ANNUAL REVIEWS

Annual Review of Immunology

Complement in the Brain: Contributions to Neuroprotection, Neuronal Plasticity, and Neuroinflammation

Suzanne S. Bohlson[1] and Andrea J. Tenner[1,2,3]

[1]Department of Molecular Biology and Biochemistry, University of California, Irvine, California, USA; email: bohlsons@uci.edu, atenner@uci.edu

[2]Department of Neurobiology and Behavior, University of California, Irvine, California, USA

[3]Department of Pathology and Laboratory Medicine, School of Medicine, University of California, Irvine, California, USA

Annu. Rev. Immunol. 2023. 41:431–52

First published as a Review in Advance on February 7, 2023

The *Annual Review of Immunology* is online at immunol.annualreviews.org

https://doi.org/10.1146/annurev-immunol-101921-035639

Keywords

complement, C1q, neuroinflammation, microglia, synaptic pruning, Alzheimer disease

Abstract

The complement system is an ancient collection of proteolytic cascades with well-described roles in regulation of innate and adaptive immunity. With the convergence of a revolution in complement-directed clinical therapeutics, the discovery of specific complement-associated targetable pathways in the central nervous system, and the development of integrated multiomic technologies that have all emerged over the last 15 years, precision therapeutic targeting in Alzheimer disease and other neurodegenerative diseases and processes appears to be within reach. As a sensor of tissue distress, the complement system protects the brain from microbial challenge as well as the accumulation of dead and/or damaged molecules and cells. Additional more recently discovered diverse functions of complement make it of paramount importance to design complement-directed neurotherapeutics such that the beneficial roles in neurodevelopment, adult neural plasticity, and neuroprotective functions of the complement system are retained.

1. INTRODUCTION

Widely recognized for its ability to mediate innate immune responses to invading microbes, the complement system consists of over 40 soluble and membrane-associated components, regulators, and receptors that also contribute to directing an appropriate adaptive immune response to infection or injury and restricting microbial infection. Classically described antimicrobial functions include (*a*) enhancing phagocytic clearance of microbes, (*b*) increasing vascular permeability to enable rapid and targeted local access of immune components to the site of challenge, (*c*) recruiting a cellular response to infection, and (*d*) destroying the invading pathogens through generation of the lytic membrane attack complex (MAC). While activation of complement is critical to the immediate host response to infection, additional functions for the complement system and individual components, both independent and dependent on complement pathway activation, continue to be discovered and described (1–3). Some of the complement components are pattern recognition proteins that recognize microbes and damaged/dying self, including apoptotic cells or cellular debris, as well as misfolded or aggregated protein, leading to activation of the complement system. For example, the classical complement system initiation complex, C1, is activated by apoptotic cell surface markers and immune complexes, as well as fibrillar amyloid beta (Aβ) (a component of amyloid plaques) and neurofibrillary tangles (4–6), both hallmark pathologies of Alzheimer disease (AD) (**Figure 1**).

Many of the complement components circulate as inactive proenzymes, and the activation cascades are assembled following recognition of a permissible surface, often microbial or altered self. While classically considered to be liver derived, essentially all complement components can be synthesized in the central nervous system (CNS) with expression induced or enhanced upon infection or injury. Activation pathways that were initially described in serum are similarly activated in the CNS when the activators and complement components are present. While activation of the three pathways occurs via three different mechanisms, all pathways converge at complement component C3 and terminate with C5b-9, the components of the terminal MAC (**Figure 1**). Cleavage of C3 and C5, by C3 and C5 convertases, respectively, results in production of the anaphylatoxins C3a and C5a, which bind to seven-transmembrane-spanning G-protein-coupled receptors C3aR and C5aR1, respectively, to mediate leukocyte migration and activation. Cleavage of C3 also results in generation of C3b and subsequent Factor I–mediated degradation to iC3b, both major opsonins of the complement system that mediate recognition and engulfment through complement receptors such as complement receptor 1 (CR1) and CR3 on phagocytic cells. Since activation of the complete complement cascade is proinflammatory, overproduction (or ineffective regulation) of complement cleavage products such as C3a and C5a can result in pathologic inflammation and/or a misdirected lytic MAC, which in the CNS contributes to neuroinflammation and cognitive decline associated with many neurodegenerative diseases. While inappropriate autoantibodies that activate complement result in direct neuronal damage in diseases such as myasthenia gravis, neuromyelitis optica, and neuropsychiatric lupus (8–10), this review highlights recently described inflammatory, as well as noninflammatory and anti-inflammatory, functions of the complement system in the CNS. Particular focus is given to C1q, the recognition component of the classical complement pathway, as well as activation products C3a and C5a and their receptors, which are central to inflammatory and noninflammatory functions of complement in the brain (**Figure 1**).

2. COMPLEMENT IN CENTRAL NERVOUS SYSTEM HOMEOSTASIS AND NEUROPROTECTION

2.1. Complement Synthesis in the Brain

Traditionally considered an immune-privileged site, the CNS is discontinuous with the peripheral complement system due to the presence of the blood-brain barrier, which largely excludes cellular

and humoral components of blood, including the soluble complement components. However, virtually all complement components can be synthesized locally in the brain by a variety of cell types (reviewed in 11), and this increases with aging as well as with neurodegenerative diseases in humans and mouse models (12–15; reviewed in 16–18). Earlier studies of synthesis of complement components using cell lines and in vitro systems that can be misrepresentative of in vivo physiology have now been enhanced by in vivo studies in multiple models and human systems. Recent RNA-sequence analyses of complement component expression in human and animal models confirm that select complement proteins are expressed in major cell types in the CNS. Indeed, complement component expression in the CNS is modulated in response to injury/infection, developmental cues, and aging (11, 17, 19–21). Moreover, there is regional and temporal regulation of complement component expression in the CNS, which likely dictates the roles of the specific components and controls/prevents the generation of inappropriate downstream effector mechanisms (19, 22; reviewed in 23). For example, microglia, brain-resident macrophages, are the major producers of complement component C1q (24), although C1q is synthesized by neurons during specific developmental periods (34). C1q expression is often an early response to disease/injury independent of C1r and C1s, the proteases required to generate the C1 complex and hence activate the classical complement pathway (26). This suggests a role for C1q independent of the activation of the rest of the complement pathway and is supported by previously described roles for C1q in neuroprotection and regulation of inflammation discussed below (see Section 3.1). In contrast to the predominant microglial production of C1q, activated astrocytes are the major producers of C3 (27–29), particularly in response to challenge such as the inflammatory signal LPS (lipopolysaccharide), but more recent reports show strong induction of C3 in microglia in diverse neurodegenerative disorders as well (27, 30), although whether all consequences of C3 are detrimental remains to be determined. Cell-specific deletion of C3 could help to dissect these issues. To further mechanistically define potential targets for regulation, it will be important to determine the signaling pathways, including transcription factors, that regulate complement gene expression over time and in response to injury in the different cell types and specific regions of the brain. This is made feasible with the emergence of new technologies including spatial transcriptomics. The recent release of analyzed transcriptomic data from the Seattle Alzheimer's Disease Brain Cell Atlas consortium by the Allen Brain Institute is a major step forward in collating cell-specific gene expression, including complement components in AD (**https://portal.brain-map.org**).

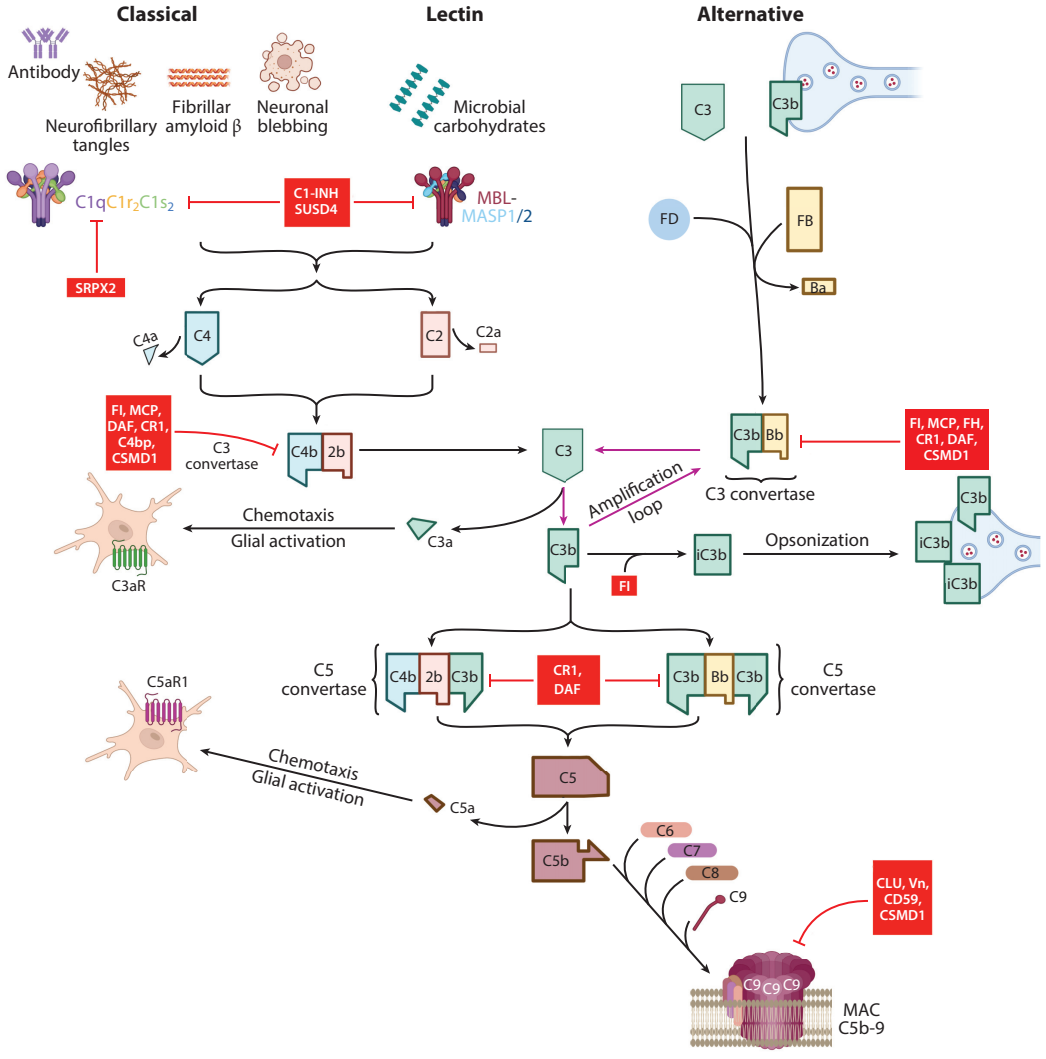
2.2. Regulation of Complement Pathway Activation

Inappropriate activation of the complement system on host tissue can lead to tissue damage, and therefore the system needs to balance activation and inactivation to clear pathogens or altered self while limiting damage to bystander cells. Both soluble (such as Factor H, Factor I, C4bp, clusterin) and membrane-associated (such as CR1, CD46/MCP, CD55/DAF, CD59) regulators of complement protect the host from complement-mediated damage by disrupting the cascades at multiple points, including proteolytic inactivation of generated C3a and C5a, and C4b and C3b, and dissociation of convertases (**Figure 1**). The C1 complex and the MAC are also targets of complement inhibitors (**Figure 1**). Failure to regulate complement at host surfaces can lead to pathologic inflammation and autoimmunity (31). Mutations in complement regulatory proteins shed light on the significance of tissue and region-specific regulation of the complement system, including that in the CNS. For example, sushi repeat protein X-linked 2 (SRPX2), a C1 inhibitor expressed by neurons, is required for normal brain development, and mutation of *SRPX2* is associated with seizures, cognitive impairment, and speech disorders (32, 33) (discussed below in Section 2.4). The sushi domain, also known as a complement control protein (CCP) domain or

short consensus repeat (SCR), is found in many of the described complement inhibitors and has helped in identification of CNS-associated complement regulatory proteins (34).

2.3. Complement and Synaptic Pruning in Normal Development and the Adult Brain

The complement system is required for multiple developmental processes including cell migration and proliferation during normal CNS development (35–37). These properties have been extensively reviewed elsewhere (17, 38). In addition, the targeted removal of synaptic connections, termed synaptic pruning, is required for normal brain development and appropriate sculpting of neuronal circuitry and maintenance of neuronal plasticity. In a seminal discovery, Stevens et al. (39) demonstrated that the classical complement pathway mediates synaptic pruning in



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Complement activation pathways. Three pathways, the classical, lectin, and alternative pathways, are distinguished based on recognition molecules (C1q, MBL/ficolins, and C3-H_2O, respectively). The recognition molecules sense and bind to different surfaces, triggering downstream pathway activation. While antibody/antigen complexes are the classic mediators of C1q recognition and classical pathway activation, C1q also binds damaged self-associated ligands that are then indicators of neurodegenerative diseases, such as the neurofibrillary tangles and amyloid plaques in Alzheimer disease as well as apoptotic cells. The lectin pathway is activated in response to the recognition components MBL/ficolins binding to microbial carbohydrates, and structures exposed by damaged cells (7). The alternative pathway is spontaneously activated when C3 is hydrolyzed, resulting in generation of a reactive thioester bond, subsequent covalent attachment to an accepting surface, and exposure of sites leading to C3 and C5 convertase formation. Activation of any of the three pathways leads to generation of a C3 convertase, C4b2b for the classical and lectin pathways and C3bBb for the alternative pathway, and further downstream activation of the pathways leads to generation of a C5 convertase and generation of the membranolytic MAC. The alternative pathway can amplify activity of the classical or lectin pathway because it is initiated with cleaved C3. Cleavage of C3 or C5 by the C3 or C5 convertase, respectively, leads to production of anaphylatoxins C3a and C5a, which regulate glial cell activation and chemotaxis. C3b and particularly iC3b, a Factor I–mediated cleavage product of C3b, tag synapses and facilitate synaptic pruning. The pathways are regulated in part by complement inhibitors (*red rectangles*) that regulate inappropriate activity. Abbreviations: C1-INH, C1 inhibitor; C4bp, C4-binding protein; CLU, clusterin; CR1, complement receptor 1; CSMD1, CUB and sushi multiple domains 1; DAF, decay accelerating factor (CD55); FB, Factor B; FD, Factor D; FH, Factor H; FI, Factor I; MAC, membrane attack complex; MASP1/2, MBL-associated serine protease 1/2; MBL, mannose-binding lectin; MCP, membrane cofactor protein (CD46); SRPX2, sushi repeat protein X-linked 2; SUSD4, sushi domain–containing protein 4; Vn, vitronectin. Figure adapted from Reference 18 and images created with BioRender.com.

postnatal CNS since mice deficient in C1q or C3 have defects in synapse elimination. C1q, produced in the CNS, tags synapses for subsequent activation of the classical pathway, leading to deposition of C3 cleavage products and ultimately CR3-mediated engulfment of the tagged synapse by microglia. Synaptic refinement through pruning is regulated by synaptic activity since microglia preferentially engulf synapses in a CR3- and C3-dependent manner when synaptic activity is decreased (40). The developing mouse retinogeniculate circuit, encompassing the dorsal lateral geniculate nucleus (LGN), has been widely utilized to investigate activity-dependent synaptic pruning (41). During development of this circuit, the LGN is initially innervated by both eyes, and activity-dependent, complement-mediated pruning contributes to the shift to monocular innervation seen in the mature LGN (39, 40). C1q-mediated synaptic pruning is regulated in space and time since C1q is required for synapse elimination in the developing retinogeniculate circuit, but it is not required for development and plasticity in the primary visual cortex despite its expression in the primary visual cortex during development (42). In addition to its role in development of the retinogeniculate circuit, complement-mediated synaptic elimination is required for plasticity (43) and forgetting in the adult brain. Forgetting is mediated by elimination of synapses between engram cells, the neurons involved in memory storage (44). Depletion of microglia in mice inhibited forgetting as measured using contextual fear conditioning (45). Using a Cre-dependent adeno-associated virus expressing decay-accelerating factor (DAF/CD55), an inhibitor of classical and alternative pathway C3 and C5 convertases, CD55 was selectively expressed in engram cells in the dentate gyrus. CD55-expressing mice showed higher freezing (less forgetting) compared to control mice, indicating a requirement for complement in forgetting. Elimination of synapses is also associated with the aging process, as C3-deficient C57Bl/6 mice fail to demonstrate region-specific synaptic loss associated with aging observed in wild-type mice (46). However, dysregulation in complement-mediated synaptic pruning is associated with disorders such as epilepsy and schizophrenia (47, 48). These studies indicate a highly regulated role for complement in normal CNS development and continuous neuroplasticity in the adult and aging brain.

2.4. Complement Regulators in the Brain

SRPX2 is a soluble C1 inhibitor that is secreted by neurons and regulates synapse density in the cortex (32, 49). Deficiency in SRPX2 is associated with epilepsy, cognitive impairment, and

speech dysfunction (33). Cong et al. (32) demonstrated that SRPX2 coimmunoprecipitated with C1q in mouse brain lysates as well lysates from HEK293T cells transfected with C1qa/b/c. In vitro, SRPX2 inhibited complement activation in serum, and the inhibition was at the level of C1 since SRPX2 binds to C1q and blocked C1-dependent cleavage of C2. Retinogeniculate synapses are overpruned in the SRPX2-deficient mice, and this overpruning is dependent on the presence of C3. SRPX2-mediated regulation of synaptic pruning in the dorsal LGN also required C1q, consistent with inhibition of complement activation at the level of C1 (50). While SRPX2 and C1q are expressed throughout the retino-geniculo-cortical pathway, deletion of C1qa or SRPX2 did not affect synaptic pruning in the cortex (50). This system further demonstrates the temporal and region-specific activity of the complement system in synaptic pruning.

The sushi domain–containing protein (SUSD) family and the CUB and sushi multiple domains (CSMD) family are expressed in the brain and have demonstrated complement-inhibitory activity (34). The sushi domain–containing protein 4 (SUSD4) gene is predicted to encode two isoforms; one is a type 1 membrane-spanning protein containing four CCP repeats (SUSD4a) and the other a secreted protein containing three CCP repeats (SUSD4b). The SUSD4a isoform is highly expressed in the CNS and inhibits formation of the classical/lectin and alternative C3 convertases in vitro (51). The SUSD4 knockout mouse has elevated C1q levels in the brain and demonstrates defective motor skills and increased anxiety behavior, consistent with defective CNS development (52). González-Calvo et al. recently demonstrated a defect in AMPA receptor turnover in SUSD4 knockout mice, and they postulate that SUSD4 may interact with C1q or C1q globular domain–containing molecules that are known to regulate synapses, such as CBLN1 and other C1q-like proteins (53). Thus, the mechanism by which SUSD4 regulates synapse function, and the role of complement as well as C1q-like proteins (54–56), in synapse organization requires further elucidation. Similar to SUSD4, CSMD1 is highly expressed in the CNS, and mutations in CSMD1 are linked to neurological disorders (34). These observations are supported by the finding that the CSMD1 knockout mouse displays neuropsychological deficits (57). CSMD1 promoted Factor I–mediated cleavage of C4b and C3b and inhibited deposition of the MAC (58). It is yet to be determined whether CSMD1 regulates complement-mediated synaptic pruning.

2.5. Balancing Signals in Synaptic Pruning

The tagging of synapses by C1q for removal by microglia or astrocytes is akin to the targeted removal of apoptotic cells and cellular debris, which has been well described in the periphery (59). Engulfment of debris occurs when eat-me signals engaging phagocytic receptors overcome don't-eat-me signals that engage receptors that downregulate phagocytosis. C1q is a well-characterized eat-me signal that stimulates engulfment of apoptotic cells through multiple mechanisms, including activation of the classical complement pathway and subsequent opsonization by C3 cleavage fragments, as well as direct C1q-mediated engulfment (60). Therefore, it could be expected that mechanisms identified in the periphery for clearance of debris are conserved in the CNS for engulfment of inactive synapses. Indeed, microglia preferentially engulf apoptotic neurons and neuronal blebs in the presence of C1q in vitro (61), and C1q preferentially tags synapses that have upregulated apoptotic markers in vivo (62). Among other surface markers of apoptotic cells, C1q binds phosphatidylserine (PS), the best-studied eat-me signal. PS is temporally upregulated in the hippocampus and at retinogeniculate synapses during developmental periods of high microglia-mediated synaptic pruning, and PS-labeled material is detected within microglial lysosomes during these periods (63). Further, there was reduced microglia-mediated engulfment of PS in the absence of C1q (63). Complement-mediated synaptic pruning in the developing mouse retinogeniculate system is activity dependent, tagging weaker synapses for removal, and CD47 is a well-described don't-eat-me signal that localizes to the more active synapses and inhibits microglia-mediated

engulfment by binding to microglia-expressed SIRPα (64). These results demonstrate conserved engulfment pathways in the periphery and CNS that contribute to temporal and regional specificity of synaptic pruning in the CNS. An imbalance in signals due to neuroinflammation, neuronal injury, or other pathologic conditions may tip the balance toward pathologic synaptic pruning associated with neurodegeneration, as discussed below.

3. COMPLEMENT IN ALZHEIMER DISEASE AND OTHER NEURODEGENERATIVE DISEASES

3.1. C1q Is Neuroprotective

An estimated 6.5 million people in the United States have AD, the most common form of dementia, which results in the progressive decline of cognitive function, loss of independence, and death (65). Pathological hallmarks of AD are fibrillar Aβ plaques and neurofibrillary tangles, as well as neuroinflammation and synaptic and neuronal loss. Rogers and colleagues (66) provided critical evidence for complement activation in AD in 1992, and advancements over the last three decades have continued to dissect the pathways and consequences of the complex complement cascade in AD, including multiple genome-wide association studies that support a role for neuroinflammation and complement in AD (67). Upon sufficient induction of complement component synthesis as discussed above, the proinflammatory complement cascade can be activated and amplified immediately upon exposure to a permissible substrate. However, there is compelling evidence that various components of the complement system maintain normal tissue homeostasis in the absence of significant injury. Increased hippocampal expression of C1q is observed as early as two months of age in the 3xTg mouse model of AD when compared to control mice (26). The increase in C1q expression precedes AD pathology (plaque deposition, gliosis, tau pathology, and cognitive impairment) (68). In contrast, expression of C1r and C1s, the proteases required to generate the C1 complex and thus to activate the classical complement pathway, is not upregulated at two months of age in this model, demonstrating a lack of coordinated expression of the C1 complex components, similar to that previously reported in peripheral myeloid cells and during development (19, 69). C1r and C1s expression showed a significant increase only after 10–13 months of age when AD pathology is apparent (26). These data as well as multiple subsequent transcriptome analyses suggest that C1q, produced early and locally in response to injury, without the coordinated expression of other complement components, may serve a protective role and limit progression of disease (**Figure 2**).

C1q is an ancient molecule, with a vertebrate ortholog expressed in a primitive chordate lineage that existed prior to jawed vertebrates (70), indicating ample time to coevolve and be utilized for multiple functions. Indeed, C1q in the absence of C1r and C1s (and other complement pathway components) has both neuroprotective and anti-inflammatory activities that promote resilience and resolution of injury (reviewed in 1). C1q enhances the viability of primary rodent neurons in culture when the cells are subjected to nutrient stress or toxic Aβ peptide (fibrillar Aβ or oligomeric Aβ) (26, 71, 72). Under these conditions, C1q increased the activation and nuclear translocation of cAMP response element–binding protein (CREB), a transcription factor that promotes neuron survival and neurite outgrowth (26). CREB likely functions as a central transcription factor regulating C1q-mediated neuroprotection since inhibition of CREB blocked C1q-mediated neuroprotective activity. Interestingly, C1q also stimulates CREB phosphorylation and activation in human monocytes, which are associated with anti-inflammatory signaling (73). Thus, CREB may serve as a downstream target in regulation of neuroprotection and resolution of inflammation, which may be critical processes in slowing the progression of neurodegenerative diseases such as AD. C1q suppresses proinflammatory cytokine production from microglia in vitro while enhancing phagocytosis of apoptotic neurons (61), and it decreases inflammasome

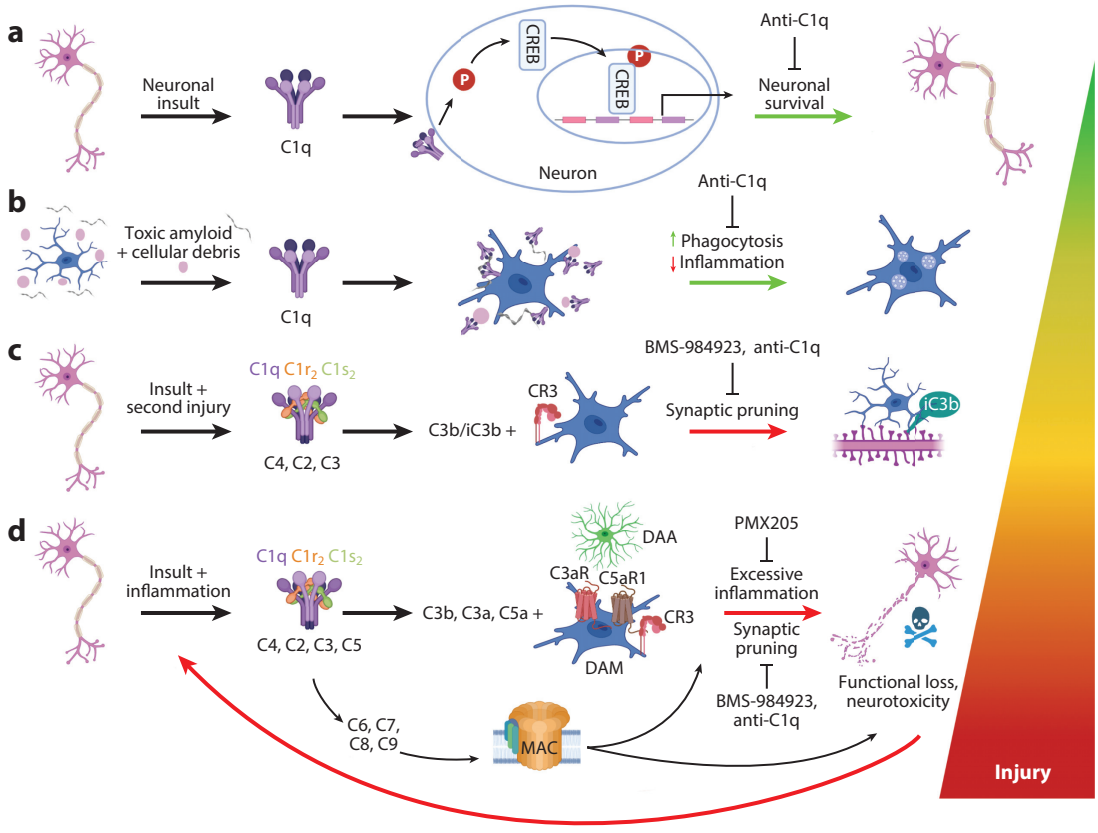


Figure 2

Selective targeting of complement activation–induced signaling may preserve the neuroprotective functions of C1q while inhibiting neurodegenerative functions. (*a*) C1q promotes survival of neurons in vitro when they are challenged with fibrillar or oligomeric Aβ. (*b*) C1q enhances phagocytosis and limits proinflammatory cytokine production in microglia. The C1q-mediated neuroprotective pathways in panels *a* and *b* could be inhibited with a therapeutic antibody directed at C1q. (*c*) With prolonged insult, induced synthesis of C1s and C1r leads to generation of the C1 complex and ability to activate the classical complement pathway. Subsequent deposition of iC3b on injured neurons leads to synaptic pruning by microglia and astrocytes associated with cognitive decline. (*d*) Chronic injury and thus complement activation, as in the brain with Alzheimer disease due to fibrillar Aβ, hyperphosphorylated tau, and damaged neurons, induce the terminal pathway components and additional C3a/C5a production, leading to induction of detrimental disease-associated microglia and disease-associated astrocytes and neurotoxicity. BMS-984923 inhibits neuronal injury and subsequent C1-dependent synaptic pruning. PMX205 inhibits C5a/C5aR1 signaling, limiting generation of DAM and neurotoxicity. Inhibition of downstream complement-mediated neurodegeneration in panels *c* and *d* could preserve the neuroprotective function of C1q early during the disease process while limiting the neurodegenerative functions later in the disease. Abbreviations: Aβ, amyloid beta; CREB, cAMP response element–binding protein; DAA, disease associated astrocyte; DAM, disease-associated microglia; MAC, membrane attack complex. Figure adapted from Reference 18 and images created with BioRender.com.

caspase 1 activation and production of IL-1β in human macrophages (74), all suggesting a role for C1q in sensing injury and limiting pathology associated with neurodegenerative disease (**Figure 2**). Neural stem cells (NSCs) are multipotent undifferentiated cells that can generate many cells of the CNS, including neurons, glia, and oligodendrocytes (75). C1q binding to CD44 on NSCs triggers ERK phosphorylation and migration of NSCs in vitro and migration and repair after spinal cord injury in vivo (76), again supporting a role for C1q in sensing injury and mediating resolution and repair in multiple cell types within the CNS.

3.2. Downstream Complement Activation Leads to Neurotoxicity in Alzheimer Disease

Complement-mediated damage in AD is complex and may result from multiple mechanisms including but not limited to neuroinflammation accompanied by (*a*) excessive synaptic pruning (27, 62, 77, 78), (*b*) direct neurotoxicity by C5a (79, 80), and (*c*) gliosis directly or indirectly (81–83). The role of complement in these processes, and their contribution to pathology and dysfunction, is complicated by region- and time-specific activation as well as differences in animal models used to investigate these processes. For example, genetic ablation of C3 or overexpression of a C3 inhibitor was reported to prevent age-related hippocampal decline while increasing amyloid plaques and altering gliosis in the J20 mouse model (46, 84). Subsequently, it was shown that despite the increase in plaques, constitutive C3 ablation prevented synaptic loss and, importantly, cognitive decline in the APP/PS1 mouse model (83). The loss of synapses has long been associated with cognitive decline in AD (85, 86). Support for this was provided by Stevens and colleagues, who demonstrated that AD-associated synapse loss, or excessive synaptic pruning, was inhibited in the absence of C1q, C3, and CR3 in AD mouse models, and that complement-mediated synaptic pruning by microglia occurred early in disease, prior to plaque deposition (78). Carpanini et al. (87) recently expanded on the mechanism of complement-mediated synaptic pruning in AD by demonstrating a role for the terminal complement components C6 and C7 in synapse elimination using two AD mouse models, thus implicating the MAC in pathogenic synapse elimination in AD. It is important to note the localization, age, and disease stage of changes in these models of neurodegeneration. The lack of such extensive region- and time-specific analysis in earlier studies may contribute to perceived discrepancies between reports. Kinetic studies correlating pathology, gene expression, neuronal integrity, and behavior are critical in comparing models and arriving at causative events of dysfunction. Finally, given the substantial influence of complement during development of the nervous system, as recently reviewed (17, 20, 88), the use of newly developed inducible complement component knockout mice rather than constitutive genetic ablation is critical to avoid confounding issues due to developmental deficiencies that affect adult function (89), and it has the added advantage of mimicking more closely a pharmacologic intervention in adults.

A novel recent study by Spurrier et al. (90) demonstrated that blocking oligomeric Aβ–mediated neuronal damage prevented C1q deposition at the neuronal synapse and limited pathology and cognitive decline in mouse models of AD. The same group previously reported that oligomeric Aβ exerts neurotoxic activity through an interaction with the glycosylphosphatidylinositol (GPI)-anchored cellular prion protein (PRP^C) that couples with the transmembrane metabotropic glutamate receptor 5 (mGLR5) to induce neurotoxic signaling (91). The mGLR5 silent allosteric modulator BMS-984923 prevents oligomeric Aβ–mediated neurotoxicity but does not alter glutamate signaling required for optimal neuron function, and thus it is a potential therapy for at least some subtypes of AD (92, 93). Spurrier et al. (90) used two aged mouse models of AD, the amyloidogenic *APPswe/PS1ΔE9* overexpressing transgenic mouse and the *AppNL-G-F/hMapt* double knock-in mouse, and demonstrated that BMS-984923 restored synaptic density and alleviated memory deficits after a one-month treatment. C1q expression was elevated in the brain in these AD models as expected, and BMS-984923 did not influence total C1q expression levels. However, C1q only accumulated at synapses in the dentate gyrus of vehicle-treated AD animals, and not in animals treated with BMS-984923 where synaptic density was restored. Interestingly, BMS-984923 also reversed alterations in neuronal gene expression associated with AD but had little impact on microglial gene expression as measured by single-nucleus RNA sequencing of combined cortex and hippocampus. These data suggest that damage- or injury-induced changes in neurons may result in C1q tagging of synapses for subsequent engulfment by astrocytes and/or

microglia. In addition, these findings demonstrate that the presence of activated microglia and elevated C1q is not sufficient to drive pathologic synaptic pruning in these AD mouse models. The data further emphasize the importance of monitoring specific locations/regions of protein expression and induction of drivers of activation to define precise contributions of complement components to physiology and pathophysiology, as well as more fully describing the now apparent different states of activated glia.

In addition to altered synapses, the classical complement system is activated by both fibrillar Aβ plaques and hyperphosphorylated tau found in extracellular neurofibrillary tangles (4, 5) (**Figure 1**), and activation of the classical complement pathway leads to deposition of C3b, formation of the C3 and C5 convertases, and production of anaphylatoxins C3a and C5a. C5a has been shown to be directly toxic to primary rodent neurons in vitro via C5aR1, as toxicity was prevented by addition of the C5aR1-specific antagonist PMX53, and primary neurons from C5aR1 knockout mice were not killed by C5a treatment (80, 94). C1q deficiency in the Tg2576 and APP/PS1 AD mouse models results in limited gliosis in older animals where fibrillar plaque pathology is apparent (81), suggesting downstream consequences of classical complement pathway activation in AD-associated neuroinflammation. C3a and C5a mediate glial cell activation and migration via binding to C3aR and C5aR1, respectively (**Figure 1**). Importantly, treatment of Tg2576 and 3xTg with PMX205, a cyclic hexapeptide C5aR1 antagonist, at the beginning stage of plaque deposition results in decreased accumulation of fibrillary Aβ and activated glia (95, 96), consistent with a role for the complement system, and C5a-C5aR1 signaling specifically, in propagating glial-mediated inflammation in AD. Subsequent analysis of gene expression showed that PMX205 decreases selective inflammatory gene expression, reduces the activation of a unique microglial subpopulation associated with synapse pruning in the hippocampus of the Tg2576 mouse model of AD, and increases disease-mitigating microglial activation (96). Furthermore, genetic deletion of C5aR1 in the Arctic AD mouse model prevented behavioral deficits and inflammatory gene expression and rescued hippocampal neuronal complexity seen in the Arctic AD mouse relative to wild type. Interestingly, in this model there was no effect on amyloid plaque accumulation (79), suggesting that rather than the plaques themselves causing neurotoxicity, it is either the response to plaques or the response to some other injury that is causing the detrimental effects associated with plaque deposition. C5a/C5aR1 signaling enhances detrimental microglial activation pathways, as selective gene expression was prevented when C5aR1 was deleted or selectively enhanced when C5a was overexpressed in AD mice (15). Interestingly, early and intermittent treatment of the 5xFAD model of amyloidosis with a peptide, EP67, with select C5aR1 agonist activity resulted in increased amyloid phagocytosis and decreased synapse (synaptophysin) and neuronal (NeuN) loss (97). Whether these results are due to the timing of treatment or to the receptor-selective engagement of EP67 will be informative in directing therapeutic manipulation of these systems. It is important to note that C5a and C5a-desArg (the result of carboxypeptidase cleavage of C5a) can also bind to a second receptor C5aR2 (previously known as C5L2), which, unlike C5aR1, lacks the ability to signal via G-proteins and has often been considered a negative regulator of C5a-C5aR1 activity acting as a scavenger receptor for C5a and promoting anti-inflammatory properties (reviewed in 98). C5aR2 is often expressed similarly to C5aR1 but at lower levels. C5a binds to C5aR2 with less affinity than C5a binding to C5aR1, although C5a-desArg binds to C5aR2 with a tenfold higher affinity than to C5aR1 (99). C5aR2 can form heterotrimers with C5aR1, promoting C5aR1 internalization via recruitment of β-arrestin and thus downregulating ERK signaling (98). C5aR2 agonism reduces C5a-C5aR1-mediated inflammatory response to Toll-like receptors, C-type lectin receptors, or cytosolic DNA sensor stimulation of interferon genes (100).

C3a, generated upon complement activation, binds to C3aR, expressed on both immune and nonimmune cells. C3aR expression is induced in microglia in mouse models of amyloidosis,

demonstrating tissue and temporal induction as recently reported (15) as well as human AD. However, the role of C3aR in neurodegeneration is clearly complex, as reported data have supported both protective and detrimental effects. For example, genetic ablation of *C3ar1* ameliorates disease in tauopathy models (27) and West Nile virus–induced synapse loss (101), but it also enhances neurogenesis in the adult brain (102) and has been shown to mitigate inflammatory cytokine and chemokine production, particularly in acute injury in the periphery (103). An excellent review of the complexity of C3a-C3aR signaling, which can be either pro- or anti-inflammatory, summarizes data implicating C3a binding to receptors and ligands other than C3a for C3aR, as well as cellular differences in signaling pathways induced by C3a interaction with C3aR (104). For example, C3aR signaling via the alternative ligand TLQP-21 (derived in vivo from VGF processing) reduced amyloid plaques and dystrophic neurites in the 5xFAD mouse model of AD (105). In addition, C3a can be cleaved by a carboxypeptidase forming C3a-desArg. While it is known that C3a can bind C3aR and C5aR2, C3a-desArg only interacts with C5aR2 (106), suggesting another avenue for differential anti-inflammatory effects of C3a/C3a-desArg. Combined, these studies suggest that targeting downstream complement activities (e.g., C5aR1 signaling) should limit pathologic neuroinflammation in AD without blocking potential beneficial effects of C3aR and C5aR2, or C1q, which have both neuroprotective and anti-inflammatory functions that may limit AD-associated pathology (**Figure 2**) as well as peripheral beneficial host defense and clearance functions.

Additive insults over time in AD lead to increased neuroinflammation, due at least in part to heightened expression of complement components triggered by damage-associated molecular signaling, and subsequent activation of the classical complement cascade (**Figure 2**). Synergy between the complement activation products and other proinflammatory pathways increases production of proinflammatory cytokines and additional toxic inflammatory mediators resulting in further tissue damage, eventually leading to neuronal loss and cognitive decline. Identification of temporal and regional regulation of expression of inflammatory mediators, transcription factors induced during the disease state that propagate expression of complement proteins and inflammatory mediators, as well as mechanisms of pathway synergy should inform and direct future therapeutics for AD. For example, similar to recognition components in the complement system, the Toll-like receptor (TLR) family of pattern recognition receptors evolved to rapidly sense injury and/or infection and direct immune responses via modulation of gene expression. Single-nucleotide polymorphisms (SNPs) in *TLR4* that are associated with attenuated inflammatory responses protect against susceptibility to AD (107). Microglia, expressing TLR4, respond to aggregated Aβ with enhanced production of proinflammatory cytokines in vitro (108). Synergy between proinflammatory TLR4 signaling and C5aR1 signaling has been demonstrated in the periphery (reviewed in 109) and is likely to contribute to neuroinflammation in AD (82). Indeed differential gene expression in the Arctic C5aR1 knockout model is consistent with this additive or synergistic effect on inflammation (15, 79).

In addition to inflammation within the brain, aging, the greatest risk factor in AD, is accompanied by systemic changes in innate and adaptive immune responses including chronic low-grade inflammation, termed inflammaging. Since alterations in immune system pathways are associated with susceptibility to AD, it is important to consider the contribution of both central and peripheral immune responses to progression of AD (reviewed in 110).

3.3. Conserved Complement Functions in Diverse Neurodegenerative Processes

Since inflammation is at the genesis of many disease processes, and the complement system is a key contributor and regulator of inflammation, the "clinical complement revolution," initiated with the anti-C5 mAb eculizumab for treatment of complement-mediated hemolysis in

paroxysmal nocturnal hemoglobinuria (PNH) in 2007 (111, 112), and more recently for treatment in neurological disorders myasthenia gravis and neuromyelitis optica spectrum disorders (113, 114), is well poised to expand into other disorders, including AD and other currently incurable neurodegenerative processes and diseases. Chronic inflammation is associated with indirect secondary neurodegeneration that accompanies delayed adverse outcomes in traumatic brain injury (TBI) (115–117). Acute damage to the cortex leads to secondary injury in the thalamus accompanied by complement-mediated neuroinflammation and neurodegeneration (118, 119). In mice, C1q gene expression was upregulated in the cortex following acute experimental TBI, and elevated microglial C1q expression extended along neural pathways into the thalamus. Targeting C1q globular heads with a monoclonal antibody reduced neurologic impairment associated with the TBI (119). Interestingly, the vulnerable neurons in this TBI model were in the GABAergic reticular thalamus, and not cortical or excitatory thalamic neurons, again illustrating a region- and neuron-specific impact of complement. In another approach, C3 was targeted in a TBI system using CR1-related gene/protein Y (Crry), a complement inhibitor expressed in rodents that, similar to CR1, is a cofactor for Factor I–mediated degradation of C3b and C4b and also accelerates the decay of C3 convertases (120). Crry was fused to CR2, which binds at sites of complement activation, facilitating the inhibition of C3 convertases and thus suppressing local generation of C3a, C5a, and the MAC. Such treatment improved learning and memory and reduced glial activation at extended time points after the injury (115, 116). Ischemic stroke leads to a similar pattern of acute injury followed by secondary damage associated with complement-dependent neurodegeneration and cognitive decline (121; reviewed in 23). Revascularization therapy associated with ischemic stroke limits some pathology (e.g., infarct size) and is standard of care; however, it is accompanied by downstream complement-dependent, neuroinflammatory, and neurodegenerative processes (122). Alawieh et al. (121) targeted C3 convertase, using Crry linked to a single-chain antibody that recognizes the damage-associated molecular pattern (DAMP), annexin IV, expressed specifically in damaged tissue from both human and experimental stroke. Experimental reperfusion after ischemic stroke in the presence of targeted complement inhibition blocked microglia-mediated synaptic pruning and cognitive decline after injury. However, C5a was increased in a mouse model of ischemia-reperfusion, and mice lacking C5aR1 showed smaller lesion size and improved neurological score compared to C5aR1-sufficient mice (80), so it is not yet clear how much of the benefit of inhibiting C3 cleavage is due to reduced synaptic pruning or the suppression of neuronal injury by blocking the generation of C5a and thus C5aR1 signaling. C3 deficiency or inhibition also suppresses neuronal loss in mouse models of tauopathy and multiple sclerosis (27, 30, 123). While further investigation remains to be done to clarify the precise detrimental processes (MAC, C3a, C5a, opsonization), these data demonstrate additional neurodegenerative processes that are likely to respond to complement-directed therapeutics, and the additional advantage of targeting therapy to the site of injury. For a review of additional CNS pathologies for which C1q and complement have been implicated, including age-related macular degeneration and viral infection, see References 18, 23, 124, and 125.

4. EMERGING AREAS AND THERAPEUTIC TARGETS

4.1. Precision Targeting of Complement Components

As discussed above, and illustrated in **Figure 2**, the complement system mediates both neuroprotective and neurodegenerative functions in the CNS. Therefore, efforts to strategically target destructive neuroinflammatory functions while preserving neuroprotective pathways should be a priority for drug development for neurodegenerative diseases, including proteinopathies such as AD, Parkinson disease, and Huntington disease, all of which are associated with

complement-dependent clearance of aggregated proteins, as well as excessive complement-dependent neurotoxicity (reviewed in 126, 127) and lack disease-modifying treatments at this time. Aging-associated alterations in the systemic immune system may impair the normal clearance processes, leading to accumulation of toxic protein aggregates such as Aβ, α-synuclein, and huntingtin protein in AD, Parkinson disease, and Huntington disease, respectively, and leading to neuronal loss (110, 128). Therefore, boosting the beneficial clearance mechanisms and neuroprotective functions of complement components may be useful in prevention of disease progression. While C1q has been considered as a target in neurodegenerative diseases, the selective targeting of C1q could block beneficial C1q-mediated synaptic pruning in circuit refinement, complement activation–independent neuroprotective and anti-inflammatory functions of C1q, as well as classical pathway–mediated clearance of toxic protein aggregates and pathogen destruction. Moreover, systemic depletion of C1q may trigger autoimmune-type reactions, as has been recently reported in a phase 2 Huntington disease trial of an anti-C1q antibody (ANX005, Annexon, Inc.). Therefore, targeting downstream complement activation products or their activating receptors, such as C5a or C5aR1, respectively, would maintain the protective functions of complement in the brain while limiting pathogenic inflammation associated with neurodegeneration. In addition to the preclinical studies in AD mice mentioned above, PMX205 was successfully tested in the hSOD1G93A mouse model of amyotrophic lateral sclerosis (129), and clinical trials are now being proposed to test this in human patients with amyotrophic lateral sclerosis. It should be noted that eculizumab, an inhibitor of C5 cleavage, has been used successfully for more than 15 years for treatment of MAC-mediated hemolysis in acquired anemias (111, 112). A drawback of C5 inhibition is the enhanced susceptibility to infection, and particularly to *Neisseria* infection, that results from the accompanying systemic inhibition of MAC-dependent lysis. Therefore, in some diseases selective targeting of C5aR1 would maintain the beneficial antimicrobial function of the terminal complement components, since C5b generation would not be impaired, while limiting pathogenic neuroinflammation associated with C5a activity. Moreover, selective targeting of C5aR1 would preserve C5a signaling through C5aR2, which may be neuroprotective (130). Importantly, selective targeting of C5aR1 in the Arctic mouse model of AD limited proinflammatory gene expression while enhancing expression of prophagocytic genes, suggesting that not only do beneficial phagocytic clearance mechanisms remain intact (131) but the absence of C5aR1 signaling enables the resolving or disease-mitigating programs to be optimally engaged (79, 96, 132).

Another potential complement-directed therapeutic that should be considered is CR1, a multifunctional transmembrane glycoprotein expressed on a variety of cell types that, among other activities, binds to C3b/C4b to mediate clearance of opsonized immune complexes from blood. Erythrocyte CR1, the majority of CR1 expressed in humans, traffics C3b/C4b opsonized immune complexes to liver and spleen for degradation in a process called immune adherence (133). SNPs in CR1 that are associated with decreased CR1 surface density on red blood cells are associated with an increased risk for late-onset Alzheimer disease, suggesting a requirement for CR1 in limiting disease susceptibility (67, 134–136). Whereas thus far, monoclonal antibody therapies targeting Aβ for clearance have been largely ineffective (137), it has been postulated that enhancing erythrocyte CR1-mediated clearance of Aβ-anti-Aβ immune complexes by treatment with a bispecific antibody to Aβ and CR1 may facilitate peripheral Aβ clearance in AD and hence limit pathologic accumulation of Aβ plaques in the brain (138, 139; reviewed in 23, 140). However, since this CR1 immune adherence mechanism is present only in primates, the hypothesis can be tested only in mice transgenic for human CR1 (141) or potentially in the recently generated humanized CR1/CR2 mouse (142). As mentioned above, CR1 is also a cofactor for Factor I–mediated degradation of C3b, facilitates the dissociation of C3 and C5 convertases, and thus suppresses the generation of downstream complement effectors (C3a, C5a, MAC). In addition, CR1 is a phagocytic

receptor, mediating engulfment of C3b-opsonized particles. These and other functions of CR1 may also contribute to the association of loss of function (and/or lower expression) of CR1 variants with increased susceptibility to AD (139, 143).

In considering complement-directed therapeutic strategies, complement components, functioning within or independent of the complement cascades, have traditionally been investigated in the context of the extracellular space as described above. However, recently a role for intracellular complement, coined the complosome, has been described as a metabolic regulator for cells of the immune system (144; reviewed in 145). Intracellular C3 in T cells is cleaved by cathepsin L into C3a and C3b, and intracellular C3a triggers activation of lysosomal C3aR, activating mTOR, a central regulator of nutrient sensing and metabolism (146, 147). C3a and C3b are also shuttled to the cell surface, where C3b engages cell surface CD46, further driving metabolic activity in humans (reviewed in 148). Astrocytes and microglia are the major producers of C3 in the injured or sensitized brain (27, 28), but whether glial-derived C3 is involved in intracellular signaling and/or metabolic reprogramming, as hypothesized (148), is yet to be determined. Niyonzima et al. (149) recently provided evidence supporting the expression of an intracellular C5 convertase in monocytes and macrophages that generates C5a for subsequent proinflammatory signaling via mitochondrion-expressed C5aR1. Whether this system is similarly active in microglia and contributes to neuroinflammation remains to be explored; however, both intracellular and extracellular complement signaling should be considered in the context of future therapeutic targeting.

4.2. Targeted Delivery in the Central Nervous System

In addition to the US Food and Drug Administration–approved monoclonal anti-C5 antibodies (eculizumab and the subsequent improved versions) and small-molecule C5aR1 receptor antagonist avacopan, there are some notable preclinical successes in delivering biological inhibitors of complement (115, 116, 121, 123, 150, 151). Multiple challenges exist toward effective targeting of the complement system in the brain. The blood-brain barrier restricts drug access to the site of injury, although emerging strategies to overcome challenges associated with complement-targeted drug delivery across the blood-brain barrier in neurodegenerative diseases have been recently reviewed (125). The continued advances in viral delivery of engineered inhibitors, engineered use of transport receptors to enable stealth receptor-mediated transcytosis of therapeutics, and nanobody technology including complement component–specific targets (123, 152–154) not only will be useful for targeting specific activities of these complex multifunctional proteins but also could inform the development of small molecules that can selectively target unwanted or aberrant function. As discussed above, complement has both neuroprotective and neurodegenerative activities, so selective targeting of specific pathways while maintaining function of other pathways is critical. In addition, expression of complement proteins is temporally and spatially regulated, and it likely evolved to orchestrate specific functional activity, such that timing and location of drug delivery need to be controlled. For example, as described above, specific populations of GABAergic neurons were vulnerable to complement-mediated attack in TBI and were therefore protected in the presence of anti-C1q therapy, which would suppress downstream complement mediators as well (119). However, while acutely beneficial for protection of this vulnerable population of neurons, prolonged loss of the C1q-mediated neuroprotective and anti-inflammatory functions beneficial in promoting neuronal resilience in other populations and/or in disease stages (reviewed in 1) may not be as helpful for chronic disorders. Therefore, caution should be used in directing therapeutics to appropriate locations and at specific times. Further elucidation of the gene expression programs, signaling pathways, and cellular communication during the entirety of disease processes should further illuminate targetable pathways and components in the complement

system to effectively enhance and/or inhibit the complement system to better human health and longevity.

SUMMARY POINTS

1. Complement is synthesized and complement pathways are active in the brain.
2. Complement expression and function in the brain are regulated in time and space according to extracellular cues and intracellular signaling pathways.
3. Complement mediates neuroprotection, neuroplasticity, and neurodegeneration in the central nervous system (CNS).
4. Neuroinflammation, including activation of the complement system, is a driver of neurodegenerative processes.
5. CNS-specific complement regulatory molecules regulate complement activity in the brain.
6. Complement-mediated synaptic pruning refines neural circuits, contributing to neuroplasticity in the developing brain and adult brain.
7. Pathologic complement-mediated synaptic pruning contributes to cognitive decline in neurodegenerative disease.
8. The design of precision human therapeutics such as targeting downstream proinflammatory complement signaling (e.g., C5aR1) in neurodegenerative diseases would preserve systemic host defense and clearance mechanisms as well as neuroprotective functions of upstream components (e.g., C1q, C3).

FUTURE ISSUES

1. Complement-directed therapeutics in neurodegenerative diseases should preserve or enhance neuroprotective/beneficial functions of complement while inhibiting neurotoxic proinflammatory signaling.
2. Use of inducible genetic ablation of complement components to confirm stage-specific roles for specific complement components will be informative in designing effective targeted therapeutics for neurodegenerative diseases.
3. Identification of signaling pathways and transcriptional programs regulating complement expression in the brain should inform additional potential modes of therapeutic intervention.
4. Further identification of brain-specific complement regulatory proteins will provide new therapeutic targets in neurodegenerative diseases.
5. Combining new technologies in spatial transcriptomics with validated/specific protein expression will provide critical insight into regulation of complement expression in time and space. Precision targeting of specific complement components in time and space should be considered in the development of CNS-targeted therapeutics.
6. Defining conserved roles for complement in multiple neurodegenerative diseases (e.g., Alzheimer disease, Huntington disease, Parkinson disease) should inform and improve treatment strategies for these incurable diseases.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This work was supported in part by NIH R01 AG060148, NIA R21 AG061746, and NIH U54 AG054349 (A.J.T.), and the Edythe M. Laudati Memorial Fund (A.J.T.).

LITERATURE CITED

1. Thielens NM, Tedesco F, Bohlson SS, Gaboriaud C, Tenner AJ. 2017. C1q: a fresh look upon an old molecule. *Mol. Immunol.* 89:73–83
2. Hawksworth OA, Li XX, Coulthard LG, Wolvetang EJ, Woodruff TM. 2017. New concepts on the therapeutic control of complement anaphylatoxin receptors. *Mol. Immunol.* 89:36–43
3. Peterson SL, Anderson AJ. 2014. Complement and spinal cord injury: traditional and non-traditional aspects of complement cascade function in the injured spinal cord microenvironment. *Exp. Neurol.* 258:35–47
4. Velazquez P, Cribbs DH, Poulos TL, Tenner AJ. 1997. Aspartate residue 7 in amyloid beta-protein is critical for classical complement pathway activation: implications for Alzheimer's disease pathogenesis. *Nat. Med.* 3:77–79
5. Shen Y, Lue L, Yang L, Roher A, Kuo Y, et al. 2001. Complement activation by neurofibrillary tangles in Alzheimer's disease. *Neurosci. Lett.* 305:165–68
6. Jiang H, Burdick D, Glabe CG, Cotman CW, Tenner AJ. 1994. beta-Amyloid activates complement by binding to a specific region of the collagen-like domain of the C1q A chain. *J. Immunol.* 152:5050–59
7. Zhang M, Takahashi K, Alicot EM, Vorup-Jensen T, Kessler B, et al. 2006. Activation of the lectin pathway by natural IgM in a model of ischemia/reperfusion injury. *J. Immunol.* 177:4727–34
8. Nestor J, Arinuma Y, Huerta TS, Kowal C, Nasiri E, et al. 2018. Lupus antibodies induce behavioral changes mediated by microglia and blocked by ACE inhibitors. *J. Exp. Med.* 215:2554–66
9. Howard JF Jr., Utsugisawa K, Benatar M, Murai H, Barohn RJ, et al. 2017. Safety and efficacy of eculizumab in anti-acetylcholine receptor antibody-positive refractory generalised myasthenia gravis (REGAIN): a phase 3, randomised, double-blind, placebo-controlled, multicentre study. *Lancet Neurol.* 16:976–86
10. Wingerchuk DM, Fujihara K, Palace J, Berthele A, Levy M, et al. 2021. Long-term safety and efficacy of eculizumab in aquaporin-4 IgG-positive NMOSD. *Ann. Neurol.* 89(6):1088–98
11. Veerhuis R, Nielsen HM, Tenner AJ. 2011. Complement in the brain. *Mol. Immunol.* 48:1592–603
12. Shen Y, Li R, McGeer EG, McGeer PL. 1997. Neuronal expression of mRNAs for complement proteins of the classical pathway in Alzheimer brain. *Brain Res.* 769:391–95
13. Singhrao SK, Neal JW, Rushmere NK, Morgan BP, Gasque P. 1999. Differential expression of individual complement regulators in the brain and choroid plexus. *Lab. Investig.* 79:1247–59
14. Cribbs DH, Berchtold NC, Perreau V, Coleman PD, Rogers J, et al. 2012. Extensive innate immune gene activation accompanies brain aging, increasing vulnerability to cognitive decline and neurodegeneration: a microarray study. *J. Neuroinflamm.* 9:179
15. Carvalho K, Schartz ND, Balderrama-Gutierrez G, Liang HY, Chu SH, et al. 2022. Modulation of C5a-C5aR1 signaling alters the dynamics of AD progression. *J. Neuroinflamm.* 19:178
16. Boche D, Gordon MN. 2022. Diversity of transcriptomic microglial phenotypes in aging and Alzheimer's disease. *Alzheimer's Dement.* 18(2):360–76
17. Lee JD, Coulthard LG, Woodruff TM. 2019. Complement dysregulation in the central nervous system during development and disease. *Semin. Immunol.* 45:101340
18. Petrisko TJ, Gomez-Arboledas A, Tenner AJ. 2021. Complement as a powerful "influencer" in the brain during development, adulthood and neurological disorders. *Adv. Immunol.* 152:157–222
19. Jeanes A, Coulthard LG, Mantovani S, Markham K, Woodruff TM. 2015. Co-ordinated expression of innate immune molecules during mouse neurulation. *Mol. Immunol.* 68:253–60

20. Gorelik A, Sapir T, Haffner-Krausz R, Olender T, Woodruff TM, Reiner O. 2017. Developmental activities of the complement pathway in migrating neurons. *Nat. Commun.* 8:15096
21. Lee JD, Levin SC, Willis EF, Li R, Woodruff TM, Noakes PG. 2018. Complement components are upregulated and correlate with disease progression in the TDP-43^{Q331K} mouse model of amyotrophic lateral sclerosis. *J. Neuroinflamm.* 15:171
22. Stephan AH, Madison DV, Mateos JM, Fraser DA, Lovelett EA, et al. 2013. A dramatic increase of C1q protein in the CNS during normal aging. *J. Neurosci.* 33:13460–74
23. Schartz ND, Tenner AJ. 2020. The good, the bad, and the opportunities of the complement system in neurodegenerative disease. *J. Neuroinflamm.* 17:354
24. Fonseca MI, Chu SH, Hernandez MX, Fang MJ, Modarresi L, et al. 2017. Cell-specific deletion of C1qa identifies microglia as the dominant source of C1q in mouse brain. *J. Neuroinflamm.* 14:48
25. Deleted in proof
26. Benoit ME, Hernandez MX, Dinh ML, Benavente F, Vasquez O, Tenner AJ. 2013. C1q-induced LRP1B and GPR6 proteins expressed early in Alzheimer disease mouse models, are essential for the C1q-mediated protection against amyloid-beta neurotoxicity. *J. Biol. Chem.* 288:654–65
27. Wu T, Dejanovic B, Gandham VD, Gogineni A, Edmonds R, et al. 2019. Complement c3 is activated in human AD brain and is required for neurodegeneration in mouse models of amyloidosis and tauopathy. *Cell Rep.* 28:2111–23.e6
28. Zhou J, Fonseca MI, Pisalyaput K, Tenner AJ. 2008. Complement C3 and C4 expression in C1q sufficient and deficient mouse models of Alzheimer's disease. *J. Neurochem.* 106:2080–92
29. Habib N, McCabe C, Medina S, Varshavsky M, Kitsberg D, et al. 2020. Disease-associated astrocytes in Alzheimer's disease and aging. *Nat. Neurosci.* 23(6):701–6
30. Bourel J, Planche V, Dubourdieu N, Oliveira A, Séré A, et al. 2021. Complement C3 mediates early hippocampal neurodegeneration and memory impairment in experimental multiple sclerosis. *Neurobiol. Dis.* 160:105533
31. Zipfel PF, Skerka C. 2009. Complement regulators and inhibitory proteins. *Nat. Rev. Immunol.* 9:729–40
32. Cong Q, Soteros BM, Wollet M, Kim JH, Sia GM. 2020. The endogenous neuronal complement inhibitor SRPX2 protects against complement-mediated synapse elimination during development. *Nat. Neurosci.* 23:1067–78
33. Roll P, Rudolf G, Pereira S, Royer B, Scheffer IE, et al. 2006. SRPX2 mutations in disorders of language cortex and cognition. *Hum. Mol. Genet.* 15:1195–207
34. Gialeli C, Gungor B, Blom AM. 2018. Novel potential inhibitors of complement system and their roles in complement regulation and beyond. *Mol. Immunol.* 102:73–83
35. Shinjyo N, Stahlberg A, Dragunow M, Pekny M, Pekna M. 2009. Complement-derived anaphylatoxin C3a regulates in vitro differentiation and migration of neural progenitor cells. *Stem. Cells* 27:2824–32
36. Coulthard LG, Hawksworth OA, Conroy J, Lee JD, Woodruff TM. 2018. Complement C3a receptor modulates embryonic neural progenitor cell proliferation and cognitive performance. *Mol. Immunol.* 101:176–81
37. Rahpeymai Y, Hietala MA, Wilhelmsson U, Fotheringham A, Davies I, et al. 2006. Complement: a novel factor in basal and ischemia-induced neurogenesis. *EMBO J.* 25:1364–74
38. Coulthard LG, Hawksworth OA, Woodruff TM. 2018. Complement: the emerging architect of the developing brain. *Trends Neurosci.* 41:373–84
39. Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, et al. 2007. The classical complement cascade mediates CNS synapse elimination. *Cell* 131:1164–78
40. Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, et al. 2012. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* 74:691–705
41. Shatz CJ. 1990. Competitive interactions between retinal ganglion cells during prenatal development. *J. Neurobiol.* 21:197–211
42. Welsh CA, Stephany C, Sapp RW, Stevens B. 2020. Ocular dominance plasticity in binocular primary visual cortex does not require C1q. *J. Neurosci.* 40:769–83
43. Wu Y, Dissing-Olesen L, MacVicar BA, Stevens B. 2015. Microglia: dynamic mediators of synapse development and plasticity. *Trends Immunol.* 36:605–13

44. Josselyn SA, Köhler S, Frankland PW. 2015. Finding the engram. *Nat. Rev. Neurosci.* 16:521–34
45. Wang C, Yue H, Hu Z, Shen Y, Ma J, et al. 2020. Microglia mediate forgetting via complement-dependent synaptic elimination. *Science* 367:688–94
46. Shi Q, Colodner KJ, Matousek SB, Merry K, Hong S, et al. 2015. Complement C3-deficient mice fail to display age-related hippocampal decline. *J. Neurosci.* 35:13029–42
47. Chu Y, Jin X, Parada I, Pesic A, Stevens B, et al. 2010. Enhanced synaptic connectivity and epilepsy in C1q knockout mice. *PNAS* 107:7975–80
48. Sekar A, Bialas AR, de Rivera H, Davis A, Hammond TR, et al. 2016. Schizophrenia risk from complex variation of complement component 4. *Nature* 530:177–83
49. Sia GM, Clem RL, Huganir RL. 2013. The human language–associated gene SRPX2 regulates synapse formation and vocalization in mice. *Science* 342:987–91
50. Cong Q, Soteros BM, Huo A, Li Y, Tenner AJ, Sia GM. 2022. C1q and SRPX2 regulate microglia mediated synapse elimination during early development in the visual thalamus but not the visual cortex. *Glia* 70:451–65
51. Holmquist E, Okroj M, Nodin B, Jirstrom K, Blom AM. 2013. Sushi domain-containing protein 4 (SUSD4) inhibits complement by disrupting the formation of the classical C3 convertase. *FASEB J.* 27:2355–66
52. Zhu H, Meissner LE, Byrnes C, Tuymetova G, Tifft CJ, Proia RL. 2020. The complement regulator Susd4 influences nervous-system function and neuronal morphology in mice. *iScience* 23:100957
53. González-Calvo I, Iyer K, Carquin M, Khayachi A, Giuliani FA, et al. 2021. Sushi domain-containing protein 4 controls synaptic plasticity and motor learning. *eLife* 10:e65712
54. Suzuki K, Elegheert J, Song I, Sasakura H, Senkov O, et al. 2020. A synthetic synaptic organizer protein restores glutamatergic neuronal circuits. *Science* 369:eabb4853
55. Matsuda K. 2017. Synapse organization and modulation via C1q family proteins and their receptors in the central nervous system. *Neurosci. Res.* 116:46–53
56. Yuzaki M. 2017. The C1q complement family of synaptic organizers: not just complementary. *Curr. Opin. Neurobiol.* 45:9–15
57. Steen VM, Nepal C, Ersland KM, Holdhus R, Nævdal M, et al. 2013. Neuropsychological deficits in mice depleted of the schizophrenia susceptibility gene *CSMD1*. *PLOS ONE* 8:e79501
58. Escudero-Esparza A, Kalchishkova N, Kurbasic E, Jiang WG, Blom AM. 2013. The novel complement inhibitor human CUB and Sushi multiple domains 1 (CSMD1) protein promotes factor I-mediated degradation of C4b and C3b and inhibits the membrane attack complex assembly. *FASEB J.* 27:5083–93
59. Mehrotra P, Ravichandran KS. 2022. Drugging the efferocytosis process: concepts and opportunities. *Nat. Rev. Drug Discov.* 21(8):601–20
60. Galvan MD, Greenlee-Wacker MC, Bohlson SS. 2012. C1q and phagocytosis: the perfect complement to a good meal. *J. Leukoc. Biol.* 92:489–97
61. Fraser DA, Pisalyaput K, Tenner AJ. 2010. C1q enhances microglial clearance of apoptotic neurons and neuronal blebs, and modulates subsequent inflammatory cytokine production. *J. Neurochem.* 112:733–43
62. Gyorffy BA, Kun J, Torok G, Bulyaki E, Borhegyi Z, et al. 2018. Local apoptotic-like mechanisms underlie complement-mediated synaptic pruning. *PNAS* 115:6303–8
63. Scott-Hewitt N, Perrucci F, Morini R, Erreni M, Mahoney M, et al. 2020. Local externalization of phosphatidylserine mediates developmental synaptic pruning by microglia. *EMBO J.* 39:e105380
64. Lehrman EK, Wilton DK, Litvina EY, Welsh CA, Chang ST, et al. 2018. CD47 protects synapses from excess microglia-mediated pruning during development. *Neuron* 100:120–34.e6
65. Alzheimer's Assoc. 2022. 2022 Alzheimer's disease facts and figures *Alzheimer's Dement.* 18(4):700–89
66. Rogers J, Cooper NR, Webster S, Schultz J, McGeer PL, et al. 1992. Complement activation by beta-amyloid in Alzheimer disease. *PNAS* 89:10016–20
67. Carpanini SM, Harwood JC, Baker E, Torvell M, Gerad Consort., et al. 2021. The impact of complement genes on the risk of late-onset Alzheimer's disease. *Genes* 12(3):443
68. Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, et al. 2003. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Aβ and synaptic dysfunction. *Neuron* 39:409–21

69. Bensa JC, Reboul A, Colomb MG. 1983. Biosynthesis in vitro of complement subcomponents C1q, C1s and C1 inhibitor by resting and stimulated human monocytes. *Biochem. J.* 216:385–92
70. Gao Z, Li M, Ma J, Zhang S. 2014. An amphioxus gC1q protein binds human IgG and initiates the classical pathway: implications for a C1q-mediated complement system in the basal chordate. *Eur. J. Immunol.* 44:3680–95
71. Benoit ME, Tenner AJ. 2011. Complement protein C1q-mediated neuroprotection is correlated with regulation of neuronal gene and microRNA expression. *J. Neurosci.* 31:3459–69
72. Pisalyaput K, Tenner AJ. 2008. Complement component C1q inhibits beta-amyloid- and serum amyloid P-induced neurotoxicity via caspase- and calpain-independent mechanisms. *J. Neurochem.* 104:696–707
73. Fraser DA, Arora M, Bohlson SS, Lozano E, Tenner AJ. 2007. Generation of inhibitory NFκB complexes and phosphorylated cAMP response element-binding protein correlates with the anti-inflammatory activity of complement protein C1q in human monocytes. *J. Biol. Chem.* 282:7360–67
74. Benoit ME, Clarke EV, Morgado P, Fraser DA, Tenner AJ. 2012. Complement protein C1q directs macrophage polarization and limits inflammasome activity during the uptake of apoptotic cells. *J. Immunol.* 188:5682–93
75. Galiakberova AA, Dashinimaev EB. 2020. Neural stem cells and methods for their generation from induced pluripotent stem cells in vitro. *Front. Cell Dev. Biol.* 8:815
76. Benavente F, Piltti KM, Hooshmand MJ, Nava AA, Lakatos A, et al. 2020. Novel C1q receptor-mediated signaling controls neural stem cell behavior and neurorepair. *eLife* 9:e55732
77. Gyorffy BA, Toth V, Torok G, Gulyassy P, Kovacs RA, et al. 2020. Synaptic mitochondrial dysfunction and septin accumulation are linked to complement-mediated synapse loss in an Alzheimer's disease animal model. *Cell Mol. Life Sci.* 77:5243–58
78. Hong S, Beja-Glasser VF, Nfonoyim BM, Frouin A, Li S, et al. 2016. Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science* 352:712–16
79. Hernandez MX, Jiang S, Cole TA, Chu SH, Fonseca MI, et al. 2017. Prevention of C5aR1 signaling delays microglial inflammatory polarization, favors clearance pathways and suppresses cognitive loss. *Mol. Neurodegener.* 12:66
80. Pavlovski D, Thundyil J, Monk PN, Wetsel RA, Taylor SM, Woodruff TM. 2012. Generation of complement component C5a by ischemic neurons promotes neuronal apoptosis. *FASEB J.* 26:3680–90
81. Fonseca MI, Zhou J, Botto M, Tenner AJ. 2004. Absence of C1q leads to less neuropathology in transgenic mouse models of Alzheimer's disease. *J. Neurosci.* 24:6457–65
82. Yang J, Wise L, Fukuchi KI. 2020. TLR4 cross-talk with NLRP3 inflammasome and complement signaling pathways in Alzheimer's disease. *Front. Immunol.* 11:724
83. Shi Q, Chowdhury S, Ma R, Le KX, Hong S, et al. 2017. Complement C3 deficiency protects against neurodegeneration in aged plaque-rich APP/PS1 mice. *Sci. Transl. Med.* 9:eaaf6295
84. Wyss-Coray T, Yan F, Lin AH, Lambris JD, Alexander JJ, et al. 2002. Prominent neurodegeneration and increased plaque formation in complement-inhibited Alzheimer's mice. *PNAS* 99:10837–42
85. Selkoe DJ. 2002. Alzheimer's disease is a synaptic failure. *Science* 298:789–91
86. Mecca AP, O'Dell RS, Sharp ES, Banks ER, Bartlett HH, et al. 2022. Synaptic density and cognitive performance in Alzheimer's disease: a PET imaging study with [^{11}C]UCB-J. *Alzheimer's Dement.* 18(2):2527–36
87. Carpanini SM, Torvell M, Bevan RJ, Byrne RAJ, Daskoulidou N, et al. 2022. Terminal complement pathway activation drives synaptic loss in Alzheimer's disease models. *Acta Neuropathol. Commun.* 10:99
88. Fatoba O, Itokazu T, Yamashita T. 2022. Complement cascade functions during brain development and neurodegeneration. *FEBS J.* 289:2085–109
89. Pozo-Rodrigalvarez A, Ollaranta R, Skoog J, Pekny M, Pekna M. 2021. Hyperactive behavior and altered brain morphology in adult complement C3a receptor deficient mice. *Front. Immunol.* 12:604812
90. Spurrier J, Nicholson L, Fang XT, Stoner AJ, Toyonaga T, et al. 2022. Reversal of synapse loss in Alzheimer mouse models by targeting mGluR5 to prevent synaptic tagging by C1Q. *Sci. Transl. Med.* 14:eabi8593
91. Um JW, Kaufman AC, Kostylev M, Heiss JK, Stagi M, et al. 2013. Metabotropic glutamate receptor 5 is a coreceptor for Alzheimer Aβ oligomer bound to cellular prion protein. *Neuron* 79:887–902

92. Haas LT, Salazar SV, Smith LM, Zhao HR, Cox TO, et al. 2017. Silent allosteric modulation of mGluR5 maintains glutamate signaling while rescuing Alzheimer's mouse phenotypes. *Cell Rep*. 20:76–88
93. Neff RA, Wang M, Vatansever S, Guo L, Ming C, et al. 2021. Molecular subtyping of Alzheimer's disease using RNA sequencing data reveals novel mechanisms and targets. *Sci. Adv.* 7:eabb5398
94. Hernandez MX, Namiranian P, Nguyen E, Fonseca MI, Tenner AJ. 2017. C5a increases the injury to primary neurons elicited by fibrillar amyloid beta. *ASN Neuro* 9:1759091416687871
95. Fonseca MI, Ager RR, Chu SH, Yazan O, Sanderson SD, et al. 2009. Treatment with a C5aR antagonist decreases pathology and enhances behavioral performance in murine models of Alzheimer's disease. *J. Immunol.* 183:1375–83
96. Gomez-Arboledas A, Carvalho K, Balderrama-Gutierrez G, Chu S-H, Liang HY, et al. 2022. C5aR1 antagonism alters microglial polarization and mitigates disease progression in a mouse model of Alzheimer's disease. *Acta Neuropathol. Commun*. 10:116
97. Panayiotou E, Fella E, Andreou S, Papacharalambous R, Gerasimou P, et al. 2019. C5aR agonist enhances phagocytosis of fibrillar and non-fibrillar Aβ amyloid and preserves memory in a mouse model of familial Alzheimer's disease. *PLOS ONE* 14:e0225417
98. Li XX, Lee JD, Kemper C, Woodruff TM. 2019. The complement receptor C5aR2: a powerful modulator of innate and adaptive immunity. *J. Immunol.* 202:3339–48
99. Cain SA, Monk PN. 2002. The orphan receptor C5L2 has high affinity binding sites for complement fragments C5a and C5a des-Arg^{74}. *J. Biol. Chem.* 277:7165–69
100. Li XX, Clark RJ, Woodruff TM. 2020. C5aR2 activation broadly modulates the signaling and function of primary human macrophages. *J. Immunol.* 205:1102–12
101. Vasek MJ, Garber C, Dorsey D, Durrant DM, Bollman B, et al. 2016. A complement-microglial axis drives synapse loss during virus-induced memory impairment. *Nature* 534:538–43
102. Stokowska A, Atkins AL, Moran J, Pekny T, Bulmer L, et al. 2017. Complement peptide C3a stimulates neural plasticity after experimental brain ischaemia. *Brain* 140:353–69
103. Wei L-L, Ma N, Wu K-Y, Wang J-X, Diao T-Y, et al. 2020. Protective role of C3aR (C3a anaphylatoxin receptor) against atherosclerosis in atherosclerosis-prone mice. *Arterioscler. Thromb. Vasc. Biol.* 40:2070–83
104. Coulthard LG, Woodruff TM. 2015. Is the complement activation product C3a a proinflammatory molecule? Re-evaluating the evidence and the myth. *J. Immunol.* 194:3542–48
105. El Gaamouch F, Audrain M, Lin WJ, Beckmann N, Jiang C, et al. 2020. VGF-derived peptide TLQP-21 modulates microglial function through C3aR1 signaling pathways and reduces neuropathology in 5xFAD mice. *Mol. Neurodegener.* 15:4
106. Kalant D, Cain SA, Maslowska M, Sniderman AD, Cianflone K, Monk PN. 2003. The chemoattractant receptor-like protein C5L2 binds the C3a des-Arg^{77}/acylation-stimulating protein. *J. Biol. Chem.* 278:11123–29
107. Zhou Y, Chen Y, Xu C, Zhang H, Lin C. 2020. TLR4 targeting as a promising therapeutic strategy for Alzheimer disease treatment. *Front. Neurosci.* 14:602508
108. Walter S, Letiembre M, Liu Y, Heine H, Penke B, et al. 2007. Role of the Toll-like receptor 4 in neuroinflammation in Alzheimer's disease. *Cell Physiol. Biochem.* 20:947–56
109. Hajishengallis G, Lambris JD. 2010. Crosstalk pathways between Toll-like receptors and the complement system. *Trends Immunol.* 31:154–63
110. Bettcher BM, Tansey MG, Dorothee G, Heneka MT. 2021. Peripheral and central immune system crosstalk in Alzheimer disease—a research prospectus. *Nat. Rev. Neurol.* 17:689–701
111. Brodsky RA, Young NS, Antonioli E, Risitano AM, Schrezenmeier H, et al. 2008. Multicenter phase 3 study of the complement inhibitor eculizumab for the treatment of patients with paroxysmal nocturnal hemoglobinuria. *Blood* 111:1840–47
112. Parker C. 2009. Eculizumab for paroxysmal nocturnal haemoglobinuria. *Lancet* 373:759–67
113. Asavapanumas N, Tradtrantip L, Verkman AS. 2021. Targeting the complement system in neuromyelitis optica spectrum disorder. *Expert Opin. Biol. Ther.* 21:1073–86
114. Dhillon S. 2018. Eculizumab: a review in generalized myasthenia gravis. *Drugs* 78:367–76

115. Mallah K, Couch C, Alshareef M, Borucki D, Yang X, et al. 2021. Complement mediates neuroinflammation and cognitive decline at extended chronic time points after traumatic brain injury. *Acta Neuropathol. Commun.* 9:72
116. Alawieh A, Chalhoub RM, Mallah K, Langley EF, York M, et al. 2021. Complement drives synaptic degeneration and progressive cognitive decline in the chronic phase after traumatic brain injury. *J. Neurosci.* 41:1830–43
117. Alawieh A, Langley EF, Weber S, Adkins D, Tomlinson S. 2018. Identifying the role of complement in triggering neuroinflammation after traumatic brain injury. *J. Neurosci.* 38:2519–32
118. Scott G, Hellyer PJ, Ramlackhansingh AF, Brooks DJ, Matthews PM, Sharp DJ. 2015. Thalamic inflammation after brain trauma is associated with thalamo-cortical white matter damage. *J. Neuroinflamm.* 12:224
119. Holden SS, Grandi FC, Aboubakr O, Higashikubo B, Cho FS, et al. 2021. Complement factor C1q mediates sleep spindle loss and epileptic spikes after mild brain injury. *Science* 373:eabj2685
120. Kim YU, Kinoshita T, Molina H, Hourcade D, Seya T, et al. 1995. Mouse complement regulatory protein Crry/p65 uses the specific mechanisms of both human decay-accelerating factor and membrane cofactor protein. *J. Exp. Med.* 181:151–59
121. Alawieh AM, Langley EF, Feng W, Spiotta AM, Tomlinson S. 2020. Complement-dependent synaptic uptake and cognitive decline after stroke and reperfusion therapy. *J. Neurosci.* 40:4042–58
122. Alawieh A, Langley EF, Tomlinson S. 2018. Targeted complement inhibition salvages stressed neurons and inhibits neuroinflammation after stroke in mice. *Sci. Transl. Med.* 10:eaao6459
123. Werneburg S, Jung J, Kunjamma RB, Ha SK, Luciano NJ, et al. 2020. Targeted complement inhibition at synapses prevents microglial synaptic engulfment and synapse loss in demyelinating disease. *Immunity* 52:167–82.e7
124. Schulz K, Trendelenburg M. 2022. C1q as a target molecule to treat human disease: What do mouse studies teach us? *Front. Immunol.* 13:958273
125. Zelek WM, Morgan BP. 2022. Targeting complement in neurodegeneration: challenges, risks, and strategies. *Trends Pharmacol. Sci.* 43:615–28
126. Carpanini SM, Torvell M, Morgan BP. 2019. Therapeutic inhibition of the complement system in diseases of the central nervous system. *Front. Immunol.* 10:362
127. Garred P, Tenner AJ, Mollnes TE. 2021. Therapeutic targeting of the complement system: from rare diseases to pandemics. *Pharmacol. Rev.* 73:792–827
128. Santra M, Dill KA, de Graff AMR. 2019. Proteostasis collapse is a driver of cell aging and death. *PNAS* 116:22173–78
129. Lee JD, Kumar V, Fung JN, Ruitenberg MJ, Noakes PG, Woodruff TM. 2017. Pharmacological inhibition of complement C5a-C5a$_1$ receptor signalling ameliorates disease pathology in the hSOD1^{G93A} mouse model of amyotrophic lateral sclerosis. *Br. J. Pharmacol.* 174:689–99
130. Biggins PJC, Brennan FH, Taylor SM, Woodruff TM, Ruitenberg MJ. 2017. The alternative receptor for complement component 5a, C5aR2, conveys neuroprotection in traumatic spinal cord injury. *J. Neurotrauma* 34:2075–85
131. Matcovitch-Natan O, Winter DR, Giladi A, Vargas Aguilar S, Spinrad A, et al. 2016. Microglia development follows a stepwise program to regulate brain homeostasis. *Science* 353:aad8670
132. Keren-Shaul H, Spinrad A, Weiner A, Matcovitch-Natan O, Dvir-Szternfeld R, et al. 2017. A unique microglia type associated with restricting development of Alzheimer's disease. *Cell* 169:1276–90.e17
133. Hess C, Schifferli JA. 2003. Immune adherence revisited: novel players in an old game. *News Physiol. Sci.* 18:104–8
134. Johansson JU, Brubaker WD, Javitz H, Bergen AW, Nishita D, et al. 2018. Peripheral complement interactions with amyloid β peptide in Alzheimer's disease: polymorphisms, structure, and function of complement receptor 1. *Alzheimer's Dement.* 14:1438–49
135. Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, et al. 2009. Genome-wide association study identifies variants at *CLU* and *PICALM* associated with Alzheimer's disease. *Nat. Genet.* 41:1088–93
136. Lambert JC, Heath S, Even G, Campion D, Sleegers K, et al. 2009. Genome-wide association study identifies variants at *CLU* and *CR1* associated with Alzheimer's disease. *Nat. Genet.* 41:1094–99

137. Hettmann T, Gillies SD, Kleinschmidt M, Piechotta A, Makioka K, et al. 2020. Development of the clinical candidate PBD-C06, a humanized pGlu3-Aβ-specific antibody against Alzheimer's disease with reduced complement activation. *Sci. Rep.* 10:3294
138. Taylor RP, Lindorfer MA, Atkinson JP. 2020. Clearance of amyloid-beta with bispecific antibody constructs bound to erythrocytes. *Alzheimer's Dement*. 6:e12067
139. Fonseca MI, Chu S, Pierce AL, Brubaker WD, Hauhart RE, et al. 2016. Analysis of the putative role of CR1 in Alzheimer's disease: genetic association, expression and function. *PLOS ONE* 11:e0149792
140. Tenner AJ. 2020. Complement-mediated events in Alzheimer's disease: mechanisms and potential therapeutic targets. *J. Immunol.* 204:306–15
141. Repik A, Pincus SE, Ghiran I, Nicholson-Weller A, Asher DR, et al. 2005. A transgenic mouse model for studying the clearance of blood-borne pathogens via human complement receptor 1 (CR1). *Clin. Exp. Immunol.* 140:230–40
142. Jackson HM, Foley KE, O'Rourke R, Stearns TM, Fathalla D, et al. 2020. A novel mouse model expressing human forms for complement receptors CR1 and CR2. *BMC Genet*. 21:101
143. Mahmoudi R, Kisserli A, Novella JL, Donvito B, Drame M, et al. 2015. Alzheimer's disease is associated with low density of the long CR1 isoform. *Neurobiol. Aging* 36:1766.e5–e12
144. Merle NS, Singh P, Rahman J, Kemper C. 2020. Integrins meet complement: the evolutionary tip of an iceberg orchestrating metabolism and immunity. *Br. J. Pharmacol.* 178(14):2754–70
145. Kolev M, Kemper C. 2017. Keeping it all going—complement meets metabolism. *Front. Immunol.* 8. **https://doi.org/10.3389/fimmu.2017.00001**
146. Arbore G, West EE, Spolski R, Robertson AAB, Klos A, et al. 2016. T helper 1 immunity requires complement-driven NLRP3 inflammasome activity in $CD4^+$ T cells. *Science* 352:aad1210
147. Kolev M, Dimeloe S, Le FG, Navarini A, Arbore G, et al. 2015. Complement regulates nutrient influx and metabolic reprogramming during Th1 cell responses. *Immunity* 42:1033–47
148. Kunz N, Kemper C. 2021. Complement has brains—do intracellular complement and immunometabolism cooperate in tissue homeostasis and behavior? *Front. Immunol.* 12:629986
149. Niyonzima N, Rahman J, Kunz N, West EE, Freiwald T, et al. 2021. Mitochondrial C5aR1 activity in macrophages controls IL-1β production underlying sterile inflammation. *Sci. Immunol.* 6:eabf2489
150. Vlaar APJ, de Bruin S, Busch M, Timmermans S, van Zeggeren IE, et al. 2020. Anti-C5a antibody IFX-1 (vilobelimab) treatment versus best supportive care for patients with severe COVID-19 (PANAMO): an exploratory, open-label, phase 2 randomised controlled trial. *Lancet Rheumatol*. 2(12):e764–73
151. Woodruff TM, Shukla AK. 2020. The complement C5a-C5aR1 GPCR axis in COVID-19 therapeutics. *Trends Immunol*. 41:965–67
152. De la Obecerra KI, Oosterheert W, van den Bos RM, Xenaki KT, Lorent JH, et al. 2022. Multifaceted activities of seven nanobodies against complement C4b. *J. Immunol.* 208:2207–19
153. Ullman JC, Arguello A, Getz JA, Bhalla A, Mahon CS, et al. 2020. Brain delivery and activity of a lysosomal enzyme using a blood-brain barrier transport vehicle in mice. *Sci. Transl. Med.* 12(545):eaay1163
154. Wouters Y, Jaspers T, De Strooper B, Dewilde M. 2020. Identification and in vivo characterization of a brain-penetrating nanobody. *Fluids Barriers CNS* 17:62