OPINION

Complement — tapping into new sites and effector systems

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Abstract | Complement is traditionally known to be a system of serum proteins that provide protection against pathogens through direct cell lysis and the mobilization of innate and adaptive immunity. However, recent work indicates that the complement system has additional physiological roles beyond those in host defence. In this Opinion article, we describe the new modes and locations of complement activation that enable it to interact with other cell effector systems, such as growth factor receptors, inflammasomes and metabolic pathways. We propose that the location of complement activation dictates its function.

The complement system, which was discovered more than 100 years ago, is one of the oldest components of immunity and is central to the detection and destruction of invading pathogens[1–5]. Complement is a system of fluid-phase proteins (found in the blood, lymph and interstitial fluids) and cell membrane-bound proteins. The serum-circulating proteins, which are generally synthesized in the liver, are mostly present in an inactive pro-enzyme state, and the membrane-bound proteins comprise receptors and regulators of complement activation fragments. The detection of microorganisms that have breached the host environmental barriers by fluid-phase complement components leads to the activation of the complement cascade and the elimination of the microbial target (FIG. 1a). The complement cascade can be activated by three pathways: the classical, alternative and lectin pathways[1–5] (FIG. 1a). All activation pathways lead to the generation of the C3 and C5 convertase enzyme complexes, which cleave C3 into the anaphylatoxin C3a and the opsonin C3b, and C5 into the anaphylatoxin C5a and into C5b, respectively. Deposition of C5b onto a target initiates membrane attack complex (MAC) formation and target lysis[1]. The opsonins and anaphylatoxins promote phagocytic uptake of pathogens by scavenger cells, and activate neutrophils, monocytes and mast cells, respectively[1–5]. On the basis of these effector functions, complement has long been considered as an innate immune pathway.

However, the discovery that receptors for complement activation fragments are expressed by almost all immune cells — including B cells and T cells — and that these cells can sense and convert the levels of complement activation into tailored responses[6] led to the appreciation that complement directs both innate and adaptive immune responses. For example, complement receptor activation lowers the threshold for B cell activation, directs antigen handling by follicular dendritic cells (FDCs) and contributes to the maintenance of B cell tolerance and memory[7,8]. Similarly, complement has a non-redundant role in $CD4^+$ and $CD8^+$ T cell activation and function, either directly through stimulating complement receptor-mediated signalling events in T cells or indirectly through modulating antigen-presenting cell (APC) function[9–11]. The appreciation of the role of complement in adaptive immunity coincided with the understanding that complement detects not only pathogenic microorganisms but also potentially harmful self molecules, such as those that are exposed by stressed, injured, apoptotic or necrotic tissues and cells[4]. The discovery that complement aids in the disposal of cellular debris and instructs the adaptive immune system provided the missing mechanistic explanations for the long-known but poorly understood finding that complement deficiencies predispose to autoimmune disease[12–15].

Recent studies are also providing a new dimension to our understanding of complement. Unexpectedly, it was shown that complement can be activated not only at the cell surface, as traditionally thought, but also in intracellular compartments[16]. Moreover, it is now becoming clear that systemic serum complement has different functions from local immune cell-derived complement. Rather than being a mostly pro-inflammatory effector system, complement is emerging as a central player in cell and tissue development, homeostasis and repair. Studies of the molecular mechanisms underlying these new functions of complement have led to the discovery of new crosstalk between complement components and other cell effector systems, including growth factor receptors, inflammasomes, metabolic sensors and the Notch system. In this Opinion article, we propose a model to explain how the different locations of complement activation dictate its diverse functions and how complement engages other effector systems at these locations to regulate immune-related and non-immune-related processes.

Novel aspects of complement activation

Immune cell-derived local complement. It is now clear that almost all cells in the human body can produce complement proteins (for example, bone marrow-derived cells contribute 40% of total C3 in the serum)[17], and that in some immune-privileged organs, such as the brain and eye, local production by astrocytes and epithelial cells, respectively, is the main source of complement[18,19].

The production of local complement can be induced and/or increased by pro-inflammatory cytokines, which suggests that extrahepatic complement production evolved to respond to environmental cues that signal the requirement or presence of an immune response[20–23]. In turn, increases in local complement can modulate cytokine production by immune cells[24–26], indicating the existence of bidirectional feedback loops.

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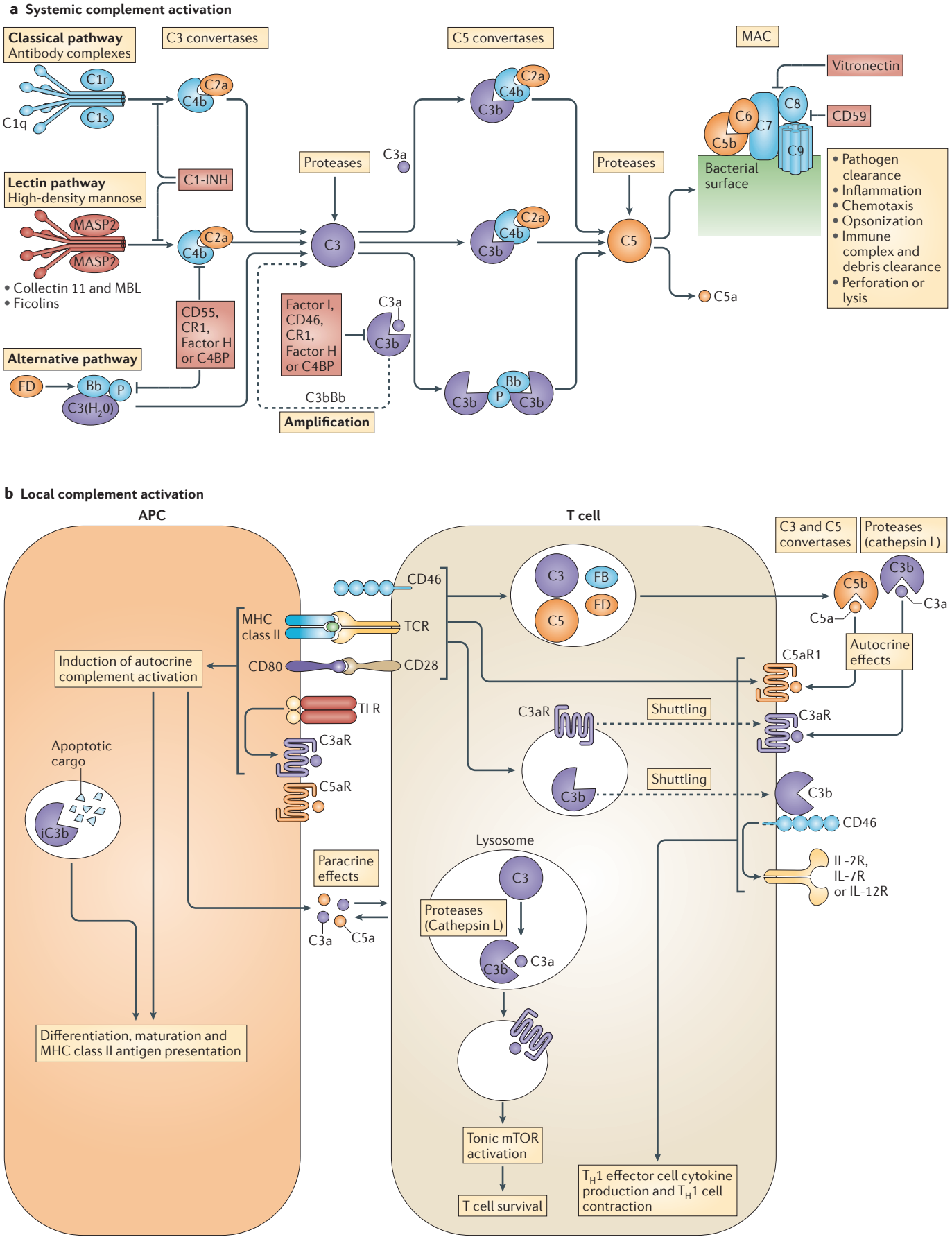

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The biological importance of extrahepatic local complement production was initially suggested by the finding that C4 produced by monocytes restores the impaired humoral response against tumour-derived antigens in serum C4-deficient mice[27], and that the local synthesis of C3 by kidney epithelial cells has a crucial pathophysiological role during renal transplant rejection[28]. The general assumption was that the activation of locally produced C4 (or C3 and C5) occurs via serum-derived convertases that form in the fluid phase and/or on the cell surface. However, studies using T cells and APCs as a model system in which to delineate the contributions of immune cell-derived complement during T cell activation demonstrated that these cells not only secrete C3 and C5 upon cognate interaction but also produce Factor B and Factor D, thereby providing the intrinsic activation machinery for local C3 and C5 convertase formation[9,29,30].

The C3a and C5a that are generated following a cognate T cell–APC interaction engage their respective G protein-coupled receptors (GPCRs), C3aR and C5aR1 (also known as CD88), on the T cell and APC in an autocrine manner (FIG. 1b) and mediate effector responses. These include the potentiation of Toll-like receptor (TLR)-driven APC maturation, the secretion of pro-inflammatory cytokines — such as interleukin-2 (IL-2), IL-12 and IL-23 — by APCs, and the induction of effector function in T helper 1 (T_H1) cells or T_H17 cells.

Intracellular complement. Both systemic and local complement activation were thought to be confined to the extracellular space; however, this view has recently been challenged by the finding that complement activation also occurs intracellularly[16] (FIG. 1b). Resting human $CD4^+$ T cells contain intracellular stores of C3 and the protease cathepsin L in endosomal and lysosomal compartments, and cathepsin L cleaves C3 into C3a and C3b. Intracellular C3 activation is not limited to T cells but is observed in a wide array of immune and non-immune cell types[16], suggesting that this novel location of complement activation may be of broad physiological significance. Interestingly, the complement-activating protease is likely to be cell type specific because although cathepsin L also processes C3 in monocytes, C3 in epithelial cells is activated by another currently undefined protease. This unexpected finding of intracellular complement activation has opened the door for the discovery of new functions for complement.

◀ Figure 1 | **Distinct location-directed functions of complement activation. a** | Liver-derived, systemically circulating complement forms the first line of defence against invading pathogens and can be activated through three pathways: the classical pathway, the lectin pathway and the alternative pathway, with the initial deposition of C3b on a surface also initiating a feedback amplification loop. Through the formation of C3 convertases (C4bC2a for the classical and lectin pathways, and C3bBb for the alternative pathway), these pathways culminate in the generation of the opsonin C3b and the anaphylatoxin C3a. Subsequent C5 convertase formation (C4bC2aC3b for the classical and lectin pathways, and C3bBbC3b for the alternative pathway) leads to C5b and anaphylatoxin C5a generation, with C5b initiating the formation of the membrane attack complex (MAC) and its insertion into target membranes. C3 and C5 can also be activated directly via activating proteases (see BOX 1). Self tissue is protected from complement deposition through fluid-phase and cell-bound regulators; C1 inhibitor (C1-INH) inhibits the functions of C1r, C1s and mannan-binding lectin-associated serine protease 2 (MASP2). C3b (and C4b) are inactivated by the serine protease complement Factor I and one of several cofactor proteins (surface-bound CD46 and complement receptor type 1 (CR1) or fluid-phase Factor H and C4b-binding protein (C4BP)). Convertases are regulated through disassembly by regulators that have decay-accelerating activity — surface-bound CD55 and CR1 or fluid-phase Factor H and C4BP — and the formation of the MAC is controlled by CD59 and vitronectin (also known as S protein)[113]. **b** | Locally occurring complement activation is triggered when a cell-activating signal (such as T cell receptor (TCR) stimulation) initiates the generation and secretion of C3, C5, Factor B (FB) and Factor D (FD), leading to C3 and C5 convertase formation in the extracellular space and/or on the cell surface, and ultimately to the generation of the complement activation fragments C3a, C3b, C5b and C5a. C3a, C3b and C5a bind to their respective receptors on the T cell and induce cellular responses. Intracellular complement activation in resting $CD4^+$ T cells (and possibly other cell types) occurs continuously through the action of the C3-cleaving protease cathepsin L. The resulting C3a fragment engages the intracellular lysosome-localized receptor C3aR, which sustains tonic mammalian target of rapamycin (mTOR) activation and T cell survival (resting T cells express C3aR only intracellularly). TCR activation induces cell-surface translocation (shuttling) of this intracellular C3 activation system (indicated by the dashed arrows), where engagement of surface C3aR and CD46 induce intracellular signalling events (for details on these signalling events, see REFS 11,35) that ultimately mediate upregulation of key growth factor receptors — including the receptors for interleukin-2 (IL-2), IL-7 and IL-12 (IL-2R, IL-7R and IL-12R, respectively) — as well as proliferation and the induction of effector function. Autocrine complement receptor activation in antigen-presenting cells (APCs) is triggered by Toll-like receptor (TLR) activation and mediates APC maturation and the expression of MHC class II and co-stimulatory molecules, as well as cytokine production. The sum of autocrine and paracrine effects of local complement activation during cognate APC and T cell interactions defines the functional outcome of T cell activity. Although not depicted here, the cell-surface expression of complement regulators affects these processes by regulating local complement activation[9,30,36]. Furthermore, the C3 activation fragments inactive C3b (iC3b) and C3dg are deposited extracellularly on apoptotic cells and are then taken up by APCs; here, they regulate lysosomal fusion, processing of apoptotic cell debris and subsequent antigen presentation by an as yet undefined mechanism[48]. MBL, mannose-binding lectin; P, properdin; T_H, T helper.

Location dictates complement function

Functions of extracellular complement. The location of complement activation substantially influences the functional outcome of this reaction. For example, it is clear that systemic C3 and C5 are key for providing protection against invading pathogens through the direct detection, opsonization and induction of phagocytic uptake of microorganisms, and the recruitment of immune cells (FIG. 1a). Accordingly, patients with a deficiency in these components suffer from recurrent infections[12].

However, an emerging paradigm indicates that locally produced and autocrine-functioning complement has a crucial role in the induction and modulation of T cells, particularly in the differentiation of T_H1 cells. For example, in humans, stimulation of C3aR and the complement regulator CD46 by T cell-generated C3a and C3b, respectively, during T cell receptor (TCR) activation is a requirement for the induction of T_H1 cells (FIG. 1b), and CD46-deficient or C3-deficient patients are unable to generate T_H1 cell responses, whereas T_H2 cell responses remain intact[31–33]. Interestingly, although CD46-deficient patients have compromised T_H1 cell responses throughout life, C3-deficient patients suffer from recurrent infections only during childhood and not adulthood[34], which suggests that either C4 (C4b is also a CD46 ligand) and/or other adaptive immune pathways may compensate for C3 deficiency over time. The contribution of local complement activation to normal human T_H1 cell generation is achieved at least in part through CD46-mediated regulation of IL-2 receptor (IL-2R) assembly, the induction of protein kinase B (PKB; also known as AKT) phosphorylation and the regulation of *IL2* promoter activity[31,35].

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Studies using bone marrow chimaera experiments have demonstrated that systemic complement cannot functionally substitute for immune cell-derived complement. Accordingly, in contrast to chimeric mice that had received bone marrow from wild-type mice, chimeric mice that had received C3-deficient bone marrow cells did not respond to alloantigenic stimuli[29,36]. Furthermore, naive T cells from *C3ar1*$^{-/-}$ *C5ar1*$^{-/-}$ animals were unable to assume a T_H1 cell phenotype, even in the presence of systemic C3 (REFS 9,29,37). In mice, anaphylatoxin receptor-mediated signals in T cells induce the upregulation of expression of the IL-12 receptor β1 chain and the initiation of an anti-apoptotic programme[29], both of which are required for T_H1 cell development and T cell proliferation[9,29]. Moreover, the absence of anaphylatoxin receptor-transduced signals in APCs and T cells during their cognate interaction in *C3ar1*$^{-/-}$*C5ar1*$^{-/-}$ animals triggered the production of transforming growth factor-β (TGFβ) by both T cells and APCs, and the subsequent differentiation into forkhead box P3 (FOXP3)-expressing regulatory T (T_{Reg}) cells[38,39]. Conversely, forced anaphylatoxin receptor signalling in T_{Reg} cells — achieved by adding exogenous C3a and/or C5a to cultures — diminishes the suppressive function of T_{Reg} cells by inducing the phosphorylation of forkhead box protein O1 (FOXO1), which reduces FOXP3 expression[40].

It should be noted that the presence of C5aR1 on mouse T cells is debated; some groups (see above) have reported C5aR1 expression in activated T cells in mice[9,29,38,40], whereas other groups cannot observe C5aR1 expression on resting or stimulated T cells[41–43]. We can confirm intracellular C5aR1 expression in resting and activated human $CD4^+$ T cells (A. Fara, G. Arbore and C. Kemper, unpublished observations). The reasons for the observed discrepancies in C5aR1 expression by mouse T cells are unclear but could reflect species-specific differences, or differences in the activation conditions used and/or the sensitivity of the C5aR1 detection reagents used.

Besides T cells, other immune cells also respond to complement in an autocrine manner. For example, upregulation of the expression of MHC class II molecules and the co-stimulatory molecules CD40 (also known as TNFRSF5) and CD86 in response to TLR activation is defective in C3-deficient mouse and human dendritic cells (DCs)[44,45]. In addition, a lack of C5aR1 signalling in TLR2-stimulated mouse DCs increased their secretion of TGFβ, IL-6 and IL-23, leading to amplified T_H17 cell development[46].

Immune cell-derived complement can also function in a paracrine manner, in particular during cognate cell interactions, during which APC-derived anaphylatoxins support naive T cell activation[29,31,32] (FIG. 1b). Of note, rodents lack CD46 expression in somatic tissues[35] and a functional homologue has not been identified, indicating that there are substantial differences in the complement signalling pathways that regulate T cell immunity between species (as reviewed in REFS 10,11,35).

Functions of intracellular complement. In principle, the tonic generation of intracellular C3a and C3b by cathepsin L (FIG. 1b) may arm the cell for a rapid response to 'danger' (for example, through TCR activation) and provide an explanation for the observed appearance of autocrine C3 fragments on T cell surfaces within minutes of activation[16,33]. It is likely that TCR-induced surface shuttling of intracellularly generated C3 activation fragments and extracellular complement activation do not operate in isolation, and that each system is affected by the other — these effects remain to be explored. Other areas for future investigation include understanding how intracellular C3 compartmentalization and shuttling of C3 activation fragments are regulated. However, intracellular complement activation also has another non-immune-related purpose. Intracellularly generated C3a engages the C3aR, which is mainly located in lysosomes of resting T cells, and sustains homeostatic T cell survival through basal activation of mammalian target of rapamycin (mTOR)[16] (FIG. 1b). Exactly how lysosomal C3aR expression links to mTOR activity still needs to be defined. Inhibition of intracellular C3a generation by a cell-permeable cathepsin L inhibitor or reduction in C3aR expression induces T cell apoptosis[16]. Importantly, serum-derived extracellular C3a cannot rescue T cell survival in the absence of intracellular C3a[16]. Upon TCR engagement, intracellularly generated C3a and C3b shuttle to the T cell surface (as does C3aR), where they induce protective T_H1 cell responses by stimulating surface-bound C3aR and CD46 (REF. 31). This suggests that C3aR-mediated signalling events that are induced within intracellular compartments differ from those that are triggered by cell-surface engagement of C3aR, and that it is the cellular location of the receptor that defines the outcome of complement activation. Functional differences between inside-in versus outside-in receptor signalling have recently been shown for another GPCR, the α2-adrenoceptor[47], and are also in keeping with the observations that intracellular C3a activation fragment-induced signalling (via the engagement of receptors that are yet to be defined) regulates lysosomal fusion and apoptotic cargo trafficking in mouse DCs[48]. In addition, intracellular sensing of C3 fragments that are deposited onto cell-invading pathogens triggers a mitochondrial antiviral signalling protein (MAVS)-driven immune response and degradation of the pathogens by the proteasome[49]. Also, intracellular CD59 guides insulin exocytosis in mouse pancreatic islets through interactions with soluble NSF attachment protein receptor (SNARE) proteins[50]. Further support for the idea that C3aR has different functions at distinct cellular locations is the finding that individuals with a serum C3 deficiency do not secrete C3 or C3 fragments, and they have severely diminished T_H1 cell responses but can generate sufficient amounts of intracellular C3a to mediate T cell survival[16]. Thus, combined extracellular and intracellular C3 deficiency may not exist in humans.

The discovery of intracellular complement activation may have implications for the design of next-generation therapeutics that target complement. Indeed, T cells from patients with autoimmune arthritis have substantially increased levels of intracellular complement activation and mTOR activity[16]. Importantly, inhibition of intracellular cathepsin L normalizes T_H1 cell responses in these patients, indicating that this intracellular system is amenable to therapeutic intervention and that proteases that activate intracellular complement are potential therapeutic targets (BOX 1).

Thus, complement activation is not confined to the extracellular space but occurs within cellular compartments, where it engages intracellular complement receptors to trigger functions that are distinct from those that are triggered by the same receptors at the cell surface. The identification of distinct functions for intracellular complement and the recent descriptions of new links between complement and other cell effector systems explain how complement can have such a broad range of immune-related and non-immune-related functions.

Crosstalk with other effector systems

The functional importance of the intersection of the complement system with the coagulation system and TLR pathways (FIG. 2) has long been known. For example, C3 and

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C5 can be activated by several proteases of the coagulation cascade (BOX 1), mannan-binding lectin-associated serine protease 2 (MASP2) converts prothrombin to thrombin[51], and C5aR1 signalling induces tissue factor expression in endothelial cells, which in turn triggers the initiation of the coagulation pathway by activating factor VII[52]. Such bidirectional crosstalk also occurs between complement and TLRs; these two systems can synergize to induce and shape innate and adaptive immune responses and also, anaphylatoxin receptor signalling can negatively regulate TLR activity (for detailed reviews, see REFS 6,52,53).

Novel crosstalk serving immune-related functions. Studies of T cells from CD46-deficient patients have shown that CD46 regulates the expression of CD127 and CD132 (also known as IL-7RA, and IL-2RG and γc, respectively), which together form the receptor for IL-7 (REF. 31). IL-7 sustains T cell homeostasis, and enhances T_H1 cell and T_H17 cell responses[54], and importantly, the *CD127* gene has been identified as a strong risk factor for the T cell-driven disease multiple sclerosis[55]. In addition, dysregulation of CD46-mediated signals on T cells has been linked to the progression of multiple sclerosis[56]. However, CD132 is also a component of the receptors for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 (the IL-2R family)[54], most of which are involved in normal lymphocyte function. As IL-2-mediated signalling through the high-affinity IL-2R — composed of CD25 (also known as IL-2RA), CD122 (also known IL-2RB) and CD132 — is required for T_H1 cell induction[54], CD46 contributes to normal T cell function by regulating the expression of cytokine receptors that are required for homeostasis and activation[57] (FIG. 2). Interestingly, simultaneous ligation of CD46 and IL-2R drives IL-10 production and the contraction of T_H1 cell responses[33] (FIG. 2), and the lack of upregulation of a specific CD46 isoform that is required for IFNγ and IL-10 production in effector T cells characterizes human non-IL-10-producing thymus-derived T_{Reg} cells[16]. As mentioned, C3aR-mediated and C5aR1-mediated signals regulate the expression of the IL-12R β1 chain by mouse T cells[9,29], but it is not known whether complement regulates the expression of the IL-2R family in mice, as has been shown in humans.

The study of complement-driven pathways that mediate T cell contraction[23,33] has contributed to our understanding that complement, which is commonly thought to be pro-inflammatory, also has many key anti-inflammatory roles (BOX 2). A recent finding that the complement pathway intersects the Notch pathway further supports this. On resting T cells, CD46 sequesters the Notch ligand Jagged 1, thereby preventing the T cell-activating interaction between Jagged 1 and Notch[31]. In the presence of TCR activation, CD46 is engaged by autocrine C3b, which promotes the upregulation of IL-2R that is required for T_H1 cell differentiation[31] (FIG. 1b). Simultaneously, CD46 activation also induces shedding of CD46 from the T cells, which releases Notch for a T_H1 cell-activating interaction with Jagged 1 (REF. 35). Furthermore, CD46 regulates the expression of several Notch receptors and ligands[31], and we anticipate that the connection between these two systems will have additional roles, particularly in developmental processes (see below).

Recent work has revealed links between complement and another ancient system, the inflammasome (FIG. 2). C3aR-mediated signals induce activation of the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3; also known as NALP3) inflammasome and the subsequent production of IL-1β (which is encoded by *IL1B*) by human monocytes[25]. This is achieved by

Box 1 | **The emerging importance of protease-mediated complement activation**

Recent work has discovered unexpected new locations for complement activation. What is also becoming apparent is that 'alternative' C3 and C5 activation by specific proteases (that is, activation in a convertase-independent manner) is likely to be of greater physiological importance than previously anticipated. Most bacteria generate proteases that proteolytically inactivate C3, C4 and/or C5, thereby protecting them from lytic complement attack[92]. Similarly, malignantly transformed human cells have been shown to secrete C3-degrading proteases to prevent their complement deposition-mediated killing and clearance[93]. However, several proteases belonging to the co-agglutination system[94,95] and the evolutionarily old cathepsin and granzyme families activate C3 and C5 efficiently[16,96,97] (see the table). Such convertase-independent complement activation has a role in trauma and sepsis, in which the increase in C3-activating and C5-activating granzyme B, cathepsin D and factor VII-activating protease (also known as HABP2) correlates with anaphylatoxin generation and injury score in patients[97], and with thrombin-mediated C5 activation in the genetic absence of C3 in mice[95].

Activation of complement by a 'single' protease is rapid, requires less energy expenditure and may have evolved before convertase enzyme complex-mediated 'activation', suggesting that it may have important far-reaching functions that are yet to be discovered. Indeed, a recent study showed that neutrophil cathepsin G, elastase and protease 3 inactivate the C5a receptor C5aR1, thereby modulating neutrophil function[98]. Lastly, proteolytic activation may not be the only way in which to activate complement, as phospholipids on the surface of liposomes can also induce C3 activation by hydrolysis of C3 to $C3(H_2O)$[99]; this C3 activation mechanism is also observed on other cell interfaces and *in vitro* systems[100]. Thus, research into convertase-independent complement activation poses an interesting area to explore and may deliver some novel and unexpected therapeutic targets to control complement.

Protease	Species	Complement proteins cleaved	Active fragments generated?	Study type	Refs
Cathepsin L	Human and mouse	C3	Yes	*In vitro*	16
Cathepsin D	Human	C5	Yes	*In vitro*	96
Factor VII-activating protease	Human	C3 and C5	Yes	*In vitro* and *in vivo*	94
Thrombin	Human and mouse	C3 and C5	Yes	*In vitro* and *in vivo* (mice)	95
Factor Xa	Human	C3 and C5	Yes	*In vitro*	52
Factor XIa	Human	C3 and C5	Yes	*In vitro*	52
Granzyme B	Human	C3 and C5	Yes	*In vitro*	97
Trypsin	Human	C3	Yes	*In vitro*	101
Snake venom metallo-proteinases	Snake	C3, C4 and C5	Yes	*In vitro*	102
Kallikrein	Rabbit and human	C3 and C5	Yes	*In vitro*	103
Elastase	Human	C3	Yes (C3d and C3c)	*In vitro*	104

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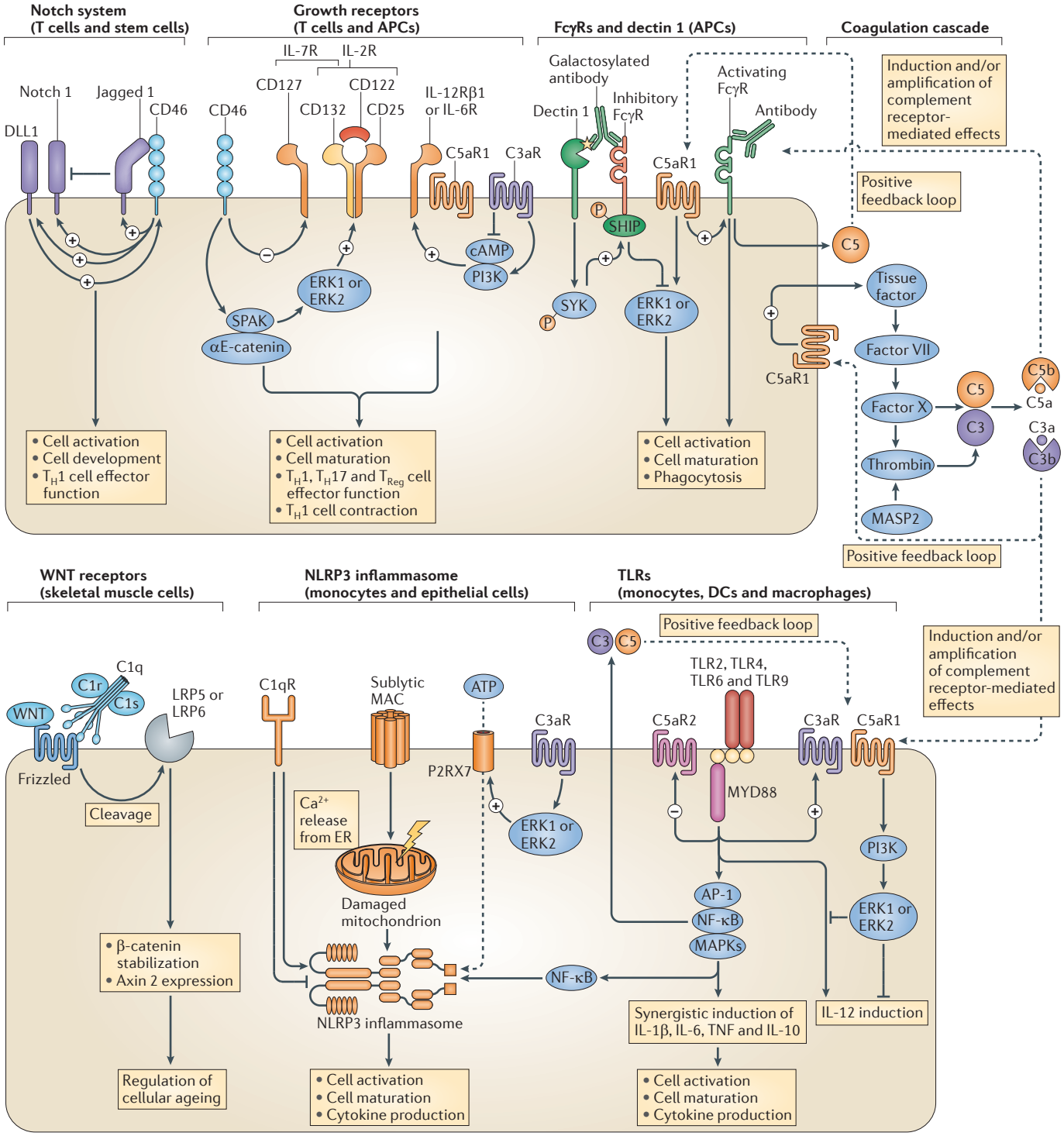

Figure 2 | **Functional crosstalk between complement and other cell effector systems.** The functional crosstalk between the complement system and Toll-like receptors (TLRs) and the coagulation cascade has long been acknowledged. The recent developments in the field have led to the discovery of additional direct crosstalk with key effector systems, including the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome, carbohydrate receptors (such as dectin 1), Fc receptors for IgG (FcγRs), and cytokine and growth factor receptors, as well as the WNT and Notch systems. Cell populations in which this crosstalk occurs are indicated and, where identified, the signalling pathways driving the functional outcome of the crosstalk between complement and effector systems are shown. The regulation of the mammalian target of rapamycin (mTOR) metabolic sensing system by complement is not included here but the current knowledge about this crosstalk is summarized in REFS 23,39. '+' denotes upregulation; '–' denotes downregulation; AP-1, activator protein 1; APC, antigen-presenting cell; cAMP; cyclic AMP; DC, dendritic cell; DLL1, delta-like ligand 1; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; IL, interleukin; LRP, low-density lipoprotein receptor-related protein; MAC, membrane attack complex; MAPK, mitogen-activated protein kinase; MASP2, mannan-binding lectin-associated serine protease 2; MYD88, myeloid differentiation primary response protein 88; NF-κB, nuclear factor-κB; P2RX7, P2X purinoceptor 7; PI3K, phosphoinositide 3-kinase; SHIP, SH2 domain-containing inositol-5-phosphatase; SYK, spleen tyrosine kinase; R, receptor; SPAK, ST20/SPS1-related proline-alanine-rich protein kinase; T_H, T helper; TNF, tumour necrosis factor; T_{Reg}, regulatory T.

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triggering the activation of extracellular signal-regulated kinase 1 (ERK1) and ERK2 downstream of C3aR ligation, which leads to increased efflux of ATP that then engages its cell-surface receptor P2X purinoceptor 7 to activate the NLRP3 inflammasome[25]. C5aR1-mediated signals also contribute to inflammasome activation in monocytes, as cholesterol crystal-triggered activation of the classical complement pathway induces IL-1β production in a C5a-dependent manner by increasing *IL1B* gene transcription, as well as caspase 1 activation[58]. Sublytic MAC formation mediates NLRP3 activation in mouse DCs[59] and in human lung epithelial cells by mediating the release of Ca^{2+} from the endoplasmic reticulum[60], and complement component C1q has been identified as an NLRP3 activator in human retinal epithelial cells[61]. Importantly, complement also has negative effects on the inflammasome, as C1q suppresses NLRP3 activation during apoptotic cell uptake by macrophages by an as yet undefined mechanism[62].

Other evidence suggests that there may be similar interactions between complement and other inflammasomes, such as the NLRP1, NOD-, LRR- and CARD-containing 4 (NLRC4) and absent in melanoma 2 (AIM2) complexes[63], or other intracellular danger sensors (besides intracellular TLRs[64]), such as the nucleotide-binding oligomerization domain (NOD)-like receptors and the retinoic acid inducible gene-I (RIG-I)-like receptors[65]. Indeed, virus-induced expression of the receptor for the globular heads of C1q (gC1qR) on mitochondria negatively regulates antiviral responses that are dependent on RIG-I and myeloid differentiation-associated protein 5 (MDA5; also known as IFIH1)[66], whereas C1qa expression positively regulates RIG-I activation and type I IFN production, thus supporting host antiviral responses[67].

Effector system crosstalk is complex and often multifactorial (FIG. 3), as recently shown for the interaction between Fc receptors for IgG (FcγRs), complement and the carbohydrate-binding receptor dectin 1 (also known as CLEC7A). Activating FcγRs and C5aR1 have been shown to promote inflammation in experimental autoimmune nephritis, arthritis and peritonitis[68] owing to a C5aR1-mediated increase in the number of activating versus inhibitory FcγRs[6]. Karsten *et al.*[69] demonstrated that this relationship is bidirectional and that dectin 1 has a central role in this crosstalk. High *N*-glycan galactosylation of IgG1 molecules promotes cooperative signalling of the inhibitory receptor FcγRIIB and dectin 1. This leads to the phosphorylation of SH2-containing inositol-5-phosphatase (SHIP; also known as INPP5D) downstream of FcγRIIB and spleen tyrosine kinase downstream of dectin 1, with subsequent blockade of C5aR1-mediated activation of ERK1 and ERK2, and C5aR1-induced effector functions[69] (FIG. 2). This finding illustrates how post-translational modifications of antibodies can affect complement receptor pathways and underpins the important relationship between complement and the carbohydrate-recognition network.

Novel crosstalk serving non-immune-related functions. Beyond its immune-related functions, complement activity is also linked to non-immune-related pathways, such as development and tissue homeostasis. During normal development, C3a mediates the mutual cell attraction that is required for collective cell migration in *Xenopus laevis*[70], and collectin 11 (also known as CLK1) of the lectin complement pathway regulates the migration of neural crest cells[71]. Other studies suggest a role for complement in stem cell or progenitor cell fate decisions. It has been shown that CD46 expression induced by the Notch ligand Delta-like ligand 1 (DLL1) is crucial for human epidermal progenitor cell proliferation and self-renewal[72], and that anaphylatoxins support the maintenance of the pluripotent state of human embryonic stem cells[73], mediate the mobilization of haematopoietic stem cells from the bone marrow[74], and promote the migration and proliferation of cardiac pluripotent progenitor cells[75]. C3a and C5a receptors also facilitate osteoclast differentiation and bone formation[76,77].

At the level of whole organs, C1q is required for normal neuron maturation as it directs the elimination of excitatory synapses in the brain via 'synapse pruning' (REF. 78), and it can also protect neurons against fibrillar amyloid β-mediated injury through the induction of phosphorylated cAMP-response element-binding protein and activator protein 1 (AP-1), which are transcription factors that are associated with neuronal survival and neurite outgrowth[79]. By contrast, increased C1q levels correlate with a decline in synaptic plasticity, cognitive function and tissue regeneration during ageing by directly increasing the induction of WNT pathways[80,81], which suggests that targeting C1q could be a potential approach for the treatment of dementia.

The desarginated form of C3a (C3a-desArg; also known as acylation-stimulating protein)[82] and, as shown more recently, C3a itself[83] can be produced by adipocytes and stimulate triglyceride synthesis in adipocytes[84], and systemic complement has an important homeostatic

Box 2 | The 'anti-inflammatory face' of complement

The pro-inflammatory effects of complement are well acknowledged, but its important anti-inflammatory properties in immunity are less well appreciated. The anti-inflammatory effects of complement are commonly associated with the lack of complement activation fragment generation, and the recent finding that the absence of anaphylatoxin receptor stimulation during T cell priming induces regulatory T cell development supports this notion[38,40]. However, new studies suggest that complement has more active, negative regulatory roles in immune responses, in cell and tissue homeostasis, and in tissue repair[105]. A picture emerges in which cells provide not only the autocrine activating signals, via local complement activation, but also simultaneous inhibitory signals. Thus, the generation of initial activation fragments C3a, C5a and C3b mediates the immune cell-mobilizing effects of complement, but further processed forms of these fragments — for example, inactive C3b (iC3b), desarginated C3a (C3a-desArg) and C5a-desArg — then engage pathways with negative, tolerogenic and tissue reconstructive capacity[10]. Therefore, it is the balance between these distinct complement signals and the integration of environmental cues (such as growth factor availability and serum complement activation) that instructs cells to keep going or shut down.

Listed below are key observations underpinning the active anti-inflammatory role of complement (for detailed reading on this subject, see REF. 4).

- Complement receptor activation mediates T helper 1 cell contraction, and disturbances in this process contribute to pathology in multiple sclerosis and rheumatoid arthritis[33,56].
- Complement and Notch system crosstalk sustains $CD4^+$ T cell homeostasis[31].
- Complement receptor engagement on activated dendritic cells can induce a tolerogenic phenotype[106].
- Complement receptors restrain natural killer cell and neutrophil activation[107].
- Complement receptor-mediated signals are crucial for liver and retina regeneration after injury, and for bone healing[108–112].

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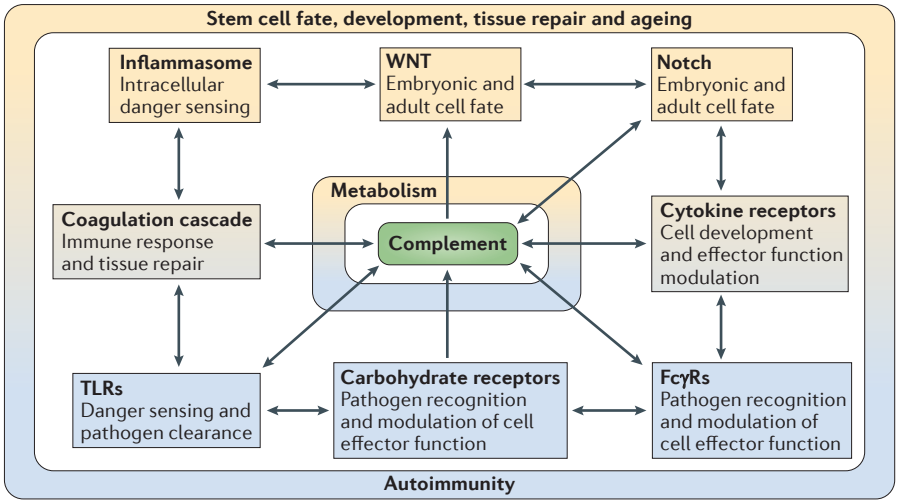

Figure 3 | **Complement at the nexus of the extensive crosstalk between cell effector systems.** The interaction between complement and other key cell effector systems involved in innate and adaptive immunity is multifactorial and in most cases bidirectional. Furthermore, the functional impact of complement on effector systems with primarily non-immune functions (for example, the Notch and WNT systems) is more substantial than previously thought, and indicates that complement contributes to normal development, and possibly to ageing and behaviour. We suggest that the emerging role of complement in core physiological metabolic pathways may be the crucial functional intersection point in this network. FcγR, Fc receptor for IgG; TLR, Toll-like receptor.

and regulatory role in metabolic organs such as the pancreas, liver and adipose tissue (as reviewed in REF. 85). The recently discovered link between intracellular C3aR-mediated signals and the mTOR network suggests that complement may also participate in metabolic sensing. mTOR integrates signals from pathways that sense cellular nutrient, oxygen and energy levels, and translates these signals into appropriate responses, such as apoptosis induction, inhibition of autophagy, and/or cell activation and proliferation[86,87]. We have recently found that CD46 controls the expression of amino acid and glucose transporters and the assembly of mTOR complex 1 (mTORC1) in activated $CD4^+$ T cells (M. Kolev, S. Dimeloe, C. Hess and C. Kemper, unpublished observations). Furthermore, C3a regulates proteasome activity and thereby normal protein turnover in human retinal epithelial cells[88]. This suggests that the connection between complement and metabolism may extend to sensing nutrient and/or cellular stress, and subsequent modulation of catabolic and anabolic metabolism at the single-cell level.

Conclusions and future perspectives

Complement has traditionally been defined as an innate and systemic system that functions in the defence against pathogens. However, it is now considered to be a central regulator of innate and adaptive immunity, with new functions that extend beyond protective immunity, including roles in cell generative, degenerative and regenerative processes. The finding that complement is activated within cells and not only engages intracellular complement receptors but also intersects with several other cell effector systems helps to explain its unexpectedly wide-reaching effects.

Nevertheless, there is still much to discover about this ancient system and key future questions include: how is intracellular complement generation and activation regulated? Does this novel pathway contribute to disease? Are additional complement components, including regulators, functionally active inside cells? In this regard, we have detected intracellular C5a (A. Fara and C. Kemper, unpublished observations) and several studies have reported intracellular expression of Factor D, complement receptor type 1 (CR1; also known as CD35) and the positive regulator properdin in resting cells[89,90]. Thus, one could envision the existence of an intracellular 'Complosome' — somewhat analogous to the inflammasome[91] — that has novel functions in cell survival and activation. Furthermore, a unifying feature of the new roles and interactions for complement is their reliance on appropriate sensing of cellular integrity and balanced control of energy and substrate metabolism. Therefore, the emerging cooperation between complement and the metabolic pathway network may arise as a core intersection point for the diverse functions of complement in immunity and beyond (FIG. 3).

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doi:10.1038/nri3761
Published online 14 November 2014

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Acknowledgements

The authors acknowledge their funding sources: the Medical Research Council, The European Union and The Wellcome Trust. Their research was also funded by the National Institute for Health Research (NIHR) Biomedical Research Centre at Guy's and St Thomas' NHS Foundation Trust and King's College London, UK. The views expressed are those of the authors and not necessarily those of the National Health Service, the NIHR or the Department of Health. The authors also acknowledge and thank P. Garred (University of Copenhagen, Denmark). The authors apologize to the groups whose work they were unable to cite owing to space constraints.

Competing interests statement

The authors declare no competing interests.

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