mutations in the genes encoding individual regulators can result in defective control and inappropriate delivery of complement activation products onto the surface of host cells. This results in complement attack at self-cell surfaces and, depending on the intensity of complement activation, in pathology (see below and FIG. 2 and TABLE 2).

Mutations of a single complement gene can result either in the absence of the protein in the circulation or in the dysfunction of the inhibitor. Changes in a single component can disturb the whole regulatory network. In a normal setting, when complement activation occurs at low levels, the absence or dysfunction of a single component can be tolerated or compensated for by the presence and concerted action of multiple additional regulators. However, when a local complement

Opsonization
The deposition of activation products, for example the C3 activation fragment C3b, on the surface of a target to mark the target and facilitate and enhance recognition and uptake by phagocytic cells. Phagocytosis eliminates the particle from the circulation and aids in its destruction.

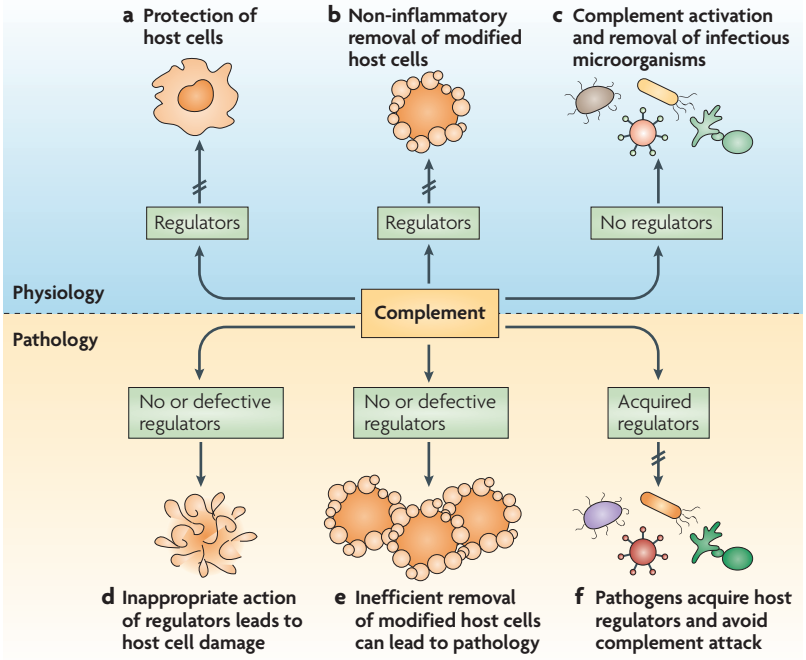

Figure 2 | **The benefits and risks of complement.** Complement activation has multiple effects in a physiological setting, which can either benefit the host (**a**–**c**) or be detrimental to the host and possibly lead to pathology (**d**–**f**). **a** | Owing to the spontaneous nature of complement activation, intact host cells must protect their surfaces from the action of effector compounds. This protection is mediated by multiple regulators. **b** | Complement is activated in a controlled manner on the surface of damaged or modified host cells, such as apoptotic particles and necrotic cells. This allows complement activation to proceed until C3b surface deposition, but further progression (generation of complement component 5 (C5) convertase and terminal complement complex assembly) is blocked. This results in the non-inflammatory removal of modified self cells. **c** | Complement is activated and amplified on the surface of invading microorganisms. Activation results in recognition and damage of the invading microorganisms and in the removal of the cellular debris. **d** | Inappropriate action of complement regulators results in damage to the surface of intact host cells. **e** | Inappropriate tagging of modified self cells, which can lead to inefficient removal and accumulation of apoptotic particles, can result in secondary necrosis and pathology. This is thought to be a contributing factor to the initiation of autoimmune diseases. **f** | Some pathogenic microorganisms can evade recognition and actively block immune attack by the activated complement system. In this way, pathogens cross the first layer of immune defence, leading to the establishment of an infection.

response is initiated and amplified at the site of an infection the concerted action of membrane-bound and recruited regulators is required for the protection of bystander host cells from complement-mediated lysis. The absence or the defective function of a single component results in incomplete local protection. This also demonstrates how complement acts as a powerful weapon against a bacterial or viral infection. When complement activation is not properly controlled or inhibited at the host surfaces it can progress and lead to inflammation, pathology and ultimately autoimmune diseases (FIG. 2).

Role of complement in clearance of modified self cells. Complement serves an additional function in the human body as complement activation products tag modified self cells such as apoptotic particles and necrotic cells and allow non-inflammatory removal or clearance of immune complexes[42–45]. The membrane of an apoptotic cell undergoes dramatic morphological changes, resulting in the breakdown of membrane integrity and the 'flip-flop' of the membrane, which is accompanied by the loss of the membrane complement regulators at the cell surface. Consequently, such modified self structures serve as activating surfaces for complement. To avoid complement-mediated attack and adaptive immune responses to self antigens, modified self surfaces acquire the soluble effector C1q in combination with the regulator factor H. This initiates early steps of the classical pathway but blocks the formation and amplification of the C5 convertase[46–48].

In this situation the initiation of the complement cascade by C1q allows C3b generation and deposition, but progression beyond the C3 convertase stage is blocked by the complement regulators. This ultimately results in the C3b-mediated non-inflammatory removal of cell debris by phagocytosis[48,49] (FIG. 2). Inappropriate tagging of modified self surfaces and inefficient clearance result in the accumulation of cellular debris, and self antigens can then serve as autoantigens in autoimmune diseases[50] (FIG. 2).

Complement activation on the surface of microorganisms. Most infectious agents are efficiently eliminated by a moderately activated complement system, even in the absence of inflammatory mediators. Newly released C3a recruits immune effector cells, and C3b or C3 activation fragments deposited on the foreign surfaces promote phagocytosis through opsonization. In addition, the activated terminal pathway forms the TCC, which damages the microorganism. Most microorganisms are eliminated at this stage[8,9,51]. However, during millions of years of evolution, pathogenic microorganisms have developed ways of avoiding complement recognition, evading host complement attack and surviving in an immunocompetent host[51]. A recent study described the crystal structure of Staphylococcal complement inhibitor (SCIN) from *Staphylococcus aureus* bound to the alternative pathway C3 convertase C3bBb[52]. This provided insights into the action of C3 convertases as well as information on the mechanisms of inhibition by SCIN.

Distribution of complement regulators

The importance of complement restriction is highlighted by the large number of regulators identified to date, which exceeds that of the components of the complement cascade (C1 to C9) (TABLE 1).

Complement regulators operate at all levels and are categorized in three major classes: fluid phase, attached to the surface of host cells and membrane-integral complement clearance receptors. Surprisingly, several regulators have additional activities beyond complement, as they mediate cell adhesion and extracellular matrix interactions or link the complement cascade with other important physiological networks such as the coagulation cascade.

Table 2 | **Complement deficiencies and complement-mediated diseases**

Disease	Major defects	Causes	Genes affected	Refs
Atypical haemolytic uraemic syndrome (aHUS) and possibly some cases of thrombotic thrombocytopenic purpura (TTP)	Defective C3 convertase, stabilization of the convertase, defective regulation, and increased stability and turnover	Mostly heterozygous mutations, genetic defects and autoantibodies	Deficiency of CFHR1, CFHR3, factor B, factor H and factor I	9,91, 92,99
Membranoproliferative glomerulonephritis, type II (MPGN II; also known as dense-deposit disease)	Defective C3 convertase	Mostly homozygous mutations, genetic defects, autoantibodies and C3 nephritic factor	Factor H and C3	93,99
Systemic lupus erythematosus (SLE)	Defective clearance of apoptotic cells and bodies	Hereditary homozygous deficiency and genetic defects	C1q, C1r, C1s, C2, C3 and C4	49,96, 110,111
Pyogenic infections	Inappropriate complement attack	Infections with *Neisseria meningitidis* and *Streptococcus pneumoniae*	C3, factor H, factor I, properdin and TCC	99
	Deficiency of properdin	Infections with *Neisseria* spp.	Gene mutation	51
	Deficiency of factor I	Infections with *N. meningitidis* and *S. pneumoniae* and other respiratory tract infection	Gene mutation	5
	Deficiency of factor H	Infection with *N. meningitidis*	Gene mutation affecting protein secretion	53
Haemolysis and thrombosis	Erythrocyte lysis and thrombus formation	Unknown	CD59, factor H and deficiency of CFHR1 and CFHR3	9
Partial lipid dystrophy	Loss of adipose tissue	C3 nephritic factor	Unknown	9
Hereditary angioedema	Recurrent spontaneous non-allergic oedema of the subcutaneous tissues and mucous membranes	Mostly heterozygous mutations	C1 inhibitor	9
Paroxysmal nocturnal haemoglobinuria (PNH)	Failure of CD55 and CD59 expression	Genetic deficiency of PIG-A, and failure to form GPI anchor	PIGA	9
Age-related macular degeneration (AMD)	Drusen formation and chronic inflammation	Unknown	Factor H, C3, C2, deficiency of CFHR1 and/or CFHR3, factor B and factor I	94,99, 107
Tumour cells	Overexpression of membrane and secreted regulators, and enhanced binding of soluble regulators	Unknown	Unknown	97

C, complement component; CFHR, complement factor H-related protein; GPI, glycosylphosphatidylinositol; PIGA, phosphatidylinositol glycan anchor biosynthesis, class A; TCC, terminal complement complex.

Fluid phase complement regulators. Fluid phase complement regulators are distributed in human plasma and in body fluids such as the synovial and vitreous fluids. These regulators are grouped according to their major activity and include the alternative pathway regulators factor H[53], FHL1 (REF. 54) and the activator protein properdin[55] (FIG. 3a). Carboxypeptidase N, which cleaves and partly inactivates the anaphylactic peptides C3a and C5a, acts in all three major complement activation pathways[56,57] (TABLE 1). Soluble classical and lectin pathway regulators include C1 inhibitor (C1INH)[58] and C4BP[59]. C1q has a crucial role in host defence and in clearance of immune complexes[60]. The known soluble inhibitors of the terminal pathway include the recently identified CFHR1, clusterin and vitronectin[61–63], which have additional biological functions beyond complement. Clusterin, for example, is associated with high-density lipoprotein particles and acts as an adhesion protein and as a potent inducer of cell aggregation. Vitronectin binds to the extracellular matrix, functions in cell attachment or cell spreading and acts as an adhesion protein with fibronectin-like activity[64]. Properdin can directly bind to and initiate complement activation on a microbial surface and triggers local assembly and action of the alternative pathway C3 convertases[65,66].

Membrane-bound complement regulators. The membrane of host cells is also equipped with complement regulators[67], including CR1 (REF. 68), CR2 (also known as CD21)[69,70], CD55 (also known as DAF)[71], CD46 (also known as MCP)[72], CD59 (also known as protectin)[73] and complement receptor of the immunoglobulin superfamily (CRIg; also known as VSIG4)[74]. CRIg is expressed in a long and a short form. The long proteins CRIg and CR1 act further away from the cell membrane than the short membrane proteins CD46, CD55 and CD59. Membrane-bound regulators control the three major complement activation pathways and inactivate both C3 and C4 (CR1 and CD46). By contrast, fluid phase regulators are more specific and control either the alternative, the classical or the lectin pathway and act almost exclusively on either C3 or C4 (FIG. 3a).

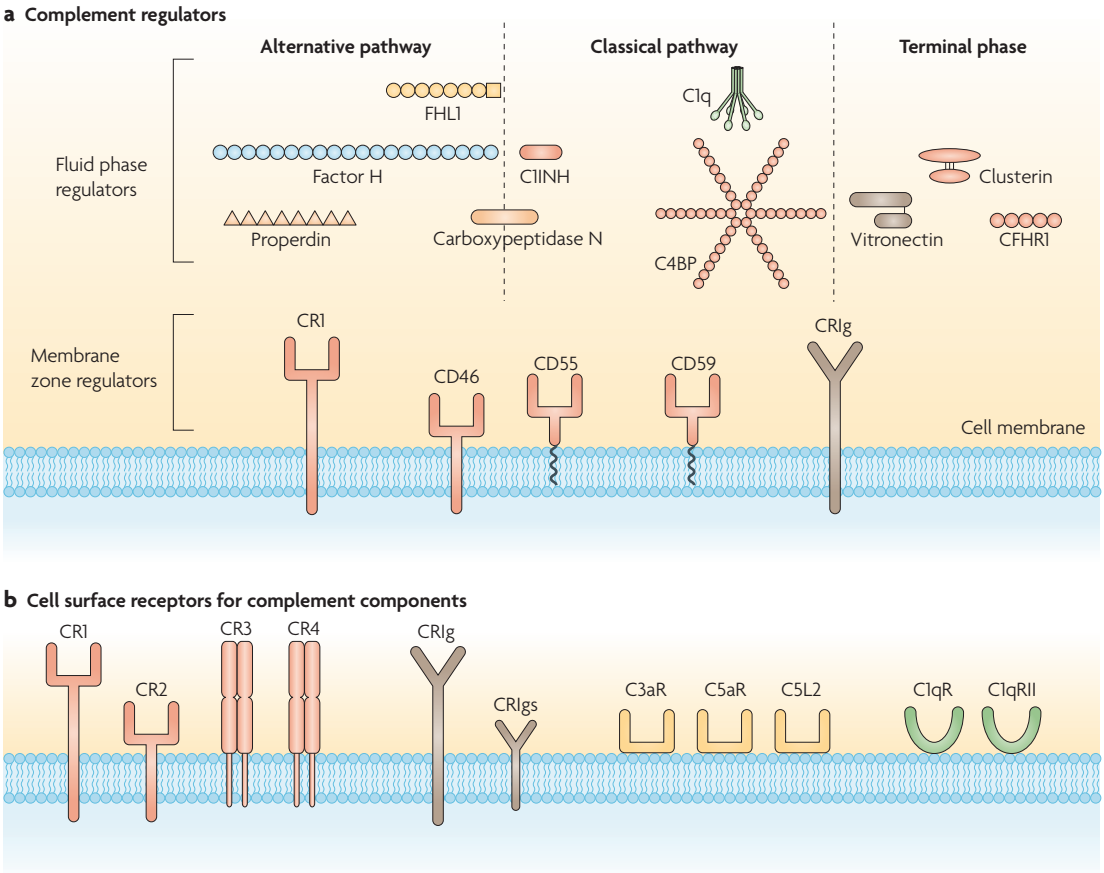

Figure 3 | **Complement regulators and surface receptors. a** | Complement regulators can exist either in the fluid phase or at the cell membrane. There are multiple fluid phase complement regulators, which control different steps of the cascade and are distributed in different compartments. Alternative pathway regulators distributed in the plasma and body fluids are factor H, factor H like protein 1 (FHL1) and the activator protein properdin. Carboxypeptidase N has regulatory activity in the alternative pathway, the classical pathway and the lectin pathway. The soluble classical and lectin pathway regulators include complement component 1q (C1q), C1 inhibitor (C1INH) and C4b-binding protein (C4BP). The soluble inhibitors of the terminal pathway include clusterin, vitronectin and complement factor H-related protein 1 (CFHR1). Membrane zone complement regulators include CR1 (also known as CD35), CD46 (also known as MCP), CD55 (also known as DAF), CD59 (also known as protectin) and complement receptor of the immunoglobulin superfamily (CRIg; also known as VSIG4). The complement regulators act in various ways. For example, C1INH induces the dissociation of the C1 components. CD35, CD55 and C4BP displace a component of the C3 convertase in the classical pathway. CD59 prevents final assembly of the membrane attack complex. **b** | The membrane of host cells is equipped with complement receptors, including CR1, which binds C3b, iC3b and C4b, and CR2 (also known as CD21), which binds C3d and C3dg, CD46, CD55, CD59 (not shown) and CRIg. However, the distribution and number of the individual membrane-bound receptors varies between different cell types and on different surfaces. On the membrane of B cells, both CR1 and CR2, which act as co-receptors for surface-bound immunoglobulin, regulate B cell differentiation and maturation and instruct B cells to respond to C3d-coupled foreign antigens. So, C3b attached to the antigen interacts with CR2 and acts as a molecular adjuvant[74]. CR3 (also known as MAC1, αMβ2 integrin and CD11b–CD18) and CR4 (also known as αXβ2 integrin and CD11c–CD18) are also integrin receptors. CR3 and CR4 bind iC3b, C3dg and C3d and mediate phagocytosis of C3b-opsonized cells or particles. In addition, these receptors bind multiple other ligands including the matrix protein fibrinogen[75,76] (TABLES 1,3). The recently identified receptor CRIg, and the shorter form CRIgs, has a major role in the clearance of C3d- and iC3b-tagged particles, including microorganisms and autologous cells[77]. C5L2, C5a receptor-like 2.

Receptors for complement effector molecules. Complement activation products and effector compounds have potent biological activity. They induce and modulate inflammation, control inflammatory responses, direct multiple cellular responses and induce effector functions, such as tagging of cellular surfaces and induction or enhancement of phagocytosis[46,48].

There are many receptors for complement effector molecules, which bind and respond to effector compounds such as C3a and C5a, C3b-opsonized surfaces, modified self surfaces and tagged microorganisms, toxins or antigens. Five major membrane-bound effector receptors, CR1, CR2, CR3 (also known as CD11b–CD18), CR4 (also known as CD11c–CD18)

and CRIg, bind C3b or C4b deposited on the surface of the target and drive effector functions corresponding to the specific form of the C3 and C4 activation compounds[75–77] (FIG. 3b; TABLE 1).

The action of the anaphylactic fragments C3a and C5a is mediated by members of the seven transmembrane spanning receptor family, including C3aR and the two surface receptors C5aR and C5L2. The biological role of the newly described C5L2 is still under discussion. At present it is unclear whether C5L2 mediates effector functions in response to C5a or whether the receptor acts as a decoy and attenuates C5a function[12,32,78–82]. In addition, C1q is recognized on the cell surface by two receptors: C1qR and the dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN) homologue SIGN-related 1 (SIGNR1; also known as CD209)[83–85].

An important aspect of the role of membrane receptors in regulation and the response to effector compounds is their expression and distribution on individual cell lineages or cell types. Surface-attached complement and effector receptors are not uniformly expressed by nucleated host cells, erythrocytes or platelets. Consequently, the distribution and number of individual receptors and the complete receptor repertoire on a given cell membrane are important parameters for defining the protective surface coat and the cellular response to complement.

Surface-attached complement regulators. It is now clear that several fluid phase complement regulators also attach to cell surfaces and to biomembranes such as the glomerular basement membrane of the kidney and the Bruch's membrane of the retina[86–88]. In addition, these regulators bind to modified self surfaces such as apoptotic particles, necrotic bodies and the extracellular matrix[48,59]. Thus, factor H, FHL1, C4BP, CFHR1, clusterin and vitronectin, which control complement in the fluid phase, also attach to lipid bilayers or to biomembranes and maintain complement-regulatory activity. This surface attachment forms an additional protective layer (known as the surface zone) for intact host cells that controls complement and limits its formation of complement activation products and effector compounds at the cell surface. This flexible and protective shield cooperates with the membrane zone, an inner shield that is formed by membrane-anchored regulators. At present, the ligands on the cell surface and the biomembrane that anchor the fluid phase complement regulators have not been identified at the molecular level but, as heparin modifies and inhibits this interaction, it has been proposed that glycosaminoglycans act as cell and biomembrane surface receptors.

The simultaneous attachment of multiple regulators creates an additional protective surface zone. The approximate dimension of the surface zone depends on the length and the density of the attached proteins. For example, the length of a fully extended complement factor H protein was calculated to be 73 nm and that of membrane bound CD46 to 14 nm[89]. A single, extended factor H molecule can control an area corresponding to a hemisphere with a radius of approximately 70 nm. In addition, membrane-bound regulators such as CD46 and CD59 are released or shed from the membrane and thus contribute to the protective shield[90].

Complement regulators in disease

Diseases caused by defective complement functions mirror the central surveillance roles of complement in the human body. Defective complement regulation and deficiency of particular components acting early in the cascade can result in both host cell damage and accumulation of immunological debris. These defects are associated with the renal disorder atypical haemolytic uraemic syndrome (aHUS)[91,92]. Similarly, a defective surface zone and accumulation of debris have been reported to be a cause of dense-deposit disease (DDD; also termed membranoproliferative glomerulonephritis, type II (MPGN II))[93], the retinal disease age-related macular degeneration (AMD)[94,95] and systemic lupus erythematosus (SLE)[96]. By contrast, tumour cells and pathogenic microorganisms attach host complement regulators and mimic a self surface zone to escape complement surveillance, resulting in unrestricted growth and infections, respectively[4,5,53,97,98].

The three distinct disorders aHUS, DDD and AMD manifest in different organs and have different pathology. In aHUS, thrombi form in the glomerulus, whereas DDD and AMD involve formation of deposits at renal and retinal sites, respectively. However, these disorders are caused by mutations in the same genes, and the same set of genes is associated with each of the three disorders. Each disease is clearly associated with surface damage occurring at platelets, renal endothelial cells, the glomerular basement membrane of the kidney or the Bruch's membrane in the retina. The affected genes encode C3 and factor B (the two components of C3 convertase) or the regulators of C3 convertase factor H, CD46 and factor I[99–101]. The absence of CFHR1 and CFHR3 in plasma as a result of a chromosomal deletion has opposite effects on the progression of the disorders[102–105]: in aHUS, the deletion seems to represent a risk factor and is associated with generation of autoantibodies to factor H[92,102,103], whereas in AMD it is described as having a protective effect[104]. Deletion of single components causes pathology and drives the balanced complement regulatory network in opposing directions. The crystal structure of the first four domains of factor H in complex with its target, C3b, was recently identified, providing information on the mechanisms of action of factor H and a structural basis for the effects of disease-associated mutations[106].

The fact that this set of genes can induce diseases that manifest in different organs suggests that the quantity of protein at local sites is relevant and that mutated proteins attach to different surfaces in distinct organs. For example, factor H gene mutations can be either homozygous or heterozygous and thus can result in various concentrations of functional protein both in the circulation and at the cell surface. In addition, mutations that result in amino acid exchanges occur within distinct sites and affect different functional domains of this

multifunctional factor H protein. So, single mutations as well as polymorphic exchanges translate into different effects, cause different conditions and can define whether the defect manifests in renal or retinal sites.

Atypical haemolytic uraemic syndrome. In aHUS, most patients have one defective allele and one intact allele of factor H. This results, in general terms, in a ~50% reduction in complement activity. This reduced level of functional protein allows complement control in the fluid phase. However, following an immunological insult, amplified local complement activation requires maximal protection of bystander cells from lysis. In this situation, damage to host structures can occur. Such a scenario may explain the incomplete penetrance of heterozygous mutations and may explain why the disease frequently develops after an episode of infection.

In aHUS, most of the heterozygous factor H gene mutations cluster in the carboxy-terminal surface recognition region and leave the complement-regulatory region, in the amino-terminus, intact. Reduced surface binding of mutant factor H weakens the protective surface zone. In DEAP (deficient for CFHR proteins and factor H autoantibody positive)–HUS the deficiency for CFHR1 and CFHR3 is frequently associated with the development of autoantibodies, which bind to and block the C-terminal recognition region of factor H[92].

Dense-deposit disease. In DDD, mutations in the gene encoding factor H occur mostly in homozygous or compound heterozygous conditions and result in defective protein secretion and the absence of the protein in the plasma[93]. This leads to unrestricted complement activation in the plasma. An additional aspect of DDD is the presence of autoantibodies (termed C3 nephritic factor) that bind to a neo-epitope of the C3 convertase, stabilize the enzyme and enhance C3 cleaving[105]. Both predispositions result in C3 consumption in the fluid phase and local C3 deposit formation at surfaces that lack endogenous regulators, such as the glomerular basement membrane of the kidney and the Bruch's membrane of the retina.

Age-related macular degeneration. The Bruch's membrane in the retina, similar to the glomerular basement membrane of the kidney, requires membrane-bound soluble regulators for control. Thus, the absence or defective surface binding of factor H may lead to uncontrolled complement activation at such unprotected sites.

The AMD-associated factor H gene polymorphism (H402Y) is located in the heparin- and CRP-binding domain 7 and affects surface attachments of factor H to the Bruch's membrane and retinal epithelial cells as well as protein deposits known as drusen[107].

Further evidence for a common disease mechanism for DDD and AMD is based on recent proteomic analyses of glomerular dense deposits and retinal drusen[108,109]. The deposits formed in the two distinct organs comprise almost the same proteins, including complement activation products and inflammatory components (such as C3, C3d and iC3b), terminal pathway components (C5, C6, C7, and C8) and terminal pathway regulators (CFHR1, clusterin and vitronectin, CFHR2 and CFHR5). This overlap highlights the similarity of AMD and DDD, shows that complement is associated with the pathology of both diseases and suggests that defective complement regulation results in chronic inflammation.

Autoimmune diseases. Defective clearance of self antigens or apoptotic particles and accumulation of cellular debris can occur in the absence of C1q or C4. This can lead to exposure of self antigens to lymphocytes and can also lead to inappropriate activation of self-reactive B and T cells[47]. This is considered a major susceptibility factor for development of autoimmune diseases. Defective complement action can result in the accumulation of cellular debris or the formation of pathological deposits (FIG. 2). Mutations and homozygous deficiency of genes of the classical pathway of complement activation and the presence of autoantibodies are associated with the pathogenesis of the systemic autoimmune disease SLE[110,111]. Most homozygous deficiencies for genes encoding components of the early classical pathway, including C1q, C1r and C1s as well as C2, C3 and C4, are strongly associated with the development of SLE. The strongest association is observed with C1q deficiencies and over 90% of these cases develop rheumatic diseases. In addition, deficiencies of C5, C8 and mannose binding lectin (MBL) are associated with SLE or lupus-like syndromes. Similarly acquired factors, such as autoantibodies — in particular autoantibodies to C1q, C3 and CR1, but also C3 nephritic factor and autoantibodies to C4b2a and C1INH — are reported in cases of SLE. These defects or newly acquired factors probably result in the failure to clear C3b-opsonized apoptotic cells or particles and lead to secondary necrosis and pathology.

Tumour cells. Cancer cells are another type of modified self cell that actively escape complement and immune surveillance. The expression of membrane-bound complement inhibitors (CD46, CD55 and CD59) is upregulated in various primary tumours and tumour lines[112]. Similarly, lung, ovarian, glial and colon cancer cells show enhanced expression and surface binding of soluble regulators, including factor H, FHL1, C4BP and factor I. In addition, complement activation products such as C5a suppress the antitumour response mediated by $CD8^+$ T cells and inhibition of complement activation has been suggested as an option for treatment of cancer[113,114].

The recent concept of the role of complement in diseases has already been translated from the bench to the bedside, as defective complement regulators or the absence of regulators is efficiently overcome by plasma transfusion. Similarly, the humanized monoclonal antibody specific for C5 (eculizumab), which has been efficiently used as a treatment for aHUS, provides an excellent recent example of promising translational research[115,116].

Infectious pathogens. The protective complement shield is also relevant in infectious diseases as pathogenic microorganisms, by exploiting soluble host complement regulators, generate a protective shield on their surfaces.

Table 3 | **Host and pathogen complement regulators**

Point of action	Host regulators		Pathogen-encoded regulators and host regulator binding proteins	Refs
	Fluid phase regulators	Membrane bound regulators		
C3 convertase	Factor H, FHL1, C4BP, properdin and C1INH	CR1, CD55, CD46 and CRIg	CBPA, CRASP1–CRASP5, GPM, EFB, FBA, FHA, GNA1870, HIC, IDES, LOS, M protein, OMP100, PAAP, PAE, PLA, PLY, POR, PRA1, PREH, PRTH, PSPA, PSPC, RCA, RCK, REC, SAK, SBI, SCIN, SPEB, STCE, SSCL, TUF and YADA	4,5,52–55, 58,59,68, 71,72,74,98
C5 convertase	CFHR1	None	CHIPS, SCPA/SCPB and SSL-7	4,5,52,61,98
Terminal pathway	CFHR1, clusterin and vitronectin	CD59	CD59-like, FHA, OMP A, SIC, TRAT, USPA1 and USPA2	4,5,52, 61–63,73,98

C, complement component; C1INH, C1 inhibitor; C4BP, C4b-binding protein; C5L2, C5a receptor-like 2; CFHR1, complement factor H-related protein 1; CHIPS, chemotaxis inhibitory protein; CRASP, complement regulator-acquiring surface proteins; CRIg, complement receptor of the immunoglobulin superfamily; EFB, extracellular fibrinogen binding protein; FBA, fibronectin binding protein; FHL1, factor H-like protein 1; GMP, phosphogycerate mutase; GNA1870, genome-derived neisserial antigen 1870; HIC, factor H-binding inhibitor of complement, IDES, IgG-degrading enzyme of *Streptococcus pyogenes*; LOS, Lipooligosaccharide; M protein, membrane protein; OMP, outer membrane lipoprotein; PAAP, *Pseudomonas aeruginosa* protease; PAE, *P. aeruginosa* elastase; PLY, streptococcal pneumolysin; POR, outer membrane porins; PRA1, pH regulated antigen of *Candida albicans*; PRTH, *Porphyromonas* protease; PSPA/C, pneumococcal surface protein A/C; SAK, staphylokinase; SBI, *Staphylococcus aureus* IgG binding protein; SCIN, staphylococcal complement inhibitor; SIC, streptococcal inhibitor of complement; SPEB, streptococcal pyogenic exotoxin B; SSL7, staphylococcal superantigen like protein 7; STCE, streptococcal E protease; TUF, translation elongation factor of *P. aeruginosa*; USPA, universal stress protein A; YADA; *Yersinia* adhesion protein A.

Acquisition of soluble host regulators converts the foreign, non-self surface of the pathogen to a 'self-like' surface. Such a camouflaged pathogen remains invisible to the host innate immune system. Pathogens can also mimic features of host cells by expressing endogenous regulators that block complement activation. The ability of the conserved mechanism of complement to damage many different membrane types is illustrated by the fact that diverse human pathogens, including Gram-negative bacteria, Gram-positive bacteria, fungi, viruses, multicellular parasites and helminths, use similar, and even identical, strategies to evade host complement attack[4,5,51,98] (TABLE 3). As complement forms one of the first barriers of innate immunity any pathogenic microorganism has to cross this important layer of immune defence in order to establish an infection. Interestingly, the innate immune responses are constant and all human pathogens face the same complement attack. This may explain why different classes of pathogens use similar strategies to evade human complement attack.

Many different mechanisms of action for individual inhibitors have been defined; for example, *Borrelia burgdorferi* expresses five different complement regulator-acquiring surface proteins, termed CRASP1–CRASP5, for immune evasion[117]. Structural data explain the interaction in molecular terms; for example, the *S. aureus* extracellular fibrinogen binding protein (Efb) blocks the conformational activation of the C3b molecule[118], and the *Neisseria meningitidis* factor H-binding protein genome-derived neisserial antigen 1870 (GNA1870) mimics host surfaces by binding host factor H[119]. The success of microbial complement regulator GNA1870 binding proteins as vaccine candidates demonstrates the clinical relevance of these types of proteins[120–122] (TABLE 3).

Concluding remarks and perspectives

Complement-mediated innate immune function not only recognizes and eliminates infectious agents but also controls homeostatic processes such as organ and neuron development, clearance of cellular debris and elimination of infectious particles. In the future new functions will probably be identified for other cleavage products, such as Ba, C2a and C4a.

Because of its central role in multiple physiological processes, dysregulation or imbalance of the complement system can result in pathology and diseases that manifest in different organs. Interestingly, disturbances of the alternative pathway in particular, a master regulator of homeostasis, are associated with several autoimmune diseases. So, the mechanistic characterization of complement-mediated clearance and the identification of associated genes and gene products represent a major challenge for the future.

In addition, an important aspect of future work is to precisely define the link between the complement system and other important immune and homeostatic effector circuits, such as the coagulation cascade, and the integration of complement receptor signalling with other pathways, such as the Toll-like receptor signalling network. The interplay between complement and coagulation systems is expected to provide new clues to the pathological mechanisms of various diseases, for example the various disorders that are associated with thrombus formation.

Additional new methods for targeting complement are now being developed[122]. One of the great challenges of future complement research is to specifically interfere with, and block, the damaging inflammatory reactions that lead to pathology, but at the same time maintain the protective effects that are benefical for the host.

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Acknowledgements
The work of both the authors is supported by the Deutsche Forschungsgemeinschaft, Germany. P.F.Z.'s work is also supported by Kidneeds, Iowa, USA, the National Institutes of Health, USA, and ProRetina, Germany .

DATABASES
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C1INH | C1qR | C3 | C3aR | C4a | C4b | C4BP | C5 | C5aR | C6 | C7 | C8 | C9 | CD46 | CD59 | CFHR1 | CR2 | CR1 | CRIg | DAF | factor B | factor D | factor H | factor I | FHL1 | properdin | SIGNR1 | vitronectin

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