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Author

Tenner, Andrea J

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Complement-mediated events in Alzheimer's Disease: Mechanisms and Potential Therapeutic Targets

Andrea J. Tenner

Department of Molecular Biology and Biochemistry, Department of Neurobiology and Behavior, Department of Pathology and Laboratory Medicine, Institute for Memory Impairment and Neurological Disorders, University of California Irvine, Irvine, CA

Abstract

An estimated 5.7 million Americans suffer from Alzheimer's disease in the United States with no disease modifying treatments to prevent or treat cognitive deficits associated with the disease. GWAS studies suggest that an enhancement of clearance mechanisms and/or promotion of an anti-inflammatory response may slow or prevent disease progression. Increasing awareness of distinct roles of complement components in normal brain development and function and in neurodegenerative disorders align with complement-mediated responses and thus thorough understanding of these molecular pathways is needed to facilitate successful therapeutic design. Both beneficial and detrimental effects of C1q as well as contributions to local inflammation by C5a-C5aR1 signaling in brain highlight the need for precision of therapeutic design. The potential benefit of β -amyloid clearance from the circulation via CR1-mediated mechanisms is also reviewed. Therapies that suppress inflammation, while preserving protective effects of complement could be tested now to slow the progression of this debilitating disease.

Keywords

Alzheimer's disease; Complement; Brain; Innate Immunity; Neurodegeneration; Neuroprotection; TLR; microglia; macrophage

The complement system is an evolutionarily ancient system contributing to defense from pathogens and injury (1). While the protective effector functions of the complement system in the vasculature were the first to be described, it is now clear that the components of the system have a plethora of roles in immune mechanisms of homeostasis, the development and retraction of the adaptive immune responses, intracellular metabolism and regeneration of injured tissue (reviewed in (2–4)), and that over activation or dysregulation contributes to disease. There has been an explosion of new findings of the role of complement in the nervous system, from contributions to migration of cells and synapse elimination during development (5, 6), to detrimental damage of nerve cells in autoimmunity (7) and stroke (8), and aberrant synapse pruning in neurological disorders (reviewed in (9, 10)). In this brief review, the systems involved will be introduced, followed by discussion of data suggesting

modulatory roles of complement in Alzheimer's disease. Finally, potential approaches to therapies targeting specific elements of the complement system will be presented.

Alzheimer's disease

Alzheimer's disease (AD) is the most prevalent progressive neurodegenerative disorder of the elderly, and the sixth leading cause of death in the United States (11). Clinical symptoms of the disease include an unrelenting progressive decline of both memory and executive cognitive function. Unfortunately, there are still no treatments that limit the pathology or, more importantly, slow the progressive cognitive loss characteristic of this disorder. Alois Alzheimer first described AD in 1906 as clinically dementia with brain pathology defined by the presence of extracellular plaque deposits and neurofibrillary tangles. The plaques contain aggregated β -amyloid peptide ($A\beta$) and the tangles are aggregated hyper-phosphorylated tau (a microtubule associated protein) (12). It is now clear that "reactive" microglia and astrocytes indicative of a glial neuroinflammatory response surround the fibrillar plaques (plaques containing multimers of $A\beta$ in a β -sheet fibrillar conformation, $fA\beta$). This, in conjunction with measured elevated levels of inflammatory mediators in post-mortem AD brains, suggests a role for inflammation in the progression of the disease ((13, 14) and reviewed in (15, 16)).

Most AD cases (about 95–98%) are diagnosed after the age of 60 and are classified as "sporadic" or, perhaps a better term, "late onset" (LOAD). While age is the greatest risk factor for the disease, large genome-wide association studies (GWAS) have identified greater than 25 genetic risk loci including two complement associated genes, clusterin (CLU) and complement receptor 1 (CR1) (17–21). While most variants confer low risk, many have relatively high prevalence in the population, and thus can combine to generate significant polygenic risk scores (22, 23). These data strongly suggest that multi-genetic as well as environmental factors contribute to the disease. Concurrently, large unbiased bioinformatics analyses reproducibly point to clusters of genetic risk factors in pathways that suggest overlapping processes contribute to synapse loss, neuronal degeneration and cognitive dysfunction (21). Importantly, complement, microglia, astrocytes and immune response pathways involving inflammation and/or clearance mechanisms align with the genetics and pathological features of LOAD (13). Gene expression studies in mouse models of AD (24–27) support these critical pathways. A somewhat unexpected finding from models that perturb inflammatory pathways via deletion of C5AR1 and TYROBP, was that normalization of gene expression pathways and cognitive deficits occurred in the absence of decreased amyloid plaque deposition (25, 26). However, those data are consistent with reports of cognitively normal individuals with substantial plaque pathology at autopsy (28, 29) and indicate that it is an induced response to plaque that is detrimental in AD, rather than the plaques alone. Thus, these dysregulated "response" pathways can provide potential therapeutic targets to ameliorate or slow the progression of disease.

Current therapeutics thus far have shown limited efficacy (reviewed in (30, 31)) with recent disappointing results from anti- $A\beta$ and BACE1 inhibitor therapies. While steroids, non-steroidal anti-inflammatories, and anti-oxidants target inflammation, these broad, non-selective drugs have had limited success in AD clinical trials and are accompanied by

substantial side effects (32). These negative clinical results may have several possible inadvertent design caveats, but combined with the GWAS studies and more recently gene expression data, have led to new conceptual frameworks suggesting that there are many pathways to the cognitive loss associated with AD pathology. Thus, more consideration is now being given to the development of drugs with highly specific targets that would be disease modifying in subsets of AD patients or that would reduce excessive detrimental inflammation or promote neuronal resilience broadly.

Complement

The complement system, composed of over 40 interacting proteins in blood and other tissues, is a part of the innate immune response that is critically important for quickly recognizing and clearing pathogens, dying cells and misfolded proteins (33). It is also a prominent effector in antibody-mediated pathogen killing and clearance, and contributes to the generation of the pathogen-specific adaptive immune system response (34, 35). The complement system is activated by three different recognition pathways (classical, lectin and alternative), all of which lead to sequential enzyme activation, protein cleavage and conformational change to mediate effector functions (33). The classical complement pathway is activated by binding of C1q, a component of the C1 complex, to the Fc domain of antibody in immune complexes or to nonimmunoglobulin activators such as fibrillar A β . The interaction-induced conformational change induces activation of the proenzymes C1r and C1s within the C1 complex. C1s then cleaves complement component C4 generating C4a and C4b, the latter of which is covalently deposited on the activator surface by a thioester bond. C4b binds C2 and the C1s protease then cleaves C2 into C2a and C2b. (Here C2b refers to the larger cleaved fragment of C2 that contains the enzymatic activity (36, 37).) The nascently formed C3 convertase, C4b2b, cleaves C3 into C3a and C3b. C3a influences inflammation and cellular responses via C3aR (38–40), and C3b stably associated with the activator is a potent opsonin engaging phagocytic cells, as well as mediating clearance of immune complexes. Some of the cleaved C3b remains associated with C4b2b forming C4b2b3b, the classical pathway C5 convertase. The C5 convertase then cleaves C5 into C5a and C5b. C5a can bind to receptors expressed on myeloid, mast cells, and other cell types (41, 42), inducing chemotaxis, increased vascular permeability and stimulating proinflammatory pathways. C5b initiates the formation of the membranolytic pore-forming C5b-9 complex, capable of lysing pathogens.

The lectin pathway is quite similar to the classical pathway except that the recognition components ficolins, mannan binding lectin (MBL) or other collectins are activated by specific repeating carbohydrate structures and the associated serine proteases are MASP1/2. The alternative pathway of activation is the result of the direct association of C3b with an activating surface (43), which is often a pathogen but can also be fibrillar A β (44). The serine proteases Factor D and Factor B result in the generation of the C3 cleaving enzyme complex C3bBb and the alternative C5 cleaving enzyme C3bBb3b. The alternative pathway can also amplify the classical pathway activation, and thus could be targeted to decrease excessive downstream complement activation.

Regardless of the activation mechanism, the same major effector functions can result: (1) opsonization, via tagging of activators with cleavage fragments C3b/iC3b (and to a lesser extent C4b and C1q) resulting in more efficient receptor-mediated clearance of pathogens, apoptotic cells, and cellular debris by phagocytes; (2) recruitment of immune cells, including microglia, to the site of injury via production of chemotactic peptides, C3a and C5a, and their subsequent engagement of specific cell membrane receptors; (3) targeted death of pathogens due to the sequential interaction of activation cleavage product C5b with C6, C7, C8, C9 generating the lytic membrane attack complex (MAC); (4) increased vascular permeability; and (5) polarization of cells via induction of specific gene expression patterns (reviewed in (33, 45)).

Noncanonical roles of Complement in the Central Nervous System (CNS)

In 2007, Beth Stevens, Ben Barres and colleagues “rocked” the complement and neurodevelopment world using high resolution confocal microscopy to quantify synaptic puncta in mice genetically deficient in the complement proteins C1q and C3 to demonstrate a substantial role for the upstream classical complement pathway in eliminating “inactive” synapses during the refinement of the developing retinogeniculate system (6). Microglia ingested C1q- and iC3b/C3b-tagged, presumably “weak” CD47 low (46), synapses via the complement receptor CR3 (47). At the protein level, C1q was copurified with synaptosomes containing apoptosis markers (48), suggesting that synaptic pruning may involve some of the same molecular triggers as the complement mediated enhanced clearance of apoptotic cells. Thus, while very surprising at first, the nervous system appears to have merely assimilated functions normally provided by complement in the tagging of apoptotic cells and cellular material for “silent” clearance by phagocytic cells in tissue. However, subsequently, excessive complement dependent synaptic pruning and/or localized loss of spine density was demonstrated to occur in multiple mouse models of neurological disorders (49–54), often correlating with deficits in behavioral performance, indicating either aberrant activation or insufficient regulation of the cascade.

In a distinct mechanism of action, interaction of C1q with myeloid cells, including microglia, in the absence of the enzyme complex C1, suppresses proinflammatory cytokine production and enhances clearance of apoptotic cells and neuronal blebs in primary cell cultures (55–58) (Figure 1). C1q interaction with macrophages or microglia in tissue during cell turnover, tissue remodeling, or apoptotic cell death, as may occur in mild sterile injury, is a homeostasis mechanism to enable clearance without inducing an immune response to self antigens (autoimmunity) (59, 60) and as reviewed in (61, 62). This is an area of continuing investigation, as C1q can engage diverse receptors and co-receptors thereby mediating diverse cellular responses (reviewed in (63, 64)).”

C1q has direct protective effects on primary cultured neurons under nutrient stress or amyloid induced toxicity, again without the presence or activation of C1 or any of the downstream complement components (65–67) (Figure 2). Early investigations of the intracellular signaling and induced patterns of gene expression are intriguing (66, 67) and warrant further investigation in the pursuit of potential therapeutic advantage. Thus, although targeting C1q to avoid damaging downstream events due to the activation of the

classical pathway may have benefits, inhibition of these other neuroprotective functions may be counterproductive for neurodegenerative diseases in general.

Synthesis of Complement Proteins

The liver had long been recognized as the site of complement protein production (reviewed in (68), both constitutively and in some cases (for example, C3, MBL) as inducible acute phase proteins (69, 70). However, the synthesis of complement proteins is now recognized to be differentially induced in multiple cell types, including myeloid cells throughout the body, and importantly can be transcriptionally regulated in CNS resident neurons, astrocytes, oligodendrocytes, and microglia with injury or aging (reviewed in (9, 71)). CNS expression of most complement components increases with aging, and further increases in AD patients and animal models of AD consistent with a role for complement in the response to injury and progression of disease (72–75). However, evidence thus far suggests that complement system regulators such as C1 Inhibitor levels in the aging, AD brain are decreased especially relative to the increased activators, such as misfolded proteins, apoptotic cells/damaged neurons, or cell debris (76, 77) and reviewed in (78), leading to a potentially significant imbalance in control of inflammatory activation.

More is known about C1q and C3 synthesis in the brain. An increase in C1q mRNA results in the dramatically increased C1q protein in the normal aging of mouse and human brain (79). During development of the visual system, synthesis of C1q is upregulated in neurons (6). In this case, C1q synthesis by neurons is dependent on TGF β secreted by astrocytes (80). However, in a microglial specific conditional C1q knock out mouse, blood C1q levels remain unchanged while C1q in the adult brain was absent, Implicating microglia as the predominant CNS source of C1q (81). Thus, this complement component is present in aging brain even without a breakdown of the blood brain barrier. In contrast to the dominant expression of C1q by microglia, C3 is largely produced by reactive astrocytes in brain and C4 appears to be synthesized in oligodendrocytes as demonstrated by immunohistochemistry (82) and RNA-seq (51, 83, 84). C3 has been used as a marker of A1 activated astrocytes (83).

Over 35 years ago it was demonstrated that C1q, the initiating component of the classical complement cascade, could be synthesized in the absence of the C1 serine proteases C1r and C1s in peripheral myeloid cells (85). In the brain, upregulation of C1q expression at the mRNA and protein level is an early response to injury or disease and in many cases, is robustly produced in the absence of the classical pathway serine proteases (as reviewed in (86) and more recently (67)) supporting C1-independent C1q-mediated functions. C1r, C1s and C3 are upregulated later in models of AD when plaques become more abundant (67). Wu and colleagues report differential synthesis of several complement components in murine astrocytes and microglial as a function of amyloidosis or tauopathy with age (51). The temporal and cellular regulation of individual component cascade proteins in normal, aging and diseased brains is clearly important to define as several components contribute to different disease processes and tissue repair or resilience. How transcriptional and translational control of complement protein availability contributes to disease progression

will become more clear as single cell RNA-Seq and proteomic studies assessing complement component genes in regional and temporal dimensions are completed (84).

Complement and Alzheimer's disease

As early as 1982, complement proteins were found associated with amyloid plaques in human AD brain (87). It was then demonstrated that the complement cascade could be directly activated *in vitro* by fibrillar A β and neurofibrillary tangles (hyperphosphorylated tau) (44, 88–90). C1q, C3b, C4b and properdin were verified as being readily associated with fibrillar A β plaques in humans and animal models providing *in vivo* correlates to the activation of both classical and alternative pathway, as reviewed in (91) and in (92), clearly implicating the complement system as a player in the inflammatory scenario.

Clearance of A β plaques is either impaired in AD or not sufficient to overcome an accumulation of A β (or both). While a portion of A β is transported out of the brain via LRP-1 and microglia have been shown ingesting amyloid, the mechanisms and relative contributions to clearance of A β plaques remains to be clarified (93). While oligomeric A β directly stresses neurons, the β -sheet fibrillar amyloid plaque (and likely protofibrils) is the complement activating conformation (94). A beneficial result of complement activation in the case of AD could be opsonization and clearance of misfolded proteins (95, 96). In addition, glutamate containing vesicular blebs generated by damaged neurons and apoptotic cells bind C1q and are cleared by microglia (58) (Figure 1). Both membrane-bound and soluble complement regulators, including, for example, CD55, Factor H and C4 binding protein (C4BP), limit the formation of the downstream C3 and C5 convertases. Factor H is a cofactor for Factor I mediated cleavage of C3b whereas C4BP is a cofactor for cleavage of C4b by factor I, leading to inactivation of C3b and C4b, respectively and thus inhibiting further C3 cleavage activity. Both Factor H and C4BP are colocalized with plaques and dead cells in AD brains, demonstrating that there is some level of inhibition at the C3 convertase level (78, 97–99) that could permit clearance of apoptotic cells, with concomitant attenuation of inflammation, with limited generation of downstream mediators C5a and C5b-9. However, the MAC, C5b-9, has also been found in human AD brain in areas containing fibrillar plaques and tangles (100). While this could result in MAC-induced damage by insertion into cells (adjacent to the plaques) that may not be well protected by host cell CD59 (76), the contribution to AD dysfunction by the C5b-9 complex remains to be established. The presence of the MAC nevertheless demonstrates that complete activation of the cascade does occur within the AD brain, thereby inferring the generation of the generally proinflammatory fragment, C5a. A large body of previous investigations of C5aR1 signaling (reviewed in (101)) indicate that the anaphylatoxin receptor C5aR1 produces a pro-inflammatory environment by acting on MAPKs and leading to generation of inflammatory cytokines many of which are known to be expressed in AD brain. While carboxypeptidase N (CPN) reduces C3a and C5a affinity for their receptors and thus reduces corresponding pro-inflammatory actions by rapidly cleaving their C-terminal Arg, little is known of the expression and role of CPN in the CNS (102). C5aR2, an alternate receptor for C5a, is thought to have C5a scavenger and neuroprotective functions (103). C5aR2 can also heterodimerize with C5aR1, resulting in modulation of that receptor expression/activity (104), again demonstrating multiple potential levels of regulation.

To explore functions and consequences of complement activation *in vivo*, mouse models of AD were crossed to C3 knockout mice or to a transgenic mouse overexpressing the C3 convertase inhibitor, Crry. Greater amyloid accumulation and greater cognitive deficits were observed in the C3 deficient or Crry-overexpressed mice (95, 105), suggesting a beneficial role for activated C3. In contrast, in the Tg2576 transgenic mouse, genetic ablation of C1q resulted in reduced plaque and glial activation, less loss of synaptophysin in stratum lucidum of CA3 (where mossy fibers synapse CA3 pyramidal dendrites), less loss of MAP2 in CA3 pyramidal neurons dendrites, and less cognitive decline relative to the C1q sufficient Tg2576 (106). In more recent studies, deletion of C1q or C3 in other AD models was found to be beneficial in cognitive studies (53, 107). This protection was attributed to suppression of excessive synaptic pruning. Similar evidence of the involvement of early complement components in detrimental excessive synapse elimination has been reported in models of frontal temporal dementia (FTD) and West Nile Virus infection (49, 54). However, the complex role of complement in the brain complicates interpretation of the results of either knocking out or inhibiting the early components of complement as that can influence the generation of multiple downstream functions, which must be considered as potential mechanisms for the observed results.

Toll-like receptors (TLRs) are pattern recognition receptors which are important sensors for innate immune system that can synergize with or antagonize the complement system to initiate and enhance the response to pathogens and misfolded proteins (reviewed in (108)). In the periphery, C5a-C5aR1 signaling was found to synergize with TLR2 and TLR4 stimulation and enhance pro-inflammatory cytokine responses (TNF- α and IL-1 β) in mouse models (109, 110), mouse macrophages, and human monocytes all of which can be beneficial in the resolution of infections (reviewed in (108)). Although it remains to be determined if similar synergy occurs in CNS, A β binding to microglia has been found to involve TLR4 and TLR2 (111). The early *in vitro* findings that C5a increased the release of IL-1 β and IL-6 in A β -primed human monocytes (112) and induced chemotaxis in microglia (113), was consistent with a scenario in which fibrillar amyloid plaque complement activation generates C5a which then recruits microglia to the plaque. Upon interaction of fibrillar plaques with TLR (114) on the recruited microglia would synergistically initiate an inflammatory response leading to a neurotoxic environment (Figure 1). An unbiased integrated systems approach identified immune functions and microglial activation products including complement, TLR and cytokine networks as key nodes correlating with attributes of human late-onset AD (13), and thus as in the periphery, C5a may play a synergistic role with other damage associated molecular patterns (DAMPs) in the response to perceived danger in the brain.

Role of C5a and Inflammation in AD

Evidence from multiple systems suggests that inflammation due to the complement system is triggered by the activation-induced cleavage product, C5a. C5a is chemotactic for phagocytes (including microglia) and leads to an alteration of their functional states (reviewed in (115)). In AD, the complement system may be continually activated by both fibrillar A β (89, 116) and extracellular tangles formed by hyperphosphorylated tau (88). This

could contribute to a chronic inflammatory state mediated substantially by the complement activation product C5a upon binding to its receptor C5aR1 on microglia (Figure 1 and 2).

Evidence for the concept of microglial priming has long been proposed with proponents advocating peripheral or CNS origin of the priming and/or secondary inflammatory stimuli (117, 118). Many groups have recognized the dichotomous roles of microglia, both detrimental and beneficial, for some time (119). RNA-Seq and immunohistochemical data provide evidence that microglia near $\text{A}\beta$ plaques adopt different functional states that enable them to produce pro-inflammatory cytokines as well as reactive oxygen species (ROS) while other microglial subsets enhance phagocytosis and generate neuroprotective growth factors (24, 26, 27, 120–122). The functional state of microglia is critical because the location and “balance” of different microglia populations may have significantly different effects on astrocytes and neurotoxicity vs neurons and resiliency during CNS diseases (83, 123). If microglia can be inhibited in their differentiation to the disease activated microglial (DAM) state or “repolarized” to perform a more beneficial role in neurodegenerative and/or neuroinflammatory diseases such as AD (27, 121), it would certainly be a valuable therapeutic treatment target.

Recently, in an additional AD mouse model (Arctic), genetic ablation of C5aR1 prevented spatial specific memory deficits (26) independent of changes in amyloid load. Importantly, Sholl analysis of neurons in the CA1 region of the hippocampus revealed a profound decrease in neuronal complexity temporally aligning with cognitive deficits in the C5aR1-sufficient AD model mice, whereas no such loss of neuronal branching was seen in the AD mice lacking C5aR1. Gene expression data from adult microglia isolated from brain at four different ages was examined to investigate the effect of complement C5a on microglial gene expression. Indeed, a lack of C5aR1 prevents the polarization of microglia to a more inflammatory state seen with age and amyloid accumulation, while expression of genes involved in phagocytosis and lysosomal degradative enzymes were enhanced (26), consistent with induction of clearance and repair functions. Since C5aR1 knock out Arctic mice maintained neuronal integrity and had no behavior deficit as seen in the C5aR1 sufficient Arctic mice, treatment with a C5aR1 antagonist, without blocking C1q or C3 may be an effective treatment for slowing the progression of cognitive loss in AD and AD-related dementias. The data also confirm that amyloid plaques may be necessary (by definition), but not sufficient, to cause the cognitive decline seen in this AD mouse model (26) and in high amyloid pathology cognitively intact patients (29). A potential caveat of all mouse models of AD studied thus far is that they are more closely aligned with the early onset AD, that is the result of mutations in the amyloid precursor protein, APP, or the presenilins that cleave APP to form $\text{A}\beta$, overexpress these proteins under a variety of promoters that lack endogenous regulation, and lack genetic diversity seen in the human population (124, 125). New candidate models of late onset AD are currently being generated, with a few currently distributed commercially. Thus, while accumulation of misfolded proteins, complement activation and inflammation are hallmarks of AD dementia pathology, utilization of these models will certainly accelerate the path to effective therapeutic intervention.

C5aR1 as a Therapeutic Target

PMX53 and related PMX205 are cyclic hexapeptide analogs of the C-terminal region of C5a, and function as antagonists of C5aR1. PMX205 has a more lipophilic nature than PMX53, and thus more easily gains access to the brain than PMX53 (126, 127). Treatment with PMX205 led to less fibrillar amyloid accumulation in 2 mouse models of Alzheimer's disease, less activation of microglia and astrocytes, and reduction of cognitive loss (128) (Figure 2). In the 3xTg model that acquires tangles, PMX205 treatment resulted in a 70% decrease in hyperphosphorylated tau. PMX205 and PMX53 have been protective in other models of neurodegeneration (reviewed in (9)), and prevented neuronal death *in vitro* (129, 130). Such a therapy would be less immunocompromising than complete inhibition of C1q, C1 or C3, as it leaves opsonization and the ability to form the MAC on pathogens intact in the brain and periphery. Complement activation by fA β leading to C5a generation also produces C5b and in the presence of C6, C7, C8 and C9 would lead to the formation of MAC. Given that the plaque would not be damaged by MAC and that MAC would be susceptible to neutralization by clusterin or vitronectin complexes in solution (20, 131–133), only insertion into membranes of bystander cells could be detrimental. While in the described mouse models of AD C5aR1 receptor antagonist treatment was protective, suggesting a limited consequence of generated MAC, it of course remains to be seen if the protection would also be effective in humans, and at all stages of disease.

PMX53 and another orally available selective antagonist of C5aR1, Avacopan, have been tested in human clinical trials for safety. PMX53 was found to be safe in a human Phase 1 clinical trial for autoimmune diseases (reviewed in (115)). Thus, PMX53 and PMX205 could have an accelerated path to human clinical trials. It is important to note that PMX53 binds to human C5aR1 with much higher affinity than mouse C5aR1 (134), suggesting that beneficial results in murine models may underestimate that in humans. Avacopan (135) is a small molecule specific antagonist for C5aR1 that was effective in mouse models of anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis (136) and is currently in Phase 3 clinical trial for ANCA-associated vasculitis. Brain permeability has not yet been reported. As mentioned above, while a C5a receptor antagonist would block the proinflammatory effects of C5a (and perhaps enhance phagocytosis and clearance pathways), the complement activation fragment, C5b, would still be intact and able to participate in the MAC formation during a bacterial infection. Thus, this protective function of complement as well as opsonization, normal synaptic pruning and neuroprotective effects will not be compromised during systemic treatment with the antagonist. Finally, it should be noted that 16 years of clinical experience with Eculizumab (an anti C5 therapeutic antibody that blocks generation of C5a) have indicated a lack of toxicity/adverse effects resulting from the absence of C5a-C5aR1 signaling. In summary, the mouse and human data together predict that C5aR1 inhibition is safe and may suppress inflammation and enhance homeostatic processes in AD.

CR1, a multi-functional protein

CR1 is a host cell-associated regulatory protein, which binds C3b, and more weakly C4b and C1q (137). A major CR1 function is to promote the dissociation of C3b containing C3

convertases and to act as cofactor for Factor I cleavage of C3b to iC3b (which can no longer participate in C3 or C5 convertase activity), thereby, preventing the accumulation of C3b and formation of cell lytic C5b-9 on host cells (138). In humans, erythrocyte CR1 plays a major role in the clearance of C3b-opsonized immune complexes from the circulation via a mechanism called “immune adherence”. C3b linked to immune complexes via its thioester bond, binds to erythrocyte CR1 which then transports the immune complexes to the liver and spleen for ingestion, degradation and thus clearance (139). Polymorphisms in CR1 have been associated with AD risk although most SNPs are located in non-coding regions of the CR1 gene. Keenan and colleagues identified a SNP that is within the coding region of CR1 (18) resulting in an amino acid change (S1610T) within the protein domain that has been attributed to C1q binding (137). However, interestingly these SNPs often co segregate with another genetic polymorphism that is associated with decreased expression of CR1 on erythrocytes (140–142). As a result, it has been hypothesized that this lower density of CR1 on red cells, lowers peripheral clearance of A β , and thus may contribute to the phenotype of CR1 risk variants, although a contribution of CR1 as an extrinsic regulator of complement is not excluded. In a small study of 36 individuals, AD patients had significantly lower levels of C3b-opsonized A β bound to their erythrocytes than age-matched controls or mild cognitively impaired individuals, consistent with a defect in peripheral amyloid clearance mechanisms (143). In a series of reports, Rogers and colleagues provided further evidence of decreased erythrocyte A β levels in AD vs cognitively intact individuals, that A β and erythrocytes were colocalized with Kupffer cells in human liver (indicated of immune adherence mediated clearance), and that anti-A β in the presence of serum complement enhanced capture of A β by human erythrocytes *in vitro* and *in vivo* (140, 144, 145). Thus, facilitating A β immune complex association with CR1 (146) may be a part of a therapeutic strategy to enhance peripheral clearance of amyloid, if this leads to lower brain amyloid and cognitive improvement. While in humans CR1 and CR2 are coded for by distinct genes, mice express CR1 and CR2 proteins that result from differential splicing of a single Cr2 gene. In addition, CR1 in the mouse is not expressed on erythrocytes as in humans, making mouse models of the role of red cell CR1 in AD more challenging. However, a transgenic mouse expressing CR1 in mouse erythrocytes has been reported (147) and a new humanized CR1 knockin mouse has been generated by the MODEL-AD consortium. Both mice are available through Jackson Labs, and may enable preclinical testing of this novel therapeutic approach.

Conclusions

Prevalence of AD in the United States is projected to reach 13 million by 2050 creating a great financial as well as emotional burden. With no cure or disease-modifying therapeutics available, there is currently a significant unmet medical need to develop therapies for AD. The relatively recent awareness of the role of the early complement components in synapse pruning in multiple neurological diseases adds another level of complexity and/or opportunity for selective modulation of this process. In addition, colocalization of complement components with both plaques and tangles, and the detection of C5b-9 in brain provide clear evidence of activation of the entire complement cascade by a variety of activators within the AD brain. In animal models, direct and specific inhibition of the

function of C5aR1 had beneficial effects on both pathology and cognitive behavior in several murine models of AD. This strategy would leave the beneficial functions of other complement components such as C1q and C3b, and C5b-9 intact. How suppressing C5aR1 would affect synaptic pruning is yet unknown. Quantitative studies on the balance between activators and inhibitors of complement in injured brain and the influence of the complement (148) on progression of AD could be useful in designing personalized therapies in the future. While much remains to be clarified, targeting specific effector pathways of complement is justified now as a potential therapeutic strategy for this debilitating neurodegenerative disease.

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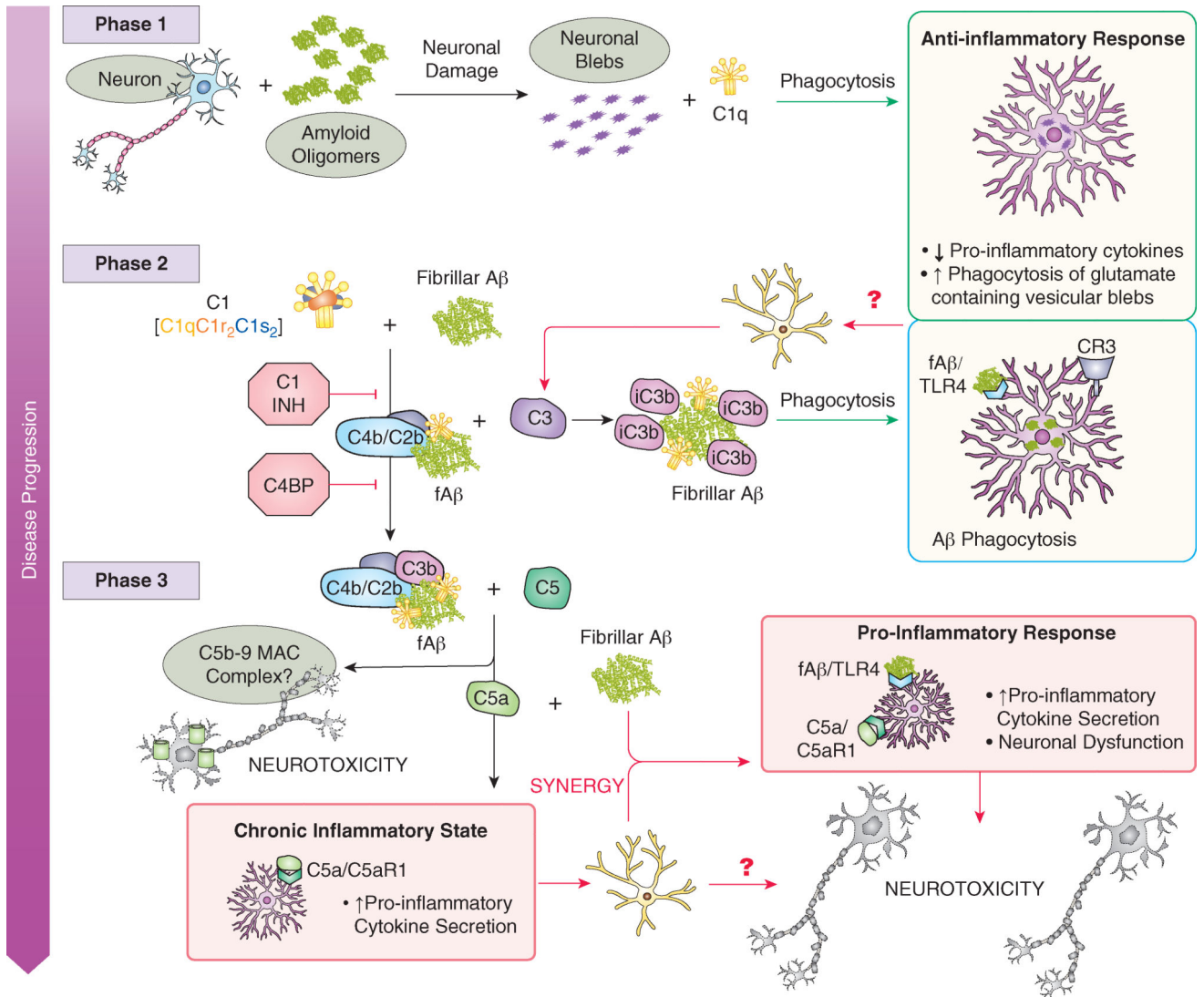


Figure 1: Complement-mediated functions change with disease progression.

Early disease (Phase 1): C1q expression is upregulated by “injury” independently of other complement cascade proteins. C1q binds apoptotic neurons and neuronal blebs, thereby enhancing phagocytosis by microglia (pink) while also suppressing inflammatory response in the absence of other PAMPs/DAMPs. Phase 2: The complement cascade is chronically activated as fA β and other activators accumulate. C1r, C1s, C4, and C3 synthesis is induced in response to more local damage, while the complement regulators C1-INH and C4BP are not comparatively upregulated. Generated C3b/iC3b covalently links to fA β and may lead to phagocytosis. However, in Phase 3, C5a is also generated, diffuses from the plaque, engages C5aR1 on microglia and induces chemotactic activity recruiting microglia to the plaques. fA β binds to microglial TLR receptors (or others) inducing a synergistic response including pro-inflammatory cytokine secretion and reactive oxygen species production. Since large fA β plaques are not efficiently cleared, a chronic inflammatory environment develops contributing to greater neuronal damage conducive to more fA β production and ultimately

neuronal dysfunction and death. The contribution of all these events to AD onset and progression in humans remains to be determined.

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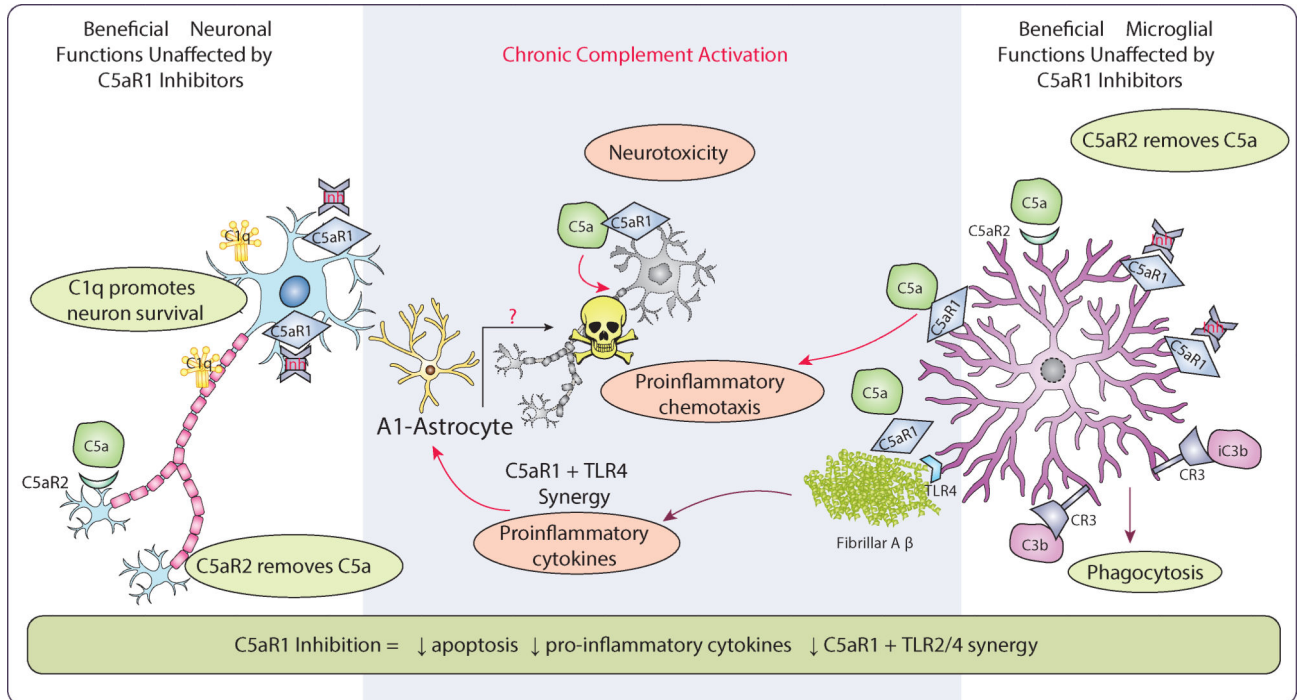


Figure 2: Beneficial complement-mediated functions in the brain are retained in the presence of C5aR1 antagonists.

A specific C5aR1 antagonist (inhibitor, INH) will prevent induction of detrimental pro-inflammatory responses to chronic fibrillar A β via blocking C5a-induced microglia responses (and possibly induction of A1 astrocytes), as well as block C5a-induced neuronal apoptosis. The antagonist will not affect the direct interaction of C1q with neurons that promotes survival and resistance to fA β induced death (left), nor the enhancement of microglial uptake of apoptotic cells and neuronal blebs with suppression of inflammatory cytokine release (right). CR3 will remain functional mediating phagocytosis by engaging iC3b and C3b bound to A β , apoptotic cells or other debris (right). C5aR2 would be unaffected and can act as a scavenger of C5a to contribute to the suppression of pro-inflammatory responses (left and right). Not pictured: Formation of membranolytic C5b-9 on pathogens will not be affected.