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Review article

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A guide to complement biology, pathology and therapeutic opportunity

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Abstract

Complement has long been considered a key innate immune effector system that mediates host defence and tissue homeostasis. Yet, growing evidence has illuminated a broader involvement of complement in fundamental biological processes extending far beyond its traditional realm in innate immunity. Complement engages in intricate crosstalk with multiple pattern-recognition and signalling pathways both in the extracellular and intracellular space. Besides modulating host–pathogen interactions, this crosstalk guides early developmental processes and distinct cell trajectories, shaping tissue immunometabolic and regenerative programmes in different physiological systems. This Review provides a guide to the system-wide functions of complement. It highlights illustrative paradigm shifts that have reshaped our understanding of complement pathobiology, drawing examples from evolution, development of the central nervous system, tissue regeneration and cancer immunity. Despite its tight spatiotemporal regulation, complement activation can be derailed, fuelling inflammatory tissue pathology. The pervasive contribution of complement to disease pathophysiology has inspired a resurgence of complement therapeutics with major clinical developments, some of which have challenged long-held dogmas. We thus highlight major therapeutic concepts and milestones in clinical complement intervention.

Sections

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Introduction

"Nothing endures but change", *Heraclitus of Ephesus*

The complement system constitutes a fast-acting and versatile protein interaction platform of innate immunity that assembles to tackle sterile or non-sterile inflammatory insults[1]. This highly conserved immune sentinel elicits activities that intertwine with inflammatory circuits under a broad range of pathophysiological contexts[2]. Complement proteins mediate tissue homeostasis and immune surveillance against a wide spectrum of pathogens[3]. Complement is one of the oldest innate immune systems with a pervasive presence throughout evolution that has predated mammals and humans by more than 900 million years[4]. Traditionally associated with pathogen phagocytic clearance, removal of immune complexes and clearance of apoptotic cell debris, complement is now appreciated as a system-wide modulator of biological processes extending beyond the realm of traditional innate immunology[1,5]. This Review focuses on new insights and paradigm shifts that have transformed our knowledge of complement biology, opening new windows of opportunity for innovative therapeutic approaches in many debilitating human diseases in which complement dysregulation has a key pathogenic role.

Overview of the complement system

Protein interactions within a complex proteolytic cascade

The complement system comprises more than 50 soluble or membrane-bound glycoproteins that engage in multi-tiered protein–protein interactions, resulting in the assembly and activation of enzymatic complexes and the generation of bioactive fragments that initiate diverse cellular responses through binding to complement receptors and regulators[6,7] (Fig. 1). These interactions promote microbial innate sensing or 'altered self' recognition (microorganism-associated molecular pattern (MAMP) and damage-associated molecular pattern (DAMP) recognition, respectively), opsonophagocytic responses, inflammatory and immunomodulatory functions, and crosstalk mechanisms in different tissues, both extracellularly and within intracellular compartments[1,8]. Complement activation proceeds through a finely regulated cascade of proteolytic reactions that initially engages soluble pattern-recognition molecules and leads to the assembly of multiprotein enzymatic complexes termed C3 and C5 convertases[6]. These proteolytic reactions result in the release of bioactive fragments that mediate a wide array of functions on complement receptor-expressing cells (Fig. 1).

The complement system is activated through three pathways — the classical, alternative and lectin pathways — with distinct initiation but intercommunicating nodes[7,9]. Distinct pattern-recognition steps trigger each of these pathways into action: the classical pathway is triggered by the binding of C1q to the Fc portion of immunoglobulins bound to their antigen; the lectin pathway is activated through binding of soluble pattern-recognition molecules, such as mannose-binding lectin, collectins or ficolins, to carbohydrate signatures present on the cell wall of bacteria or fungi or to damage-associated ligands on altered host cells[10]; and the alternative pathway is triggered by both MAMPs and the steady-state, spontaneous hydrolysis of C3 in the fluid phase (known as C3 tick over), which maintains this pathway in a state of low-level activation, forming initial alternative pathway C3 convertases and allowing for rapid amplification of effector responses on targeted surfaces[11].

Studies using cryo-electron microscopy and x-ray crystallography led to the elucidation of the critical structural determinants underpinning the recognition of immune complexes by the active C1 complex $C1q(C1s)_2(C1r)_2$ and its Fc-binding component C1q[12,13]. The discovery that C1q latches onto a hexameric antibody platform that is stabilized by reciprocal Fc–Fc interactions between the antibody molecules has not only provided insight into the molecular basis of pattern recognition by the classical pathway but has also fuelled efforts to develop next-generation biotherapeutics based on modified antibody scaffolds that can potentiate classical pathway activation and complement-dependent cytotoxic responses against tumour cells[12,14].

Regardless of the initial trigger, all complement pathways converge at the proteolytic cleavage of C3, the most abundant complement protein in circulation, by C3 convertases[15,16] (Fig. 1a). C3 activation induces conformational rearrangements in the protein resulting in the generation of the opsonin C3b, the larger of the two C3 fragments, which tags target surfaces, such as those of microorganisms. C3b opsonization involves covalent binding of its exposed thioester to amino or hydroxyl groups on the targeted surface. The smaller C3 fragment generated in the process, termed C3a anaphylatoxin, has diverse immunomodulatory functions in both immune and non-immune cells[17]. Clustered deposition of C3b molecules at high surface densities facilitates the assembly of C5 convertases, the complexes responsible for the proteolytic cleavage of C5. C5 activation generates the anaphylatoxin C5a, which has potent immunomodulatory properties, and C5b, which initiates the sequential assembly of the multiprotein, cell membrane-perforating complex C5b–C9 or membrane attack complex (MAC)[18].

Non-canonical activation routes

Complement activation may also occur in a non-canonical, convertase-independent manner. Proteases of the kallikrein–kinin, coagulation and fibrinolytic cascades, such as plasmin, thrombin, kallikrein and coagulation factors Ia, Xa and IXa, can cleave C3, C5 or factor B, thereby generating fragments that can either assemble functional C3 convertases or participate in downstream effector responses[19,20] (Fig. 1a). Tissue-enriched proteases, such as renin, can also contribute to non-canonical complement activation by cleaving C3 and generating C3a and C3b[21]. In fact, tissue 'tropism' of certain complement-cleaving enzymes may have important implications as an amplifying mechanism in kidney glomerular disorders associated with complement dysregulation such as C3 glomerulopathy. Additionally, non-canonical routes of C3 activation, likely driven by coagulation proteases, have been implicated in a subset of patients with severe COVID-19 and pronounced thrombo-inflammation[22]. In a further layer of complexity, lysosomal enzymes, such as cathepsin L, can cleave C3 intracellularly[23], contributing to non-canonical routes of complement activation (Fig. 1b). Recently, the contact activation pathway was shown to modulate complement activity through the ability of activated factor XIa to cleave and neutralize the alternative pathway regulator factor H[24]. Furthermore, neutrophil-derived or bacteria-derived serine or cysteine proteases, such as elastase and gingipains, respectively, can cleave C3 and C5, generating bioactive fragments[25,26]. These convertase-bypass pathways have been implicated as either stand-alone or amplifying mechanisms of complement activation. However, their physiological relevance remains context dependent and should be considered in relation to disease pathophysiology. For instance, recent studies have placed the capacity of thrombin to cleave native C5 under scrutiny, showing that this cleavage occurs only with the purified C5 protein and after a pH-dependent conformational change of C5 protein[27].

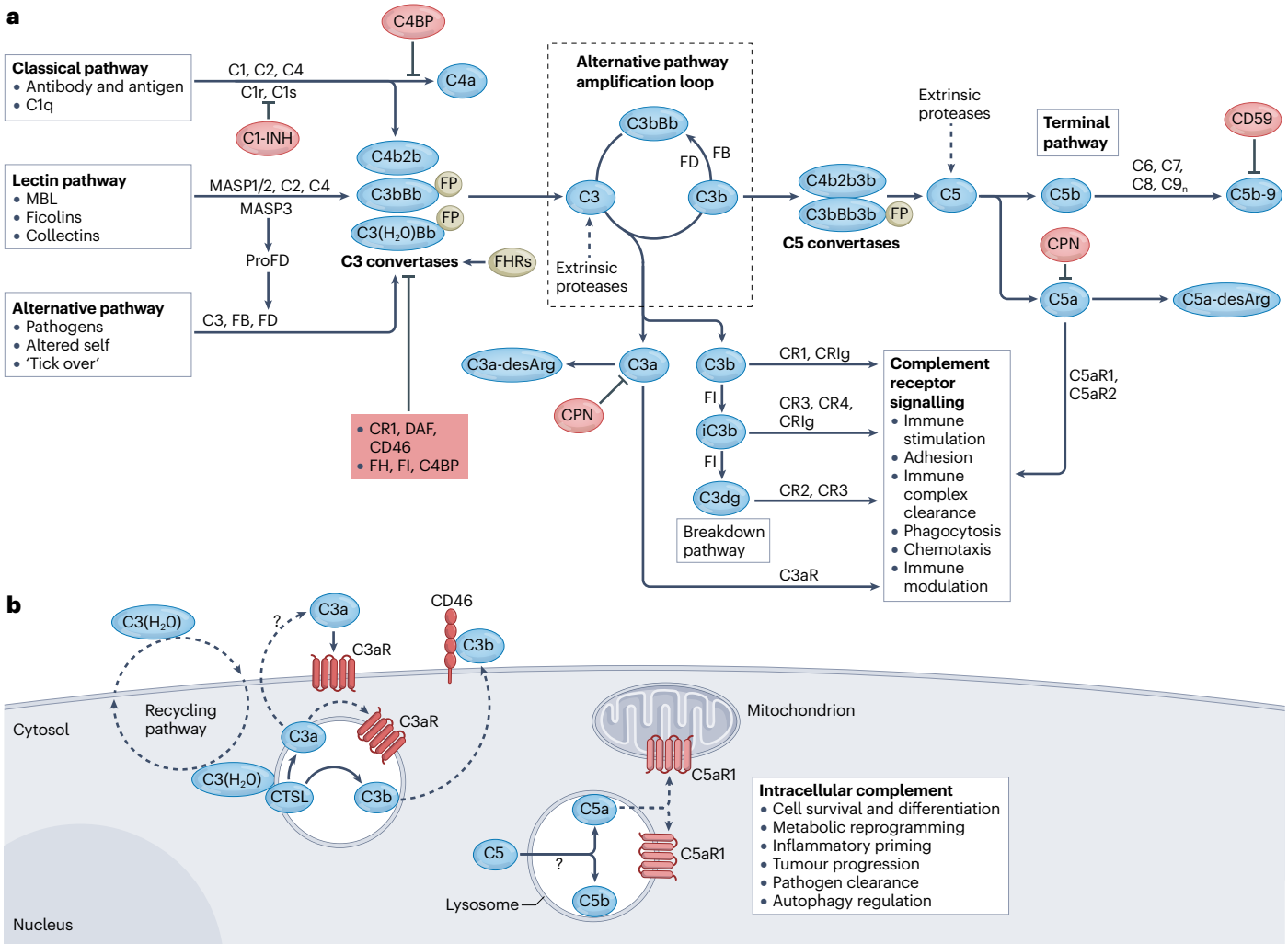

Fig. 1 | Overview of the complement cascade, its topology, regulation and key effector functions. a, This schematic depicts the main protein–protein interactions involved in the initiation, amplification and regulation of complement responses and in downstream effector functions. Both canonical and non-canonical routes of activation are shown, including key complement-driven interactions taking place in the extracellular and intracellular space. Canonical activation proceeds through the classical, lectin, and alternative pathways and involves distinct initial steps of pattern recognition and sequential activation of protein substrates that lead to the formation of multiprotein enzymatic complexes, termed C3 and C5 convertases. C3 and C5 convertases cleave C3 and C5, respectively, into their bioactive fragments. Non-canonical complement activation is mediated by extrinsic serine proteases of the coagulation, contact activation and fibrinolytic cascades that can cleave C3, C5 and factor B, thereby bypassing the action of conventional convertases. Additional 'bypass' pathways operate alongside the canonical routes providing the system with essential signalling redundancy for tissue immunosurveillance. Downstream signalling involving the interaction of C3 fragments and anaphylatoxins with complement receptors expressed on immune cells results in a wide array of immunomodulatory and effector functions. **b**, The expansion of the complement circuitry into the intracellular space of various cell types has revealed crucial interactions with pattern recognition, immunometabolic and inflammatory processes that shape cell fate decisions in health and disease. C1-INH, C1 esterase inhibitor; C4BP, C4b-binding protein; CPN, carboxypeptidase N; CR, complement receptor; CRIg, complement receptor of the immunoglobulin superfamily; CTSL, cathepsin L; DAF, decay-accelerating factor; desArg, desarginated; FB, factor B; FD, factor D; FH, factor H; FHRs, factor H-related proteins; FI, factor I; FP, properdin; MASP, mannan-binding lectin-associated serine protease; MBL, mannose-binding lectin; proFD, pro-factor D.

Functional pathway plasticity

Besides these extrinsic routes of complement activation, there is also functional plasticity and 'leakage' between the main three initiation pathways. The lectin and alternative pathways converge via crosstalk involving shared enzymes that can cleave protein substrates of either pathway. For example, the lectin pathway protease mannan-binding lectin-associated serine protease 3 (MASP3) activates pro-factor D and generates the active enzyme that mediates the formation of the alternative pathway C3 convertase C3bBb[28,29] (Fig. 1a). Moreover, a mannose-binding lectin-dependent C2-bypass pathway that can support alternative pathway-mediated C3 activation has been described, suggesting a functional compensation that operates in distinct complement deficiency states such as C2 or C4 deficiency[30]. Similarly, increased binding of collectin 11 to L-fucose exposed on stressed renal

Box 1

In the light of evolution

The complement system represents one of the evolutionarily most ancient protein networks of the innate immune response, with its earliest members predating mammals and humans by almost 900 million years of evolution[252]. It is postulated that complement evolved very early during evolution, with protein orthologues described as early as the branching of the protostome and deuterostome lineages that led to the emergence of arthropods and vertebrates, respectively[4,16]. A primordial complement system was established more than 600 million years ago as suggested by the presence of C3-like proteins in invertebrate species like cnidarians, tunicates and sponges[16,252]. This early system is thought to have comprised a set of primitive C3, factor B (that is, a common ancestral *Bf* gene) and mannan-binding lectin-associated serine protease (MASP) proteins[253].

During this long evolutionary time, complement has co-evolved with other host defence systems and a multitude of symbiotic or pathogenic microorganisms in a process of constant selective pressure that has fostered reciprocal adaptations but also immune escape mechanisms[72]. Such immune evasion strategies are reflected in an array of secreted or membrane-bound virulence proteins that microorganisms use to subvert host complement[109,254]. Complement proteins C3, C4 and C5 belong to a highly conserved protein superfamily of thioester-containing proteins (TEPs), including α2-macroglobulin and insect TEP homologues, which mediate innate immune defence through microbial pattern recognition, phagocytic clearance or direct microbial killing[255].

Comparative phylogenetic studies suggest that C3 acquired its immunosurveillance capacity early during evolution, being endowed with immune recognition and 'non-self' discrimination properties in a primordial phagocytic system that is shared by both invertebrates and vertebrates[256]. This minimal system was retained by all deuterostomes and was further diversified through gene duplications and domain reshuffling of the *C3*, *Bf* and *MASP* genes[253]. A turning point in complement evolution is the emergence of teleost fish, which harbour multiple isoforms of C3, C5, factor B and other proteins within a single organism[257]. This plurality of isoforms likely endowed these species with an expanded innate immune recognition capacity against microbial surfaces, thereby compensating for an as-yet underdeveloped adaptive immune response and lack of immunoglobulin diversification[257]. The expanded perception of intracellular complement has spurred hypotheses that, in its most primitive form, C3 may have been retained intracellularly as a host defence protein that would tag and neutralize microorganisms ingested by primitive unicellular eukaryotic organisms (such as choanoflagellates)[256].

tubule cells can trigger the lectin pathway following kidney ischaemic injury, inducing direct MASP2-dependent cleavage of C3, independently of C2 or C4 (ref. 31). Functional redundancy has also been implied between the classical and alternative pathways through a C2-bypass mechanism that allows the formation of hybrid classical pathway C3 convertases in which C2 is substituted by factor B[32]. While these in vitro observations point to potential bypass mechanisms operating at the interface of all three pathways, they warrant further validation in vivo.

Challenging convertase formation

Our perception of how convertases are assembled during C3 and C5 activation has recently been challenged by findings suggesting that a trimeric C5 convertase (C3bBb3b) is not necessarily a prerequisite for C5 activation[33]. Indeed, under certain circumstances that favour strong complement activation and pronounced surface deposition of C3b or C4b, C5 activation can proceed through a C3 bypass that recruits a conformationally biased form of C5, 'primed' by high surface densities of C3b or C4b. This primed C5 may participate in MAC formation either through cleavage by the C4b2b and C3bBb convertases or by adopting a C5b-like conformation[33]. While the extent to which bypass pathways drive disease pathophysiology remains elusive, these mechanistic insights may help to explain cases of breakthrough haemolysis in patients on anti-C5 therapies who are exposed to triggers of potent complement activation (such as acute infections). Furthermore, they imply that complete shutdown of complement activity may not be required to achieve full therapeutic responses with anti-complement agents and that the risk of infections during pharmacological complement inhibition may be curtailed by bypass mechanisms that maintain basal complement activity for tissue immunosurveillance.

Unravelling the phylogenetic trajectory of complement pathways and components not only illustrates the evolutionary conservation of a key innate immune system (Box 1) but also serves as a base for understanding the modular structure and diverse interactions of its core components. In this regard, the evolutionary study of complement proteins has contributed immensely to protein structure resolution efforts and informed the design of target-specific and species-specific inhibitors.

Key mechanistic and functional aspects of complement

Here, we provide a summary of key mechanistic features and conceptual breakthroughs in complement biology, with an emphasis on homeostatic and immunoregulatory functions at the interface of innate and adaptive immunity and regulatory mechanisms that control the magnitude of activation. More extensive accounts of complement biology can be found in several comprehensive reviews[6,7,34].

A highly conserved function of complement proteins is their ability to sense and neutralize potential threats that come in the form of foreign abiotic (such as biomaterials) or biotic stresses (such as pathogens), or altered and diseased host cells[2]. Complement opsonins C3 and C4 bind to these surfaces, tagging them for elimination by the phagocytic system[6]. Opsonization is the process by which C3b or C4b covalently bind to targeted surfaces, triggering the subsequent binding and engulfment of these opsonized surfaces by phagocytes (such as monocytes, macrophages and neutrophils) expressing the β_2-integrin family complement receptors CR3 (also known as Mac-1; consisting of CD11b and CD18 chains) and CR4 (composed of CD11c and CD18 chains). Phagocytosis of iC3b-coated or C3b-coated foreign particles

and bacteria is also carried out by another phagocytic complement receptor, complement receptor of the immunoglobulin superfamily (CRIg), which is expressed predominantly by liver-resident macrophages (Kupffer cells)[35]. Complement opsonization of bacterial cells not only instructs phagocytic cells to eliminate circulating microorganisms intravascularly but also promotes the uptake and elimination of invasive bacteria via autophagy initiated by a direct interaction of C3 with autophagy-related protein 16-like 1 (ATG16L1)[36].

CR3, CR4 and CRIg, two complement receptors also mediate important immunoregulatory functions. CR1 (also known as CD35) is considered the prototype receptor that mediates immune complex clearance, namely the disposal of C3b-coated or C4b-coated immune complexes via binding to CR1-expressing erythrocytes. These immune complex-loaded erythrocytes are then transferred to the liver or spleen, where they are engulfed by macrophages[37]. CR1 also exerts complement regulatory activity, both as a protein that accelerates the decay of C3 and C5 convertases and as a cofactor for the cleavage of C3b or C4b by the serine protease factor I[6]. In this capacity, CR1 modulates predisposition to certain haemolytic, autoimmune or infectious diseases[38]. For instance, decreased CR1 expression on erythrocytes due to proteolytic cleavage and shedding during phagocytic engulfment may predispose to autoimmunity or complications from malaria due to impaired clearance of immune complexes and dysregulated complement deposition on red blood cells. The ability of CR1 to promote red blood cell agglutination and serve as an entry receptor for *Plasmodium falciparum* into red blood cells[38] may also contribute to its immunomodulatory roles in these diseases. Genome-wide association studies have linked certain genetic variants of *CR1* with a predisposition to late-onset Alzheimer disease[39], although their precise function in disease pathophysiology remains poorly defined.

CR2 (also known as CD21), a receptor predominantly expressed in B cells, follicular dendritic cells and a small subset of T cells, has long been recognized as the cognate signalling receptor for C3 fragments (iC3b, C3dg and C3d) but also as a signalling hub bridging innate and adaptive immune responses at the antigen-presenting cell–B cell interface[34,40]. Studies in the late 1990s established that antigen bound to multiple copies of C3d induces the co-ligation of CR2 and the B cell receptor (BCR) signalling complex (which includes the BCR signalling partners CD19 and CD81)[41]. CR2 engagement lowers the threshold for B cell activation via enhanced intracellular Ca^{2+} flux and increased B cell proliferation[34]. The role of CR2 as a molecular adjuvant that enhances humoral responses to T cell-dependent antigens has been consistently shown in mouse models but remains debated in the human system[42,43].

On the surface of follicular dendritic cells, CR2 can trap and retain C3 ligand-bound antigens and thus likely prolong their interaction with germinal centre B cells, thereby contributing to antibody affinity maturation and immunological memory[34,42]. Additionally, CR2 signalling regulates tolerance to self-antigens and dysfunctional variants of this receptor have been implicated as susceptibility traits for the development of autoimmune pathologies[44]. Recently, marginal zone B cells were shown to 'co-opt' MHC class II–C3 complexes from the surface of conventional dendritic cells through a process called trogocytosis[45]. Trogocytosis refers to the active transfer or ingestion of cell membrane fragments from antigen-presenting cells to B cells, T cells or natural killer cells[46]. CR2 recognizes C3 fragments bound to dendritic cell-expressed MHC molecules and triggers the trogocytic transfer of these complexes to the membrane of marginal zone B cells[45]. Through CR2-dependent trogocytosis, B cells can acquire dendritic cell properties and display MHC-bound antigens to T helper cells.

Another well-conserved function of complement is its ability to trigger the chemotactic recruitment of immune effector cells to sites of infection or inflammatory damage[6]. Potent chemotactic stimuli are provided by the binding of the C3a and C5a anaphylatoxins to their cognate receptors C3aR and C5aR1–CD88, respectively, on a range of myeloid and non-myeloid cells[47,48]. Besides chemotaxis, C3aR stimulation exerts various immunomodulatory functions in a wide spectrum of pathophysiological contexts[17]. Of note, the original view that C3a and C5a share similar functions as anaphylatoxins has been challenged by findings indicating that, under certain circumstances, the C3a–C3aR axis can antagonize immune cell chemotactic signals. In addition, the 'weaker' activity of C3a versus C5a on immune cells (such as neutrophils) is likely attributed to the inactivity of C3a-desArg (peptide generated by cleavage of the C-terminal arginine (desargination) of C3a) compared with C5a-desArg[49]. C5a may also bind to a second receptor, termed C5aR2 (also known as C5L2), originally thought to be a decoy receptor due to the lack of coupling to heterotrimeric G proteins[50,51]. Today, C5aR2 is considered an immunomodulatory receptor that signals through the β-arrestin pathway, finetuning C5a responses and often restraining C5aR1-induced pro-inflammatory signalling[51]. It also exerts broader immunomodulatory effects on macrophages by potentiating NLRP3 inflammasome activation and HMGB1 release and constraining signalling through Toll-like receptors, C-type lectin receptors and stimulator of interferon genes protein (STING)[52]. Of note, both C3aR and C5aR1 have been implicated in haematopoietic stem and progenitor cell (HSPC) and myelomonocytic cell trafficking. C3aR signalling sensitizes HSPCs to stromal cell-derived factor 1-mediated homing responses[53] and constrains neutrophil mobilization in response to intestinal injury[54], whereas C5aR1 signalling enhances HSPC mobilization in mice[55].

Several studies have elucidated new functions of the MAC beyond its 'classical' cytolytic (pore-forming) function. Cells exposed to sublytic MAC can instigate intracellular Ca^{2+}-dependent or Ca^{2+}-independent signalling pathways that affect cell proliferation, induction of apoptosis, cell motility, inflammasome activation and pro-inflammatory cytokine signalling[56]. Internalization of the MAC through the endosomal compartment can induce NLRP3 activation and IL-1β and IL-18 release from macrophages, providing a new framework for the design of therapeutic approaches to target MAC assembly[57].

Insights into complement regulation

Complement activation is rapidly triggered in response to environmental cues or microbial intruders, and amplification of complement responses, mainly through the alternative pathway amplification loop, can lead to a forceful pro-inflammatory reaction that, if left unchecked, can provoke collateral tissue damage[2,58]. Inappropriate self-directed complement responses are normally prevented by the concerted action of fluid-phase and membrane-bound regulatory proteins that act as checkpoints to preclude prolonged or excessive complement activity[59]. Most of these regulatory proteins belong to the regulators of complement activation (RCA) gene family, which defines a discrete cluster on chromosome 1q32.2 (ref. 59). The RCA family contains soluble regulators, such as members of the factor H family[60] (full-length factor H and six highly related proteins, including factor H-like 1 and factor H-related proteins (FHRs)) and C4b-binding protein (C4BP) but also membrane-bound regulators, such as CD46 (also known as MCP), CR1 and CR2, and the glycosylphosphatidylinositol (GPI)-anchored protein decay-accelerating factor (DAF; also known as CD55)[61,62] (Fig. 1a).

The regulatory activity of these proteins is rooted in their modular structure consisting of a tandem repeat of short consensus repeat (SCR) domains (also known as complement control protein domains). RCA proteins mediate their complement regulatory function by several mechanisms, including the acceleration of the dissociation (decay) of C3 and C5 convertases (DAF, factor H and C4BP), acting as co-factors for factor I-mediated inactivation of C3b and C4b (factor H, CR1 and C4BP), or acting as inhibitors of the assembly of the pore-forming MAC (CD59)[6]. Elegant studies have provided new insights into the structural basis of their mode of action[63], including how CD59 interferes with MAC assembly[18,64].

The magnitude of complement activation in the fluid phase is also regulated by enzymes, such as carboxypeptidases N, R or B2, that cleave off the terminal arginine from C3a and C5a, or the plasma protein C1-esterase inhibitor, which inhibits both the classical and lectin pathways by inactivating the C1r and C1s proteases and MASPs, respectively[65] (Fig. 1a). The most prominent fluid-phase alternative pathway regulator is factor H, a glycoprotein that comprises 20 SCR domains and displays decay-accelerating, cofactor activity and host-surface recognition capacities[6]. Host-recognition elements recognized by factor H include deposited C3b and polyanionic molecules such as glycosaminoglycans and proteoglycans (for example, heparan sulfate, heparin and chondroitin sulfate)[62]. Binding of factor H to altered self-ligands resulting from lipid or protein peroxidation (for example, malondialdehyde-modified epitopes) has been implicated in chronic inflammatory diseases such as age-related macular degeneration (AMD)[66]. The factor H polymorphism Y402H variant, a predisposing genetic factor for AMD, shows reduced binding to malondialdehyde-modified epitopes on host surfaces, thereby contributing to increased alternative pathway dysregulation in the diseased retina[66] (see below).

Complement regulators, such as factor H or C4BP, provide a shield against autologous complement activation by virtue of their ability to bind to the vascular endothelium, specifically to glycosaminoglycans of the glycocalyx[67]. Genetic or acquired alterations that disturb the binding of regulators to the glycocalyx may result in impaired alternative pathway regulation and uncontrollable deposition of C3 fragments on the endothelial wall, thereby fueling inflammation and pathology[2,67]. Moreover, gene variants (mutations and common or rare single nucleotide polymorphisms) or acquired alterations (autoantibodies) affecting the structure or function of complement regulatory proteins have been implicated as susceptibility factors in diseases with prominent alternative pathway dysregulation[68,69]. For example, rare and common variants of complement regulators CD46, factor I, factor H or FHRs as well as of alternative pathway components, such as C3 and factor B, have been linked to the pathogenesis of rare renal disorders, such as C3 glomerulopathy, and complement-mediated thrombotic microangiopathies with renal manifestations such as atypical haemolytic uraemic syndrome (aHUS)[69,70]. Both C3 glomerulopathy and aHUS share common pathophysiological traits but also have divergent complement gene signatures as revealed by genetic studies. Of note, factor H variants linked to aHUS pathology appear to be clustered in the host-recognition modules of the C terminus of factor H whereas, in C3 glomerulopathy, variants tend to cluster to the N-terminal C3b binding and regulatory domains of factor H, indicating a differential impact of genetic risk factors on fluid-phase C3 regulation[71].

An evolutionary arms race between the host and invading pathogens is reflected in the many ways by which viruses and bacteria subvert complement regulatory networks[3,72]. Several pathogens have evolved mechanisms by which they can either sequester complement regulators, such as factor H or factor I, to their surface using protein 'baits' or encode viral homologues of RCA proteins that display cofactor or decay-accelerating activities[3]. Two such examples are the variola virus smallpox inhibitor of complement enzymes (SPICE) and the vaccinia virus complement control protein (VCP)[73]. SPICE consists of four SCR domains and exhibits both decay-accelerating and cofactor activities, being highly homologous to human CD46 (ref. 63). VCP binds to C3b and C4b, accelerating the decay of C3 convertases generated through all complement pathways[73], and supports the factor I-mediated inactivation of C3b and C4b. Complement evasion tactics have been developed by many viral families, including herpesviruses and adenoviruses[74].

Properdin, an oligomeric plasma glycoprotein, is the only known positive regulator of complement activation[75]. It stabilizes the alternative pathway C3 and C5 convertases and serves as a pattern-recognition molecule that can recruit C3b to surfaces to initiate assembly of the alternative pathway C3 convertase via initial docking on collectin 12 (ref. 76). Non-complement immunomodulatory functions ascribed to properdin include its interaction with the activating receptor NKp46 on natural killer cells[77].

Structure-guided studies have provided unique insights into the mode of action of these complement regulators, revealing the molecular determinants of the tiered interactions taking place during convertase formation and regulation. These structural insights have provided a framework for understanding the impact of disease-related mutations on pathophysiology and microbial immune evasion tactics[63,78].

The emerging landscape of intracellular complement

Although early observations in the 1980s had documented the extrahepatic synthesis of complement proteins by lymphocytes and other cell types, alluding to non-canonical or intracellular functions of these proteins[79,80], it was not until the 2010s that this notion was revisited in studies that broadened our perception of how complement operates in the intracellular space[8,81]. Growing evidence points to the capacity of complement fragments and receptors to shuttle between the extracellular space and the interior of cells[82], forging reciprocal interactions with other immune sensing and pattern-recognition platforms to mediate a wide array of immunomodulatory functions in diverse cell types and pathophysiological contexts[8,1,40].

Intracellular pathogen clearance

Pathogen clearance relies on mechanisms that operate both extracellularly and within the cytoplasm and endosomal compartment of infected cells[1]. Mechanistic studies have revealed that C3b and C4b opsonization of viral particles and bacteria triggers intracellular pathways that mediate pathogen neutralization[1]. Internalization of C3b-coated adenoviruses can trigger the mitochondrial antiviral signalling-dependent signalling pathway inducing pro-inflammatory cytokine secretion via NF-κB, interferon regulatory factor and AP-1 signalling. This process appears to modulate both bacterial and viral pathogen clearance[83]. On the other hand, classical pathway-mediated deposition of C4b on adenoviral particles inhibits capsid disassembly, thereby preventing cytosolic viral entry and endosomal escape in a process that appears independent of C3 sensing[84]. IgG opsonization can synergize with cytosolic Fc receptor signalling to target and degrade intracellular viruses. Indeed, recent studies showed that the superior capacity of the IgG3 subclass to neutralize intracellular viruses relies

on its ability to recruit, via its flexible hinge, the cytosolic Fc receptor tripartite motif containing 21, which may synergize with C1 and C4 to promote lysosomal degradation of viral particles via direct capsid inactivation[85]. These examples describe the intracellular functions of complement proteins coupled to effector responses that originate in the extracellular space.

Modulation of T cell biology and homeostasis

Studies in $CD4^+$ T cells have provided mechanistic insights into how intracellular complement can tap into immunometabolic programmes that drive cellular phenotype expression[23]. A C3(H_2O) recycling pathway has been suggested to serve as a source of intracellular C3, which can be cleaved by lysosomal enzymes (cathepsin L) to generate C3a and C3b[23,82] (Fig. 1b). Intracellularly generated C3a binds to C3aR on lysosomal membranes triggering the mTOR pathway that promotes homeostatic T cell survival[86]. T cell receptor engagement also modulates the translocation of intracellular C3 activation fragments to the cell membrane, where combined C3b-induced CD46 and C3a-induced C3aR signalling triggers sustained activation of mTORC1 (ref. 87). These molecular events potentiate glycolysis and oxidative phosphorylation, thereby contributing to induction of the T helper 1 (T_H1) cell phenotype.

Human $CD4^+$ T cells contain intracellular stores of C5, although their exact origin is still debated. Combined T cell receptor and CD46 stimulation triggers intracellular generation of C5a, inducing C5aR1-dependent production of reactive oxygen species, which in turn potentiates the NLRP3 inflammasome-driven release of IL-1β that sustains induction of T_H1 cells[88]. Autocrine activation of CD46 in conjunction with IL-2 receptor signalling leads to the production of IL-10, which drives homeostatic contraction of the T_H1 cell response[89].

Cell-intrinsic C3 signalling influences immunometabolic signalling and phenotypic trajectories not only in $CD4^+$ T cells but also in $CD8^+$ T cells. C3b generation and autocrine CD46 signalling modulate optimal cytotoxic activity and interferon-γ secretion in conjunction with T cell receptor and CD28 co-stimulation by augmenting nutrient influx and fatty acid synthesis[90]. Intracellular C3 activation in $CD8^+$ T cells appears to be partly mediated by cathepsin L. In contrast to $CD4^+$ T cells, CD46 signalling in $CD8^+$ T cells appears to be uncoupled from the formation of a canonical NLRP3 inflammasome[90], thus revealing a divergent crosstalk with immunometabolic pathways regulating T cell effector function.

Moreover, during transendothelial migration of T cells and monocytes to peripheral tissues, interaction between the integrin lymphocyte function-associated antigen 1 on immune cells and intercellular adhesion molecule 1 on endothelial cells induces C3 expression, indicating a functional crosstalk between integrin signalling and complement biology[91]. Consistently, T cells and monocytes with defective expression of lymphocyte function-associated antigen 1 (specifically, obtained from patients with leukocyte adhesion deficiency type 1) display impaired effector function, which is improved by intracellular C3 provision[91].

Tapping into myeloid cell biology

Besides T cell responses, intracellular complement modulates myeloid and stromal cell responses triggered by sterile inflammatory insults[92]. Exposure of macrophages to cholesterol crystals induces C5aR1 signalling on mitochondrial membranes, which in turn enhances the generation of reactive oxygen species and anaerobic glycolysis, culminating in pro-inflammatory IL-1β secretion[93]. While colocalized staining for C3b and Bb within C5-positive macrophages suggests possible assembly of C3 and C5 convertases, further validation by biochemical and functional assays is warranted before definitive conclusions can be drawn.

Exacerbating tissue inflammation from 'within'

Inflammatory tissue priming contributes to the refractoriness of chronic inflammatory diseases such as rheumatoid arthritis[92]. A recent study identified a novel mechanistic link between intracellular C3a–C3aR signalling and the NLRP3 inflammasome in synovial fibroblasts[92,94]. Autocrine C3aR stimulation upregulated the activity of the mTOR and hypoxia-inducing factor-1α pathways, leading to metabolic reprogramming of fibroblasts (increased oxidative phosphorylation and glycolysis) and NLRP3 inflammasome-mediated IL-1β production, which sustains a prolonged inflammatory response that exacerbates tissue inflammation[94].

Collectively, these tightly regulated interactions between intracellular complement proteins and various receptors and immune signalling complexes have illuminated new aspects of the involvement of complement in T cell homeostasis and survival as well as in immunometabolic programmes driving T cell effector functions and stromal cell inflammatory priming.

Extending the scope of immunomodulatory functions

In addition to C3 fragments, regulators and anaphylatoxin receptors, other complement components contribute to both canonical and non-canonical functions within the intracellular space, including roles of C1q and factor H in cancer cell proliferation, immunosuppression in the tumour microenvironment, and tumour progression (discussed in the next section). Intracellular complement activity has also been linked to autophagy regulation and insulin secretion in pancreatic islets[81]. Of note, cytosolic C3 is highly expressed in human islet β-cells and mediates protection from oxidative stress by interacting with ATG16L1, a component of the autophagic machinery[95]. Recent studies have provided mechanistic insights into the generation of intracellular C3 by showing that alternatively translated C3 variants lacking the signal peptide are produced and retained in the cytosol[96]. Cytosolic C3 is functionally active and has the capacity to opsonize invasive *Staphylococcus aureus* cells within infected epithelial cells[96]. Similarly, intracellular splice variants of CD59, lacking the C-terminal GPI-anchoring signal, have been identified in both mouse and human pancreatic islets[97]. Their expression modulates insulin secretion from β-cells, thus identifying a new connection between intracellular complement biology and metabolic regulation.

Despite this surge of studies ascribing new roles to intracellular complement, there are still several controversies that need to be addressed with regard to the precise origin of complement fragments, the contribution of canonical complement pathways to intracellular activation and the ability to assemble functional enzymatic complexes within restrictive microenvironments such as the acidic lysosomal compartment.

Complement interactions with the microbiome

Millions of years of evolutionary pressure have placed complement effector pathways at the heart of a balancing act between the host immune response and its commensal eubiotic or dysbiotic microbiota[98]. Moreover, a wide range of pathogens (fungi, viruses, protozoa and bacteria) have evolved evasion tactics aimed at neutralizing complement activities that lead to microbial destruction[3,72]. For example, *S. aureus*, a commensal Gram-positive bacterium that

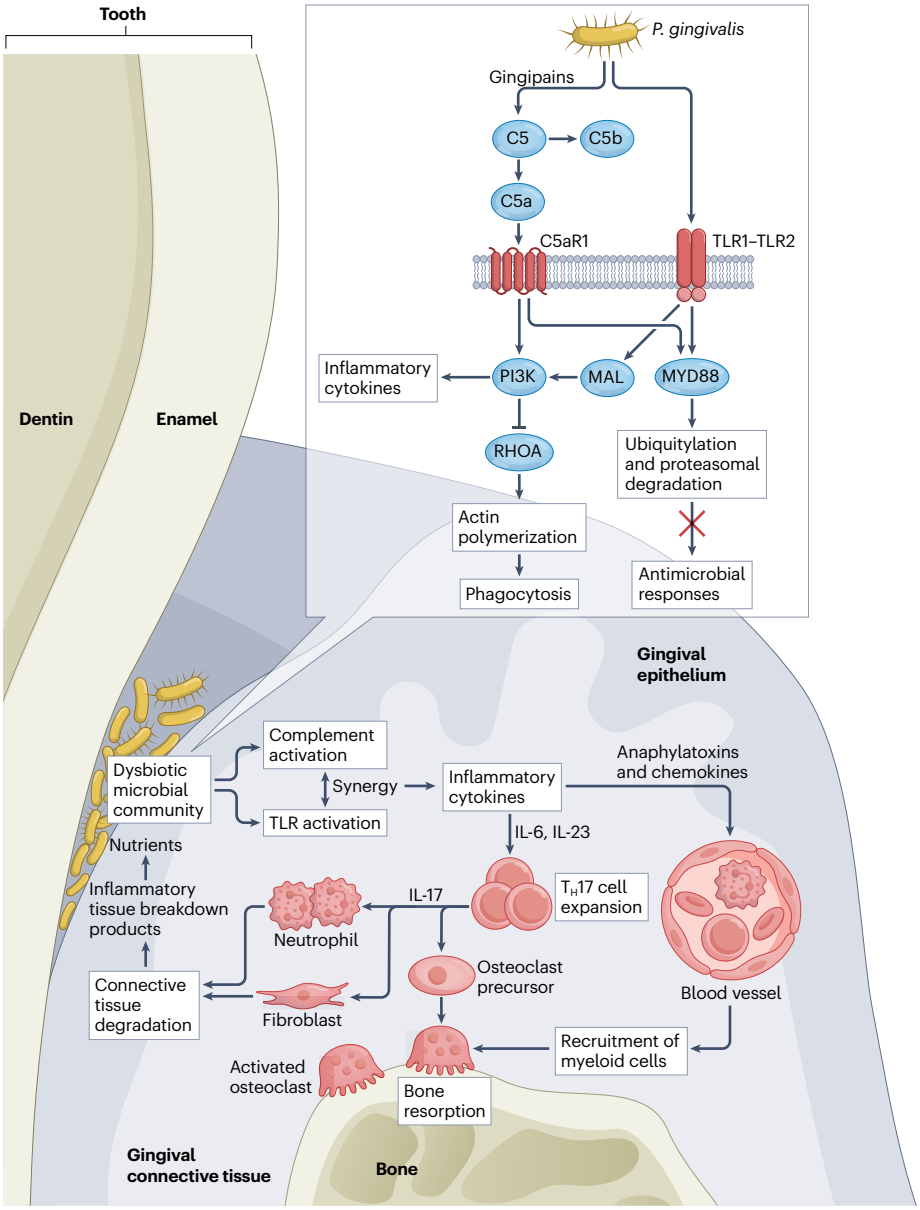

Fig. 2 | Role of complement in microbial dysbiosis-driven inflammatory disease. Periodontitis is a typical example of a dysbiosis-driven inflammatory disorder, where an imbalanced microbiome and the host inflammatory response engage in reciprocally reinforced interactions. The ability of certain periodontal pathogens, such as *Porphyromonas gingivalis*, to subvert complement function contributes to the dysbiosis of the microbial community, in part through impaired immune surveillance that enables the outgrowth of pathobionts. Specifically, *P. gingivalis* expresses Toll-like receptor 2 (TLR2) ligands and uses arginine-specific gingipain enzymes that act on C5 to generate high levels of C5a. *P. gingivalis* can therefore co-activate complement C5aR1 and the TLR2–TLR1 complex in neutrophils, and the resulting signalling crosstalk results in ubiquitylation and proteasomal degradation of the TLR2 adaptor protein MYD88, which would otherwise lead to antimicrobial responses that control this and other pathogens. Furthermore, the C5aR1–TLR crosstalk activates phosphoinositide 3-kinase (PI3K) via the MYD88-like adaptor protein (MAL). Activated PI3K inhibits the RHOA GTPase and hence actin polymerization, which in turn impairs phagocytosis. These functionally integrated signalling pathways, as exploited by *P. gingivalis*, benefit the entire microbial community by undermining innate immune surveillance while perpetuating inflammation (a source of nutrients for the bacteria). Complement-mediated inflammation amplifies the recruitment of inflammatory myeloid cells (via production of anaphylatoxins and chemokines) and causes local expansion of IL-17-secreting $CD4^+$ T cells (T_H17 cells) in periodontal tissue (via production of IL-6 and IL-23). These inflammatory events lead to the degradation of connective tissue and resorption of alveolar bone but also generate a nutritionally favourable environment for the persistence of dysbiotic microbiota. This is because inflammatory tissue breakdown products are selectively used as a source of nutrients by inflammophilic pathobionts. The depicted feedforward loop connecting dysbiosis and inflammation is self-sustained and contributes to the chronicity of periodontal disease.

can subvert immune homeostasis causing invasive disease, employs a multitude of virulence factors[99] that target all stages of the complement cascade[3]. Among them, members of the SCIN family and extracellular fibrinogen-binding protein (Efb) target the central step of C3 activation[100]. SCIN proteins target both the classical and alternative pathway C3 convertases and stabilize these complexes, trapping them in a catalytically inactive state[101]. Efb-C, a proteolytically stable C-terminal fragment of Efb, binds to C3, C3b and C3d, acting as an allosteric inhibitor that induces conformational changes on C3b[102], greatly reducing factor B binding and thus impairing formation of the alternative pathway C3 convertase[103].

Inflammation is a major ecological factor that contributes to the remodelling of a health-associated commensal microbial community to a disease-provoking dysbiotic community with an overrepresentation of pathobionts[104,105]. In this regard, the activation of complement signalling pathways and their crosstalk with MAMP-activated pattern-recognition receptors synergistically enhance inflammation that can selectively fuel the outgrowth of pathobionts, which feed off nutrients derived from inflammatory tissue breakdown products[106,107] (Fig. 2). Dysbiosis-driven inflammatory diseases, such as periodontitis, have helped us to understand the key role of complement in promoting the dysbiotic transformation of polymicrobial communities[105,108]. Besides contributing to the generation of a nutritionally favourable inflammatory environment, complement is also manipulated by periodontitis-associated bacteria for immune evasion[109]. For instance, the keystone periodontal pathogen *Porphyromonas gingivalis* subverts C5aR1–Toll-like receptor crosstalk signalling in phagocytes in ways that impair antimicrobial immunity (extracellular killing and phagocytosis),

while potentiating inflammatory pathways; this uncoupling of immune bacterial clearance from inflammation promotes the fitness of the entire dysbiotic community[110–112]. Complement-driven inflammation and dysbiosis may thus engage in mutually reinforcing interactions and generate a self-sustained feedforward loop that underlies the chronicity of the disease[105] (Fig. 2).

A series of genetic and pharmacological intervention studies in both rodent and non-human primate models of ligature-induced, *P. gingivalis*-driven or naturally occurring periodontitis have established a key role of complement C3 activation and downstream C5aR1 signalling in promoting periodontal inflammation and alveolar bone resorption[113–116]. Local C3 modulation with the compstatin analogue Cp40 in aged non-human primates with severe natural periodontitis results in a marked reduction of key pro-inflammatory cytokines (IL-1β, IL-6 and IL-17) and osteoclastogenic markers such as RANKL[115]. Of note, dysbiosis-driven T_H17 cell expansion and associated neutrophil accumulation in periodontal tissues drive immunopathology and inflammatory tissue destruction in both human and experimental mouse periodontal disease[117]. In this regard, complement activation by the dysbiotic periodontal microbiota is required for pathogenic T_H17 cell expansion through the production of critical T_H17 cell-inducing cytokines IL-6 and IL-23 (ref. 118) (Fig. 2). In particular, IL-6 production was dependent on cooperation between MAMP-activated Toll-like receptor and C3a–C3aR signalling pathways in the gingival epithelium[118]. In summary, complement activation is positioned at the heart of a dysbiotic–inflammatory vicious cycle in the oral mucosa that engages Toll-like receptor signalling, cytokine modulation and T_H17 cell expansion, thereby fueling chronic inflammatory tissue damage and alveolar bone destruction. At the same time, this complement-driven inflammatory tissue breakdown perpetuates microbial dysbiosis and growth, further amplifying microbiota-induced complement activation[105,118] (Fig. 2).

Collectively, these studies have provided fundamental insights into the intricate crosstalk of complement signalling with pattern-recognition receptors and innate immune pathways driving dysbiotic inflammatory disease as exemplified by periodontitis and may have broader implications for the modulation of systemic inflammatory disorders associated with oral dysbiosis[119,120].

Towards a new perception of complement

As a first-line defence system, complement was primarily associated with target cell opsonization, phagocytosis and pathogen destruction. However, growing evidence now indicates that complement proteins intricately regulate biological processes that extend far beyond pathogen immunosurveillance and host defence[1,8,121,122]. This section outlines the conceptual framework behind key mechanistic insights that have defined paradigm shifts in our evolving perception of complement biology.

Roles in tissue regeneration and development

Complement C3 and C5 activation and their downstream signalling pathways (C3aR and C5aR1) have been implicated as key drivers of tissue regenerative programmes in both lower vertebrates and mammals[121,123]. C3 and C5 signalling play instrumental roles in urodele limb and lens regenerative programmes[124,125], which faithfully recapitulate early morphogenesis, tissue patterning, transdifferentiation and tissue plasticity[126]. The effect of complement on tissue regeneration extends to diverse tissues and species as exemplified by the capacity of C3a to promote embryonic chick retinal regeneration by activating STAT3-dependent pro-inflammatory cytokine release in a fibroblast growth factor-independent manner[124]. These observations were largely recapitulated in models of mammalian liver regeneration[121]. Both C3 and C5 are essential drivers of pro-inflammatory cytokine signalling in Kupffer cells in a C3aR-dependent and C5aR1-dependent manner and activate transcriptional programmes driven by NF-κB and STAT3 that promote hepatocyte survival, cell cycle re-entry and regeneration[127,128] (Fig. 3). Genetic and pharmacological C3 intervention in mice subjected to ischaemia–reperfusion injury revealed that a threshold of complement activation, involving C5aR2 signalling, likely dictates a fine balance between complement-induced liver injury and regeneration[129].

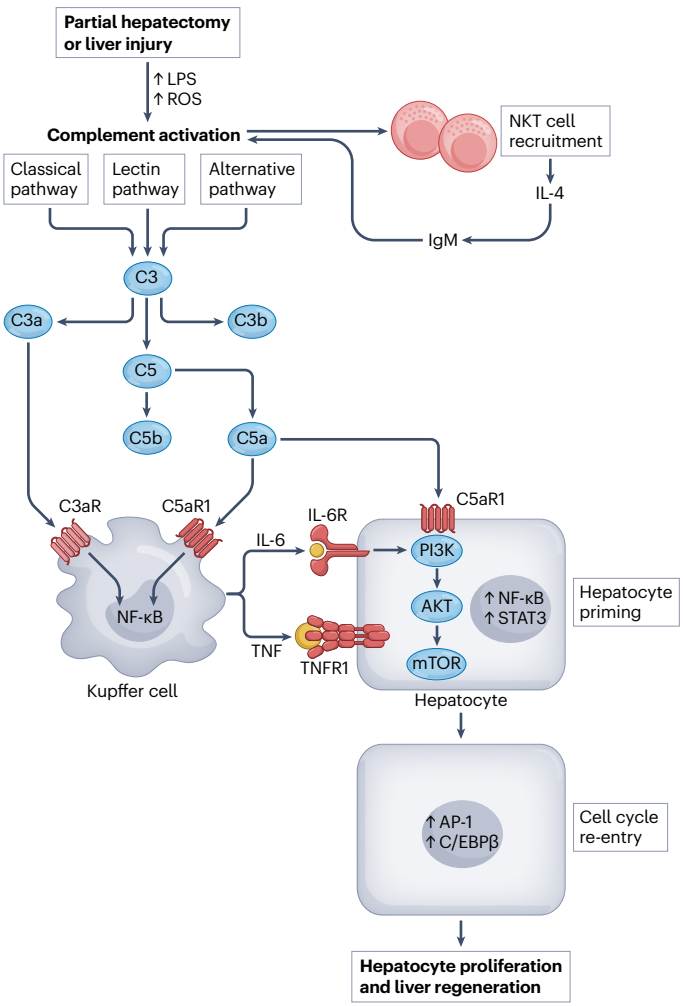

Fig. 3 | Complement shapes tissue regenerative responses across evolution. During liver regeneration in mammals, complement activation drives hepatocyte regeneration by priming quiescent hepatocytes to re-enter the cell cycle in a coordinated manner and proliferate to restore the original liver mass. Early priming events include C3aR and C5aR1 signalling in Kupffer cells and the NF-κB-dependent release of IL-6 and TNF, which together prime hepatocytes to re-enter the cell cycle through transcriptional programmes driven by STAT3 and NF-κB. Complement activation in the liver parenchyma promotes the recruitment of natural killer T (NKT) cells, which release IL-4 to sustain high IgM plasma levels during regeneration. IgM deposition in the liver fuels complement activation, thereby driving a feedforward regulatory loop that sustains complement activation, promoting liver survival, tissue repair and regeneration. LPS, lipopolysaccharide; PI3K, phosphoinositide 3-kinase; ROS, reactive oxygen species.

Consistent with a conserved role of complement in developmental processes, studies in rodents and amphibians have indicated that C3 activation and C3aR signalling can orchestrate morphogenetic processes that underpin embryonic development such as the directional or collective migration of multipotent stem cells, known as neural crest cells[130]. Enteric neural crest cell migration during early gut ontogenesis illustrates an example of how the crosstalk between C3aR signalling and N-cadherin-dependent cell-matrix adhesion promotes early mammalian development[131]. Neural progenitor cells and immature neurons express C3aR and C5aR1, and C3aR signalling in these cells promotes both basal and ischaemia-induced neurogenesis in rodent models[132,133]. Moreover, C5a regulates embryonic neural progenitor cell polarity, whereas transient C5aR1 inhibition during embryogenesis leads to abnormal brain development and behavioural deficits[134].

Roles in cancer immunity and immunotherapy

For decades, complement activation was merely considered an auxiliary mechanism that potentiates the cytolytic action of tumour-specific antibodies through the process of complement-dependent cytotoxicity[14,135]. This effector mechanism is based on the ability of therapeutic antibodies to fix the C1 complex and thus trigger classical pathway activation, leading to tumour cell destruction through assembly of the pore-forming MAC[136]. Indeed, this mechanism is exploited by currently approved immunotherapeutic agents in a range of haematological malignancies[136,137]. Structure-guided studies of C1q bound to immune complexes have revealed critical molecular determinants of classical pathway activity and informed the design of new antibody-based immunotherapies[12] that exploit tumour-directed complement-dependent cytotoxicity[135,138]. Such approaches hold therapeutic promise by overriding complement-directed escape mechanisms evolved by tumour cells such as overexpression of regulators, including factor H, DAF, CD46 or MAC[136].

The long-held dogma that complement activation elicits exclusively tumour cytotoxic responses was challenged in the late 2010s[139]. Genetic and pharmacological C3 intervention in a cervical cancer model revealed that C3 signalling in the tumour microenvironment sustains an immunosuppressive milieu characterized by myeloid-derived suppressor cell infiltration and attenuated $CD8^+$ T cell effector responses[140] (Fig. 4). C5aR1 signalling was identified as a key effector pathway that drives tumour immunosuppression[140–142]. Further studies have since added granularity to the many facets of complement biology in antitumour immunity[137]. C3a- and C5a-induced signalling through their cognate receptors C3aR and C5aR1 has been implicated in cancer cell proliferation, neoangiogenesis and tumour metastasis. As a result, receptor targeting potentiates the therapeutic efficacy of checkpoint blockade directed against PD-1 or PD-L1 in several cancer models[141–144]. Protumorigenic neutrophil C3aR signalling intertwines with procoagulant pathways that induce the formation of neutrophil extracellular traps (NETs) in the tumour microenvironment, thus promoting intestinal tumorigenesis[145]. C3 signalling and both C3a and C5a have been linked to tumour progression through immunosuppressive mechanisms that involve $CD4^+$ and $CD8^+$ T cell modulation[142,146], interactions with infiltrating myeloid cells (myeloid-derived suppressor cells, macrophages and neutrophils) and stromal cells (that is, cancer-associated fibroblasts)[147]. These interactions dictate cancer stemness, M2-like macrophage polarization and immunotherapy

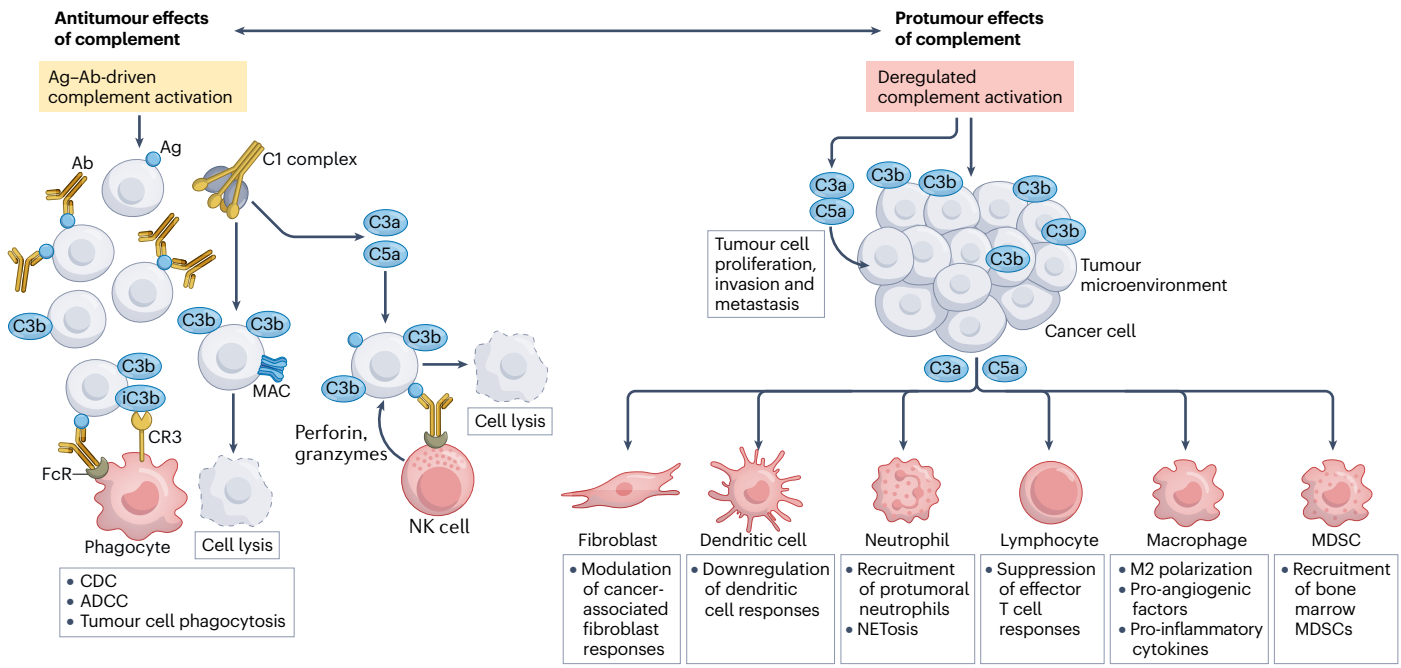

Fig. 4 | Contextual roles of complement in cancer biology and immunotherapy. Emerging mechanistic insights indicate that complement does not simply mediate a cytolytic action against tumours by acting directly (through the membrane attack complex (MAC)) and indirectly (through FcR and antibody-dependent effector mechanisms), but its dysregulation in the tumour microenvironment can promote tumorigenesis via multiple distinct or overlapping immunosuppressive mechanisms. Together, these mechanisms have broader implications for the role of complement in cancer immunity and can help design therapeutic approaches to enhance the efficacy of cancer immunotherapy. Ab, antibody; Ag, antigen; ADCC, antibody-dependent cellular cytotoxicity; CDC, complement-dependent cytotoxicity; MDSCs, myeloid-derived suppressor cells; NETosis, formation of neutrophil extracellular traps; NK cell, natural killer cell.

resistance (reviewed in refs. 136,137,142,148,149) (Fig. 4). C5aR1 blockade increases the efficacy of chemotherapy in squamous carcinoma by reversing T cell immunosuppression[150], and C3aR blockade potentiates the therapeutic efficacy of anti-PD1 treatment in spontaneous and transplantable sarcoma models[151]. Consistently, patients with sarcoma and high enrichment scores of human gene orthologues associated with a C3-deficiency transcriptional signature in mice showed better prognosis and overall survival, indicating that C3aR targeting may hold promise as an immunotherapeutic modality in certain skin cancers[151]. The combination of C3aR blockade with radiotherapy enhanced the therapeutic effects of natural killer cell-based immunotherapy in models of pancreatic cancer, opening new opportunities for complement modulation in cancer[152]. In fact, fungal dysbiosis forms a feedforward loop with complement, driving pancreatic cancer progression via lectin pathway-mediated complement activation and protumoural C3aR signalling[153]. Both renal cell carcinoma and lung adenocarcinoma are associated with an 'overactive' complement transcriptomic signature that predicts poor patient prognosis[137]. Providing mechanistic insight, recent studies have documented intracellular, non-canonical roles of factor H in fostering protumorigenic pathways in renal cancer[154]. Additionally, local classical pathway activation and C1q-expressing tumour-associated macrophages contribute to tumour immunosuppression in clear-cell renal cell carcinoma and serve as a potential prognostic marker for patient stratification[155].

Supporting a contextual role of complement in cancer, complement activation in the tumour stroma conditionally promotes antitumour immunity. C3d–CR2 signalling augments B cell responses following chemotherapy-induced immunogenic cell death and both C3a and C5a can support effector T cell responses following radiotherapy[149,156]. Collectively, these studies highlight the intricacies of the involvement of complement in tumorigenesis, with divergent effects depending on the tumour type, stage of malignancy, immune composition and therapeutic regimen[137]. The role of complement in cancer progression also depends on the magnitude, quality and spatiotemporal regulation of complement responses in the tumour stroma and distant tissues[136].

Role in CNS development and neurodegeneration

Complement activity in the brain has been traditionally associated with apoptotic cell clearance, tissue repair and defence against infectious insults[157]. It is now growingly appreciated that complement proteins constitute a vital component of brain neuroinflammatory circuits that underpin both developmental and pathological processes[122,157] (Fig. 5). Complement proteins are produced in the central nervous system (CNS) by both neuronal and glial cells (astrocytes, microglia and oligodendrocytes) forging intricate signalling networks that affect neuronal survival, axonal damage or demyelination, and astrocyte–microglial activation[157]. Emerging studies are also revealing potential roles for autocrine complement function (such as the C5a–C5aR1 axis) in neurons and glial cells that not only supports homeostatic neuronal signalling but also mediates CNS dysfunction[158]. The discovery that C1q and C3 can selectively tag neuronal synapses, instructing their phagocytic clearance by CR3-expressing microglial cells, illuminated new homeostatic functions of complement in the CNS that refine the synaptic circuitry during postnatal development[122,159] and modulate synaptic plasticity and neurogenesis in the adult brain[160].

However, complement overexpression and aberrant reactivation of the complement–microglia–astrocyte axis have been shown to fuel neuroinflammation and neurodegeneration in several diseases[5,161].

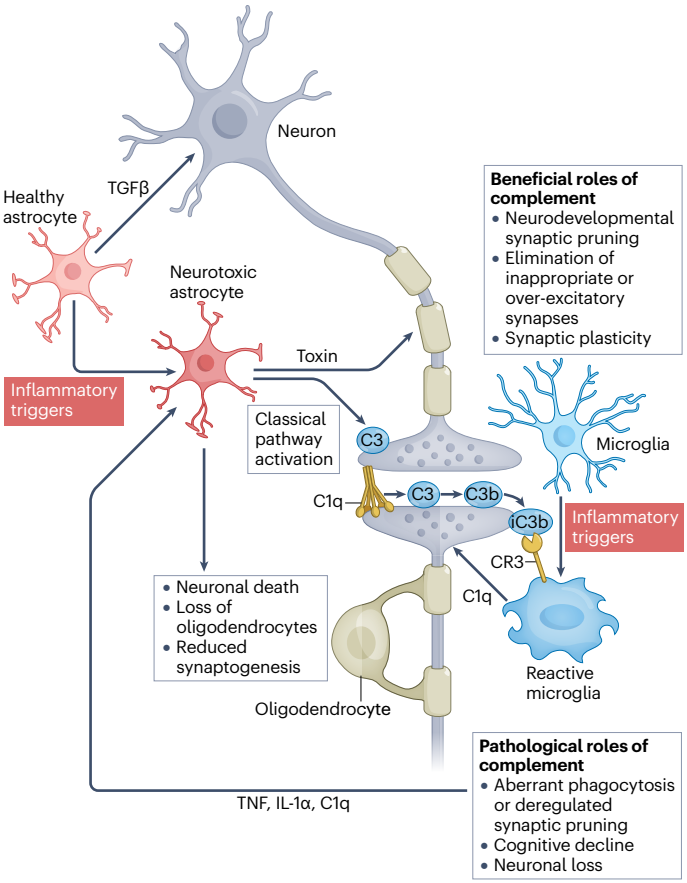

Fig. 5 | Role of complement in CNS development and neurodegeneration. Complement proteins sculpt the synaptic circuitry of the brain in health and disease. An intricate interplay involving local complement expression by glial cells and neurons, microglial-driven phagocytosis, and astrocyte activation underpins neuroinflammatory pathways that guide the developmentally regulated synaptic refinement of the central nervous system (CNS). The aberrant activation of the complement–microglia–astrocyte axis during neurodegeneration can erroneously eliminate synapses and cause neurotoxicity, contributing to cognitive decline and memory loss in neuroinflammatory and neurodegenerative diseases.

C3 activation and downstream CR3 signalling drive aberrant glial cell responses and mediate early synapse loss and cognitive decline in models of Alzheimer disease[162] (Fig. 5). C3aR signalling appears to exert opposing effects on CNS recovery following ischaemic stroke, with detrimental effects in the acute phase and beneficial effects in later stages. Consistently, intranasal C3a delivery in mice during the post-acute phase led to accelerated functional recovery, thus indicating that therapeutic C3aR modulation after stroke should be considered in a strictly time-sensitive context[163].

Neuroinvasive viral infections can also trigger complement and microglial activation and C3 or C3aR modulation attenuates viral-induced synaptic terminal loss and cognitive impairment[164]. In humans, copy number variation of the *C4A* gene isotype is associated with an increased risk of schizophrenia[165,166]. Genetic intervention studies have recently shown that overexpression of the human gene *C4A* in mice mediates abnormal synaptic engulfment by microglia, resulting in cognitive and behavioural aberrations that recapitulate the

schizophrenic phenotype, thus providing evidence for a causal role of complement activation in schizophrenia[167]. Besides CR3, C3aR has also been implicated as a driver of aberrant glial responses in neurodegenerative diseases. A reciprocal interaction between reactive astrocytes overexpressing C3 and C3aR-positive microglia fuels tau pathology and accumulation of neurofibrillary tangles in both humans and mice, revealing a novel mechanistic link between C3a–C3aR signalling and glial STAT3 activation[168]. A recent study has coupled complement biology to nitric oxide signalling during Alzheimer disease pathogenesis, elucidating a sex-dependent mechanism that partly explains the female predominance of the disease. Aberrant post-translational *S*-nitrosylation of C3 was more pronounced in the brains of women with Alzheimer disease, promoting aberrantly activated synaptic engulfment by human microglia in a β-oestradiol-regulated manner[169].

These studies illustrate how rapidly this field of research is progressing, offering both new mechanistic insights and an expanded understanding of the development of complement-based therapeutic approaches for diseases of the CNS[170].

The 'dark side' of complement

While complement has a key role in innate immune recognition, danger sensing and tissue immunosurveillance, it can also cause extensive collateral damage when it is erroneously activated or dysregulated[2]. This inappropriate complement response can lead to aberrant thrombo-inflammatory activation, immune dysregulation, vascular endothelial inflammation and organ damage[171]. As described above, complement responses can be rapidly amplified in the vicinity of an activating surface with detrimental consequences. However, in the steady state, this is avoided by the expression of a range of membrane-bound or fluid-phase regulators in host tissues that promote C3b breakdown into inactive fragments and also accelerate the disassembly of C3 and C5 convertases, thereby limiting the extent of complement activation and resulting tissue damage[59]. Genetic or acquired alterations in complement regulatory proteins may subvert tissue homeostasis, leading to uncontrollable C3 activation both in the fluid phase and on surfaces, persistent C3b opsonization, alternative pathway amplification, and deleterious consequences such as MAC-mediated cell lysis, oxidative tissue damage and prolonged non-resolving inflammation[2,67]. Complement dysregulation fuels a range of immune-mediated and inflammatory diseases of the ocular, renal, haematological and neurological systems[172,173].

Here, we provide an overview of complement-mediated pathophysiology, using as examples disease areas in which extensive research has established the involvement of complement in disease pathogenesis and in which therapeutic proof-of-concept has been established with complement inhibitors already clinically approved or advancing through late-stage human trials[172] (Figs. 6–8).

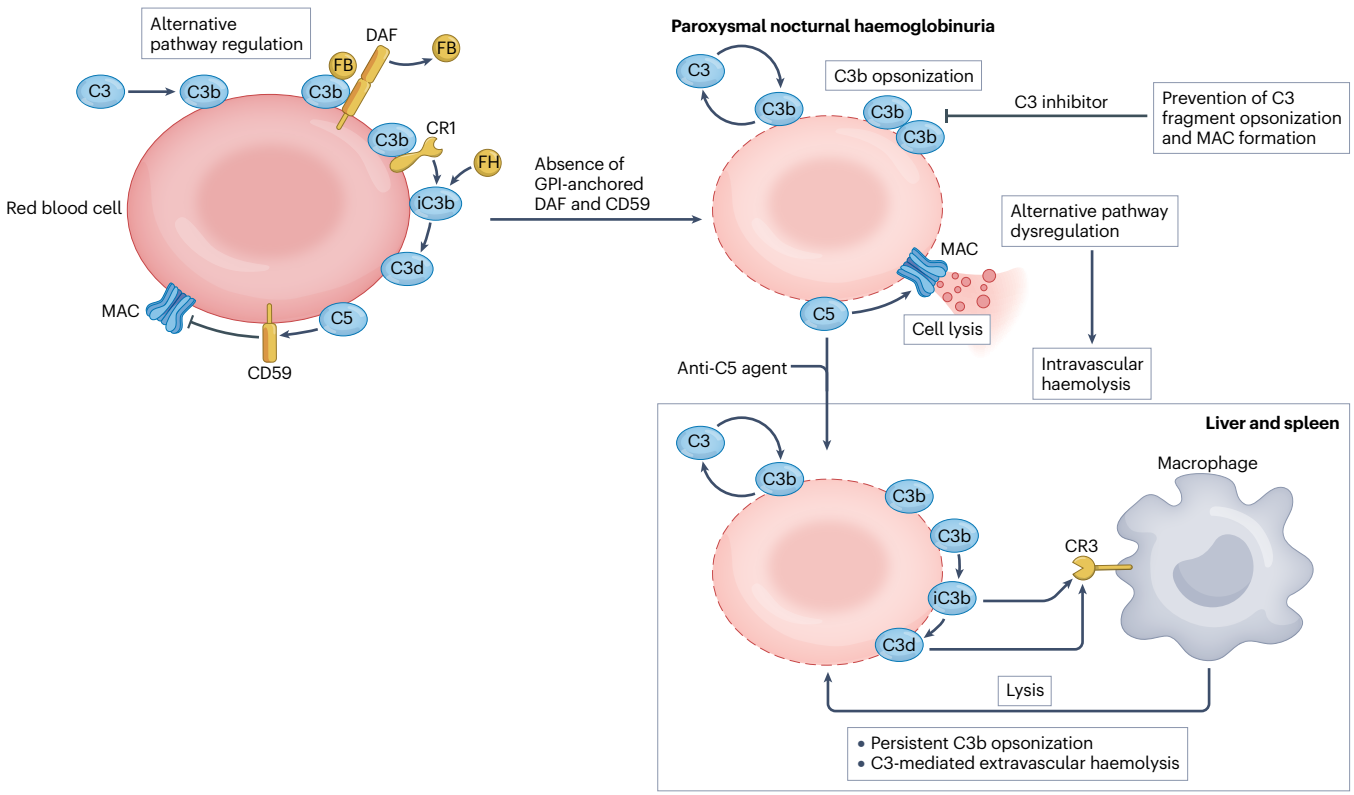

Fig. 6 | Complement dysregulation drives pathology in PNH. The alternative pathway is regulated by the glycosylphosphatidylinositol (GPI)-anchored complement regulators decay-accelerating factor (DAF) and CD59. Patients with paroxysmal nocturnal haemoglobinuria (PNH) have a genetic aberration that results in the loss of GPI-anchored DAF and CD59 on red blood cells. This leads to dysregulated activation of the alternative pathway, which results in intravascular haemolysis via membrane attack complex (MAC) formation. Treatment with an anti-C5 agent can protect PNH cells from MAC-mediated intravascular haemolysis but C3b opsonization still occurs due to the absence of DAF. This leads to C3-mediated extravascular haemolysis by $CR3^+$ macrophages in the liver and spleen. In the presence of a C3 inhibitor, C3b opsonization is prevented and PNH red blood cells are protected from both intravascular and extravascular haemolysis. FB, factor B; FH, factor H.

Haematological disorders

Paroxysmal nocturnal haemoglobinuria (PNH) is a rare haematological disorder characterized by the clonal expansion of a haematopoietic stem cell population that bears an acquired somatic mutation in *PIGA*, the gene encoding phosphatidylinositol *N*-acetylglucosaminyltransferase subunit A[174,175], an enzyme required for the biosynthesis of GPI anchors. This genetic aberration leads to the release of PIGA-deficient erythrocytes into the circulation that are devoid of the GPI-anchored complement regulators DAF and CD59. By virtue of its aetiology, PNH constitutes an archetypal complement-mediated disease (Fig. 6). PNH erythrocytes are susceptible to spontaneous rupture owing to autologous complement attack and MAC-mediated intravascular haemolysis[176]. The predominant role of alternative pathway dysregulation in PNH pathophysiology made PNH a testbed for the evaluation of novel complement therapeutics[174]. Of note, the clinical development of therapeutic agents that target C5 not only addressed the mechanism of intravascular haemolysis but also revealed an elusive pathological mechanism that is largely refractory to treatment with anti-C5 agents, termed C3-mediated extravascular haemolysis[177]. PNH cells exposed to anti-C5 blockade sustain persistent C3b deposition on their surface due to the lack of the C3 regulator DAF. These C3b-opsonized erythrocytes are transferred to the liver and spleen, where CR3-expressing macrophages phagocytose them, causing residual anaemia[178]. Proof-of-concept therapeutic studies with C3 inhibitors of the compstatin family revealed the broader benefit of C3 modulation in PNH in attenuating both intravascular and extravascular haemolysis[179].

Besides PNH, intravascular haemolysis characterizes a range of other diseases, including hyperhaemolysis syndrome, the delayed haemolytic transfusion reactions in sickle cell disease and rare thrombotic microangiopathies with renal manifestations like aHUS[180,181]. Recent studies have shown that, besides alloantibody-mediated classical pathway activation, there is a marked contribution of alternative pathway activation to delayed haemolytic transfusion reactions owing to cell-free haem-dependent C3 activation[182,183]. Haem release following intravascular haemolysis due to microthrombosis can promote alternative pathway dysregulation in aHUS in the context of pre-existing genetic susceptibility[181]. Intravascular haemolysis and haem release triggered complement activation and C3 deposition in vivo in a mouse model of sickle cell disease, whereas pronounced C3 and C5b–C9 deposition was observed in kidney biopsies of patients with sickle cell disease[182]; these findings provided a rationale for the evaluation of therapeutic complement inhibition in this clinical setting (see Table 1 for drug candidates tested in this setting).

Additional mechanisms by which haem may act as a secondary trigger of alternative pathway dysregulation include its direct interaction with factor I and inhibition of factor I-mediated C3b degradation[184] as well as its capacity to downregulate expression of the regulators CD46 and DAF on vascular endothelial cells[181]. Moreover, haem can trigger Toll-like receptor 4-dependent expression of P-selectin on endothelial cells, which can, in turn, amplify C3 deposition via the alternative pathway amplification loop, using P-selectin as a scaffold to anchor C3($H_2$0) or C3b on the modified endothelium[185]. These mechanistic insights offer a conceptual platform for our understanding of the broader role of complement dysregulation in a range of haemolytic and thrombo-inflammatory diseases. Complement not only mediates direct erythrocyte destruction (intravascular haemolysis) but also orchestrates several tissue destructive processes that are amplified by secondary triggers, such as haem or erythrocyte microvesicles, and are perpetuated by an inflamed and thrombogenic vascular endothelium with reduced complement regulatory capacity[67].

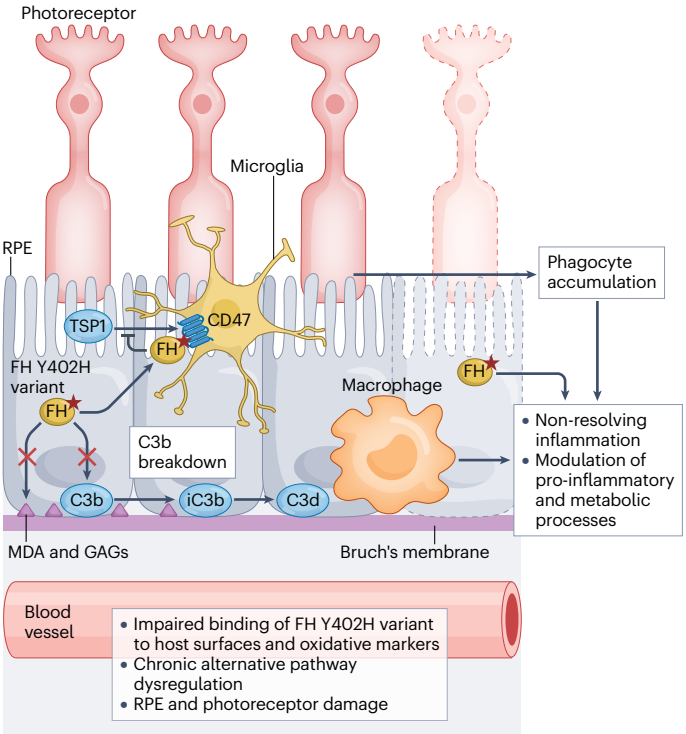

Fig. 7 | Pathogenic role of complement dysregulation in ocular inflammatory diseases. A polymorphism in factor H (FH), Y402H, confers increased risk to the development of age-related macular degeneration. The FH Y402H variant shows impaired binding to markers of lipid oxidation (malondialdehyde (MDA)) and polyanionic surface markers (glycosaminoglycans (GAGs)), which contributes to alternative pathway dysregulation. The FH variant also blocks CD47-mediated elimination of phagocytes in the subretinal space, thus promoting non-resolving inflammation and damage to the retinal pigmented epithelium (RPE) and photoreceptors. Lastly, cell-intrinsic FH Y402H affects immunometabolic and inflammatory processes in RPE cells, favouring age-related macular degeneration pathogenesis.

Ocular disorders and AMD

AMD is a chronic, inflammatory retinal disorder and a leading cause of irreversible vision loss among the elderly in the industrialized world[186,187]. Its early or intermediate form is marked by the presence of dense protein-rich and lipid-rich deposits (called drusen) in the subretinal epithelial space[187]. Early AMD progresses to two advanced stages of the disease: the 'dry' form, called geographic atrophy, which is characterized by the progressive loss of photoreceptors and retinal pigmented epithelium (RPE) cell death owing to choroidal vessel deterioration[186], and the more advanced form, 'wet' or neovascular AMD, largely driven by vascular endothelial growth factor-induced neovascularization that promotes inflammatory damage resulting in RPE atrophy and vision loss[188].

Genome-wide association studies have revealed a linkage between common and rare polymorphisms in complement genes (such as *C3*, *CFB*, *CFI* and *C9*) and disease predisposition[189,190]. The discovery that a common polymorphism of factor H (Y402H) confers an elevated

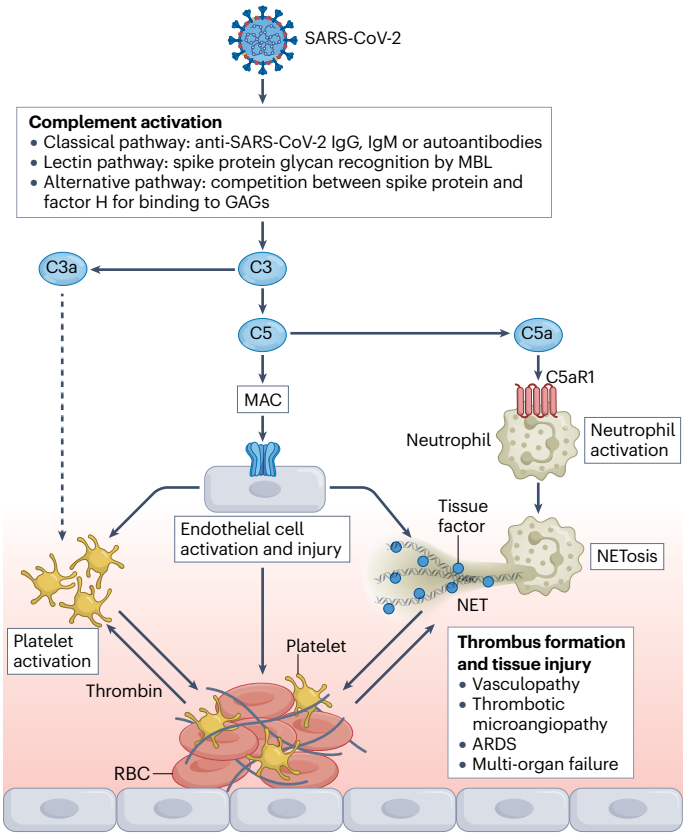

Fig. 8 | Complement dysregulation supports COVID-19-associated immunothrombosis. A maladaptive host thrombo-inflammatory response is considered a key driver of thrombotic microangiopathy and multi-organ failure in severe COVID-19. SARS-CoV-2-triggered complement activation, via all three pathways, results in the release of C3 fragments, generation of C5a and assembly of the membrane attack complex (MAC), which contribute to persistent endothelial cell activation, vascular injury and immunothrombosis. Thrombotic microangiopathy is fuelled by crosstalk signalling and a feedforward loop involving reciprocal interactions between activated platelets, neutrophils and the inflamed endothelium. Complement C3 signalling potentiates the formation of tissue factor-decorated neutrophil extracellular traps (NETs) and has broad thrombo-inflammatory effects in severe COVID-19. ARDS, acute respiratory distress syndrome; GAGs, glycosaminoglycans; MBL, mannose-binding lectin; RBC, red blood cell.

risk of developing AMD stimulated efforts to better understand the pathophysiology and develop targeted therapeutic interventions[191,192]. Residing within a region of factor H (SCR6–8) involved in host tissue recognition through its binding to surface glycosaminoglycans, the Y402H variant likely skews the ability of factor H to bind polyanionic surfaces, thereby resulting in alternative pathway dysregulation in retinal tissue[193]. Other potential pathogenic mechanisms for the Y402H variant include its role in obstructing CD47-mediated elimination of phagocytes from the subretinal space, which is mediated by thrombospondin 1, thereby promoting non-resolving inflammation[194] (Fig. 7). Furthermore, RPE cells expressing the Y402H variant display increased C3 turnover, cathepsin D leakage into drusen-like deposits and impaired lysosomal function, which is restored by C3 inhibition[195]. Beyond its classical regulatory activity, factor H appears to modulate key immunometabolic and inflammatory processes in RPE cells. For instance, knockdown of cell-intrinsic factor H fuels inflammatory cytokine expression, increases C3 and factor B levels through dysregulated NF-κB signalling[196], and impairs RPE glycolysis, mitochondrial respiration and lysosomal turnover. Altogether, impaired factor H function appears to have broad effects on the retina, rendering the RPE susceptible to oxidative damage (such as lipid peroxidation) and shifting these cells to a dysregulated metabolic state, which likely contributes to AMD pathogenesis[197] (Fig. 7).

Emerging evidence points to an even more complex pathophysiological landscape in AMD with plasma complement proteins, including FHRs (such as FHR4), accumulating into the choriocapillaris and Bruch membrane and likely contributing to ocular alternative pathway dysregulation by competing with factor H for surface binding and regulation of C3b breakdown[198]. While the relative contribution of systemic complement stores versus local complement biosynthesis in the retina is still greatly debated, there is evidence for the contribution of both local and systemic mediators to AMD pathogenesis[199], likely fuelled by an impaired blood–retina barrier allowing for 'leakage' of systemic factors into the choroid–RPE interface[187].

COVID-19

The COVID-19 pandemic has reshaped our understanding of the reciprocal interactions between the host immune response and SARS-CoV-2, a viral pathogen with formidable immune evasion tactics[200,201]. It is increasingly appreciated that severe COVID-19 is associated with a maladaptive host inflammatory response that engages multiple innate immune mechanisms and pattern-recognition pathways[201]. Complement dysregulation has been implicated as a key driver of thrombo-inflammation that perturbs vascular endothelial function in multiple organs and enhances reciprocal platelet–neutrophil interactions and NET formation, thereby driving COVID-19 immunothrombosis[202–204] (Fig. 8). Multiple mechanisms have been suggested to fuel complement dysregulation during COVID-19 (refs. 205–207). The classical pathway may be triggered by promiscuous autoantibodies or antibodies to SARS-CoV-2-encoded epitopes[208] and the lectin pathway through direct recognition of carbohydrate signatures on the viral nucleocapsid and spike proteins by mannose-binding lectin or MASPs[209]. The alternative pathway amplifies this response and is also triggered through competition of viral spike protein with factor H for binding to surface glycosaminoglycans like heparan sulfate, thereby potentiating the activity of alternative pathway C3 convertases[210]. Intracellular C3 signalling and C3aR stimulation have also been implicated in the disease process. Virus-triggered interferon receptor signalling in lung epithelial cells activates the JAK1–JAK2–STAT1 pathway, which in turn upregulates the transcription of C3 and factor B, thereby enhancing C3 activation, C3a–C3aR-mediated leukocyte recruitment and lung inflammation[211].

Other organ-specific pathologies

Complement dysregulation, fuelled predominantly by fluid-phase or tissue-directed alternative pathway overactivation and the contribution of multiple triggering pathways, has been linked to many renal pathologies, including C3 glomerulopathy, aHUS, IgA nephropathy, immune complex-mediated glomerulonephritis, antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis and lupus nephritis[67,212]. Of note, while therapeutic complement inhibition has markedly changed the treatment paradigm in aHUS (C5 inhibition) and afforded clinical gains in ANCA-associated vasculitis (C5aR1 blockade), there is still a gap to be addressed in other renal diseases such as C3 glomerulopathy or lupus nephritis, where complement blockers have thus far yielded mixed or

Table 1 | Complement therapeutics currently in the clinic and in various stages of clinical development

Drug candidate[a] (Brand name; Company)	Target	Drug class; mode of administration	Mechanism of action	Stage of clinical development, targeted indications
Approved complement therapeutics				
Therapeutic targets in the initiation pathways				
C1-esterase inhibitor (Cinryze (Shire), Berinert (CSL Behring), Cetor (Sanquin), Ruconest (Pharming))	C1s, C1r, MASPs, other proteases	Protein; IV	Inhibits classical and lectin pathways, and blocks serine proteases of coagulation and fibrinolytic pathways	Approved: hereditary angio-oedema; phase II/III: kidney ischaemia–reperfusion injury, kidney transplantation, sepsis
Sutimlimab (also known as BIV009 or TNT009) (Enjaymo; Sanofi/Bioverativ)	C1s	mAb; IV	Inhibits classical pathway and inhibits C1s protease	Approved: cold agglutinin disease; completed phase I: chronic and refractory immune thrombocytopenia
Therapeutic targets of the central step of C3 activation and alternative pathway amplification loop				
APL-2 (also known as pegcetacoplan) (Empaveli (systemic use) and Syfovre (IVT); Apellis Pharmaceuticals)	C3	PEGylated peptide, compstatin-based C3 inhibitor; SQ (for systemic use), IVT	Inhibits C3 activation by C3 convertases	Approved: paroxysmal nocturnal haemoglobinuria, geographic atrophy (dry age-related macular degeneration); phase III: cold agglutinin disease, C3 glomerulopathy and other nephropathies; phase II: HSCT-TMA
Therapeutic targets in the terminal effector pathways				
Eculizumab (Soliris; Alexion/ AstraZeneca)	C5	mAb; IV	Blocks C5 activation by C5 convertases	Approved: paroxysmal nocturnal haemoglobinuria, atypical haemolytic uraemic syndrome, generalized myasthenia gravis, NMOSD
Ravulizumab (also known as ALX1210) (Ultomiris; Alexion/ AstraZeneca)	C5	mAb; IV, SQ	Blocks C5 activation, long-acting version of eculizumab that exploits FcRn recycling technology	Approved: paroxysmal nocturnal haemoglobinuria, atypical haemolytic uraemic syndrome, generalized myasthenia gravis; phase II/III: dermatomyositis, HSCT-TMA; filed market approval: NMOSD
Avacopan (also known as CCX168) (Tavneos; ChemoCentryx/Amgen)	C5aR1	Small molecule; oral	Antagonist of C5aR1	Approved: ANCA-associated vasculitis; phase I/II: C3 glomerulopathy, hidradenitis suppurativa, lupus nephritis, IgA nephropathy
Complement therapeutics in clinical development				
Therapeutic targets in the initiation pathways				
ANX005 (Annexon Biosciences)	C1q	mAb; IV	Binds to C1q and inhibits classical pathway activation	Phase III: Guillain–Barré syndrome; phase II: Huntington disease, amyotrophic lateral sclerosis
ARGX-117 (Argenx)	C2	mAb; IV, SQ	Binds to C2 and inhibits classical and lectin pathway activation; exploits FcRn recycling technology	Phase II: multifocal motor neuropathy; delayed graft function or transplantation, dermatomyositis
Narsoplimab (also known as OMS721) (Omeros)	MASP2	mAb; IV	Inhibits lectin pathway by blocking MASP2 activity	Phase III: atypical haemolytic uraemic syndrome, IgA nephropathy, HSCT-TMA; phase II: lupus nephritis, severe COVID-19
OMS906 (Omeros)	MASP3	mAb; IV	Blocks MASP3 activity and inhibits alternative pathway	Phase I: paroxysmal nocturnal haemoglobinuria and other alternative pathway-driven diseases
Therapeutic targets of the central step of C3 activation and alternative pathway amplification loop				
AMY-101 (Amyndas Pharmaceuticals)	C3	Non-PEGylated peptide, third-generation compstatin-based C3 inhibitor; IV, SQ (local delivery)	Inhibits C3 activation by C3 convertases	Completed phase II: periodontal disease; interim results of phase II: severe COVID-19; stroke, haemodialysis, ABO incompatible-transplantation
ALXN2030 (Alexion/ AstraZeneca)	C3	C3-targeting siRNA; SQ	Downregulates hepatic C3 expression	Phase I: chronic alloantibody-mediated rejection
NGM621 (NGM Biopharmaceuticals)	C3	mAb; IVT	Inhibits C3 activation	Phase II: geographic atrophy (dry age-related macular degeneration)
ADX-097 (Q32 Bio)	C3d, factor H	Bifunctional fusion protein	C3d-targeting mAb fused to factor H (SCR1–5) for local complement modulation in diseased tissue	Phase I: alternative pathway-driven clinical disorders
GT005 (Gyroscope Therapeutics/Novartis)	Factor I	AAV2-based gene therapy driving expression of factor I	Increases C3b breakdown, inhibits alternative pathway	Phase II: geographic atrophy
Mirococept (also known as APT070) (Medical Research Council, UK)	C3 and C5 convertases	Protein; IV	Inhibits classical and alternative pathway C3 and C5 convertases	Phase II: kidney transplantation

Table 1 (continued) | Complement therapeutics currently in the clinic and in various stages of clinical development

Drug candidate[a] (Brand name; Company)	Target	Drug class; mode of administration	Mechanism of action	Stage of clinical development, targeted indications
Therapeutic targets of the central step of C3 activation and alternative pathway amplification loop (continued)				
Iptacopan (also known as LNP023) (Novartis)	Factor B	Small molecule; oral	Inhibits alternative pathway C3 convertase	Completed phase III: paroxysmal nocturnal haemoglobinuria; phase III: C3 glomerulopathy, IgA nephropathy, atypical haemolytic uraemic syndrome; phase II: membranous nephropathy, lupus nephritis, cold agglutinin disease
IONIS-FB-L_{Rx} (also known as RG6299) (Ionis Pharmaceuticals/Roche)	Factor B	Antisense oligonucleotide; SQ	Inhibits alternative pathway through downregulation of hepatic factor B expression	Phase II: IgA nephropathy
Danicopan (also known as ALXN2040 or ACH-4471) (Achillion/Alexion)	Factor D	Small molecule; oral	Inhibits alternative factor C3 convertase	Phase III: paroxysmal nocturnal haemoglobinuria (first market filing acceptance received); phase II: geographic atrophy
Vemircopan (also known as ALXN2050 or ACH-0145228) (Achillion/Alexion)	Factor D	Small molecule; oral	Inhibits alternative pathway C3 convertase	Phase II: paroxysmal nocturnal haemoglobinuria, lupus nephritis, IgA nephropathy, generalized myasthenia gravis
CLG561 (Alcon/Novartis)	Properdin	mAb; IVT	Inhibits alternative pathway activation and amplification	Phase II/III: geographic atrophy, age-related macular degeneration
Therapeutic targets in the terminal effector pathways				
Crovalimab (also known as RG6107; formerly SKY59/RO7112689) (Chugai/Roche)	C5	mAb; IV, SQ	Blocks C5 activation (different C5 epitope from eculizumab), exploits antibody recycling technology	Phase III: paroxysmal nocturnal haemoglobinuria; phase II: sickle cell disease
Tesidolumab (also known as LFG316) (Novartis)	C5	mAb; IV, IVT	Blocks C5 activation (different C5 epitope from eculizumab)	Completed phase II: paroxysmal nocturnal haemoglobinuria
Pozelimab (also known as REGN3918) (Regeneron)	C5	mAb; IV or SQ	Blocks C5 activation (different C5 epitope from eculizumab)	Phase III: paroxysmal nocturnal haemoglobinuria; phase II/III: CD55-deficient protein-losing enteropathy, generalized myasthenia gravis
Zilucoplan (also known as RA101495) (Ra Pharmaceuticals/UCB)	C5	Peptide macrocycle; SQ	Allosteric inhibition of C5 activation	Completed phase III: generalized myasthenia gravis (drug application under review by FDA); phase II/III: paroxysmal nocturnal haemoglobinuria
Cemdisiran (also known as ALN-CC5) (Alnylam)	C5	RNAi therapeutic; SQ	Inhibits hepatic expression of C5	Phase II/III: paroxysmal nocturnal haemoglobinuria, IgA nephropathy
Avacincaptad pegol (Zimura; Iveric Bio)	C5	PEGylated RNA aptamer; IVT	Inhibits C5 expression	Completed phase III: geographic atrophy, age-related macular degeneration; phase IIb: Stargardt disease
Nomacopan (also known as coversin) (Akari Therapeutics)	C5, leukotriene B4	Protein; SQ, IVT	Inhibits C5 activation and leukotriene B4 signalling	Phase III: paediatric HSCT-TMA; preclinical stage: geographic atrophy
ABP959 (Amgen)	C5	mAb; IV	Biosimilar to eculizumab (Soliris)	Phase III: paroxysmal nocturnal haemoglobinuria; preclinical stage: other complement-mediated diseases
Vilobelimab (also known as IFX-1) (Gohibic; InflaRx)	C5a	mAb; IV	Binds to C5a and blocks its activity	Completed phase III: critical COVID-19 (received EUA from FDA); phase II: cutaneous squamous cell carcinoma, pyoderma gangrenosum
Avdoralimab (also known as IPH5401) (Innate Pharma)	C5aR1	mAb; IV	Blocks C5aR1 signalling	Phase I/II (terminated): solid tumours (in combination with anti-PDL1); phase II: bullous pemphigoid; completed phase II: severe COVID-19
ALS-205 (also known as PMX205) (Alsonex/Teva)	C5aR1	Cyclic peptide; oral, SQ	Selective C5aR1 antagonist	Phase Ib: amyotrophic lateral sclerosis; phase I: Alzheimer disease; preclinical stage: Parkinson disease, Huntington disease
HMR59 (Hemera Biosciences/Janssen)	CD59	AAV2-based gene therapy driving expression of soluble CD59; IVT	Inhibits MAC assembly	Phase I/II: geographic atrophy, wet (neovascular) age-related macular degeneration, dry age-related macular degeneration

AAV, adeno-associated virus; ANCA, antineutrophil cytoplasmic antibody; EUA, emergency use authorization; HSCT-TMA, haematopoietic stem cell transplant-associated thrombotic microangiopathy; IV, intravenous; IVT, intravitreal; MAC, membrane attack complex; MASPs, mannan-binding lectin-associated serine proteases; mAb, monoclonal antibody; NMOSD, neuromyelitis optica spectrum disorder; RNAi, RNA interference; SCR, short consensus repeat; siRNA, small interfering RNA; SQ, subcutaneous. [a]The list of drug candidates is not exhaustive but rather aims to reflect the breadth and scope of targets in the complement cascade and the various drug classes currently under clinical investigation.

mediocre results[213], likely reflecting the complex pathophysiology of these disorders, the heterogeneity of clinical phenotypes and the need for robust biomarker-guided patient stratification[212].

The organ transplantation field has also seen significant strides in our understanding of the contribution of complement to multiple facets of pathology, including donor organ storage, ischaemia–reperfusion injury, acute antibody-mediated rejection and delayed graft function[214]. Recent studies have also provided new insights into the pathogenic role of complement activation in conditions requiring treatment in the intensive care unit such as acute polytrauma with multi-organ failure and rhabdomyolysis-induced acute kidney injury[215,216].

In addition, new insights have been gained in chronic inflammatory and autoimmune diseases in which complement has long been recognized as a contributing or disease-exacerbating factor, such as systemic lupus erythematosus, antiphospholipid syndrome and rheumatoid arthritis[217]. While preclinical rheumatoid arthritis models have shown promise for complement modulation, thus far, this has not been translated in the clinical setting. A deeper understanding of the early immune events and inflammatory processes driving synovial inflammation and disease flares will help to elucidate the relative contribution of local versus systemic complement factors to rheumatoid arthritis pathophysiology, their spatiotemporal distribution in the inflamed synovium, and their correlation with disease activity and therapy response in patients[218]. These new mechanistic and translational studies have only begun to reveal a new field of opportunity for therapeutic complement modulation.

Therapeutic modulation of complement

Besides its integral role in human pathophysiology, complement has attracted considerable interest as a target of therapeutic modulation in inflammatory, ageing-related and immune-mediated diseases. Today, the clinical arsenal of complement therapeutics features a panel of approved drugs with distinct targets, modes of action and delivery routes that have drastically changed the treatment landscape in rare haematological, renal and neurological disorders as well as in more prevalent ocular inflammatory diseases with no therapy at hand until recently (Box 2 and Table 1).

Systemic modulation of complement

The complement cascade features multiple points of therapeutic intervention, depending on the distinct role of each target in disease pathophysiology. Intervention at the level of classical or lectin pathway initiation offers the advantage of blocking disease-promoting activities of early components (such as C1q, C2 and MASP2) whilst preserving the core functionality of the cascade and its multiple downstream effectors[2]. Terminal pathway inhibition at the level of C5 may tackle the deleterious effects of pro-inflammatory C5a signalling and MAC-mediated cytolysis, while targeted blockade of the C5a–C5aR1 axis

Box 2

The clinical arsenal of complement-targeting inhibitors

Approval of the C5-targeting monoclonal antibody eculizumab (Soliris, Alexion) revolutionized the treatment of patients with paroxysmal nocturnal haemoglobinuria[258]; its development was spearheaded by the monoclonal antibody BB5.1[259]. It also bolstered confidence in the development of a new generation of therapeutic agents targeting various components of the complement cascade[172,173,260] (Table 1). Besides eculizumab and its long-acting successor ravulizumab (Ultomiris)[261], the clinical arsenal now includes three more complement-specific drugs with distinct targets and modes of action: Empaveli (APL-2 or pegcetacoplan), a C3-targeted inhibitor and the first member of a mechanistically distinct class of peptide C3 inhibitors of the compstatin family (discovered by the Lambris group at the University of Pennsylvania)[244,262–264]; Enjaymo (sutimlimab), a C1s-specific monoclonal antibody and the first classical pathway-specific inhibitor used for the treatment of the rare haemolytic disease cold agglutinin disease; and Tavneos (avacopan), a small-molecule C5aR1 antagonist that is used as an adjunctive therapeutic option in severe antineutrophil cytoplasmic antibody-associated vasculitis[265,266].

The recent approval of Empaveli for paroxysmal nocturnal haemoglobinuria, and that of its intravitreal formulation Syfovre as the only treatment for patients with geographic atrophy, marked a watershed moment in complement drug discovery[230,264], validating C3 modulation in the clinical setting. Indeed, C3 inhibition had been the subject of a long-standing debate on perceived technical issues (that is, the capacity of a drug to saturate plasma C3 levels) and concerns about the safety of chronic C3 modulation[68]. Safety concerns revolved around a purportedly increased risk of opportunistic infections by encapsulated bacteria, drawing on observations from rare primary C3 deficiencies[267]. However, this phenotype was mainly observed in younger individuals and would subside with age, probably compensated by other pathogen immunosurveillance mechanisms and a fully developed adaptive immunity[268]. Moreover, pharmacological C3 inhibition does not phenocopy a genetic deficiency and it may even attenuate pathogenic bacterial growth in certain contexts (for example, in oral dysbiotic diseases)[269]. Recently described C3 bypass pathways may further provide residual terminal pathway activity that is sufficient for microbial elimination in the context of therapeutic C3 modulation[33]. The approval of Empaveli largely dissolved these safety concerns, and long-term patient monitoring with prophylactic vaccination and a risk mitigation strategy in place, similar to anti-C5 therapy, will be integral components of its future clinical path.

At present, third-generation and fourth-generation compstatin derivatives with modifications that afford improved pharmacokinetic properties for chronic administration, longer residence in ocular compartments and better solubility are advancing through clinical development[244,270–272]. In addition to compstatins, C3 inhibitors feature members of other drug classes, including C3-directed nanobodies[273] (single-domain mini-antibodies from camelids), proteases, small interfering RNA therapeutics or C3-blocking monoclonal antibodies[172].

may abrogate C5aR1 signalling whilst preserving C5aR2 immunomodulation and the capacity for MAC assembly on complement-opsonized surfaces (for example, microorganisms)[172,173]. Therapeutic C3 modulation features a broader activity profile than C5 inhibition: C5 inhibition targets the terminal pathway blocking C5a generation and MAC formation, whereas C3 modulation can prevent both downstream effector generation and C3 signalling that affects immunomodulatory and effector B cell and T cell functions[6]. Consistent with this notion, C3 inhibition with the Cp40 analogue has shown multipronged therapeutic efficacy in a sensitized non-human primate model of kidney allograft rejection by preventing antibody-mediated rejection and beneficially modulating B cell and T cell activity[219]. While the classical pathway is considered the dominant pathway driving complement activation in the autoimmune glomerular disease membranous nephropathy[220], complement fragments from all three pathways are detected in renal biopsies, implying a more complex contribution to pathogenesis[221,222]. Therapeutic targeting of C3 attenuated glomerular injury and disease severity in a mouse model of membranous nephropathy, validating the notion that C3 modulation may afford broader coverage in diseases with multifaceted complement engagement[221].

Given the contribution of multiple complement pathways to COVID-19 immunopathology, therapeutic modulation of the central protein C3 represents a more comprehensive strategy to simultaneously block the generation of all downstream pro-inflammatory effectors. It can thus also intercept NET-driven thrombo-inflammation, which fuels thrombotic microangiopathy-like pathological sequelae[202] (Fig. 8). C3 modulation in patients with severe COVID-19 attenuated plasma NETosis to a greater extent than anti-C5 blockade[223]. Translational studies unveiled a key mechanistic link between C3 dysregulation and neutrophil–platelet interactions leading to enhanced NET release and immunothrombosis[204]. Therapeutic C3 modulation was evaluated in a phase II randomized controlled trial in patients with severe acute respiratory distress syndrome following SARS-CoV-2 infection. C3 inhibition by the compstatin-based C3 therapeutic AMY-101 correlated with a trend towards improved clinical outcomes and a rapid anti-inflammatory response coupled to attenuation of NETosis. Additionally, despite target-saturating drug levels, residual C3 activity in a small fraction of non-responders correlated with enhanced procoagulant activity, likely supporting extrinsic protease-mediated C3 activation and providing in vivo proof-of-concept for non-canonical routes of C3 activation in COVID-19 (ref. 22).

A phase II trial of another C3 therapeutic (APL-9) in patients with mild-to-moderate acute respiratory distress syndrome reported no meaningful reduction in mortality over standard of care at the interim analysis stage. The lack of published data on drug pharmacokinetic–pharmacodynamic profiles from this trial, the small number of treated patients and divergent inclusion criteria (that is, in terms of disease severity) as well as the likely higher tissue penetration of AMY-101 than the bulkier PEGylated compound (APL-9), should be considered as potential confounding factors that limit the interpretability of these results and may have skewed clinical outcomes. While C5a-targeted blockade decreased mortality in patients being mechanically ventilated, C5aR1 inhibition failed to yield any clinical efficacy in a similar patient cohort, pointing to a knowledge gap and likely functional redundancy of targets within the C5a–C5aR1 axis[224]. More than 10 complement-specific drugs have been evaluated in clinical trials during the COVID-19 pandemic, with mixed results so far and absence of a consistent efficacy signal in clinical endpoints. These results support a more convoluted role of complement in severe COVID-19 and argue for the need to consider earlier therapeutic intervention with combination therapies. Such therapies may encompass multiple immunotherapeutic modalities against aberrantly activated innate immune pathways that fuel a vicious thrombo–inflammatory cycle in patients with severe COVID-19 (ref. 206).

Guided by the successful application of C3 inhibitors in PNH and the overarching role of alternative pathway dysregulation in disease pathophysiology, a new generation of alternative pathway-specific complement inhibitors targeting the serine proteases factor B or factor D have shown clinical efficacy in recently completed phase II clinical trials in haematological indications[174,225,226]. These orally bioavailable small-molecule inhibitors offer prospects of better patient compliance for chronic diseases compared with biologics that are given intravenously or subcutaneously – but caution should be exercised during dosing, given that even minimal residual amounts of enzyme may suffice to fuel alternative pathway activation[172].

The constant refinement of therapeutic approaches that can effectively address emerging pathophysiological aspects is readily exemplified by the recent approval of C3 inhibitors in PNH. The residual anaemia observed in a fraction of patients with PNH that remained transfusion-dependent despite anti-C5 therapy led to the discovery of C3-mediated extravascular haemolysis[177]. Additionally, the treatment-refractory phenotype of patients with PNH caused by genetic variation in C5 prompted a search for alternative therapeutic options[227]. The need to address these new pathogenic mechanisms propelled the development of both C3-targeted and alternative pathway therapeutics as new and broadly effective treatments. Similarly, the pathogenic contribution of the classical pathway to the immune complex-driven clinical manifestations of cold agglutinin disease justified the development of classical pathway-specific inhibitors such as sutimlimab[228].

Local modulation of complement

Although systemic complement modulation has shown efficacy in haematological and renal diseases, local complement modulation offers a promising therapeutic avenue in diseases associated with tissue-restricted or organ-restricted complement dysregulation[229]. In this regard, inhibitors targeted at C3, C5 or the alternative pathway are clinically developed as local treatments for ocular inflammatory diseases such as geographic atrophy[187]. Clinical efficacy of the C3 inhibitor pegcetacoplan (Syfovre) was shown in phase III trials in patients with geographic atrophy, and it has recently been granted approval by the FDA as the first and only available treatment for this debilitating eye disease[230]. C5 modulation with the synthetic C5-targeting RNA aptamer avacincaptad pegol (Zimura) also showed similar efficacy in two recent phase III studies in geographic atrophy[231]. It should be noted that both drugs carry a polyethylene glycol linker, which might entail risks associated with a high polyethylene glycol burden due to chronic tissue accumulation[232]. This aspect, as well as the long-term effect of this new class of immunomodulatory agents on clinical outcomes outside the well-defined context of clinical trials, will need to be monitored in follow-up studies.

In search for more patient-compliant options and a lower dosing frequency in chronic settings, adeno-associated virus vector-based gene therapy platforms are being explored to direct the expression of complement modulators (such as CR2–factor H and CR2–Crry) to diseased tissues. Proof of concept for the feasibility of this approach has been reported in rodent models of choroidal neovascularization-induced retinal damage and AMD-like pathology[233].

Dysbiosis-driven periodontal inflammation highlights how complement modulates both the microbiome and the host immune and inflammatory response, with the implication that complement inhibition is not simply an anti-inflammatory approach but also, indirectly, an antimicrobial approach to dysbiotic disease. Compelling evidence from proof-of-concept studies has recently led to the first phase II evaluation of the compstatin-based therapeutic AMY-101 in this oral inflammatory disease with systemic complications[234]. Local C3 modulation led to prolonged therapeutic effects by significantly attenuating clinical indices of periodontal inflammation and markers of tissue destruction (matrix metalloproteinases)[234]. The sustained therapeutic effect of C3 modulation, beyond the treatment window, suggests broader immunomodulatory effects that likely reinstate a balance between the oral microbiota and a better-regulated host immune response[108].

Orthogonal targeting approaches have also been developed to tackle complement dysregulation on opsonized host surfaces, avoiding systemic blockade of circulating complement proteins. These approaches enable the tissue-targeted delivery of complement regulatory proteins (such as factor H) as fusion constructs with a targeting moiety (such as CR2 and anti-C3d) that binds to surfaces decorated with C3 fragments (iC3b, C3b and C3dg)[235]. Such fusion inhibitors may also comprise complement regulators (such as the C3 and C5 convertase regulator Crry) combined with antibody fragments recognizing ischaemic neoepitopes in damaged tissues. Studies implementing this approach have shown promising results in models of brain ischaemic injury, retinal degeneration and cardiac ischaemia–reperfusion injury[236,237]. Mimicking pathogen immune evasion strategies, recent efforts have focused on developing coating strategies to shield biomaterials or grafted cells from complement attack. These approaches exploit protein or peptide 'baits' to capture soluble regulators, such as factor H, from the circulation[238] and have shown potential in quenching the detrimental consequences of complement activation in haemodialysis circuits or extracorporeal surgical operations (such as bypass and transplantation)[239].

In addition to synthetic small-molecule inhibitors, surface-directed modulators or larger biologics, structure-inspired miniaturized versions of endogenous regulators have also been developed as therapeutic agents. Mini-factor H, a fusion construct comprising factor H SCR1–4 and SCR19–20 retains the regulatory activity and host-surface recognition capacity of its parental protein and has shown therapeutic efficacy by outperforming factor H in ex vivo models of PNH[240].

New clinical indications and challenges

The approval of eculizumab in acetylcholine receptor antibody-positive myasthenia gravis, and aquaporin 4 antibody-positive neuromyelitis optica spectrum disorder has ignited efforts to exploit complement modulation in the neurological space[241]. In view of the emerging evidence linking complement dysregulation to neuroinflammatory pathology, the design of blood–brain barrier-permeable complement inhibitors that can effectively home into the brain is gaining momentum[161,170]. In this regard, dissecting the relative contribution of systemic versus local complement stores to complex CNS pathologies and determining the optimal timing for therapeutic intervention remain key challenges for the field. Genetic approaches using inducible conditional C3 expression have provided initial data for the temporal modulation and therapeutic potential of C3 signalling in Alzheimer disease[242]. Future studies are anticipated to provide a more robust base for the advancement of CNS-targeted complement therapeutics to the clinical stage.

Concluding remarks and outlook

Undeniably, complement research has ushered in a new era of discovery that has illuminated far-reaching implications of complement pathophysiology in diverse biological systems[1]. The wealth of data gathered from mechanistic and translational studies exploiting high-throughput multi-omics technologies, including single-cell RNA sequencing or spatial transcriptomics, has offered an unprecedented level of granularity into complement-modulated processes in health and disease[137]. Even classical roles of complement in antimicrobial immunity and phagocytic clearance are being enriched with novel mechanistic insights that consider both cell-intrinsic and systemic complement responses from a broader system-wide perspective[243]. The challenge we are now faced with is to translate these fascinating fundamental discoveries into human interventional studies and innovative therapeutic modalities for patients.

The resurgence of complement therapeutics has radically changed the clinical landscape within a very short time frame[244,245] but poses new challenges that need to be met. With discrete complement drugs approved and many more in development, personalized complement therapy moves closer to fruition but needs to be guided by rigorous diagnostic algorithms exploiting reliable and disease-specific biomarkers[246]. Subtle changes in basal complement activity driven by a set of rare or common polymorphisms, collectively known as the 'complotype', dictate susceptibility to inflammatory diseases and may influence patient stratification and therapy responses[247]. It is now widely appreciated that age, sex and metabolic status are among the factors that influence interindividual variations of complement activity, thus contributing to differential disease vulnerability[248,249]. Routes of drug administration and duration of treatment (transient versus chronic) should be tailored to accommodate specific diseases, including complex CNS pathologies[161].

Furthermore, the impact of systemic versus local complement modulation remains a fertile discussion in the field. For instance, systemic C3 inhibition may still allow for local protective C3 activity to be maintained in mucosal barriers as recently shown in a pneumonia-induced lung injury model[250]. In view of the emerging biology of intracellular complement, the design of cell-permeable inhibitors as novel tools for dissecting relevant mechanisms has gained momentum[211]. Clinical studies should strive for more transparency in pharmacokinetic–pharmacodynamic data reporting in order to enable reliable benchmarking of the efficacy of various targeting strategies[251].

In conclusion, given that currently approved complement therapies are very highly priced partly because of the market exclusivity of the orphan drug space[172], the development of lower-cost therapies that can become broadly accessible to patients, especially in low-income countries, should remain a top priority for researchers, clinicians and legislators alike.

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Author contributions
All authors contributed equally to all aspects of the article.

Competing interests
J.D.L. is the founder of Amyndas Pharmaceuticals, which is developing complement inhibitors (including third-generation compstatin analogues such as AMY-101). J.D.L. is an inventor of patents or patent applications that describe the use of complement inhibitors for therapeutic purposes, some of which are developed by Amyndas Pharmaceuticals. J.D.L. and G.H. have a joint patent that describes the use of complement inhibitors for therapeutic purposes in periodontitis. J.D.L. is also the inventor of the compstatin technology licensed to Apellis Pharmaceuticals (namely 4(1MeW)7W/POT-4/APL-1 and PEGylated derivatives such as APL-2/pegcetacoplan/Empaveli/Aspaveli/Syfovre). D.C.M. has provided paid consulting services to 4D Molecular Therapeutics and Merck KGaA.

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