New insights into the immune functions of complement

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Abstract | The recognition of microbial or danger-associated molecular patterns by complement proteins initiates a cascade of events that culminates in the activation of surface complement receptors on immune cells. Such signalling pathways converge with those activated downstream of pattern recognition receptors to determine the type and magnitude of the immune response. Intensive investigation in the field has uncovered novel pathways that link complement-mediated signalling with homeostatic and pathological T cell responses. More recently, the observation that complement proteins also act in the intracellular space to shape T cell fates has added a new layer of complexity. Here, we consider fundamental mechanisms and novel concepts at the interface of complement biology and immunity and discuss how these affect the maintenance of homeostasis and the development of human pathology.

There are two sides to every question. Protagoras.

The evolution of intricate host defence systems has allowed mammals and other organisms to protect themselves against a variety of intruders, including viruses, bacteria, fungi and foreign bodies. Prototypes of innate immune mechanisms can be found in species throughout the evolutionary tree and are mainly based on detection of pathogens and other types of insult by pattern recognition molecules, including pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) and C-type lectin receptors, and complement[1]. Homologues of complement proteins, in particular, are found in very primitive invertebrates and are surprisingly conserved throughout evolution. The multiplicity of complement genes in lower species represents a mechanism for generating immune diversity, thus underscoring a vital role for complement in immunity[2,3]. In species located at the base of the evolutionary tree, complement is mainly responsible for microbial recognition and initiation of phagocytosis and inflammation. Notably, rather than fading away during the course of evolution, the complement system became more diversified through the emergence of additional proteins constituting the classical and lytic pathways. This evolution-driven expansion of the recognition and effector mechanisms of complement accompanied the appearance of elements representing the adaptive immune system, such as MHC-like molecules and the recombination activating genes (RAGs)[2]. As a result, in vertebrates, aside from participating in immunosurveillance and inflammatory mechanisms, complement gained additional roles in the regulation of T cell and B cell responses[4,5].

The notion that complement modulates adaptive immunity first appeared in the 1970s, with the observation that lymphocytes produce complement proteins and that complement activation fragments bind to cell surface receptors on lymphocytes[6–9]. These findings were further explored in the late 1990s and early 2000s after a series of high-profile reports elucidated molecular mechanisms by which complement affects B cell and T cell function[10,11]. The role of complement in B cell responses has been extensively debated and will not be addressed here[5,10,12]. In this Review, we revisit the main findings that uncovered novel molecular pathways linking complement-mediated recognition of conserved structural motifs — microorganism-associated molecular patterns (MAMPs) or endogenous stress signals, the so-called damage-associated molecular patterns (DAMPs) — with the modulation of innate and adaptive immune responses. We also offer a critical view of the complement-dependent polarization of T cell responses and its involvement in the development of pathological conditions in humans.

Complement senses danger signals

Complement proteins are currently perceived as sensors and transmitters of exogenous and endogenous danger signals — namely, MAMPs and DAMPs[13,14]. The recognition of such molecular patterns (for example, unique carbohydrate motifs on bacteria, fungi and viruses) by soluble complement proteins elicits a proteolytic cascade culminating in the generation of protein fragments that activate complement receptors on the surface of immune cells and other cell types[15] (BOX 1).

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Box 1 | **The complement system: an overview**

The complement system comprises a network of soluble and cell membrane proteins that work in a coordinated manner towards the activation of the classical, alternative and lectin pathways. The classical pathway is initiated by the binding of C1q to immune complexes, whereas the lectin and alternative pathways are triggered by the association of C3b, properdin, mannose-binding lectin (MBL) or ficolins with microorganism-associated molecular patterns (MAMPs) or carbohydrate structures exposed on damaged cells. Activation of any of these pathways results in the formation of convertases that cleave the C3 and C5 proteins, with consequent formation of the membrane attack complex (MAC; C5b–9) and generation of active fragments such as C3a, C3b, iC3b, C3dg, C4a, C4b and C5a. While the insertion of MAC into cell membranes results in cytolysis or cell activation, other activation fragments bind to their respective complement receptors present in a variety of cell types. Triggering of complement receptors culminates in biological responses that include phagocytosis, immune adherence and removal of immune complexes, cell migration, tissue regeneration, cell activation and modulation of pattern recognition receptor (PRR)-induced responses. Notably, the alternative pathway is in a constant state of low-level activation ('tickover'), allowing for immediate response upon microbial challenge. To avoid uncontrolled activation, the complement system is tightly regulated by proteins such as carboxypeptidases, factor H, factor I, complement receptor 1 (CR1), membrane cofactor protein (MCP), decay-accelerating factor (DAF) and CD59 that accelerate the decay of the convertases or mediate the cleavage of activation fragments. System malfunction as a consequence of genetic mutations or lack of proteins inevitably leads to insufficient or excessive complement activation that triggers and/or sustains a variety of pathological conditions, including renal, autoimmune, neurological, haemolytic and inflammatory diseases[137–139].

Within the complement cascade, C1q, mannose-binding lectin (MBL), ficolins, collectins and properdin are the main sensors of MAMPs, DAMPs and other potential stress cues, including antigen–antibody complexes and acetylated or oxidized lipid moieties[14,16].

Classical and lectin pathway initiators act as danger sensors. The molecular mechanisms involved in the activation of the C1 complex ($C1qr_2s_2$) and subsequent initiation of the classical pathway have recently been elucidated by cryo-electron microscopy and small-angle X-ray scattering[17,18]. Recognition of antigen–antibody immune complexes by C1q, with the consequent generation of C5a, is a key trigger for neutrophil activation and subsequent firm adhesion to the vascular endothelium; this can lead to inflammatory reactions in the joint endothelium that characterize immune complex-induced arthritis[19]. In addition to IgG and IgM immune complexes, C1q recognizes other structures (such as lipid A, β-sheet amyloid fibrils, pentraxins and apoptotic cells) and further binds to the so-called cC1qR, gC1qR and C1qRp receptors[20,21] (TABLE 1). Whereas the role of C1qRp is not fully elucidated, cC1qR and gC1qR promote the C1q-mediated uptake and phagocytosis of apoptotic cells and immune complexes by macrophages and dendritic cells (DCs)[22]. In addition to these conventional receptors, C1q also engages DC-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) and leukocyte-associated immunoglobulin-like receptor (LAIR1) on DCs and macrophages, modulating their differentiation and activation towards an anti-inflammatory phenotype[23–25]. Supporting the concept of C1q as a promoter of homeostasis, C1q-mediated uptake of apoptotic cells has been shown to limit inflammasome activation and the production of pro-inflammatory cytokines by phagocytes[26]. Consistently, C1q-deficient patients often suffer from lupus-like autoimmune disorders, characterized by the presence of autoantibodies and aberrant activation of immune responses[22,27,28]. In addition to downregulating inflammatory responses, C1q has also been shown to trigger mechanisms that modulate pathophysiological processes such as tissue repair, embryo implantation, cancer development and neurological function[22] (BOX 2). Interestingly, C1q has the ability to promote cell proliferation and angiogenesis, and this stimulates the healing of skin lesions but may also promote tumour growth[29–31].

Like C1q, other complement-associated pattern recognition molecules, including MBL, ficolins and collectins, play a role in host defence via recognition of microorganisms and activation of the complement lectin pathway[32–34] (BOX 1). Notably, individuals with low levels of functional MBL in their plasma often suffer from recurrent infections during childhood that appear to be associated with decreased phagocytic activity. In addition to enhancing phagocytosis, MBL has been implicated in the modulation of antigen-presenting cell (APC) responses. Indeed, when added to cultures of monocytic THP-1 cells, purified MBL suppresses the induction of pro-inflammatory cytokines in response to lipopolysaccharide (LPS) challenge. Furthermore, myeloid DCs isolated from the blood of MBL-deficient patients show increased production of inflammatory cytokines after stimulation with zymosan[33,35,36]. In line with the concept that pattern recognition molecules of the lectin pathway can modulate immune responses, genetic variants of MBL, ficolins and collectins have been correlated with the development of inflammatory diseases, such as cystic fibrosis, myocardial infarction, leprosy, rheumatoid arthritis and Chagas disease. Collectins, in particular, have a key role in binding to DAMPs expressed by the renal tissue in response to tissue hypoxia, thereby modulating complement activation, inflammation and tissue destruction[33,34].

Initiators of the alternative pathway also act as danger sensors. A potential role for properdin, the positive regulator of the alternative pathway (BOX 1), as a pattern recognition molecule and initiator of the alternative pathway has been extensively investigated in the past decade. Whereas experimental evidence suggests that properdin is able to bind zymosan, LPS and apoptotic cells and further activate complement, there are conflicting data regarding the requirement for initial binding of C3b before properdin deposition[37–40]. Most recently, in a mouse model of anti-glomerular basement membrane-induced renal injury, properdin deposition was detected in the injured glomeruli of C3-deficient mice, indicating that properdin can recognize DAMPs in the absence of C3b deposition[41]. It is noteworthy, however, that direct binding of properdin to DAMPs, independently of C3b, has been demonstrated only under non-physiological conditions when C3 is lacking, for example, in C3-deficient mice or in vitro assays with purified properdin. Despite the evident challenge of investigating properdin deposition under conditions closer to physiological ones, it is still uncertain whether the role of properdin in pattern recognition and

Table 1 | **Complement receptors**

Complement receptor (alternative names)	Ligands	Cellular expression	Function	Refs
CR1 (CD35)	C3b and C4b	Erythrocytes, leukocytes, retinal pigment epithelial cells, skin keratinocytes and kidney podocytes	Accelerate decay of convertases, cofactor for factor I, facilitate removal of immune complexes or particles coated with C3b or C4b and facilitate antigen presentation by B cells	15,163
CR2 (CD21)	iC3b, C3dg and C3d	Epithelial cells, B cells and FDCs	Co-stimulation of B cells, induction and maintenance of immunological memory and B cell tolerance	5,138,164
CR3 (CD11b–CD18 or MAC1)	iC3b	Leukocytes and FDCs	Leukocyte adherence, phagocytosis of particles coated with iC3b and modulation of IL-12 production by APCs	138
CR4 (CD11c–CD18)	iC3b	Leukocytes and FDCs	Leukocyte adherence and phagocytosis of particles coated with iC3b	138
C3aR	C3a	Granulocytes, monocytes, macrophages, subsets of pulmonary and intestinal DCs and activated human T cells	Cell migration, tissue regeneration, activation of eosinophils and macrophages, regulation of neutrophil responses and upregulation of IL-10 production by APCs	59,65
C5aR1 (CD88)	C5a and C5a-desArg	Granulocytes, monocytes, macrophages, NK and NKT cells, subsets of DCs, endothelial cells, epithelial cells and human T cells	Cell migration, tissue regeneration, activation of immune cells, modulation of IL-12 production by APCs and modulation of PRR responses	59,63,66
C5aR2	C5a and C5a-desArg	Granulocytes, monocytes, macrophages, subsets of DCs and human T and B cells	Poorly defined; evidence points to regulation of C5aR1 activation	59,64
CRIg	C3b and iC3b	Macrophages and Kupffer cells	Phagocytosis of opsonized particles and pathogens and regulatory effect on convertase formation	165
cC1qR (calreticulin)	Collagen domain of C1q	Ubiquitous, with the exception of erythrocytes	Phagocytic signalling through CD91	20,21
gC1qR (p33)	Globular domain of C1q	Ubiquitous, with the exception of erythrocytes	Potential role in phagocytosis; regulates $CD8^+$ T cell responses to autoantigens	20,28
C1qRp (CD93)	C1q	Highly expressed in platelets, monocytes and endothelial cells	Potential role in regulating phagocytosis of C3b-coated and C4b-coated antigens	21
MCP (CD46)	C3b	All nucleated cells	Cofactor for factor I-mediated cleavage of C3b and C4b, role in reproduction and modulation of T cell responses	131
DAF (CD55)	C3b and C4b	Ubiquitous	Accelerate decay of convertases, co-stimulate T cell activation and indirectly modulate T cell and APC responses (via regulation of complement activity)	166,167
CD59	C8 and C9	Ubiquitous	Prevent assembly of TCC and modulate responses of T, B and NK cells	168
PAR1 and PAR4	C4a and C4a-desArg	Predominantly expressed in endothelial, immune and epithelial cells	Modulate cell activation and endothelial permeability	92

APC, antigen-presenting cell; CR, complement receptor; CRIg, complement receptor of the immunoglobulin family; DAF, decay-accelerating factor; DC, dendritic cell; FDC, follicular dendritic cell; MCP, membrane cofactor protein; NK, natural killer; NKT, natural killer T; PAR, proteinase-activated receptor; PRR, pattern recognition receptor; TCC, terminal complement complex.

initiation of complement activation is relevant in vivo in individuals showing healthy levels of C3.

Apart from binding to C3b and stabilizing the convertases, properdin has also been suggested to bind to the natural killer cell-activating receptor NKp46, which is expressed by natural killer (NK) cells and other developmentally related group 1 innate lymphoid cells[42]. Interestingly, instead of promoting responses typically associated with NK cell activation, such as degranulation and secretion of IFNγ, the triggering of NKp46 by properdin results in the secretion of X-chemokine ligand 1 (XCL1), which has antimicrobial activity. Although the ability of properdin to protect mice against *Neisseria meningitidis* infection is impaired in the absence of NKp46, the precise biological role associated with this alternative NK cell activation profile requires further investigation[42].

C3 activation caused by C3 convertases, extrinsic proteases or spontaneous hydrolysis exposes an internal thioester bond in the C3b molecule that, during its very short life, forms amide or ester bonds with amino groups and carbohydrates present on microorganisms or host cells lacking complement-regulatory proteins[2,43] (BOX 1). C3b-opsonized antigens bind to erythrocytes expressing complement receptor 1 (CR1) and are transported to the liver and/or spleen, where they are phagocytosed by macrophages via CR1 or the complement receptor of the immunoglobulin family (CRIg)[44] (TABLE 1).

Box 2 | **Complement in neuroimmune responses**

Preclinical studies and human biopsies have positioned complement activation at the heart of an inflammatory cycle in the central nervous system (CNS) that involves intricate interactions between microglia, reactive astrocytes and neuronal synaptic networks[122,140]. Pattern recognition molecules, such as C1q, and diverse complement fragments and signalling effectors have been shown to modulate neuroimmune responses that underlie both basal neurodevelopmental processes and chronic, age-related neurodegenerative conditions that invoke aberrant synapse elimination, cognitive deterioration and progressive memory loss[140–144]. The discovery that C1q-triggered complement activation promotes the complement receptor 3 (CR3)-dependent microglial engulfment of iC3b-tagged synapses, thus sculpting the synaptic circuitry of the brain during development[141,145,146], led to a surge of studies that revealed a prerequisite role for C3-dependent and CR3-dependent pathways as drivers of aberrant microglial and astrocytic responses in various neurodegenerative diseases, ranging from Alzheimer disease and Huntington disease to Parkinson disease and multiple sclerosis[139,144,147–149]. Complement–microglial crosstalk aberrantly reactivates developmental programs of synaptic pruning that culminate in memory decline and cognitive impairment in models of Alzheimer disease and age-related frontotemporal dementia. In addition, genetic ablation of C3 or C3aR mitigated microglial-dependent synaptic loss and cognitive impairment in a murine model of neuroinvasive West Nile virus infection[143,144,150–152]. A complement-driven loss of inhibitory synapses was also implicated in the neuronal hyperexcitability that is associated with human epilepsy[153]. Moreover, complement activation was recently shown to promote the polarization of astrocytes towards a neurotoxic A1 phenotype that propagates neuronal damage in various neurodegenerative diseases, including Alzheimer disease and multiple sclerosis[154]. Reactive microglia can induce A1 astrocytes via IL-1α, TNF and C1q secretion, and upregulation of C3 expression in these A1 astrocytes can fuel both complement deposition on synaptic membranes and microglial activation, thus further increasing synaptic loss via $CR3^+$ microglia[154].

Our understanding of the fundamental role of complement in the early stages of CNS neurodegeneration has been refined by studies showing that C3 activation drives early synaptic loss, preceding amyloid plaque deposition in Alzheimer disease, and that genetic ablation of C3 attenuates synaptic impairment in a transgenic model of age-related Alzheimer disease (APP/PS1 mice)[143,144]. C3 activation and downstream C3aR signalling were recently implicated as early drivers of tau pathology in PS19 tau transgenic mice (PS19), with C3aR inhibition leading to attenuation of neuroinflammation, synaptic loss and neurodegeneration[155]. Similarly, C1q instigated aberrant microglia-dependent synaptic clearance in a model of amyloid P-associated tau pathology (Tau-P301S transgenic mice), with C1q blockage effectively reversing tau pathology and ameliorating neuroinflammation[156]. Collectively, these studies identify C3 and C1q as tractable targets for developing new immunomodulatory strategies in Alzheimer disease and other tau-related pathologies. The pervasive impact of an aberrantly activated complement–microglial axis on cognitive disorders is further reflected by genome-wide association studies and cell-based models of synaptic pruning in schizophrenia. A strong association of the *C4A* gene allele with schizophrenia risk was partly attributed to increased neuronal C3 deposition and excessive synaptic pruning by microglia in a C4-dependent manner[157].

Whereas autologous cells express a variety of regulatory proteins — such as CR1, CRIg, membrane cofactor protein (MCP; encoded by *CD46*) and decay-accelerating factor (DAF) — that avert complement activation and therefore deposition of C3b on the cell surface (TABLE 1), a wealth of data indicate the presence of C3b covalently bound to the surface of APCs under physiological conditions. Such deposition on the surface of human DCs is associated with increased expression of co-stimulatory molecules and production of cytokines by these cells[43]. Consistent with these findings, absence of C3 has been shown to impair the differentiation and maturation of human monocyte-derived DCs. Furthermore, DCs isolated from a C3-deficient patient showed an immature phenotype when compared with DCs from individuals with normal plasma concentrations of C3, suggesting that C3 is important for proper DC maturation[45,46].

The C3b molecule can be further cleaved by proteases, generating iC3b and C3dg fragments that can bind to CR2 (C3dg), CR3 (iC3b and C3dg) and CR4 (iC3b)[2,43,47] (TABLE 1). Whereas the biology of CR3 is better understood than that of CR4, the structural similarity between CR3 (a CD11b–CD18 dimer) and CR4 (a CD11c–CD18 dimer) suggests that both of these receptors are responsible for the phagocytosis of iC3b-opsonized antigens and apoptotic cells[43,48]. Furthermore, iC3b has been shown to promote the differentiation of mouse bone marrow cells into potent myeloid-derived suppressor cells (MDSCs)[49]. Notably, an immune regulatory role for iC3b has also been demonstrated in a model of antigen-specific induced tolerance, and a single-nucleotide polymorphism in the CD11b chain (rs1143679) has been identified as a risk factor for systemic lupus erythematosus (SLE)[43,50]. This supports the idea that CR3-mediated signals are associated with the suppression of inflammatory responses.

Intracellular danger sensing by complement. Growing evidence from the past 5 years indicates that the danger-sensing role of complement can also be manifested intracellularly in a tight crosstalk with other MAMP-recognition and/or DAMP-recognition systems[51–54]. One line of evidence suggests that hydrolysed C3 ($C3(H_2O)$), but not native C3, can be transferred intracellularly from the extracellular space[51]. Although a specific receptor has not yet been identified, $C3(H_2O)$ uptake appears to be a generalized phenomenon found in a variety of immune and non-immune cells[51,52]. Although $C3(H_2O)$ can be cleaved by intracellular proteases to generate C3a upon its internalization, most of the protein (80%) returns to the extracellular space, suggesting the operation of a $C3(H_2O)$ recycling pathway[51].

Extracellular C3-derived fragments coating non-enveloped viruses and bacteria can also be internalized via complement receptors and trigger mitochondrial antiviral signalling (MAVS). MAVS results in the activation of the NF-κB, activator protein 1 (AP-1) and interferon regulatory factor 3 (IRF3)–IRF5–IRF7 transcription pathways, with consequent production of pro-inflammatory cytokines (FIG. 1). Propagation of C3-coated viruses can, moreover, be restricted via the engagement of transitional endoplasmic reticulum ATPase (VCP) and proteasome-mediated degradation[54] (FIG. 1). As cell activation and rapid pathogen elimination occur in response to only C3-coated virus and not virus alone, these findings suggest that an intracellular PRR senses the C3-tagged microorganism and activates specific defence pathways. A similar mechanism appears to occur during infection with *Francisella tularensis*, in which uptake of iC3b-coated bacteria by macrophages results in membrane attack complex (MAC)-independent cell death, limiting the intracellular replication of the microorganism[55]. In addition, intracellular C3 has been associated with the regulation of autophagy via an interaction with autophagy-related protein 16-1 (ATG16L1). In a rodent model of *Listeria monocytogenes* infection, C3-coated bacteria, which are sensed in the cytosol in a C3-dependent manner, trigger ATG16L1-dependent autophagy and are thus

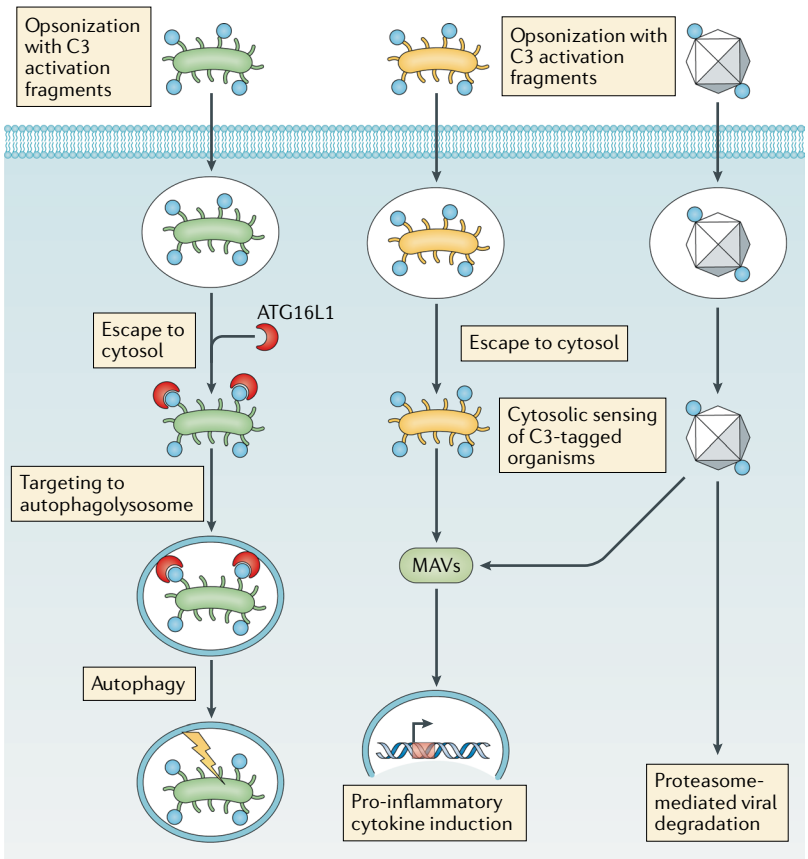

Fig. 1 | **Intracellular interactions and fates of microorganisms opsonized with C3 in the extracellular milieu.** On the left, targeting of C3-opsonized bacteria to the autophagy pathway is shown. Upon internalization, C3-coated bacteria are detected in the cytosol by autophagy-related protein 16-1 (ATG16L1), which interacts directly with C3. This interaction initiates ATG16L1-dependent autophagy, which leads to the targeting of the bacteria to autophagolysosomes for degradation. On the right, cytosolic sensing of C3-coated microorganisms is shown to trigger host defence. Bacteria and non-enveloped viruses that had been opsonized with C3 activation fragments in the extracellular setting can be sensed in the cytosol in a C3-dependent manner. This detection induces mitochondrial antiviral signalling (MAVS), resulting in the activation of pro-inflammatory responses. Cytosolic detection of C3-opsonized viruses can, moreover, lead to proteasome-mediated viral degradation.

targeted to autophagolysosomes for degradation[56] (FIG. 1). Interestingly, other bacteria such as *Shigella flexneri* and *Salmonella enterica* subsp. *enterica* serovar Typhimurium, which express membrane proteases capable of cleaving C3, are protected from autophagy-mediated killing[56]. The C3–ATG16L1 interaction has also been observed in pancreatic islet cells, regulating autophagy in insulin-secreting cells and contributing to the survival of islet cells in diabetic mice[57]. The implications of intracellular complement for immune responses will be discussed further below.

Overall, the available data indicate that multiple complement proteins recognize structural motifs present on the surface of pathogens and activate cellular pathways that contribute to the elimination of the invaders. By contrast, in the absence of infection or after an infection has resolved, complement ensures homeostasis by promoting the clearance of apoptotic cells and metabolic debris without stimulating potentially harmful inflammatory responses. Thus, the decisive element that tilts the balance between a homeostatic or a pro-inflammatory complement-mediated response appears to be the presence of other danger signals.

Complement modulates innate immune responses

Microbial infection often leads to concomitant initiation of complement pathways and activation of PRRs, such as TLRs, NOD-like receptors, NOD-, LRR- and pyrin domain-containing 3 (NLRP3) inflammasomes and C-type lectin-like receptors on immune cells[14]. As mentioned above, receptors for complement activation fragments are also present on a variety of immune cells (TABLE 1), indicating the potential for concurrent triggering of complement receptors and PRRs on the same cell during infection. Indeed, engagement of receptors for complement activation fragments triggers the recruitment of cytoplasmic adaptor molecules, allowing crosstalk with other signalling pathways[58–60].

Crosstalk between complement and Toll-like receptors. The modulation of TLR4-induced responses by C5aR1-mediated signalling has been appreciated for nearly two decades[61]. C5aR1, a seven-transmembrane-spanning G protein-coupled receptor (GPCR), is activated upon binding to the activation fragments C5a or C5a-desArg[59]. The same activation fragments also bind to C5aR2, another seven-transmembrane-spanning receptor that signals via β-arrestin and whose function is still poorly understood[59]. A third GPCR with a similar structure to C5aR1 and C5aR2 is C3aR, which is a receptor for the complement activation fragment C3a[59]. The cellular expression patterns of C3aR, C5aR1 and C5aR2 differ between resting and activated cells as well as between human and rodent cells. Considerable understanding of the expression of these receptors has been achieved using green fluorescent protein (GFP) and tandem-dye tomato fluorescent protein (tdTomato) reporter mice[62] (TABLE 1). While numerous reports agree that myeloid cells — including neutrophils, macrophages and subsets of DCs — express C3aR, C5aR1 and C5aR2, studies with reporter mice failed to confirm the presence of these receptors on T cells and B cells, suggesting that the expression of C3aR and C5aR1 on lymphocytes may be an artefact resulting from unspecific antibody staining or that the signal in reporter mice was not sensitive enough to indicate the presence of these receptors in steady state lymphoid cells[62–67].

The engagement of C3aR or C5aR1 on human monocyte-derived DCs triggers the PI3K–AKT, ERK and NF-κB signalling pathways, potentiating cell activation[68]. Synergism between TLRs and the C3aR and C5aR1 activation pathways also upregulates the expression of co-stimulatory molecules on APCs and induces their production of pro-inflammatory cytokines[58,69–71] (FIG. 2). Thus, C3a and C5a activation fragments have been designated as 'the salt and the pepper of the immune response' — that is, they are key to determining the type and magnitude of immune responses[72]. Interestingly, C5a differentially modulates TLR4-induced responses in monocytes and macrophages, upregulating the production of inflammatory cytokines in monocytes but downregulating these responses in macrophages[73].

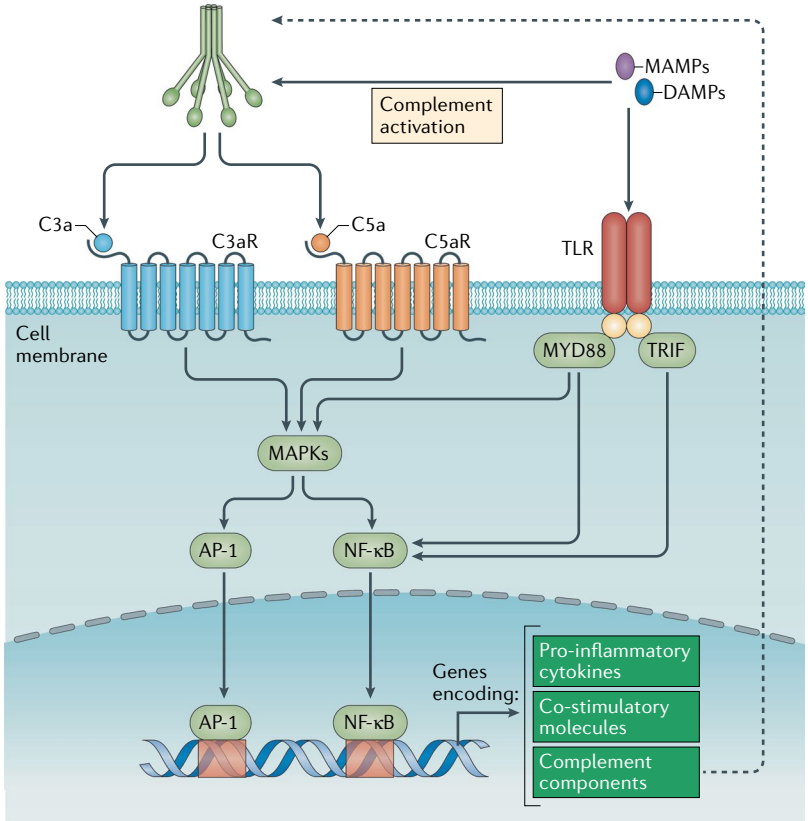

Fig. 2 | **Synergistic interactions between complement and Toll-like receptors.** Complement and Toll-like receptors (TLRs) are co-activated in response to microbial infection. Certain microorganism-associated molecular patterns (MAMPs) — such as zymosan, lipopolysaccharides and CpG (agonists of TLR2, TLR4 and TLR9, respectively) — can activate both TLRs and complement. Complement anaphylatoxin receptor signalling stimulated by C3a or C5a synergizes with TLR–MYD88 signalling induced by either MAMPs or endogenous damage-associated molecular patterns (DAMPs, for example, biglycan, hyaluronan fragments and heparan sulfate fragments). This synergy leads to enhanced activation of MAPKs and transcription factors, such as NF-κB and activator protein 1 (AP-1), resulting in upregulated expression of pro-inflammatory cytokines and co-stimulatory molecules. TLR activation can also upregulate the expression of complement proteins via a TRIF pathway (induced by TLR3 or TLR4 signalling), thereby generating a feedforward loop that further amplifies inflammatory responses.

Whereas both monocytes and macrophages play a key role in the initiation and resolution of inflammatory responses, monocytes act as danger sensors in the circulation and, upon activation, infiltrate tissues, where they differentiate into macrophages. It is likely that, in the circulation, C5a is central to potentiating the sentinel function of monocytes. In the tissues, however, amplification of inflammation would result in organ damage; hence, C5a-mediated suppression of inflammatory responses by macrophages may represent a host-protective response. In line with this concept, LPS-induced expression of C5aR1 is upregulated in monocytes but not in macrophages[74].

Apart from APCs, crosstalk between TLR4-induced and C5aR1-induced production of IFNγ and TNF has been described in NK cells and NKT cells[75]. Furthermore, in a rodent model of polymicrobial sepsis, the activation of TLR2, TLR3 and TLR4 has been shown to promote the synthesis of factor B by macrophages and cardiac cells, with consequent activation of the alternative pathway and deposition of C3 activation fragments in vital organs such as the kidney and heart[76]. Similarly, in a *Clostridium difficile* infection model, PAMP-induced IL-22 is required for the production of C3, which protects against pathogen dissemination to extra-intestinal organs, indicating a feedback mechanism between TLR-induced and complement-induced responses[77].

Activation of CR3 in macrophages has also been implicated in modulating TLR7-mediated and TLR8-mediated effector functions by inducing degradation of the adaptor protein MYD88 and the consequent downregulation of TNF production, again pointing towards a role for CR3 in the regulation of tolerogenic responses[78]. Notably, this regulatory role of CR3 in macrophages is not seen in DCs, in which the CD11b subunit of CR3 assists the endocytosis and trafficking of TLR4 to endosomes[79]. In vitro studies have also suggested that additional complement-regulatory proteins such as C4b-binding protein (C4BP) and factor H modulate TLR4-induced responses by DCs. Whereas C4BP prevents LPS-induced expression of indoleamine 2,3-dioxygenase (IDO), miR-155 and pro-inflammatory cytokines by DCs, factor H regulates the differentiation of DCs towards a tolerogenic phenotype[80,81]. Furthermore, MBL has been shown to bind to double-stranded RNA and an extracellular domain in TLR4 and suppress TLR3-induced and TLR4-induced production of inflammatory cytokines[36,82].

Given the broad evidence indicating modulation of TLR responses by complement, concomitant blockage of complement-mediated and TLR-mediated pathways has been proposed as a superior therapeutic approach for diseases in which innate immunity is overactivated. However, eritoran, a synthetic lipid A analogue that prevents LPS-induced activation of TLR4, failed to reduce mortality in patients with sepsis when administered in conjunction with standard sepsis treatment in a phase III study (NCT00334828). Interestingly, in an in vitro model of blood bacterial infection, blockade of TLR-mediated and complement-mediated pathways by dual treatment with eritoran and the C3 inhibitor Cp40 inhibited the production of inflammatory cytokines in response to *Escherichia coli* or *Staphylococcus aureus*, but inhibition of cell activation using an anti-CD14 antibody and Cp40 showed an even greater effect in suppressing inflammation[83].

Crosstalk between complement and other cell receptors. In addition to TLRs, complement-triggered pathways have also been implicated in the crosstalk with other cell receptors that modulate immune responses. A synergistic interaction between C5a-induced and NOD2-induced signalling has been reported in RAW 264.7 macrophages, in which engagement of NOD2 leads to an upregulation in the C5a-mediated expression of chemokines via phosphorylation of p38 MAPK[84]. Complement-mediated signalling also facilitates dectin 1-mediated phagocytosis by DCs and activation of NLRP3 inflammasomes[85,86]. In addition, deposition of sublytic levels of the terminal complex C5b–9 (BOX 1) on the membranes of nucleated cells promotes

the accumulation of Ca^{2+} in the mitochondrial matrix, loss of mitochondrial potential and consequent activation of inflammasomes with production of IL-1β and IL-18 in vitro and in vivo[85,87]. Crosstalk between C5aR1-induced and NLRP3-induced pathways has also been observed in a rodent model of endotoxaemia, in which triggering of C5aR1 upregulates the production of IL-1β[88].

Additional crosstalk has been observed between C5aR1 and other GPCRs such as bradykinin receptors and CC-chemokine receptor 5 (CCR5). In a rodent model of *Trypanosoma cruzi* infection, the parasite induces generation of C5a and kinins that simultaneously engage C5aR1 and the bradykinin B_2 receptor (B_2R). Such cooperation boosts anti-parasite immunity via production of nitric oxide and IFNγ[89]. In addition, an in vitro study using macrophages infected with laboratory strains of HIV shows a requirement for C5aR1 for CCR5-mediated infection of macrophages with HIV[90]. Further cooperation between C5aR1-mediated and C3aR-mediated signalling has been demonstrated in a neutrophil-dependent model of intestinal ischaemia–reperfusion injury (IRI). Engagement of C3aR in neutrophils suppresses C5aR1-induced mobilization of neutrophils and intestinal inflammation, modulating the severity of IRI pathology in mice[91]. A new line of evidence couples complement activation with endothelial barrier responses, indicating that the activation fragment C4a acts as an agonist for proteinase-activated receptor 1 (PAR1) and PAR4, thereby modulating the permeability of endothelial cells and possibly local inflammatory responses[92].

These findings collectively indicate that complement shapes the type and magnitude of immune responses by cooperating with other cell defence pathways. Although the physiological relevance of such crosstalk in humans requires further exploration, evidence acquired so far indicates that it both assists in the elimination of microbial intruders and contributes to the repair and maintenance of tissue homeostasis and immune tolerance.

Modulation of T cell responses by complement

As alluded to above, complement integrates innate and adaptive immunity and can influence the quality and magnitude of T cell activation. The stimulatory effects of complement on T cell activation may, in part, be mediated by the action of APC-generated C3a or C5a (paracrine activation). Moreover, complement fragments generated by naive $CD4^+$ T cells also appear to exert direct effects on their functional co-stimulation and differentiation (autocrine activation)[4,67,93,94].

Paracrine activation of T cells. $CD4^+$ T cell responses are regulated by three main types of signals derived from APCs and the surrounding tissue environment. APCs present antigens on MHC class II molecules that trigger the activation of T cell receptors (TCRs) and express co-stimulatory molecules, such as CD80 and CD86, that engage receptors such as CD28 on T cells (signal 1 and signal 2, respectively). A third T cell-polarizing signal comes from the cytokine milieu; IL-12 and IL-4 promote the polarization of IFNγ-producing T helper 1 (T_H1) cells and IL-4-producing T_H2 cells, respectively. Transforming growth factor-β (TGFβ), IL-6, IL-1 and IL-21 are involved in the differentiation of T_H17 cells, whereas IL-23 is important for the expansion and survival of T_H17 cell populations. FOXP3-expressing regulatory T (T_{reg}) cells share a reciprocal developmental pathway with T_H17 cells; thus, in the absence of the pro-inflammatory cytokine IL-6, TGFβ promotes the differentiation of naive $CD4^+$ T cells into T_{reg} cells[95]. Available data indicate that complement-mediated effects on APCs influence all three signals to modify the activation of T cell responses[4,96].

Concomitant engagement of PRRs and C3aR or C5aR1 on APCs is associated with upregulated expression of MHC class II and co-stimulatory molecules as well as with increased production of IL-12, ensuring the polarization of $CD4^+$ T cells towards a T_H1 cell phenotype[4,68,70,96]. Indeed, thioglycollate-elicited macrophages from DAF-knockout mice — which overexpress C3a and C5a — are more potent activators of T_H1 cell responses than are macrophages from wild-type mice[97]. In line with these findings, DCs from patients with C3 deficiency show an impaired ability to stimulate alloreactive T cell responses in vitro[45,46,98]. Furthermore, human T cells have been shown to produce C3 upon in vitro activation with anti-CD28, and the surfaces of activated T cells are known to be coated with iC3b fragments that mediate adherence between T cells and CR3-expressing DCs, inducing T cell proliferation[99]. Given that CR3-mediated responses are tolerogenic and do not initiate a potent T_H1 cell response, it is likely that an alternative polarization is achieved in this model. Supporting the idea that C3 is required for optimal proliferation of T cells, loading of C3-deficient DCs with apoptotic cells leads to accelerated fusion of the apoptotic cargo with lysosomes, resulting in impaired antigen presentation and decreased T cell proliferation. These findings again support an intracellular role for C3 as a chaperone that guides the processing of an apoptotic cargo, likely modulating T cell responses to self-antigens[53].

As discussed above, similarly to iC3b, C1q can also drive tolerogenic responses by APCs, downregulating the expression of CD86 and upregulating programmed cell death 1 ligand 1 (PD-L1) and PD-L2, with consequent differentiation of T_{reg} cells[100]. Moreover, whereas inhibition of factor H increases the ability of DCs to induce allogeneic T cell responses, inhibition of properdin leads to an impaired allostimulatory capacity. Therefore, the balance between complement regulators (such as factor H) and activators (such as properdin) appears to regulate the stimulatory capacity of DCs[101].

Autocrine activation of T cells. A new concept emerged a decade ago suggesting that locally produced complement acts in an autocrine fashion to modulate $CD4^+$ T cell responses, independent of APCs[67,93]. As previously indicated, evidence shows that activated T cells produce complement components such as C3, C5, factor B and factor D, with subsequent generation of C3a and C5a[67,99]. C3aR-mediated and C5aR1-mediated signals on T cells have been proposed to activate the PI3K–AKT signalling

pathway, leading to T_H1 cell responses with production of IFNγ. By contrast, ablation of C3aR-mediated and C5aR1-mediated signals on $CD4^+$ T cells results in the activation of an alternative signalling pathway involving the kinase PKA and consequent differentiation of $FOXP3^+$ T_{reg} cells secreting TGFβ[102,103]. Furthermore, blockade of C3aR-mediated and C5aR1-mediated signalling in thymus-derived T_{reg} cells results in increased cell-regulatory potential, abrogating autoimmune colitis in a mouse model[104]. Although there is a consensus that triggering C3aR and C5aR1 potentiates T_H1 cell responses, contradictory findings exist regarding the expression of these receptors on T cells; additional research will be required to identify the circumstances under which mouse and human T cells express receptors for C3a and C5a[62,63,65,66].

In contrast to C3aR and C5aR1, the presence of the complement regulator MCP on the surface of T cells is well characterized (TABLE 1). Engagement of MCP on $CD4^+$ T cells results in either T_H1 cell or T_{reg} cell polarization, depending on local levels of IL-2 (FIG. 3). Whereas initial triggering of MCP potentiates T_H1 cell responses with the production of IFNγ, accumulation of IL-2 induces a switch to IL-10 production, with consequent transition to a T_H1 cell population-contraction phase that represents a self-regulatory feedback mechanism during a T cell immune response[105]. It has been suggested that co-ligation of MCP and CD35 on activated $CD4^+$ T cells further potentiates the production of IL-10 by these cells and that the presence of monocytes modulates IL-10 and IFNγ production after MCP stimulation[106,107].

Whereas $CD4^+$ T cells from MCP-deficient patients show compromised induction of T_H1 cell responses upon stimulation with anti-CD3 and anti-CD28 or anti-CD3 and anti-MCP antibodies in vitro, the major clinical manifestation associated with lack of MCP, atypical haemolytic uraemic syndrome (aHUS), is not caused by an impaired T cell response but by improper complement regulation[60,108]. Impaired B cell responses, however, and consequent common variable immunodeficiency are observed in a few patients bearing homozygous MCP mutations, although this is an extremely rare condition[108]. It would be interesting, therefore, to investigate the relative contribution of MCP-mediated activation of T cells by evaluating how MCP-deficient $CD4^+$ T cells respond in the presence of APCs and immunomodulatory factors such as C3a and/or C5a. In addition to C3b, the Jagged 1 protein has been identified as a ligand for MCP. Ligation of MCP on T cells by Jagged 1 regulates the expression of Notch receptors, and crosstalk between MCP and the Notch signalling pathway further contributes to both induction of IFNγ and the switch to IL-10 production[60]. MCP-induced cytokine production by T cells is also regulated by cell metabolic pathways and dependent on the metabolism of glucose and ATP[94,109] (BOX 3; FIG. 3).

In addition to $CD4^+$ T cells, complement also regulates the activation of $CD8^+$ T cells in an autocrine fashion. In $CD8^+$ T cells, MCP delivers co-stimulatory signals by augmenting nutrient influx and the synthesis of fatty acid, potentiating the activity of cytotoxic T cells[110]. C1q also regulates the mitochondrial metabolism in effector $CD8^+$ T cells, limiting tissue damage and autoimmune responses[28].

Intracellular complement and T cell responses. As mentioned above, a rapid and saturable recycling pathway for $C3(H_2O)$ has been described in lymphocytes that allows exchange between intracellular and extracellular stores of C3 (REF.[51]). Additional evidence indicates that human resting T cells contain intracellular stores of C3 that are cleaved into C3a and C3b by the lysosomal protease cathepsin L (CTSL)[52]. Such intracellular generation of C3a has been linked with homeostatic T cell survival (FIG. 3), while externalization of C3a and binding to the C3aR on the cell surface lead to the production of pro-inflammatory cytokines. Interestingly, while T cells from patients with autoimmune arthritis show increased levels of intracellular C3a and production of IFNγ, inhibition of CTSL in vitro results in the modulation of this inflammatory response[52]. Intracellular stores of C5a have also been observed in human T cells. It has been reported that C5a binds to the C5aR1 also present in the intracellular compartment, resulting in the assembly of the NLRP3 inflammasomes and activation of T_H1 cell responses. Secretion of C5a further triggers the alternative receptor C5aR2 on the cell surface, which in turn restrains NLRP3-induced responses[94,111].

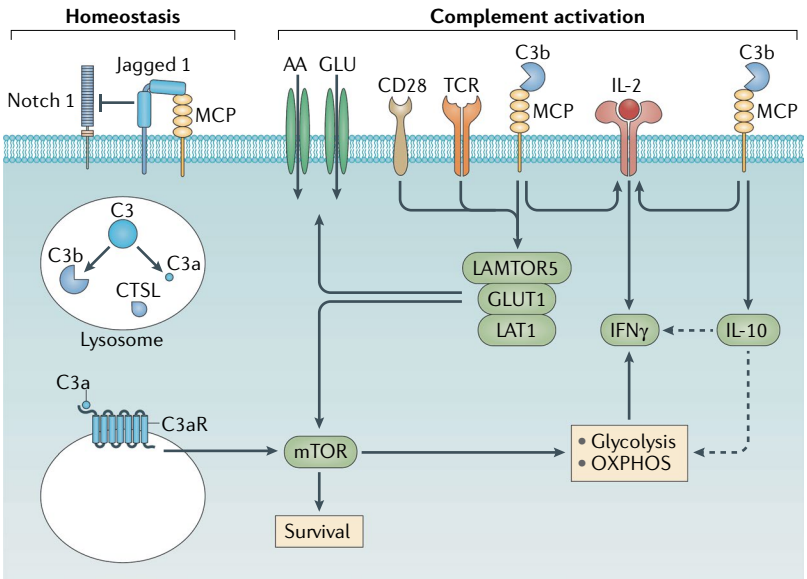

Fig. 3 | **Complement-mediated T cell activation.** Resting $CD4^+$ T cells have intracellular stores of C3 that can be cleaved intracellularly by cathepsin L (CTSL). C3aR-mediated intracellular signalling induces low levels of mechanistic target of rapamycin (mTOR) activation that regulate T cell survival. This homeostatic state is maintained by the inhibition of Notch signalling via Jagged 1–membrane cofactor protein (MCP) association. Engagement of the T cell receptor (TCR) and CD28 during conditions favouring complement activation (such as infection) results in MCP activation, assembly of the IL-2 receptor and expression of late endosomal/lysosomal adaptor and MAPK and mTOR activator 5 (LAMTOR5), glucose transporter 1 (GLUT1) and L-type amino acid transporter 1 (LAT1), leading to nutrient influx and oxidative phosphorylation (OXPHOS) and consequent induction of the T helper 1 (T_H1) cell cytokine IFNγ. MCP-mediated signalling also regulates T_H1 cell responses via collaboration with the IL-2 receptor and induction of IL-10, with subsequent downregulation of glycolysis, OXPHOS and IFNγ production. AA, amino acids; GLU, glucose.

Box 3 | **Complement in immune cell metabolism**

An association between complement and energy metabolism was initially uncovered with the observation that adipocytes, the main energy depository in the body, are a major source of factor D (also known as adipsin). Expression of factor D mRNA by adipocytes is dependent on the plasma concentrations of glucocorticoids, being upregulated during catabolic states, such as fasting and insulin-dependent diabetes, and downregulated in cases of genetically determined obesity[158]. Another complement protein, C3a-desArg, is elevated in the plasma of obese individuals and has been shown to promote the synthesis of triglycerides by adipocytes and consequent insulin resistance, indicating an association between complement and adipose tissue metabolism[159,160]. To date, the understanding of a role for complement in the regulation of cell metabolism has extended from the adipose to the pancreatic and liver tissues[161]. Additionally, in immune cells, divergent metabolic pathways associated with distinct polarization of $CD4^+$ T cell subsets are also modulated by complement[109,162]. As discussed in the main text, survival and differentiation of $CD4^+$ T cells is determined by the co-stimulation of T cell receptor (TCR) and membrane cofactor protein (MCP) with consequent activation of mechanistic target of rapamycin (mTOR) and generation of ATP via glycolysis and oxidative phosphorylation[52,109] (FIG. 3). Triggering of intracellular C5aR1 during T cell activation and subsequent NOD-, LRR- and pyrin domain-containing 3 (NLRP3)-mediated secretion of IL-1β are also impacted by the production of reactive oxygen species during oxygen metabolism in the mitochondria[111]. Similarly, in $CD8^+$ T cells, MCP-mediated signals modulate nutrient influx and the synthesis of fatty acid, potentiating the activity of cytotoxic T cells[110]. As a growing body of data associates cholesterol and glucose metabolic pathways with the production of inflammatory cytokines and consequent chronic inflammatory diseases, therapeutic modulation of specific complement proteins, such as C3a and C5a, could represent an interesting approach to interfere with the cellular metabolism with the ultimate goal of restraining the production of inflammatory factors.

It should be noted, however, that there are no indications that $CD4^+$ T cells from patients with C3 deficiency show impaired survival[112]. Because the findings discussed above indicate that intracellular C3a is required for proper T cell survival and proliferation, it has been suggested that C3a is present intracellularly in $CD4^+$ T cells from patients with C3 deficiency[52,112]. Indeed, intracellular C3 can be observed in patients with primary and secondary C3 deficiency, and C3 deficiency appears to be correlated with defects in the differentiation of memory B cells but not T cells[112]. While fascinating, these novel findings describing a role for intracellular C3 reveal numerous uncertainties about how the tertiary structure of C3, including its disulfide bonds, is maintained in the intracellular environment; whether the protein is glycosylated and originates from the Golgi apparatus; what decides whether the protein will be secreted; and the nature of intracellular C3a function in non-immune cells. Recent studies started to uncover some of these answers, showing that, in human pancreatic islet cells, intracellular C3 is transcribed from an alternative ATG codon, resulting in a protein without a secretion signal and indicating a distinct nature between intracellular and extracellular C3 (REF.[56]).

Diseases affected by complement

The vast majority of evidence linking complement-mediated T cell responses to pathological conditions comes from well-established disease models in rodents. Studies using complement gene knockout strains were instrumental in revealing that imbalanced complement activation is directly associated with maladaptive T cell responses and disease. In particular, experimental models of asthma have demonstrated that allergens drive C3a and C5a production, which in turn modulates the recruitment and activation of immune cells in the lungs and the consequent release of T_H2 cell-type and T_H17 cell-type cytokines observed in severe asthma[113–115]. A C5a-mediated T_H17 cell response is also associated with the development of chronic autoimmune arthritis in SKG mice via stimulation of IL-6 by macrophages[116]. Potentiation of T cell responses by C3a and C5a further governs T cell-mediated mechanisms of organ rejection and cancer development in mice[117,118]. Mouse models were also key to uncovering crosstalk between C5aR1–TLR2 and CR3–TLR2 and a consequent role in the development of periodontitis[119]. Furthermore, imbalanced complement activation is associated with poor disease outcome in a variety of inflammatory and autoimmune disease models[120–123]. Despite progress in unveiling such molecular pathways in mice, certain differences between the mouse and the human complement systems, such as differential expression of complement receptors, make it impossible to perfectly recapitulate human pathology using rodent models of disease[94,124–126]. For example, whereas the therapeutic blockage of C5aR1-induced inflammation is very efficient in reducing symptoms of arthritis in experimental models, the C5aR1 antagonist PMX-53 failed to reduce synovial inflammation in patients with arthritis during a proof-of-concept clinical trial[127].

Given the experimental data described above, one could expect genetic deficiency or mutations in *CD46* (encoding MCP), *CD55* (encoding DAF), *C3aR* or *CD88* (encoding C5aR1) in humans to be associated with defective T cell responses. As discussed above, however, mutations in MCP are mainly associated with the development of aHUS, indicating that, in disease states driven by persistent complement activation or dysregulation, the complement-regulatory role of MCP is more critical than the regulation of T cells[108]. DAF deficiency has been described in 11 patients showing severe protein-losing enteropathy. Interestingly, while $CD4^+$ T cells from DAF-deficient mice show increased production of IFNγ but no gastrointestinal issues, $CD4^+$ T cells isolated from patients show increased deposition of C3 activation fragments and production of TNF but not IFNγ[128]. Notably, off-label use of eculizumab in three of these patients resulted in overall improvement in disease manifestations, including diarrhoea, oedema and intestinal malabsorption[129].

Whereas mutations leading to lack of function in C5aR1 have not yet been described, a frameshift mutation in the *C3aR* gene (C3aR1 c.355-356dup, p.Asp119Alafs*19) resulting in a premature stop codon has been described in only one patient who suffered from aHUS[130]. Given that aHUS often occurs as a consequence of impaired complement regulation, this finding may underscore a previously unidentified regulatory role for C3aR in humans, supporting experimental reports that C3aR-mediated signalling inhibits C5aR1-induced responses[91,108,131]. These observations cast doubt on whether a deficiency or lack of function of C3aR or C5aR1 is extremely rare, indicating a key role for these receptors in survival, or quite common but undiagnosed owing to the absence of pathological

Table 2 | **Diseases associated with imbalanced complement activation**

Disease	Mechanism	Clinical trials
Age-related macular degeneration	Genetic variants of factor H, factor B, C3 and MCP	• NCT00935883 • NCT02247479 • NCT02247531 • NCT02503332 • NCT01527500 • NCT01535950 • NCT02515942 • NCT02686658
ANCA vasculitis	Autoantibodies lead to complement activation and generation of C5a that drives the disease	• NCT03301467 • NCT02994927
Atypical haemolytic uremic syndrome	Genetic variants of factor H, MCP, factor I and C3	• NCT00844545 • NCT00844844 • NCT03205995 • NCT02464891
Alzheimer disease	A possible mechanism described is the binding of C1q to amyloid-β deposits and consequent complement activation	–
Amyotrophic lateral sclerosis	Clusters of complement-activated oligodendroglia and degenerating neuritis positive for C3d and C4d are detected in affected areas	–
Cancer	Evidence of complement-induced inflammation resulting in tumour growth and metastasis	NCT03665129
Cold agglutinin disease	Presence of antibodies that activate complement	• NCT01303952 • NCT03347396 • NCT03347422 • NCT03226678
Diabetes	Evidence of increased levels of complement activation in the plasma	–
Epilepsy	Evidence of deposits of C1q, C3b and C5b–9 in the vicinity of affected brain tissue	–
Glomerulopathies	Genetic variants of C3, factor H, factor I and factor B; mutation in the *CFHR5* gene; and presence of autoantibodies	• NCT01221181 • NCT03124368 • NCT02682407 • NCT02384317
Guillain–Barre syndrome	Presence of antibodies that activate complement	NCT02029378
Inflammatory bowel disease	Evidence of increased complement activation in the intestinal tissue	–
Kidney transplant injury	Presence of anti-donor antibodies that activate complement and deposition of C4d in the transplanted organ	• NCT01919346 • NCT01327573 • NCT01895127 • NCT01095887 • NCT01756508 • NCT02145182 • NCT01399593 • NCT02134314
Multiple sclerosis	Evidence of increased complement activation in the cerebrospinal fluid of patients	–
Myasthenia gravis	Presence of autoantibodies that activate complement	• NCT00727194 • NCT1997229 • NCT0331530
Neuromyelitis optica	Presence of autoantibodies that activate complement	• NCT00904826 • NCT01892345
Parkinson disease	Deposition of complement activation fragments on neuronal Lewy bodies	–
Paroxysmal nocturnal haemoglobinuria	GPI anchor deficiency and defective protein anchorage, including of DAF and CD59, resulting in poor complement regulation	• NCT00122330 • NCT00122317 • NCT03056040 • NCT03157635 • NCT02534909 • NCT03115996 • NCT03078582 • NCT03030183 • NCT03225287 • NCT03053102 • NCT03181633 • NCT03472885 • NCT03439839 • NCT02588833 • NCT02264639

Table 2 (cont.) | **Diseases associated with imbalanced complement activation**

Disease	Mechanism	Clinical trials
Periodontitis	Local microbially induced complement activation	NCT03694444
Rheumatoid arthritis	Evidence of complement activation in the synovial membranes	–
Schizophrenia	Risk associated with *C4A* variant	–
Skin diseases	Evidence of local deposition of complement activation fragments	NCT00005571
Trauma	Evidence of increased complement activation systemically	–
Uveitis	Presence of autoantibodies that activate complement	NCT01526889

REFS[119,133,134]. ANCA, anti-neutrophil cytoplasmic antibody; DAF, decay-accelerating factor; GPI, glycophosphatidylinositol; MCP, membrane cofactor protein.

manifestations exclusively linked to germline mutations in these receptors. The latter suggests that, in adults with a fully developed immune system, C3aR-mediated and C5aR1-mediated immune functions may be replaced by other compensatory and/or overlapping immune mechanisms. Indeed, the role of complement throughout ageing appears to switch from defensive (maintenance of homeostasis) to offensive (promotion of pathology)[132]. Taking the deficiency of C3 as an example, in childhood, C3 deficiency is associated with severe bacterial infection that can lead to death, but susceptibility to infections does not appear to be critical in adults[2]. Supporting this concept, complete inhibition of C3 in adult cynomolgus monkeys is not associated with increased susceptibility to infections or a differential haematological profile[133].

The offensive role of complement mentioned above occurs when imbalanced complement activation leads to inflammation and consequent tissue damage, dysfunction and possible failure of a variety of organs such as the eyes, kidneys, skin, brain and vasculature[2,122,132] (TABLE 2). In cancer, in particular, excessive complement activation in the tumour microenvironment has been associated with inflammation and tumour growth[118]. An interesting approach involving dual blockage of C5a and programmed cell death 1 (PD-1), initially tested in a rodent model of lung cancer, resulted in substantial enhancement of the antitumoural efficacy of the anti-PD-1 antibody that was associated with increased numbers of $CD8^+$ T cells in the tumour[134]. These findings were used as preclinical evidence to justify a phase I clinical trial evaluating the combined use of IPH5401 (anti-C5aR1) and durvalumab (anti-PD-L1) in patients with advanced solid tumours (NCT03665129). Thus, while in the vast majority of diseases (TABLE 2) complement promotes pathogenic mechanisms by inducing inflammation, fertile ground is still available for investigating specific mechanisms linking complement and T cell responses in human diseases.

Conclusion

Decades of research have uncovered a variety of molecular mechanisms by which complement synergizes with PRR-induced pathways in immune cells to modulate the type and magnitude of immune responses. While extensive evidence obtained in experimental models suggests that complement affects T cell responses to cause diseases, recapitulation of such phenotypes in humans has proved challenging in the face of the differential expression of complement receptors in rodents and humans. Whereas common and well-characterized complement-mediated pathologies in rodents such as arthritis, asthma and transplant rejection clearly show maladjusted T cell responses, it is likely that human pathology may differ in terms of complement-induced injury[115–117]. This in no way means that T cell responses in humans are not modulated by complement but that perhaps it is time to tune the research towards human pathology. Although recent findings show surprising correlations between C3aR and aHUS, as well as between DAF and gastrointestinal disease, various other associations between complement genes and pathology may have been neglected thus far, as they were not observed in rodents[128,130]. Therefore, efforts to apply therapeutic inhibition of complement to a variety of inflammatory diseases may prove beneficial for additional pathologies not currently considered to be complement-dependent[122,129,135,136] (TABLE 2).

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Competing interests
J.D.L. and G.H. are inventors on patents or patent applications that describe the use of complement inhibitors for therapeutic purposes. J.D.L. is the founder of Amyndas Pharmaceuticals, which is developing complement inhibitors (including third-generation Compstatin analogues such as AMY-101), and the inventor of the Compstatin technology licensed to Apellis Pharmaceuticals (that is, 4(1MeW)7W/POT-4/APL-1 and PEGylated derivatives). E.S.R. and D.C.M. declare no competing interests.

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