REVIEW

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# Complement: a key system for immune surveillance and homeostasis

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**Nearly a century after the significance of the human complement system was recognized, we have come to realize that its functions extend far beyond the elimination of microbes. Complement acts as a rapid and efficient immune surveillance system that has distinct effects on healthy and altered host cells and foreign intruders. By eliminating cellular debris and infectious microbes, orchestrating immune responses and sending 'danger' signals, complement contributes substantially to homeostasis, but it can also take action against healthy cells if not properly controlled. This review describes our updated view of the function, structure and dynamics of the complement network, highlights its interconnection with immunity at large and with other endogenous pathways, and illustrates its multiple roles in homeostasis and disease.**



*A hidden connection is stronger than an obvious one.*
*—Heraclitus of Ephesus (535–475 BC)*

Traditionally, complement has been primarily viewed as a supportive first line of defense against microbial intruders, quickly tagging and eliminating them and buying the adaptive immune response time to pick up momentum. Today, we know that complement truly lives up to its name and complements, or even orchestrates, immunological and inflammatory processes, extending far beyond simple elimination of danger. Research over the past decade has drawn a picture of how complement acts as an intricate immune surveillance system to discriminate among healthy host tissue, cellular debris, apoptotic cells and foreign intruders and tunes its response accordingly (**Fig. 1a–c**). Indeed, besides its obvious involvement in eliminating microbes, complement participates in such diverse processes as synapse maturation, clearance of immune complexes, angiogenesis, mobilization of hematopoietic stem-progenitor cells (HSPCs), tissue regeneration and lipid metabolism. This versatility becomes less surprising when one considers that complement represents one of the most ancient cornerstones of immunity and has tightly coevolved with phylogenetically younger pathways[1]. Our understanding of the intricate network of effectors, receptors and regulators at the heart of complement has been continuously extended as new components have been discovered or experienced a resurrection, and new initiation pathways have been defined (**Table 1**). Structural and functional studies have provided unprecedented insight into the finely balanced machinery that underlies these versatile complement functions. However, it has also become clear that any trigger that tips this delicate balance between complement activation and regulation can induce self-attack (**Fig. 1d**). Indeed, complement can contribute to various immune, inflammatory, neurodegenerative, ischemic and age-related diseases, and complement-targeted therapeutics have recently re-entered the spotlight of drug discovery efforts. Our Review highlights recent and emerging trends that better define complement's role in physiology and pathophysiology.

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## Complement initiation and amplification

Although complement is commonly depicted as a linear cascade of separate pathways, it is essentially a hub-like network that is tightly connected to other systems (**Supplementary Fig. 1**). Depending on the trigger, several initiation and regulatory mechanisms act together to produce an anticipated result in immune surveillance (**Figs. 1** and **2**).

The classical pathway is often referred to as antibody-dependent because it is strongly initiated by IgM or IgG clusters. However, the versatile pattern recognition molecule (PRM) C1q activates complement by recognizing distinct structures directly on microbial and apoptotic cells or through endogenous PRMs such as immunoglobulins and pentraxins (for example, C-reactive protein; CRP). As part of the C1 complex ($C1qr_2s_2$), the proteases C1r and C1s are consecutively activated upon surface binding of C1q[2,3]. C1s subsequently cleaves C4 into C4a and C4b, thereby exposing a previously hidden thioester and leading to covalent deposition of C4b on surfaces in the immediate vicinity of the activation sites (opsonization). By cleaving C4b-bound C2 into C2a and C2b, C1s also mediates the generation of the classical pathway C3 convertase (C4b2b), which can cleave C3 and initiate amplification and downstream effector functions. (On the basis of recent discussions in the field and to streamline fragment nomenclature for all the complement pathways, we designate the small, nonproteolytic C2 fragment as C2a and the protease segment as C2b. As a consequence, the classical pathway and lectin pathway C3 convertase is referred to as C4b2b, by analogy to the alternative pathway C3 convertase, C3bBb.)

In the functionally similar lectin pathway, mannose-binding lectin (MBL) and ficolins act as PRMs that predominantly recognize carbohydrate patterns. Each PRM assembles with MBL-associated serine proteases (MASPs) that share structural similarity with C1r and C1s. Of

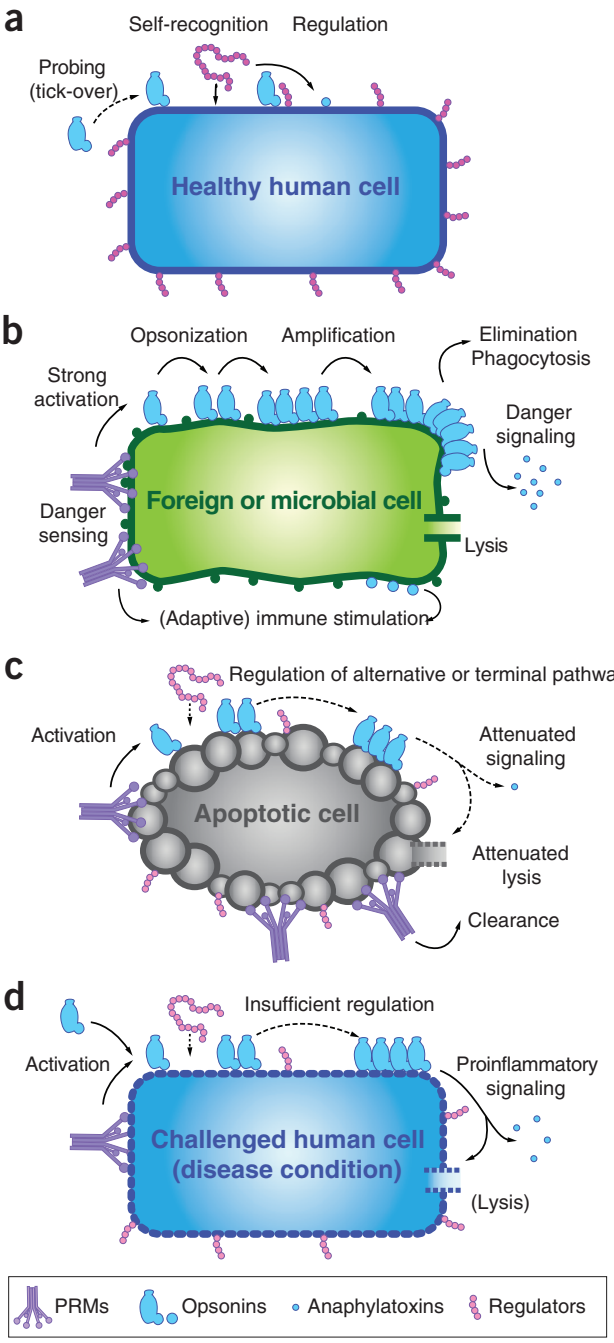


**Figure 1** Immune surveillance functions of complement. (**a**) A constant low level of complement activation (tick-over) ensures occasional probing of healthy human cells, but the presence of surface-bound regulators and self-recognition by soluble regulators prevents any amplification of the response. (**b**) In the case of microbial intruders, a strong complement response is actively induced by pattern recognition proteins and amplified in the absence of regulators. Opsonization by complement fragments and proinflammatory signaling by anaphylatoxins recruit macrophages and enable phagocytosis, and the formation of a lytic membrane attack complex on cells such as Gram-negative bacteria leads to cell death. Finally, complement degradation products stimulate downstream immune responses. (**c**) Although pattern recognition proteins also recognize the surfaces of apoptotic cells, residual and recruited complement regulators hold amplification and terminal pathways in check. Thus, opsonization facilitates elimination of the cell without triggering danger signals and further immune responses. (**d**) This fine-tuned interplay between complement recognition, activation and regulation usually allows appropriate reactions to healthy, apoptotic and foreign cells; however, any imbalance can lead to an attack on host cells and trigger immune and inflammatory diseases. Although proinflammatory and immune signaling seems to be the driving force in most complement-mediated diseases, other processes such as TCC-mediated lysis may be involved (for example, of erythrocytes in the case of paroxysmal nocturnal hemoglobinuria).

these, only MASP-2 cleaves both C4 and C2, generating the same C3 convertase as in the classical pathway. By contrast, MASP-1 cleaves C2 but not C4 and can supplement the lectin pathway response once initiated[4], thereby increasing the efficiency of convertase formation[5,6].

In contrast to the classical pathway and lectin pathway, the alternative pathway represents three distinct but partially overlapping processes. The tick-over segment keeps complement alert and allows constant probing of cells[7,8]. In its native form, C3, the central molecule of the alternative pathway, has few ligands and is relatively inert. However, a small fraction of the C3 molecules are hydrolyzed to $C3_{H2O}$, exposing new binding sites. The factor B (fB) protease binds $C3_{H2O}$ and is cleaved by factor D (fD), generating an initial, mainly solvent-based C3 convertase ($C3_{H2O}Bb$) that activates complement by cleaving C3 into its active fragments, C3a and C3b. Cleavage of C3 exposes a reactive short-lived thioester moiety in C3b, which covalently attaches to amine and carbohydrate groups on the target surface. This initial tagging is quickly amplified on foreign cells but is immediately regulated on human cells. Moreover, the reactivity of the thioester moiety to specific carbohydrates might lead to preferential opsonization of foreign particles and represent a basic pattern recognition mechanism[9,10]. The alternative pathway also includes a PRM-based initiation mechanism that resembles those found in the lectin pathway and classical pathway and involves properdin. Properdin recognizes several pathogen- or damage-associated molecular patterns (PAMPs and DAMPs, respectively) on foreign and apoptotic cells. Once bound, it initiates and propagates the complement response by attracting fluid-phase C3b to recognized surfaces[11] and allowing *de novo* convertase assembly, and by stabilizing C3 convertase complexes (C3bBbP)[12].

All surface-bound C3 convertases, regardless of origin, can induce the amplification branch of the alternative pathway by activating C3. The resulting C3b is rapidly deposited in the immediate vicinity of the activation and forms the major alternative pathway C3 convertase (C3bBb) in the presence of fB and fD, thereby creating an efficient cycle of C3 cleavage and convertase assembly that markedly amplifies the response. Despite its name, the alternative pathway might account for up to 80–90% of total complement activation, even when initially triggered by the classical pathway or lectin pathway[13]. Complement amplification can also occur in plasma when C3b forms dimeric complexes with IgG, which are partially protected from degradation and further stabilized by binding properdin[14].

Amplification by the alternative pathway increases the density of deposited C3b and gradually leads to the formation of convertases that contain an additional C3b molecule (C4b2b3b or C3bBb3b) and shift the substrate specificity from C3 to C5. These C5 convertases cleave C5 into the anaphylatoxin C5a and fragment C5b. When C5b associates with C6 and C7, the complex becomes inserted into cell membranes and interacts with C8, inducing the binding of several units of C9 to form a lytic pore, the terminal complement complex (TCC; $C5b\text{–}9_n$; also known as the membrane attack complex)[15].

Substitute routes of complement activation have emerged in recent years. Although it has long been known that proteases such as plasmin, thrombin, elastase and plasma kallikrein can cleave and activate C3, this extrinsic protease pathway (**Fig. 2a**) has gained more attention in the context of potential crosstalk between complement and coagulation

**Table 1 Proteins involved in the core complement cascade**

| Pattern recognition | Alternative names | Function |
|---|---|---|
| C1q | | Part of the C1 complex; recognizes surface-bound IgG, IgM, CRP and PAMP; initiates CP |
| MBL | | Recognizes carbohydrate patterns; initiates LP |
| Ficolin-1 | M-Ficolin | Recognizes carbohydrate patterns; initiates LP |
| Ficolin-2 | L-Ficolin; Hucolin | Recognizes carbohydrate patterns; initiates LP |
| Ficolin-3 | H-Ficolin; HAKA1 | Recognizes carbohydrate patterns; initiates LP |
| Properdin | Factor P | Recognizes PAMP and DAMP; initiates AP; stabilizes AP convertases |
| CRP[a] | | Recognizes DAMP and PAMP on apoptotic and microbial cells; binds C1q |
| CFHR-4 | FHR-4 | Recruits monomeric CRP to necrotic cells; facilitates activation of CP via C1q |
| **Proteases** | **Alternative names** | **Function** |
| C1r | | Part of the C1 complex; cleaves C1s |
| C1s | | Part of the C1 complex; cleaves C2 and C4 |
| MASP-1 | | Binds to MBL/ficolins; cleaves C2 (but not C4); may cleave MASP-2 and C3; activates pro-FD; potential role in coagulation cascade? |
| MASP-2 | | Binds to MBL/ficolins; cleaves C2 and C4 |
| MASP-3 | | Unknown (binds to MBL/ficolins, does not cleave C2 or C4) |
| C2 | | Part of the CP/LP convertases; cleaves C3/C5 |
| Factor B | CFB | Part of the AP C3/C5 convertases; cleaves C3/C5 |
| Factor D | CFD | Cleaves C3b-bound FB to form the AP C3/C5 convertases |
| Factor I | CFI | Degrades C3b and C4b |
| **Complement components** | **Alternative names** | **Function** |
| C3 | | Progenitor for anaphylatoxin C3a, opsonin C3b and signaling fragments (iC3b, C3dg, C3d); part of the AP C3 and all C5 convertases |
| C4 | | Progenitor for opsonin C4b; part of the CP/LP convertases |
| C5 | | Progenitor for anaphylatoxin C5a and C5b/TCC |
| C6 | | Part of TCC (membrane insertion) |
| C7 | | Part of TCC (membrane insertion) |
| C8 | | Part of TCC (induction of pore formation) |
| C9 | | Part of TCC (forms lytic pore) |
| **Receptors[b]** | **Alternative names** | **Function** |
| CR1 | CD35; C3b/C4b-receptor | Binds C3b/iC3b; induces phagocytosis: accelerates decay of convertases; cofactor for fI |
| CR2 | CD21; C3d-receptor | Binds iC3b/C3dg/C3d; lowers threshold for B-cell stimulation |
| CR3 | CD11b/CD18; Mac-1; integrin $\alpha_M\beta_2$ | Induces phagocytosis through interaction with iC3b; modulates IL-12 family in APCs |
| CR4 | CD11c/CD18; p150/95; integrin $\alpha_X\beta_2$ | Induces phagocytosis through interaction with iC3b |
| C3aR | | Binds C3a; triggers proinflammatory signaling |
| C5aR | CD88 | Binds C5a; triggers proinflammatory signaling |
| C5L2 | GPR77 | Binds C5a (strongly) and C5a$^{desArg}$ (weakly); might bind C3a/C3a$^{desArg}$; function not fully defined |
| CRIg | Z93Ig, VSIG4 | Induces phagocytosis through interaction with iC3b/C3c; regulatory effect on C5 convertases |
| cC1qR | Calreticulin; collectin receptor | Recognizes bound C1q; induces phagocytic signaling through CD91 |
| gC1qR | C1qbp (C1q-binding protein); p33 | Recognizes C1q; potential role in phagocytosis and signaling; modulates IL-12 on APCs |
| C1qRp | (CD93 + unknown mediator) | Part of receptor complex that binds C1q and mediates phagocytosis |
| **Regulators** | **Alternative names** | **Function** |
| C1-INH | SERPIN1 | Inhibits C1r/s and MASPs |
| sMAP | MAP19 | Binds to MBL, competes with MASPs |
| MAP-1 | MAP44 | Binds to MBL/ficolins; inhibits C4 deposition |
| C4BP | C4-binding protein | Accelerates decay of LP/CP convertases; cofactor for fI |
| Factor H | CFH | Recognizes self-surfaces; accelerates convertase decay; cofactor for fI |
| FHL-1 | Reconectin, CFHL1 | Accelerates convertase decay; cofactor for fI |
| MCP | CD46 | Cofactor for fI |
| DAF | CD55 | Accelerates decay of convertases |
| CFHR-1 | FHR-1 | Recognizes self-surfaces and C5; inhibits C5 cleavage and TCC formation |
| CD59 | Protectin | Binds to C8 and C9; prevents assembly of TCC |
| Vitronectin | S-protein; S40 | Binds to C5b-9; prevents assembly of TCC |
| Clusterin | Apolipoprotein J; SP-40,40 | Binds to C7-C9; prevents assembly of TCC |
| Carboxypeptidase-N | | Degrades C3a and C5a to their desArg forms |

AP, alternative pathway; CFHR, complement factor H-related protein; CP, classical pathway; CR, complement receptor; LP, lectin pathway; Mac-1, macrophage-1 antigen. [a]Although CRP is the best-described representative mediator of this category, other pentraxins may exert similar functions; [b]additional receptors for C1q (for example, CR1, integrin $\alpha_2\beta_1$, murine SIGN-R1) have been proposed but not yet confirmed.

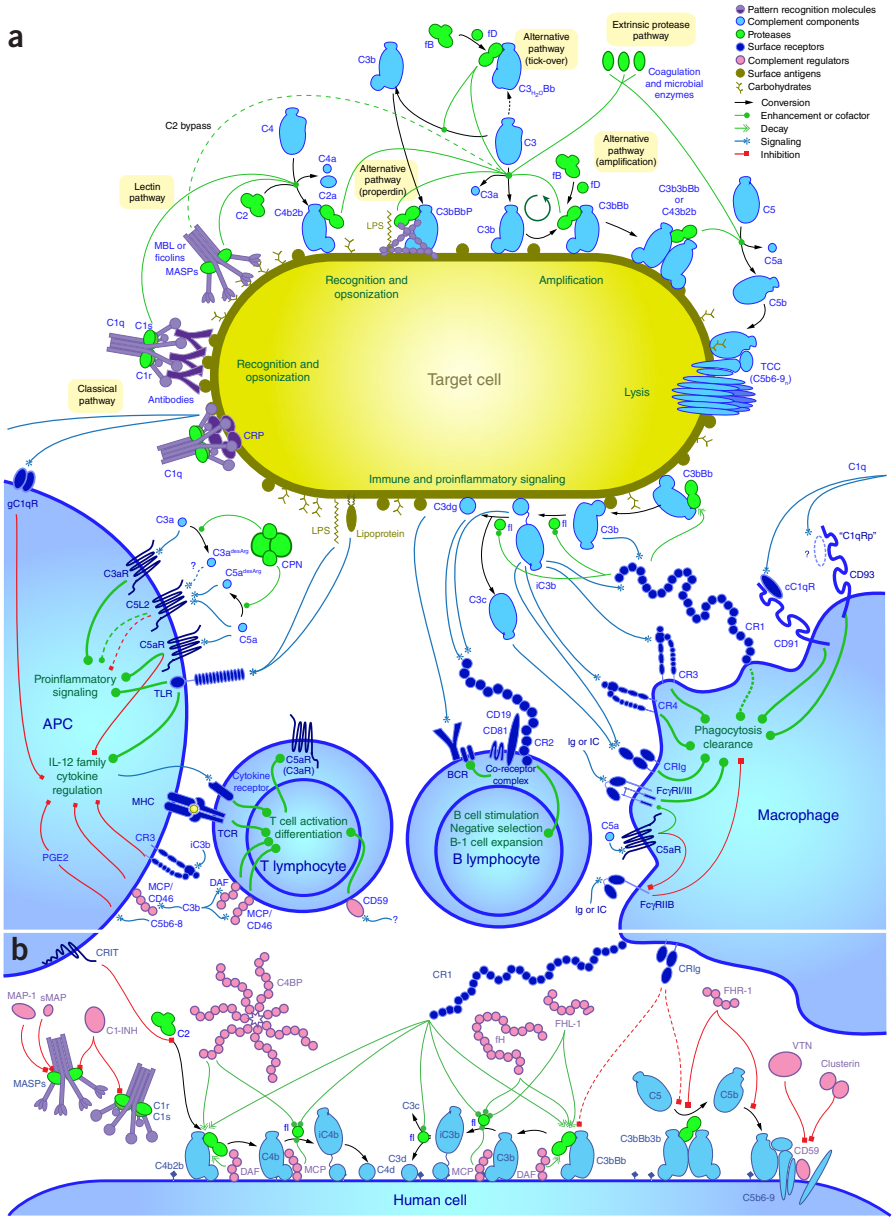


**Figure 2** Detailed view of complement activation, amplification, signaling and regulation. (**a**) A network of soluble and surface-bound proteins enables the recognition, tagging and elimination of microbial intruders and foreign cells (**a**) and stimulates downstream immune responses. In the classical pathway, C1q recognizes pathogen- or damage-associated PRMs (such as IgG, IgM and CRP) on foreign or apoptotic cells, inducing the formation of the classical pathway C3 convertase (C4b2b) through cleavage of C2 and C4 by C1s. Detection of carbohydrate patches by MBL or ficolins associated with MASP via the lectin pathway forms the same convertase, which activates the plasma protein C3, generating its active fragments C3a and C3b. Covalent deposition of C3b on nearby surfaces (opsonization) leads to the binding of factor B and conversion into the alternative pathway C3 convertase (C3bBb), which cleaves more C3 into C3b and thereby amplifies the complement response. In addition, a low level of complement activation is maintained in solution (tick-over), and resulting C3b or C3 convertases can be recruited to foreign surfaces and stabilized by properdin (P). Increasing surface densities of C3b lead to a gradual substrate shift of the convertases from C3 to C5. Cleavage of C5 into C5a and C5b initiates the assembly of the lytic TCC on susceptible cells. Opsonization by C1q and C3b, and its degradation products iC3b, C3c and C3d, induces phagocytosis by complement receptors. The anaphylatoxins C3a and C5a cause strong proinflammatory signaling through their GPCRs. C5a also co-regulates immunoglobulin (Ig)-mediated phagocytosis of immune complexes (IC) by modulating the differential expression of activating (FcγRI/III) and deactivating (FcγRIIB) Fcγ receptors. On B cells, binding of C3dg to the CR2/CD19 co-receptor complex lowers the threshold of activation by several orders of magnitude and has an important role in their maturation. Close crosstalk between TLRs, complement receptors and regulators modulates IL-12 in APCs and thereby influences the activation and differentiation of T cells. (**b**) On healthy human cells (**b**), any complement activation or amplification is attenuated by surface-bound regulators that accelerate decay of the convertases (CR1, DAF), act as a cofactor for the fI-mediated degradation of C3b and C4b (CR1, MCP), or prevent the formation of the TCC (CD59). Soluble regulators such as C4BP, FH and FHL-1 recognize self-surface pattern-like glycosaminoglycans and further impair activation. Finally, regulators enable control at the level of initiation (C1-INH, MAP-1, sMAP, C2 receptor inhibitor trispanning, FHR-4), the C5 convertases (FHR-1, CRIg) or TCC (FHR-1, VTN, clusterin).

pathways[16]. Thrombin can induce the generation of C5a in the absence of C3 (ref. 17), indicating that the extrinsic protease pathway extends to C5. Furthermore, target-bound MBL can activate C3 independently of MASP-2, C2 and C4 *in vitro*[18] (C2 bypass; **Fig. 2a**), but the physiological implications of this bypass require further investigation[19].

## Effector functions of complement

Although TCC-mediated lysis is considered to be a hallmark of complement attack, there are surprisingly few supportive examples of this. In fact, many pathogens are protected from lysis through their cell wall architecture (for example, Gram-positive bacteria) or by employing evasive strategies that interfere with TCC assembly[20]. However, even sublytic amounts of TCC or partial complexes such as C5b-8 are important for nonlethal signaling events[21].

Proinflammatory signaling and phagocytosis are essential for complement-mediated defense against most foreign cells. During activation and amplification, C3a and C5a are constantly released and trigger proinflammatory signaling through their corresponding G-protein–coupled receptors, C3a receptor (C3aR) and C5a receptor (C5aR; also called CD88). A third, G-protein–independent anaphylatoxin receptor, C5L2 (GPR77), has more recently been discovered. However, its exact roles are not yet fully determined[22], with theories ranging from decoy to regulatory or even proinflammatory functions[23,24]. Whereas C3aR binds only C3a, C5aR recognizes both C5a and (more weakly) its degradation product $C5a^{desArg}$. Interestingly, C5L2 interacts with $C5a^{desArg}$ and C5a and has comparable affinities for both[25]. Although there is evidence for a functional link between C3a or $C3a^{desArg}$ and C5L2, proof of a direct interaction remains controversial. Anaphylatoxins are highly potent effectors

and have a number of crucial roles in immune responses and inflammation, which are discussed below and reviewed elsewhere[26,27].

C3a, and especially C5a, are powerful chemoattractants that guide neutrophils, monocytes and macrophages toward sites of complement activation. Thereby, they promote phagocytosis through the interaction of opsonins with complement receptors[27,28]. Among these receptors, CR1 (CD35) is particularly relevant, as it interacts not only with C3b and C4b to promote neutrophil-mediated phagocytosis but also contributes to the regulatory degradation of its ligands by factor I (fI)[29]. Thus, CR1 attenuates complement amplification by inactivating C3b and C4b, while simultaneously rendering the opsonins accessible to other complement receptors and promoting downstream immune responses. The integrin receptors CR3 (CD11b–CD18) and CR4 (CD11c–CD18) both bind to the iC3b fragment and contribute to phagocytosis, and CR3 also regulates cytokine responses, leukocyte trafficking and synapse formation.

Recently, another phagocytic receptor, the complement receptor of the immunoglobulin family (CRIg), has been identified. CRIg is exclusively expressed on certain tissue-resident macrophages and enhances phagocytosis by recognizing cell-bound C3b and iC3b[28,30]. It is unique in that it also binds C3c, although whether this binding clears C3c or induces signaling has yet to be determined[31].

Despite their structural similarity, CR1 and CR2 (CD21) have distinct but synergistic functions: CR1 is an important contributor to surface-bound iC3b and the only cofactor that induces further cleavage of iC3b to C3dg, both of which serve as CR2 ligands for B cell activation and differentiation[32,33]. Besides initiating the complement cascade, PRMs also act as opsonins and participate in phagocytic and inflammatory signaling. Although several receptors for C1q have been described, their exact roles are not well defined. Originally designated cC1qR because of its ability to bind the collagenous part of C1q (and collectins such as MBL), cC1qR was later found to be identical to the secreted protein calreticulin and to require a transmembrane mediator, probably CD91, to induce phagocytosis[34]. The C1q receptor of phagocytosis (C1qRp or CD93) seems to behave similarly, as it apparently does not bind C1q directly and might require an unidentified bridging molecule to form a phagocytic receptor complex[35]. Finally, gC1qR, which interacts with the globular head domains of C1q, is involved in the regulation of cytokines (**Fig. 2a**). Notably, genetic deletion of either CD91 or CD93 in mouse macrophages does not substantially alter C1q-mediated phagocytosis[36,37], which underscores the complexity and potential redundancy of C1q interactions.

## Complement regulation

Soluble and cell-bound complement regulators help to control complement attack and adjust its severity, propagation and endpoints to the cellular target[38] (**Figs. 1a–c** and **2b**). In addition to C1 esterase inhibitor (C1-INH), a secreted glycoprotein of the serpin family that inhibits several proteases of the classical and lectin pathways, two other lectin pathway modulators have been identified: sMAP and MAP-1 are non-proteolytic splice products of the *MASP2* and *MASP1/3* genes, respectively, that apparently compete with MASPs for binding to MBL and ficolins. The C2 receptor inhibitor trispanning also binds to C2 and inhibits its activation by C1s[39]. In the alternative pathway, activation in solution is mainly controlled by the abundant factor H (fH) and its truncated homolog, factor H-like protein 1 (FHL-1). fH mainly acts on C3 convertases in the alternative pathway, either competitively removing Bb from the C3bBb complex (decay acceleration) or serving as a cofactor for the fI-mediated degradation of C3b. Another fluid-phase regulator, C4b-binding protein (C4BP), has similar effects on classical pathway and lectin pathway convertases. Most importantly, fH, FHL-1 and C4BP also support complement regulation on human cells by engaging host-specific surface patterns (such as sialic acid or glycosaminoglycans), thereby contributing to self-recognition and prevention of self-attack.

Most human cells also expose convertase regulators that act as decay accelerators, such as CR1 or decay-accelerating factor (DAF, also called CD55), or as cofactors for fI, such as CR1 and membrane cofactor protein (MCP or CD46)[38,40]. Only a few C5-specific regulators have been described so far; whereas fH-related protein 1 directly binds C5 and inhibits its C5 convertase activity, CRIg regulates the C3b-containing C3 and C5 convertases, although the physiological implications of this mechanism are unknown. A cell-based regulator, CD59, acts on TCC by preventing the formation of both sublytic and lytic complexes. In addition, TCC is controlled by soluble regulators such as vitronectin and clusterin. Finally, carboxypeptidase-N quickly converts anaphylatoxins to their desarginated forms; although this cleavage impairs signaling through the primary receptors C3aR and C5aR, it shifts the signaling pattern, as $C3a^{desArg}$ and $C5a^{desArg}$ themselves can trigger important functions, for example, during HSPC mobilization[41,42] or lipid metabolism[43].

## Structure and dynamics in complement

Complement must react quickly to potential danger but be selective enough to avoid wreaking havoc on the host. Molecular studies have provided a fascinating insight into how time, location, concentration and dynamics are used to achieve selectivity and orchestrate cascading events. For example, the highly abundant C3 can act as an omnipresent sentinel that has few endogenous ligands[44] but becomes transformed into one of the most versatile binding partners upon activation to C3b. Whereas the exposure of a highly reactive but short-lived acyl-imidazole moiety restricts deposition of C3b to immediate sites of activation, additional control mechanisms are responsible for keeping amplification through C3 convertases in check.

Structural studies of the C3 convertase have suggested that C3b bridges and orients the proteolytic Bb fragment of fB with C3 to allow cleavage of the substrate C3 (ref. 45). Moreover, the recent report of a C3b-fH complex[46] provided a molecular basis for important regulatory mechanisms; bound fH not only sterically interferes with contact areas of fB and Bb on C3b to accelerate convertase decay but also forms a joined binding pocket with C3b that allows fI to bind and cleave C3b, while stabilizing the domain arrangement of C3b during cleavage[46]. This conversion disrupts domains involved in amplification but exposes complement receptor–specific binding sites, thereby facilitating downstream signaling steps. These and similar studies on other components have complemented our molecular picture of the initiation, amplification, regulation and signaling of complement and revealed that many of these proteins rely on dynamic rearrangements to fulfill their roles in these processes.

## Interaction with microorganisms

When PAMPs are detected on invading microorganisms, one or several complement initiation pathways that aim to eliminate microbial intruders are triggered. As microbes normally lack complement regulators, the response is rapidly amplified by the alternative pathway and results in opsonization, proinflammatory signaling, mobilization of immune cells, phagocytosis and, on certain pathogens such as Gram-negative bacteria (mostly *Neisseria* species) or parasites, formation of TCC and subsequent cell lysis (**Fig. 1b**).

Given complement's central and early role in antimicrobial attack and the millennia of coevolution between microorganisms and this ancient component of immunity, it is not surprising that many pathogens have evolved counterstrategies to evade complement. Elaborate complement evasion mechanisms are found in bacteria, viruses, fungi and parasites[20]. These strategies most commonly involve the nonactivating capture of

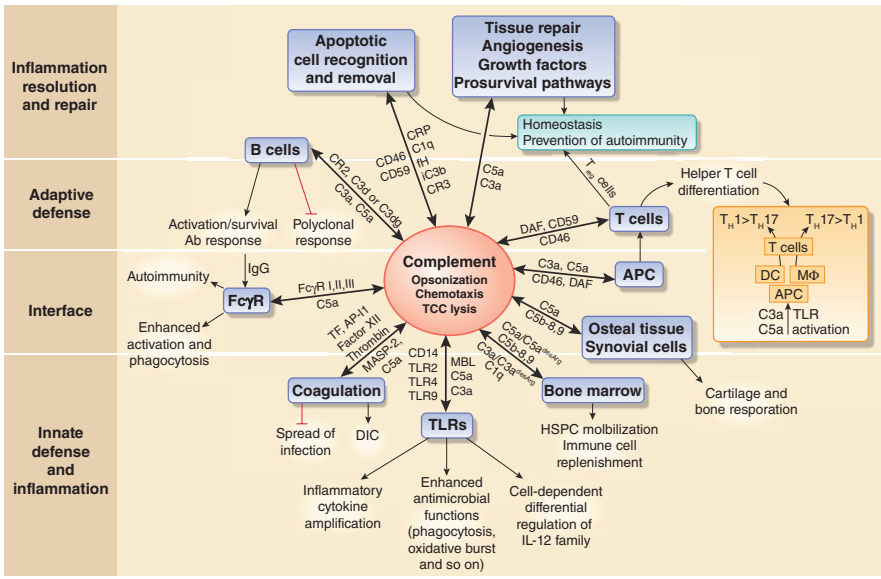


**Figure 3** Integrative role of complement in host defense and homeostasis. The traditional functions of complement (in oval) and its orchestrating role in immunity and homeostasis are shown (key participating molecules are indicated next to the arrows). Complement regulates TLR signaling to coordinate innate defenses and potentiates coagulation to provide a mechanical barrier against the spread of invading bacteria. It also facilitates IgG-mediated phagocytic killing of microbes by reducing the threshold for FcγR activation and promotes specific IgG antibody responses through B cell activation. Complement regulation of APC TLRs influences the differentiation of T cells, which can also be affected directly by complement. Regulation of T helper cell differentiation by complement is complex; however, in the context of complement-TLR crosstalk, the anaphylatoxins can either promote or inhibit $T_H1$ or $T_H17$ cell development *in vivo*, depending on whether they act through DCs or macrophages (MΦ) as APCs. To replenish the immune system during infection or injury, complement regulates the mobilization of HSPCs from the bone marrow. Most or all of the mechanisms regulated by complement can also be involved in immunopathology, such as FcγR-mediated autoimmunity, disseminated intravascular coagulation (DIC) and inflammatory bone resorption (for example, through sublytic C5b-9 signaling). The functional repertoire of complement includes the resolution of inflammation through induction of regulatory T cells, noninflammatory clearance of apoptotic cells (which permit complement activation through loss of complement regulatory proteins) and promotion of tissue repair. Most complement interactions are bidirectional, in that the production and activation of complement proteins and receptors is regulated by other receptors or system components (for example, FcγRs, TLRs, thrombin).



complement initiators (such as immunoglobulins), inactivation or depletion of complement components by secreted proteases, recruitment of complement regulators to the pathogen surface or secretion of regulator mimics, molecular inhibition of convertase activity, interference with TCC formation, or competitive and antagonistic prevention of immune signaling (**Supplementary Fig. 2**)[20]. Even more intriguingly, some pathogens take advantage of the complement system to enter cells by binding to cell-bound complement receptors and regulators, either through pathogen-expressed surface proteins or by 'voluntary opsonization' with complement fragments[20,47,48].

## Removal of apoptotic cells

The role of complement is not limited to inductive and effector phases of immunity; it also contributes to the resolution of inflammation by promoting safe clearance of apoptotic cells and immune complexes through the cooperative action of its soluble PRMs, opsonins and receptors[49,50]. Importantly, complement can differentiate between real threats and challenges that normally pose minimal danger: in contrast to a vigorous response against foreign intruders, removal of endogenous debris requires a much more tactful approach, in which complement avoids mechanisms downstream of C3 such as the inflammatory C5a-C5aR axis or TCC. Alterations in cell surface molecules during apoptosis allow dying cells to bind complement-related PRMs and become opsonized. The modified surfaces of apoptotic cells, which rapidly shed CD46 and CD59 (ref. 51), allow complement activation and their opsonization with C3b and C4b, followed by phagocytic cell uptake[38,49]. However, residual regulators on the cell surface and in circulation tame subsequent complement amplification, thereby largely preventing the generation of danger signals (**Fig. 1c**). This activity depends on the classical pathway, as macrophages show reduced ability to take up apoptotic cells in individuals deficient in C1q, C4, C2 or C3 (ref. 52). Early stage effectors are preferentially used because the modified apoptotic cell surface acquires CRP and C1q together with fH[38,53]. Specifically, CRP promotes the binding of C1q to apoptotic cells, amplifies classical pathway activation and recruits fH, which inhibits alternative pathway amplification and C5 convertase formation, thereby protecting cells from necrotic lysis and the host from unwarranted inflammation[53].

As a further safety mechanism, the clearance of apoptotic cells through iC3b opsonization and CR3 phagocytosis is accompanied by downregulation of interleukin 12 (IL-12) and a lack of oxidative burst in macrophages[54–56] or by a reduction in the expression of co-stimulatory molecules and impaired maturation of dendritic cells (DCs)[57,58]. Furthermore, even when the host sustains injury during an assault, complement can contribute to homeostasis by promoting tissue repair[59,60] (**Fig. 3**).

## Complement crosstalk with Toll-like receptors

To respond efficiently to danger, complement uses both pattern recognition and missing-self recognition strategies[61], deploys its rapid cascade, and, importantly, communicates with other biological systems (**Fig. 3**). For example, it coordinates innate immunity in cooperation with Toll-like receptors (TLRs)[61], helps to block the spread of infection by potentiating coagulation[16], links the innate response to both humoral and cellular adaptive immunity[62], and regulates the mobilization of HSPCs from bone marrow to replenish the immune system[41]. Both complement and TLRs are swiftly activated in response to infection or common microbial structures, such as lipopolysaccharides (LPS) or microbial CpG DNA. There is increasing evidence for extensive and bidirectional cooperation between the two systems, shaping the innate response through both synergistic and antagonistic crosstalk[61]. Concomitant detection of infection by distinct innate defense systems validates the presence of genuine danger, justifying and necessitating the amplification of the host's antimicrobial and inflammatory response. For instance, complement synergistically enhances TLR-induced production of proinflammatory cytokines (tumor necrosis factor (TNF), IL-1β and IL-6) *in vitro* and *in vivo* through C3aR, and especially C5aR signaling[63]. At least three TLRs (TLR2, TLR4 and TLR9) are involved in complement crosstalk, and their pathways converge with anaphylatoxin signaling at the level of mitogen-activated protein kinases (MAPKs), specifically Erk1/2 and Jnk[63]. This crosstalk partially explains earlier observations that inhibiting C5a signaling

protects against sepsis induced by high doses of LPS or by cecal ligation and puncture (CLP) peritonitis[64]. Reciprocally, activation of TLR induces the expression of complement components or receptors[65].

Intriguingly, C5a-induced TLR crosstalk might involve not only C5aR but also the enigmatic G- protein–independent C5L2, which might have both regulatory and proinflammatory roles[23,61,66] (**Fig. 2a**). Thus, C5L2 is essential for the induction of high-mobility group box-1 (HMGB1), through which C5L2 contributes synergistically with C5aR to inflammatory lethality in CLP sepsis[23]. At least *in vitro*, the induction of HMGB1 by C5a or LPS (or both) is diminished in *C5l2*$^{-/-}$ but not *C5ar*$^{-/-}$ macrophages[23]. These findings suggest that C5L2 and TLR4 might cooperate in the induction of HMGB1, perhaps through signaling crosstalk involving the MAPK and phosphatidylinositol-3 pathways[23]. Alternatively, C5L2 might act as a co-receptor for TLR4 activation.

Whether complement receptors associate with TLRs in functional multireceptor complexes is being investigated. Confocal microscopy and fluorescent resonance energy transfer experiments suggest that C5aR and TLR2 associate in activated macrophages[67]. Furthermore, in the apparent absence of C5a, blockade or genetic ablation of C5aR inhibits TLR2-induced cytokine production in DCs, suggesting that C5aR has a C5a-independent effect on TLR2 signaling[68]. MBL forms a functional complex with TLR2 in the phagosome and potentiates protective TLR2 signaling, at least in response to *Staphylococcus aureus*[69]. However, the observation that CD14 blockade inhibits complement inflammatory activities in a human whole blood model[70] could reflect either inhibition of complement-TLR crosstalk (CD14 lacks a transmembrane signaling domain and signals through TLR4 or TLR2) or extracellular interactions of CD14 with complement receptors. CD14 can physically associate with CR3 (ref. 71), although it has not been shown to interact directly with anaphylatoxin receptors.

Complement's ability to cooperate with TLRs could even undermine host immunity, if these crosstalk interactions are induced by microbial cells. For example, the oral pathogen *Porphyromonas gingivalis* induces C5aR-TLR2 crosstalk that impairs nitric oxide–dependent killing in macrophages[67]. Because this crosstalk inhibits only a subset of TLR2 signaling events[67], C5aR was characterized as a TLR modulatory receptor to distinguish it from TLR inhibitory receptors (such as IL-10R or TGF-βR), which inhibit most, if not all, inflammatory responses[72]. Moreover, although TLR2-induced transactivation of CR3 contributes to leukocyte trafficking[73], certain pathogens activate this TLR2 signaling pathway to exploit transactivated CR3 (refs. 47,48). Reciprocally, CR3 facilitates TLR2 or TLR4 signaling by promoting the recruitment of their sorting adaptor TIRAP[74]. In summary, by cooperating with other innate receptors, complement receptors can diversify their pattern recognition and signaling or regulatory capacities, and these properties can be exploited by pathogens to undermine immune defense.

## Interplay between complement and coagulation

Another crosstalk event that occurs early during infection is between complement and the coagulation system, apparently with the objective of enhancing local clotting and preventing microbial spread through the circulation[16]. Complement amplifies coagulation and inhibits fibrinolysis, mainly through C5a, which induces the expression of tissue factor[75] and plasminogen-activator inhibitor 1 (PA-I1)[16]. Moreover, MASP-2 can simultaneously activate complement and coagulation, the latter by generating thrombin from prothrombin[76]. Reciprocally, components of the coagulation cascade amplify complement activation (see also the extrinsic protease pathway above and **Fig. 2a**); for instance, activated clotting factor XII can activate the classical pathway through C1 cleavage[77], whereas thrombin directly cleaves C5 and generates biologically active C5a (ref. 17).

These procoagulatory activities are counteracted by certain pathogens, which subvert the fibrinolytic system and cause disseminating infections[78]. On the other hand, when the complement-coagulation crosstalk is activated systemically in an uncontrolled manner, as in sepsis, it can lead to life-threatening conditions such as disseminated intravascular coagulation[79]. Additional aspects of this important crosstalk, such as the interplay between complement and platelet activation, have been covered elsewhere[16].

## Complementing humoral immunity

As alluded to above, C3dg functions as a natural adjuvant, as binding of C3dg-opsonized antigen to CR2 on the B cell co-receptor complex (CR2–CD19–CD81) has a strong co-stimulatory effect on B cells[32,80] (**Fig. 2a**). Moreover, CR2 mediates antigen-independent signals that are necessary for B cells to survive in germinal centers[81]. CR1 and CR2 are the main receptors on follicular DCs for uptake and long-term retention of antigen, which contributes to B cell memory maintenance[32]. However, antibody responses to T-dependent antigens are impaired in mice with a B cell–specific CR1/CR2 deficiency (a single gene, *Cr1/2,* encodes both molecules), despite normal expression of both receptors on follicular DCs[82]. Therefore, the interaction of B cell–expressed CR2 and/or CR1 with C3 cleavage products is essential for antigen-specific antibody responses but not polyreactive antibody responses[83]. Experiments in CR1/CR2-deficient mice have confirmed the importance of complement for antibody responses to both T-dependent and T-independent antigens, including protective humoral immunity to certain bacteria and viruses[84–86]. The anaphylatoxins also have regulatory effects on B cells, including suppression of B cell polyclonal responses (C3a)[87] and promotion of the migration of naive and memory B cells (C5a)[88].

Complement promotes the effector function of the antibody response in ways that far exceed its originally identified role as a heat-sensitive activity in serum, which complements that of antibody in causing bacterial lysis. At the interface between innate and humoral immunity, C5aR signaling lowers the threshold for Fcγ receptor activation by upregulating the expression of activating Fcγ receptors (FcγR I and III) and downregulating the expression of the inhibitory FcγRIIB[89,90]. Conversely, FcγR activation enhances the synthesis of C5 for C5a generation[90]. This mutually reinforcing C5a-FcγR crosstalk (**Fig. 2a**) is important in infection as it promotes the clearance of microbial intruders by combining phagocytosis with the specificity of IgG antibodies. However, as IgG immune complexes have been implicated in autoimmune disorders (for example, rheumatoid arthritis), complement-FcγR crosstalk might exacerbate autoimmune pathology, as shown in a mouse model of autoimmune hemolytic anemia[90].

## Regulation of T cell immunity by complement

Early reports that mice treated with anti-CR1/CR2 monoclonal antibodies (mAb) show impaired antibody but intact helper T cell responses[91] could be interpreted as showing that C3 is not involved in T cell immunity. Similarly, CR1/CR2-deficient mice develop normal CD4$^+$ and CD8$^+$ T cell immunity and readily clear infection with influenza virus[92]; however, pathogen-specific T cell responses are impaired in C3-deficient mice in a CR1/CR2-independent manner[92,93]. C3 deficiency also results in impaired T cell responses in models of autoimmune disease and transplant rejection[94,95], at least in part because of lack of C3a and altered antigen-presenting cell (APC) function. For instance, C3aR-deficient DCs lose their ability to induce potent alloreactive CD4$^+$ T cell responses[96]. Moreover, C3a and C5a can be produced locally at the APC-T cell interface and can effectively determine the outcome of APC-T cell interactions[62,97–99] (**Fig. 3**).

Experiments in mice that lack complement inhibitory proteins (DAF,

CD59) highlight an important regulatory role for complement in the development of T cell immunity[62,100,101]. Interestingly, complement regulatory proteins exert both indirect (via APCs) and direct effects on T cells (**Fig. 2a**). For instance, DAF-deficient T cells show heightened cytokine responses and ligation of $CD4^+$ T cells by CD59 downregulates their activation, whereas activation of CD46-ligated $CD4^+$ T cells promotes their differentiation to a T regulatory 1 ($T_R1$) phenotype[62,100,102]. Signaling in T cells mediated by CD46 and CD59 might contribute to the resolution of the immune response, thereby preventing immunopathology[100,102]. Conversely, bacterial or viral pathogens that can directly engage these complement regulators may undermine T cell immunity as a survival tactic[62,103]. For example, the M protein of *Streptococcus pyogenes* interacts with CD46 on human $CD4^+$ T cells to induce a $T_R1$-like phenotype that suppresses bystander T cell activation[103].

Note that the effects of complement on helper T cell responses are influenced by tissue-specific microenvironmental conditions and timing variables. For instance, C5aR signaling protects the lungs against allergic asthma during the allergen-sensitization phase (C5a signaling at the DC–T cell interface leads to the induction of TGF-β and IL-10), whereas it drives a $T_H2$-mediated eosinophil and mast cell destructive response once allergic inflammation is established[104]. Intriguingly, however, the protective role of C5aR signaling during the allergen-sensitization phase is countered by C3aR signaling[105], which contributes to $T_H2$-dependent airway hyper-reactivity[106]. The emerging roles of IL-17 and the IL-17-producing helper T ($T_H17$) cells in asthma[107] and the ability of anaphylatoxins to regulate these responses in cooperation with TLRs[68,108,109] suggest that complement's influence on this disease may be more complex than originally thought.

## Impact of complement-TLR crosstalk on T cell responses

Recent work has focused on how complement-TLR crosstalk in macrophages and DCs regulates T cell immunity. The induction of C5aR (and, to a lesser extent, C3aR) signaling in TLR-activated macrophages selectively inhibits the transcription of genes that encode IL-12 family cytokines[63,110,111](**Fig. 2a**). These cytokines (IL-12, IL-23 and IL-27) have an important role in the activation and differentiation of distinct subsets of T cells. For example, IL-12 (a p35-p40 heterodimer) induces the differentiation of the $T_H1$ lineage from naive $CD4^+$ T cells, whereas IL-23 (p19-p40) drives the expansion of the $T_H17$ subset[112]. IL-27 (p28-EBI3) regulates the $T_H1/T_H17$ balance by limiting $T_H17$ development and favoring that of $T_H1$ (ref. 112). These regulatory effects might be relevant to the attenuation of T cell-mediated inflammatory tissue damage (inhibition of $T_H1$-mediated pathology by C5aR-TLR4 crosstalk). However, the relevance of this crosstalk becomes evident in microbial immune evasion. Specifically, *Leishmania major*, a macrophage intracellular pathogen, seems to benefit from C5aR-induced inhibition of $T_H1$ immunity[110].

Similar inhibition of TLR-induced IL-12 production is seen when other complement receptors (gC1qR, CD46 and CR3) are activated concomitantly with TLR4 or TLR2 in mouse macrophages or human monocytes[113–115] (**Fig. 2a**). These crosstalk pathways are likewise exploited by several pathogens to suppress $T_H1$ immunity and IL-12–IFN-γ-dependent clearance; these pathogens include hepatitis C virus, measles virus and *P. gingivalis,* which interact with gC1qR[113], CD46 (ref 114) and CR3 (ref 116), respectively. However, when the same receptors are instead activated by their natural ligands, their crosstalk with TLR pathways can have important physiological roles. For instance, interactions between macrophage CR3 and iC3b-coated apoptotic cells inhibit IL-12, preventing unwarranted inflammation during apoptotic cell phagocytosis[55,61]. Moreover, binding of C1q by gC1qR may represent a homeostatic mechanism for regulating T cell immunity; if so, this role would be consistent with observations that C1q deficiency in humans and mice causes inflammatory autoimmune pathology[117].

The crosstalk between complement and TLR that regulates IL-12 is dynamic and contextual, depending at least in part on the cell type involved. For example, activation of CD46 signaling in LPS-stimulated human DCs does not inhibit IL-12 production as it does in monocytes but rather promotes the expression of IL-12p35, IL-12-IL-23p40, and IL-23p19 (ref. 118). Moreover, in contrast to their effects on monocytes and macrophages, C5a and C3a do not inhibit TLR-induced IL-12 production in human or mouse DCs[98,99,119]. Instead, these anaphylatoxins promote IL-12 production in LPS-stimulated DCs and favor the development of $T_H1$ responses, at least in a mouse model of allostimulation[96,98,99]. These effects reflect the ability of both anaphylatoxins to inhibit immunosuppressive cAMP-dependent protein kinase A signaling, thereby releasing inhibitory constraints in DCs[98,99]. By contrast, this pathway is not inhibited by the anaphylatoxins in macrophages or neutrophils[67,79]. The findings from the allostimulation model are consistent with observations that *C3ar*$^{-/-}$ and *C5ar*$^{-/-}$ mice fail to mount $T_H1$ immune responses and succumb to *Toxoplasma gondii* infection[97]. C3a and C5a are probably generated locally by both partners at the DC–T cell interface, leading to functional co-stimulation and differentiation of naive $CD4^+$ T cells to $T_H1$ (refs. 96,97). Conversely, blockade or absence of C5aR in DCs prevents $T_H1$ differentiation, leading instead to enhanced TGF-β, IL-6 and IL-23 responses that promote differentiation of T cells to $CD25^+$ $Foxp3^+$ regulatory T cells and $T_H17$ cells *in vitro* and *in vivo*[68].

In contrast to DCs, TLR-activated macrophages require intact C5aR signaling to promote the differentiation of $CD4^+$ T cells into $T_H17$ cells[109]. *In vivo*, this $T_H17$-promoting crosstalk can induce experimental autoimmune encephalomyelitis or autoimmune arthritis and depends on synergistic induction of IL-6 by C5aR and several TLRs (TLR-2, -4 or -9)[109,120]. Thus, in the context of TLR crosstalk, complement could conceivably either promote or inhibit the development of $T_H1$ or $T_H17$ cells, depending on the type of APC involved. By acting through DCs, complement favors $T_H1$ and inhibits $T_H17$, whereas by acting through macrophages, it favors $T_H17$ and inhibits $T_H1$ (**Fig. 3**). In general, complement can reduce the threshold for T cell polarization to either $T_H1$ or $T_H17$, depending on other concomitant environmental stimuli. Indeed, *Daf1*$^{-/-}$ (but not *Daf1*$^{-/-}$*C3ar*$^{-/-}$ or *Daf1*$^{-/-}$*C5ar*$^{-/-}$) T cells show considerably higher production of IFN-γ or IL-17 than wild-type controls in response to IL-12 or IL-23, respectively[108]. In summary, complement exerts profound and complex effects on the initiation, differentiation and replenishment of immune responses (through HSPC mobilization[41]; **Supplementary Fig. 3**), necessitating a precise and contextual understanding of the signaling pathways involved.

## Complement-related diseases

Although complement's involvement in pathophysiology was historically defined by observations in patients with complement deficiencies or dysfunctions[121,122], our knowledge has been boosted by improved animal models and genome-wide association studies (GWAS). The growing list of diseases that involve complement has fueled an interest in developing complement-targeted therapies[123–125]. Despite challenges such as the high plasma concentrations of targets and a prevalence of protein-protein interactions, the first complement-directed drugs, including recombinant C1-INH (various manufacturers) for the treatment of hereditary angioedema and a therapeutic C5 antibody (Soliris, Alexion) for paroxysmal nocturnal hemoglobulinuria, are already being marketed, with more candidates in clinical trials or preclinical development. Here we address some emerging aspects of complement in health and disease, while referring to specialized reviews for detailed coverage of its role in rheumatoid arthritis[126], asthma[127] and other diseases[128,129], or in complications aris-

ing from biomaterial-induced complement activation[130] (such as during hemodialysis[131] or cardiopulmonary bypass surgery[132]).

### Inflammatory diseases

The accumulation and unsuccessful removal of cellular debris might contribute not only to autoimmune disorders such as systemic lupus erythematosus (often associated with C1q, C4 or C2 deficiencies)[50,122] but also to various age-related and neurodegenerative diseases that have chronic inflammatory components. Age-related macular degeneration (AMD) has shown particularly strong ties to complement: this leading cause of blindness in elderly people of European descent has a complex etiology and produces geographic atrophy and neovascularization of the subretinal tissue that gradually leads to loss of central vision. After several complement components were detected in subretinal lipoprotein deposits (drusen)[133,134], GWAS identified polymorphisms in the fH gene as major risk factors for AMD[135–138]. Meanwhile, additional polymorphisms and deletions that mostly affect the alternative pathway (including C3, fB and FHL-1) have been discovered, suggesting that disruption of the delicate balance between complement activation and regulation in the subretinal tissue might contribute to the progression of AMD. Although the pathogenesis of AMD is not fully understood, a slow but vicious cycle of tissue damage (for example, by oxidative stress), accumulation of debris, chronic complement activation and inflammation that perpetuates tissue damage seems to be at its heart[134]. Given the strong association between AMD and complement and the high prevalence of the disease, it is not surprising that considerable complement-targeted drug development efforts are being directed toward AMD. Complement inhibitors are among the few promising options for treating the early, dry form of AMD and potentially preventing vision loss[139].

Excessive complement activation is also involved in two rare but severe kidney diseases that often culminate in end-stage renal failure. Both membranoproliferative glomerulonephritis type II (MPGN II; dense-deposit disease) and atypical hemolytic uremic syndrome (aHUS) progress aggressively and often manifest at a very young age. In both diseases, deficiencies and polymorphisms in components of the alternative pathway (including fH, C3, fB, fI and CD46) or autoantibodies that either neutralize regulators or stabilize the C3 convertase (nephritic factor) lead to excessive complement activation[140,141]. Plasma infusion or plasma exchange therapy and renal transplantation are currently used to treat aHUS and MPGN II, but clinical trials involving treatment of patients with aHUS using complement inhibitors such as Soliris are being conducted to identify better therapeutic options[125,141].

There is also evidence that complement is involved in Alzheimer's disease, as both C1q and C3 recognize accumulating amyloid fibrils and induce persistent activation of complement. The release of anaphylatoxins might attract microglia and astrocytes that contribute to phagocytosis but can also be activated to release cytokines, proteases and reactive oxygen species (ROS), thereby contributing to inflammation and accelerating neuronal dysfunction[142]. Administration of a C5aR antagonist in a mouse model of Alzheimer's disease has substantially improved memory performance and reduced pathologic markers such as amyloid deposits[143]. Complement might also exert protective effects during the early stages of Alzheimer's disease; certain complement regulators are upregulated early in the disease, and opsonization with C1q may facilitate clearance of damaged neurons. However, with increasing damage to the brain, the deleterious effects of complement seem to prevail.

### Acute-phase disorders

In contrast to the diseases mentioned above, acute-phase disorders such as sepsis or ischemia-reperfusion injury trigger a more aggressive and distinctive complement response that can contribute to tissue damage. In sepsis, severe infection with microorganisms can trigger acute inflammatory reactions associated with a storm of cytokines and other mediators, producing hypotension, multi-organ failure and death; this overwhelming immune response causes organ damage long after the triggers have been cleared. Given complement's role in first-line defense against microbial intruders, complement activity that contributes to control of the infection is clearly beneficial in the early stages of sepsis. However, complement, and especially C5a, can contribute to organ damage in combination with the cytokine storm in the later stages of sepsis[79,144]. Thus, both the direct impact of C5a on immune cells and the induction of uncontrolled coagulation by C5a-mediated expression of tissue factor contribute to the devastating effects seen in sepsis (**Fig. 4a**). Therapeutic regimens that focus on early antimicrobial intervention and anti-inflammatory treatment during later stages therefore seem most appropriate, and inhibition of complement at the C3 level in a primate model of late-stage sepsis markedly improved organ preservation and other clinical parameters[145].

Complement-associated tissue damage is also seen in ischemia-reperfusion injuries related to myocardial infarction and stroke, or induced during procedures such as vascular surgery or organ transplantation. Reperfusion of tissue following temporary occlusion of the blood supply triggers inflammatory and immune responses, including complement activation, that can lead to self-attack, in which the contribution of individual pathways seems to depend on the affected organ[146]. Complement-mediated recognition of damaged cells further activates the cascade and enhances anaphylatoxin release, fueling inflammation and recruiting immune cells. The ensuing release of ROS and other signals generates a vicious cycle of damage and inflammation. Several complement-directed therapies have achieved only limited success in treating ischemia-reperfusion injury, especially in myocardial infarction, but recent insights into the role of complement have suggested promising therapeutic strategies[146].

### Immune connections to development and metabolism

There is growing evidence that, as in amphibians[147], complement contributes to the development and repair of mammalian tissue (in the resolution phase of inflammation). Both its role in the disposal of dead cells and the induction of growth factors that promote restoration of tissue homeostasis are considered important: C5a and C3a, for instance, induce expression of vascular endothelial growth factor, which is required for angiogenesis and tissue repair after injury[60]. The repair-promoting function of complement is apparent in liver regeneration; anaphylatoxin-induced IL-6 and TNF prosurvival signaling promotes hepatocyte growth and proliferation[148], which is severely impaired in *C3*$^{-/-}$, *C3ar*$^{-/-}$ and *C5ar*$^{-/-}$ mice[59]. However, C5 and C5a have been implicated as profibrotic factors in liver, lung and renal fibrosis[148–150], and a SNP in the gene for C5 is associated with liver fibrosis[149]. Therefore, as in immunity, the role of complement in tissue repair involves a complex and delicate process that can produce undesirable outcomes.

Complement-mediated remodeling of synaptic connections in the developing nervous system is similarly a two-edged sword. Immature astrocytes can induce the elimination of improper synaptic connections through recognition by C1q and induction of C3b- or iC3b-mediated phagocytosis[142,151] (**Fig. 4b**). The same process can be triggered by reactive astrocytes in the damaged or diseased brain, thereby contributing to the pathogenesis of neurological and neurodegenerative diseases. Complement has been linked not only to Alzheimer's disease (see above) but also to glaucoma[152], Parkinson's disease[153], multiple sclerosis[154] and schizophrenia[155,156]. Conversely, impaired complement activity early in development might be detrimental, given the surprising role for C3a-C3aR signaling in the control of neural crest migration during early

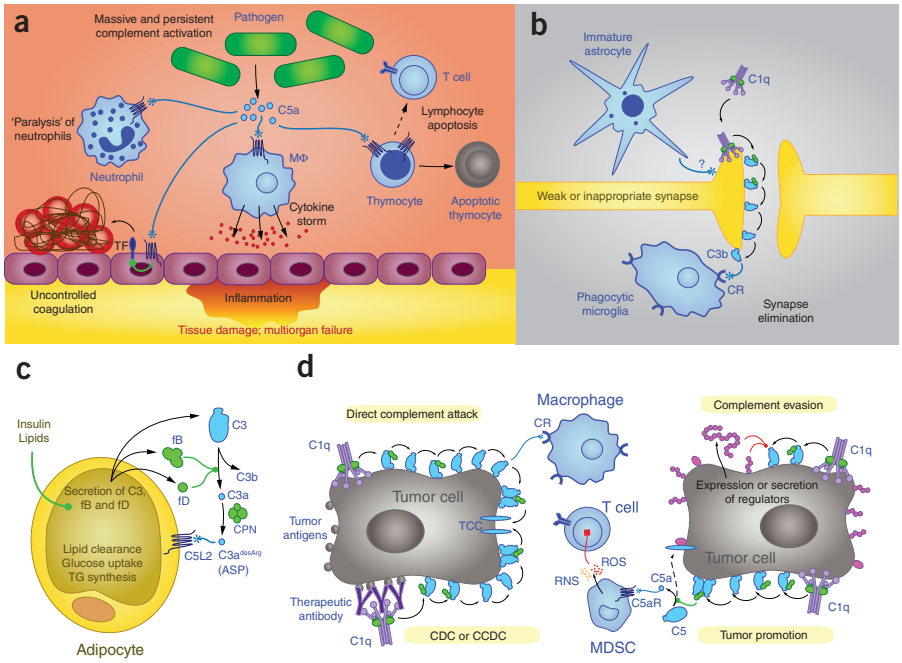


**Figure 4** Emerging roles of complement in health and disease. Besides the more 'classical' roles of complement in the elimination of microbial intruders and clearance of apoptotic debris (**Fig. 1b,c**), complement has important roles in cell homeostasis and disease. (**a**) In sepsis, high levels of infectious microorganisms in the blood cause excessive complement activation with release of C5a that contributes to devastating effects ranging from immune depletion (for example, by paralyzing neutrophils and inducing apoptosis in lymphocytes) to severe inflammation (by triggering a cytokine storm) and disseminated coagulation (partly by inducing TF), all of which may culminate in tissue damage, multi-organ failure and death. (**b**) Complement is also important in synaptogenesis, where it eliminates weak or immature synapses. An unknown signal derived from immature astrocytes promotes recognition by C1q, which leads to opsonization with C3b and iC3b and facilitates complement receptor (CR)-mediated phagocytosis by activated microglia. (**c**) Although the role of the C5L2 receptor in the immune response is unclear, it seems to be important for lipid metabolism. Adipocytes secrete C3, fB and fD, and this expression can be promoted by stimuli such as insulin or lipids, leading to a higher turnover of the alternative pathway and generation of C3a, which is transformed into the $C3a^{desArg}$ fragment (ASP). This fragment can induce lipid clearance, glucose uptake and triglyceride (TG) synthesis in adipocytes through C5L2 signaling. (**d**) Complement is likely to have a dual role in cancer. It contributes to protection through direct activation of complement or as part of the complement-dependent cytotoxicity (CDC) of tumor-directed therapeutic antibodies. However, many tumors escape complement attack by expressing and secreting complement inhibitors that largely prevent amplification, TCC formation or complement-mediated phagocytosis. The generation of C5a in the tumor microenvironment can attract myeloid-derived suppressor cells (MDSC) and induce the generation of reactive oxygen and nitrogen species (ROS and RNS, respectively) through the C5a receptor (C5aR), which impairs the tumor-directed effect of T cells.



embryogenesis[157]. Complement, and specifically C3a, also has important regulatory effects on the differentiation and migration of neural progenitor cells[158], thereby affecting neurogenesis.

Complement is also involved in prostaglandin synthesis in bone, which is considered important for the metabolism and physiological remodeling of bone[159–161]. In particular, sublytic signaling by C5b-9 or C5b-8 activates phospholipase A2, releases arachidonic acid and induces the synthesis of prostaglandin $E_2$ in synovial cells or macrophages[160,162]. However, certain autoimmune and inflammatory diseases involve complement-associated and prostaglandin-dependent bone immunopathology[124,126,163–165]; both rheumatoid arthritis and periodontitis have been associated with a SNP in the gene for C5 that is linked to increased concentrations of C5 in the serum[166,167]. Although these findings do not necessarily indicate that the C5b-9 pathway is involved, it is striking that CD59 deficiency causes enhanced cartilage and bone erosion in animal models, whereas it is reversed by C6 deficiency[168,169].

Although the immune and metabolic systems have evolved from common ancestors, functional links between these two central pillars of survival have only recently been drawn[170]. Metabolic surplus may trigger inflammatory pathways and mediators (termed metaflammation)[170], which has been associated with obesity and type II diabetes[171]. The secretion of regulatory adipokines and crosstalk between adipocytes and macrophages are crucial in this context[43], and complement seems to be an important mediator of this process. Adipocytes are the main source of fD, and its mouse homolog, adipsin, is important for the differentiation of preadipocytes. Adipocytes also produce C3 and fB, and C3 is substantially increased after exposure to insulin or dietary lipid (**Fig. 4c**). C3 has been described as a strong marker of insulin resistance in an elderly population[172]. The local secretion of components of the alternative pathway triggers a higher turnover of C3 into C3b and C3a, which is readily processed to $C3a^{desArg}$. Intriguingly, $C3a^{desArg}$ has been identified as the acylation-stimulating protein ASP, which has lipogenic activity and increases glucose uptake and triglyceride synthesis[43]. Whereas recent studies suggest that C5L2, which is expressed on adipocytes and preadipocytes, is involved in metabolic processes (**Fig. 4c**), it is unclear whether it interacts directly with $C3a^{desArg}$ (refs. 22,26,43). Importantly, *C3*$^{-/-}$ and *C5l2*$^{-/-}$ mice share a phenotype that is characterized by hypophagy, delayed lipid clearance and reduced triglyceride synthesis, and similar alterations in lipid metabolism were achieved after treatment of wild-type mice with neutralizing anti-ASP or anti-C5L2 mAbs. Anaphylatoxins may also have a direct effect on food intake regulation by the CNS; in mice, C3a promotes anorexia by influencing the prostaglandin $E_2$ pathway, whereas C5a stimulates food intake mediated by prostaglandin D and neuropeptide Y[173]. Although additional studies are warranted, the effects of complement on food intake and lipid metabolism make it an interesting target for future basic and translational studies of metabolic diseases.

## Dual role in cancer

On the basis of its involvement in immune surveillance and microbial defense, complement has long been assumed to have an active and beneficial role in the fight against malignant cells. PRMs, opsonins and effectors have been found on the surface of various tumor cells, suggesting that complement is substantially activated in the tumor microenvironment (**Fig. 4d**). Furthermore, complement-dependent cytotoxicity synergizes with tumor-directed antibody therapy[174]. However, most persisting tumors express high amounts of membrane-bound complement regulators, mainly CD46, DAF and CD59, which prevent amplification and TCC

formation (**Fig. 4d**). In addition, soluble regulators such as fH, FHL-1 and C4BP can be recruited to or secreted by tumors, thereby contributing to the tumors' complement evasion strategy[174]. Unexpectedly, the dogma that complement protects against tumors was further challenged when a recent study showed that classical pathway-induced generation of C5a in the tumor microenvironment leads to significant progression in tumor growth in a mouse model of cervical cancer[175]. This mechanism is mediated by the effects of C5a on two subpopulations of myeloid-derived suppressor cells (MDSCs) that represent immature monocytes and neutrophils[175]. In the cervical cancer model, C5a attracted neutrophil-like MDSCs to the tumor and induced the generation of ROS and reactive nitrogen species in monocyte-like MDSCs[175] (**Fig. 4d**), which interfere with the responses of T cells against tumor antigens[176]. Treatment of tumor-bearing mice with a C5aR antagonist slowed tumor progression to a similar extent as the conventional drug paclitaxel[175]. These findings suggest that C5aR-directed therapy has potential for use in certain cancers with an inflammatory component.

## Conclusions and outlook

Our perspective on the human complement system has experienced a remarkable transformation during the course of a century. After the discovery of complement as a microbial defense system at the dawn of the 20th century, the following decades provided a wealth of information about its specific function in infection and immunity. The turn of the current century seems to be similarly significant, as we now begin to envision complement as a global mediator in immune surveillance, cell homeostasis and tissue development and repair (**Fig. 3**), and we eagerly await even deeper insights into these and other roles. Ongoing improvements in animal models, systems biology, GWAS, molecular and cellular techniques and clinical diagnostics will direct the field into new endeavors. At the same time, our deepening understanding of complement's involvement in various diseases is likely to yield promising treatment options. Complement has truly come out of hiding and shown fascinating connections we had never before imagined, and these hidden connections might indeed be stronger than the original, obvious ones.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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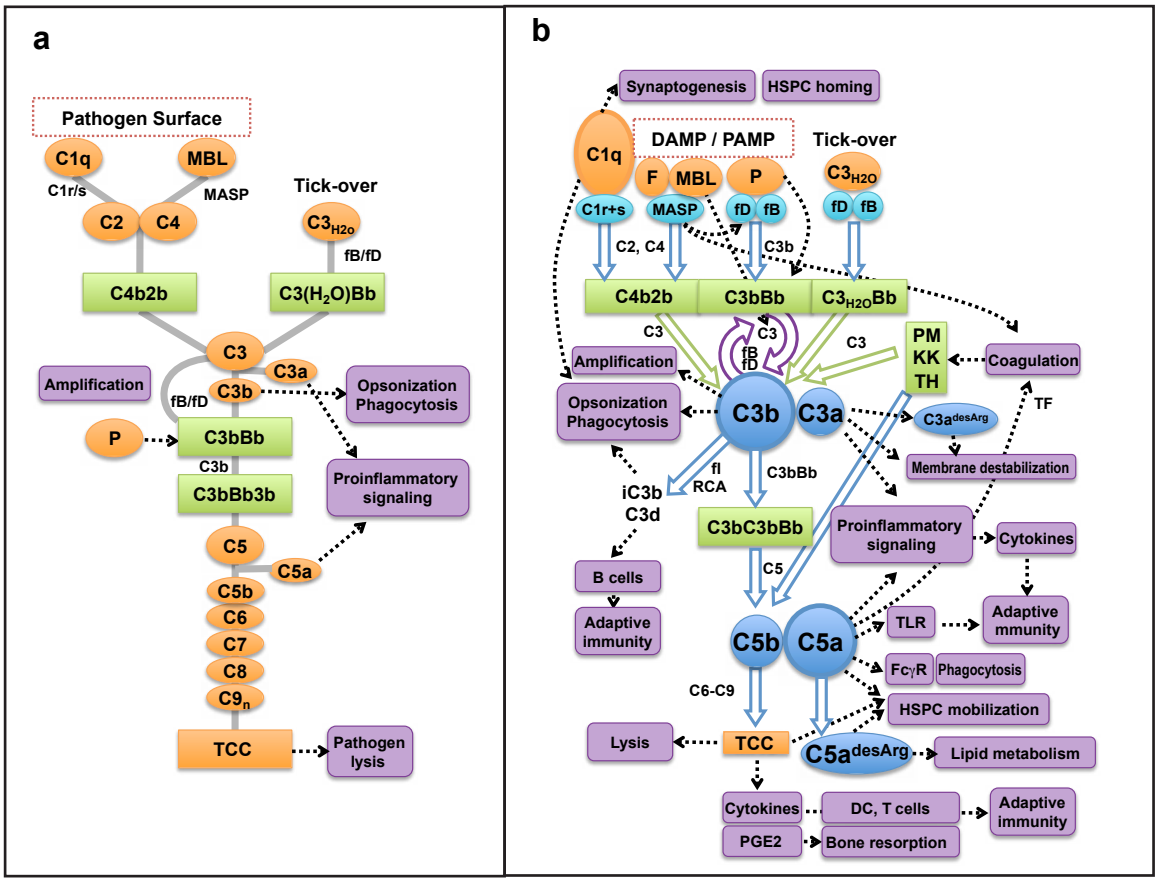
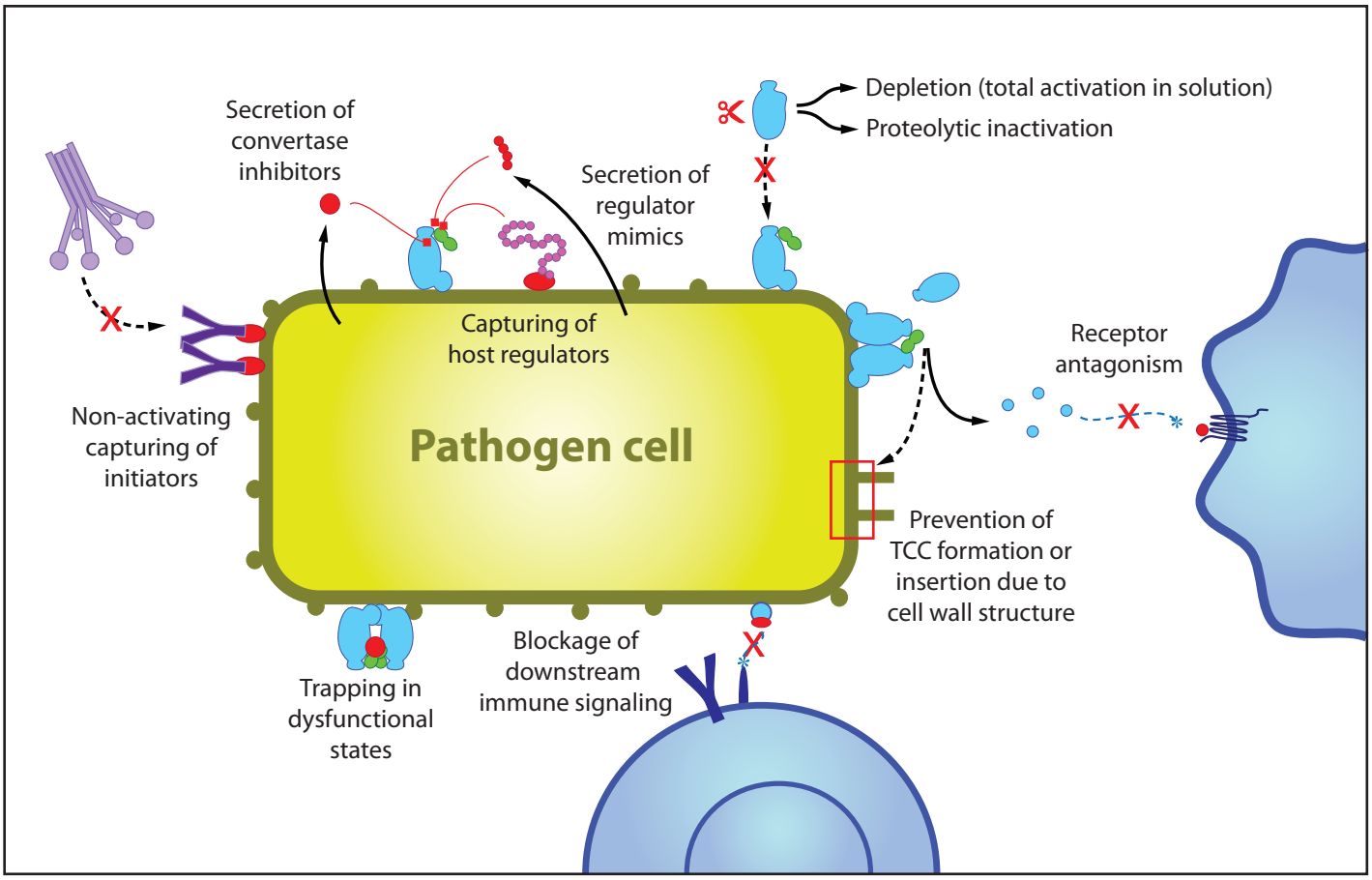
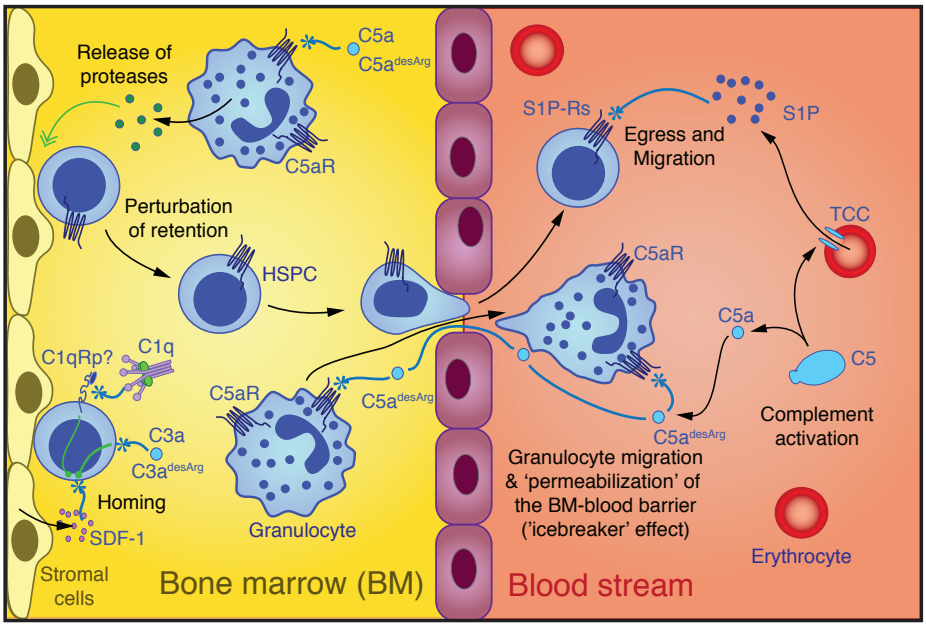
**Supp. Fig. 1** (Ricklin, Hajishengallis, Yang & Lambris: Complement - a key system for immune surveillance and homeostasis)




**Supplementary Fig. 1. Change of perspective on the complement system**. (a) The traditional view regards the complement system as a linear cascade of equal components, with the primary aim of eliminating pathogenic intruders by phagocytosis and lysis. (b) In our current perception, complement acts more like a dynamic network that contains several key functional hubs like C1q, C3b, or C5a that are primarily involved in versatile function in both immune surveillance and cell homeostasis by close crosstalk with other pathways. Both representations depict highly simplified arrangements to illustrate the change in paradigm. No individual complement regulators are shown for the purpose of clarity. Abbreviation of complement proteins can be found in Table 1. Additional abbreviations: DAMP, damage-associated molecular pattern; DC, dendritic cell; FcγR, Fc gamma receptor; KK, plasma kallikrein; HSPC, hematopoetic stem/progenitor cell; PAMP, pattern-associated molecular pattern; PM, plasmin; TF, tissue factor; TH, thrombin; TLR, toll-like receptor.




**Supplementary Fig. 2. Concepts of complement evasion strategies by human pathogens**. As part of their survival strategy, most human pathogens have developed elaborate mechanism to help them escaping the fatal grip of complement. Though each pathogen has a highly specific arsenal of evasion protein, most of them can be explained by some general concepts. The capturing of complement initiators (*e.g.*, immunoglobulins) in a manner that shields signaling sites affects both phagocytosis and activation of the cascade. While pathogen cells lack complement regulators on their surface, they often express surface proteins that recruit host regulators, or they secrete regulator mimics. Secretion of specific inhibitors may impair convertases directly or trap them in non-functional states. Whereas some inhibitors mask important sites for downstream immune signaling on complement fragments, other act as antagonists for complement receptors on immune cells. Furthermore, several pathogens secrete proteases that either cleave complement proteins to result in inactive fragments, or deplete complement response by consuming C3 in solution rather than on the pathogen surface. Finally, pathogens may avoid certain complement activities in a more passive way: for example, the cell wall chemistry of Gram-positive bacteria prevents insertion of the TCC.




**Supplementary Fig. 3. Effect of complement on hematopoetic stem/progenitor cells (HSPC).** In the current model of the involvement of complement in HSPC mobilization, various complement components exert distict roles. Complement activation (*e.g.*, during infection or inflammation) generates C5a and $C5a^{desArg}$, which act on granulocytes in the bone marrow (BM) to release proteases that perturb the retention of HSPC. $C5a^{desArg}$ also provides a chemoattraction signal for granulocytes from the BM to the blood stream. The accompanied 'permeabilization' of the BM-blood barrier paves the way for the subsequent egress of HSPC into the blood stream ('icebreaker effect'). The necessary attraction gradient is likely provided by lysis of erythrocytes through TCC(as a product of the complement activation) and release of sphingosine-1-phosphate (S1P), which is known to attract HSPC via binding to S1P receptors. On the other hand, complement is also involved in retention or homing as both C3 activation products (C3a, $C3a^{desArg}$) and C1q have been described to strengthen the retention of HSPC in the BM.