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Review

Using animal models to determine the significance of complement activation in Alzheimer's disease

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Abstract

Complement inflammation is a major inflammatory mechanism whose function is to promote the removal of microorganisms and the processing of immune complexes. Numerous studies have provided evidence for an increase in this process in areas of pathology in the Alzheimer's disease (AD) brain. Because complement activation proteins have been demonstrated *in vitro* to exert both neuroprotective and neurotoxic effects, the significance of this process in the development and progression of AD is unclear. Studies in animal models of AD, in which brain complement activation can be experimentally altered, should be of value for clarifying this issue. However, surprisingly little is known about complement activation in the transgenic animal models that are popular for studying this disorder. An optimal animal model for studying the significance of complement activation on Alzheimer's – related neuropathology should have complete complement activation associated with senile plaques, neurofibrillary tangles (if present), and dystrophic neurites. Other desirable features include both classical and alternative pathway activation, increased neuronal synthesis of native complement proteins, and evidence for an increase in complement activation prior to the development of extensive pathology. In order to determine the suitability of different animal models for studying the role of complement activation in AD, the extent of complement activation and its association with neuropathology in these models must be understood.

Background

Alzheimer's disease and complement activation

A variety of inflammatory processes are increased in regions of pathology in the Alzheimer's disease (AD) brain [1-4]. There is a reciprocal relationship between this local inflammation and senile plaques (SPs) and neurofibrillary tangles (NFTs); both SPs and NFTs, as well as damaged neurons and neurites, stimulate inflammatory responses [5], and inflammatory processes exert multiple effects, some of which promote neuropathology [6-8]. Numerous retrospective studies have shown that long-term administration of nonsteroidal anti-inflammatory drugs (NSAIDs) to individuals with arthritis significantly reduces the risk for these individuals for developing AD [9]. These findings, together with the demonstration of elevated glial cell activation [10-12], complement activation [13-15], and increased acute phase reactant production [16-19] at sites of pathology in the AD brain, support the hypothesis that local inflammation may contribute to the development of this disorder [20]. Although a short-

Table 1: Biological activities of complement activation proteins, with relevance to AD.

Name	Biological activity
C1q	Enhances Aβ aggregation [43,44]; may facilitate Aβ clearance [56]; enhances Aβ-induced cytokine secretion by microglia [49]
C3a	Anaphylatoxin (increases capillary permeability) [155] ; protects neurons vs. excitotoxicity [52]
C3b	Immune adherence and opsonization [89] (may facilitate Aβ clearance by phagocytic microglia)
C4a	Anaphylatoxin (weak) [156]
C5a	Anaphylatoxin; protects neurons vs. excitotoxicity [51]; chemotaxic attraction of microglia [46,47]; inhibits apoptosis 54; increases cytokine release from Aβ-primed monocytes [48]
C5b-9	Neurotoxicity [50]; sublytic concentrations may have both pro- and anti- inflammatory activities [157]

term trial of AD patients with the NSAID indomethacin suggested protection from cognitive decline [21], subsequent trials with other anti-inflammatory drugs have found no evidence for slowing of the dementing process [22-25]. These findings underscore the current perception of CNS inflammation as a "double edged sword" [26,27], with neuroprotective roles for some inflammatory components and neurotoxic effects for others [28-30].

The significance of complement activation, a major inflammatory mechanism, in AD is particularly problematic. The complement system is composed of more than 30 plasma and membrane-associated proteins which function as an inflammatory cascade. Complement activation promotes the removal of microorganisms and the processing of immune complexes. The liver is the main source of these proteins in peripheral blood, but they are also synthesized in other organs including the brain [31]. Protein fragments generated during activation of the system enzymatically cleave the next protein in the sequence, generating a variety of "activation proteins" with diverse activities (Table 1). Three complement pathways, the classical, alternative, and lectin-mediated cascades, have been identified (Fig. 1). Full activation results in the generation of C5b-9, the "membrane attack complex" (MAC), which penetrates the surface membrane of susceptible cells on which it is deposited and may result in cell death if present in sufficient concentration. The presence of early complement activation proteins [32-37] and of the MAC [38-42] has been demonstrated by immunocytochemical staining in the AD brain. Subsequent studies found that complement activation increases Aβ aggregation [43,44] and potentiates its neurotoxicity [45], attracts microglia [46,47], promotes microglial and macrophage secretion of inflammatory cytokines [48,49], and induces neuronal injury, and sometimes neuronal death, via the MAC [50]. These findings suggested that complement activation might contribute to the neurodegenerative process in AD. However, recent studies have also revealed neuroprotective functions for some complement activation proteins, including *in vitro* protection against excitotoxicity [51,52] and Aβ-induced neurotoxicity [53], as well as anti-apoptotic effects [54,55]. Further, C1q, the first complement protein to be deposited on cell membranes during activation of the classical complement sequence, may facilitate the clearance of Aβ by microglia [56], although this is controversial [57]. Understanding the role of complement activation in AD is of clinical relevance because some complement-inhibiting drugs are available, and others are being developed (see reviews by Sahu and Lambris [58], and Morgan and Harris [59]). Conditions for which these agents are currently being investigated include stroke [60], organ transplantation [61], glomerulonephritis [62], ischemic cardiomyopathy [63], and hereditary angioedema [64]. Modulation of CNS complement activation in experimental animal models of AD, both by treatment with complement-inhibiting drugs and by generation of AD-type pathology in complement-deficient animals, should be useful for obtaining a greater understanding of the role of this process in the development of AD-type pathology. Unfortunately, knowledge of the extent of complement activation in animal models is lacking. This paper will review (a) criteria for an optimal animal model to study this issue, (b) present knowledge about complement activation in animal models of AD, and (c) additional animal models which offer alternatives for addressing this question.

Criteria for an optimal animal model for studying AD-related complement activation

While animal models of human disease generally have similar pathological findings to the human disorders, distinct differences remain. These models may be appropriate for studying some aspects of a disease process, while less suitable for others. To determine the significance of complement activation in the development of AD-type pathology, for example, some animal models may be of value primarily for investigating the relationship between early complement activation and SP and NFT formation, whereas others may be more relevant for studying the role of the MAC in neuronal loss.

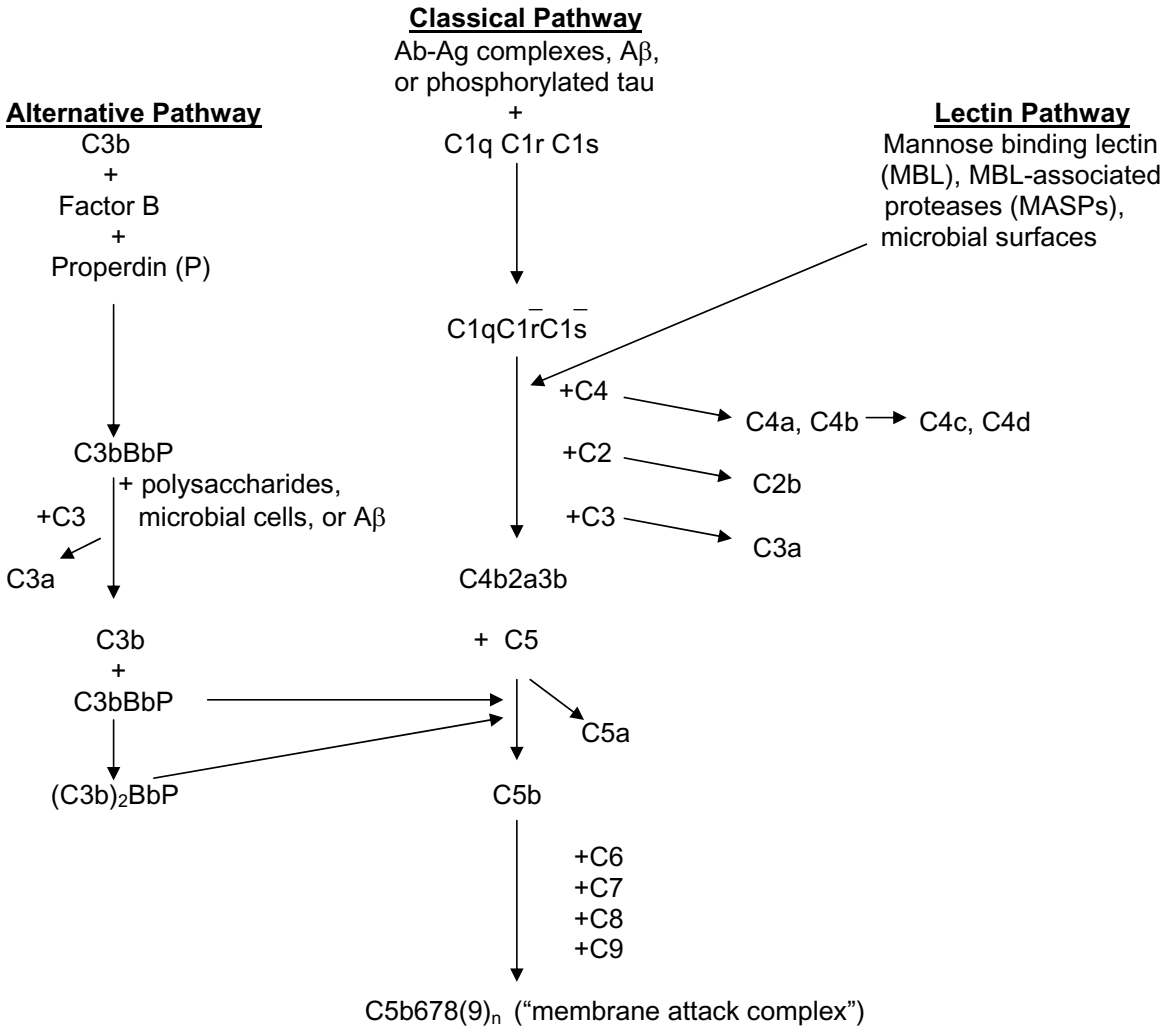

Figure 1
Schematic diagram of classical, alternative, and lectin complement activation pathways. There is evidence for activation of the classical and alternative pathways in the AD brain. (Adapted from Sahu and Lambris, 2000 [58]).

1. Complete activation of complement

Investigators at the Academic Hospital Free University in Amsterdam first reported the presence of early activation proteins in the classical complement cascade in the AD brain [32-34,36,37]. The MAC was not detected. However, further studies by other laboratories convincingly demonstrated the MAC, by a variety of techniques, in AD specimens [38-42]. The Dutch group has more recently reported detection of the MAC in brain specimens from subjects with dementia with Lewy bodies who met CERAD neuropathological criteria for AD [65]. The MAC has similarly been reported in SPs from subjects with Down's syndrome [66] and with familial British dementia [67], disorders in which typical AD-type neuropathology is present. An optimal animal model for studying AD-related complement activation should therefore have complete complement activation.

2. Association of complement activation proteins with neuropathology

Complement proteins are detectable on or closely associated with SPs, NFTs, and dystrophic neurites in the AD brain. These findings are in agreement with *in vitro* studies indicating that Aβ and tau protein, the major components in SPs and NFTs, can fully activate human complement [42,68-71]. Although the above studies suggested that complement is activated principally by the aggregated forms of Aβ and tau, soluble, non-fibrillar Aβ may also be capable of activating complement [72]. In contrast to the robust staining of complement proteins in mature plaques, immunoreactivity to these proteins in diffuse plaques has generally been below the level of detection, though it has been reported in some studies [36,73,74]. Complement activation in the AD brain is increased primarily in regions containing extensive pathology (e.g., the hippocampus and cortex), and whether early complement components are also present in the diffuse plaques that develop in the AD cerebellum is controversial [74,75]. The above findings suggest that complement activation in an optimal animal model of AD should be associated with SPs and, in those models in which neurofibrillary pathology occurs, with NFTs.

3. Initiation of complement activation early in development of pathology

How the increased complement activation in AD relates to the development of SPs and NFTs, and to neuronal loss, is unclear. Immunocytochemical staining for complement activation proteins in the aged normal human brain is generally faint, and may be below the level of detection [42,69,73]; of relevance is a recent report describing extensive neuron-associated C1q reactivity in a cognitively normal subject with neuropathological findings limited to diffuse cortical plaques [76]. Elderly "high pathology controls," lacking dementia but with increased numbers of entorhinal NFTs and neocortical Aβ deposits, have a slight increase in the percentage of C5b-9-immunoreactive plaques in comparison with aged normal subjects, though this percentage is far lower than in the AD brain [39]. A recent study in our laboratory [77] used enzyme-linked immunosorbent assay (ELISA) to measure the concentrations of two early complement activation proteins, C4d and iC3b, in brain specimens from AD and normal subjects. ELISA is more sensitive than immunocytochemical staining, though it provides no information regarding the cellular association of complement immunoreactivity. Increased concentrations of these early complement activation proteins were present in some aged normal specimens. These reports suggest that early complement activation may increase prior to the development of plaques and NFTs. Similar findings are desirable in an optimal animal model for studying AD-related complement activation.

4. Increased CNS production of native complement proteins

Both mRNA expression and protein synthesis of native complement proteins are increased in the AD brain [78-80]. (Note: the distinction between detection of native complement proteins, vs. detection of complement activation proteins, has frequently been blurred. In some studies in which immunoreactivity to complement activation proteins (C3c, C4c, C4d) has been reported, the antisera used were also capable of detecting the respective native complement proteins (C3 or C4) [40,80]. Only when antisera are used whose immunoreactivity is limited to activation-specific neo-epitopes can complement activation be confirmed. The paucity of antisera which can detect complement activation proteins in experimental animal models is a significant obstacle to determining the extent of complement activation in these models.) In addition to neurons, complement proteins are synthesized by other cells in the CNS including microglia, astrocytes, oligodendrocytes, and endothelial cells [31]. The biological effects of these activation proteins are mediated by numerous regulatory proteins including CD59, clusterin, vitronectin, C1-inhibitor, C4-binding protein, decay-activating factor, and Factor H, which inhibit different steps in the complement cascade. All of these regulatory proteins are produced in the human brain, but less is known about their CNS synthesis in other species [31]. The status of some of these regulatory proteins in AD is unclear; for example, there are conflicting reports regarding the up-regulation of C1-inhibitor [81,82] and CD59 [41,82,83]. Thus, while an optimal animal model for studying AD-related complement activation should have up-regulated CNS synthesis of complement proteins, the alterations that should be present in complement regulatory proteins are less clear.

5. Alternative as well as classical complement activation

Complement activation in the AD brain was initially thought to be limited to the classical pathway, but recent reports have also indicated increased concentrations of the alternative activation factors Bb and Ba, and Factor H, a regulatory factor for the alternative pathway, in the AD brain [84,85]. Alternative complement activation has also been reported in other familial dementias with pathologies similar to AD [67]. Therefore, while activation of the classical pathway is an absolute requirement for an optimal animal model of AD-related complement activation, an increase in the alternative pathway is also desirable.

Complement activation in animal models of AD: present knowledge

The examination of complement activation in experimental models of AD has been limited to mice and rats. The extent of complement activation and its relationship to the development of AD-type neuropathology have generally not been determined in these studies.

APP/sCrry mouse

Increased complement activation was induced by overproduction of transforming growth factor beta1 (TGF-β1) in transgenic mice expressing mutations in the human amyloid precursor protein (hAPP) gene. The APP mutations expressed in these mice have been associated with early-onset, familial AD [86]. The TGF-β1 overproduction resulted in a 50% reduction in Aβ accumulation in the hippocampus and cerebral cortex [87]. Because the production of soluble Aβ was unchanged, these results suggested that reduction in Aβ may have been due to its increased clearance by microglia. A subsequent study by the same investigators [88] found that the mRNA level of C3 in the cerebral cortex was 5-fold higher in APP/TGF-β1 mice than in APP mice at 2 months of age (prior to deposition of Aβ) and 2-fold higher at 12–15 months, when senile plaques are present. Thus, in this model, increased CNS synthesis of C3 precedes senile plaque formation. Because C3b, an activation protein produced by cleavage of C3, functions as an opsonin [89], the increased C3 levels together with the reduced Aβ deposition in the APP/TGF-β1 mice suggested a neuroprotective role for complement in this model. To investigate this possibility, the APP mice were crossed with mice expressing soluble complement receptor-related protein y (sCrry), a rodent-specific inhibitor of early complement activation [90]. APP/sCrry mice had a 2- to 3- fold increase in Aβ deposition in the neocortex and hippocampus at 10–12 months of age, together with a 50% loss of pyramidal neurons in hippocampal region CA3. The authors concluded that complement activation may protect against Aβ-induced toxicity, and may reduce the accumulation or promote the clearance of amyloid and degenerating neurons [88]. Neuroprotective functions (protection against excitotoxicity) have been demonstrated *in vitro* for C3a [52], and the increased neuronal loss in the APP/sCrry mouse may be due to decreased production of C3a as well as the opsonin, C3b. However, whether inhibition of complement activation in the AD brain would similarly result in increased neuropathology is unclear, because complement activation in AD is likely to be more extensive than in the APP mouse. Although no peer-reviewed articles have appeared in which the extent of complement activation in the APP mouse has been examined, two abstracts have dealt with this issue. Yu et al. [91] reported C3, C5, and C6 immunoreactivity to thioflavin-S-reactive plaques, whereas McGeer et al. [92] found only weak complement staining of plaques and slight upregulation of complement proteins. Significantly, neither study reported detection of the MAC. At least two factors, in addition to the lack of NFTs, mitigate against complement activation in the APP mouse being equivalent to that in AD: (a) the mouse complement system is functionally deficient, as mouse C4 lacks C5 convertase activity [93] and many mouse strains have low complement levels relative to other mammals [94], and (b) mouse C1q binds less efficiently to human Aβ than does human C1q, resulting in less activation of mouse complement than of human complement in the presence of human Aβ [95].

PS/APP mouse

In addition to APP, mutations in the gene encoding for presenilin-1 (PS-1) have also been associated with familial AD [96]. The PS/APP mouse carries both of these transgenes and has been extensively used as a model for studying processes relating to the formation of SPs. Aβ deposition occurs more rapidly in these mice than in the single transgenic APP mouse [97]. In neither model does NFT formation occur. Aβ deposition in PS/APP mice is initially detected at 3 months of age, and increases with age; total Aβ burden peaks at one year of age, although the percentage of Aβ that is fibrillar (thioflavin-S reactive) increases up to 2 years of age. Matsuoka et al. [98] described the CNS inflammatory response to Aβ in these animals. Activated astrocytes and microglia increased in parallel with total Aβ and were closely associated with both diffuse and fibrillar plaques. C1q immunoreactivity was detected at both 7 and 12 months of age, co-localizing with activated microglia and fibrillar Aβ. These findings were similar to those in the AD brain in that complement activation was associated with SP formation. The extent of complement activation was not addressed in this study.

APP (Tg2576)/C1q-deficient mouse

Fonseca et al. [99] investigated the role of C1q in AD by crossing Tg2576 (APP) mice [100] and APP/PS1 mice with C1q knockout mice [101]. C1q immunoreactivity was associated with plaque formation in the APP Tg2576 animals, as previously reported by Matsuoka et al. [98]. In both the Tg2576/C1q$^-$ and APP/PS1/C1q$^-$ animals, lack of C1q did not alter either plaque density or the time course of plaque deposition. Neuronal cell numbers (NeuN$^+$ cells), assessed only in the Tg2576 (APP) mouse, were not changed by the absence of C1q; however, immunoreactivity to MAP-2 (a marker for neuronal dendrites and cell bodies) and synaptophysin (a marker for presynaptic terminals) in the hippocampus (region CA3) was increased 2-fold in the APP/C1q$^-$ animals, compared with APP mice. Microglial and astrocytic activation was significantly reduced in the APP/C1q$^-$ animals. These results were interpreted to suggest that in these animal models of AD, (1) early complement activation (as indicated by C1q deposition) in response to fibrillar Aβ deposition might be responsible for the chemotactic attraction of activated glial cells, and (2) the activated microglia, while unable to clear fibrillar Aβ, may have contributed to the loss of neuronal integrity indicated by reduced MAP-2 and synaptophysin staining in the APP mice. By recruiting activated microglia, complement activation could potentially con-

tribute to neuronal injury even if full activation (MAC formation) does not occur.

Postischemic hyperthermic rat model

Coimbra and colleagues [102] described progressive neuronal loss in the hippocampus and cerebral cortex in rats subjected to common carotid artery occlusion to produce transient forebrain ischemia, as an animal model for stroke. The post-surgical hyperthermia which occurs spontaneously in these animals was suggested to promote the infiltration of microglia, whose secretory products increased the subsequent neuronal loss. A later study by the same group [103] found that subjecting the rats to post-surgical hyperthermia (38.5 – 40°C) increased microglial and astrocytic infiltration and accompanying neuronal loss, and resulted in the formation of AD-type pathology. Aβ-reactive diffuse plaques were detected in the cerebral cortex at 2 months post-surgery, with more compact plaques in the hippocampus and cortex by 6 months. Increased ubiquitin and phosphorylated tau immunoreactivity was observed at both time points, together with staining for C5b-9 in the somatosensory cortex. The MAC immunoreactivity co-localized with acid fuchsin staining, a marker for neuronal death [104]. Other complement proteins were not evaluated in these studies. This is apparently the only animal model of AD in which full complement activation has been reported. It is noteworthy that while both SPs and neurofibrillary pathology were present in these animals, the MAC apparently did not co-localize with these structures, unlike in AD.

Acute lesioning

Alterations in native complement mRNA and protein levels have been evaluated in the rat hippocampus following experimental induction of acute neuronal injury. These surgical and pharmacological procedures result in neuronal loss in the entorhinal cortex, and deafferentation of hippocampal neurons, similar to that which occurs in AD [105]. Selective damage to the rat hippocampus has been induced by surgical transection of the perforant pathway, which runs between the entorhinal cortex and the molecular layer of the dentate gyrus [106,107], systemic administration of the excitotoxin kainic acid [108,109], or injection of the neurotoxin colchicine into the dorsal hippocampus [109]. Surgical transection of the perforant pathway increased C1qB mRNA in the entorhinal cortex and hippocampus [106] and C9 immunoreactivity in the hippocampus [107]. Injection of kainic acid similarly increased C1qB and C4 mRNA expression and C1q immunoreactivity in the hippocampus [108,109]. Colchicine infusion into the dorsal hippocampus, which selectively damages granule cells of the dentate gyrus, produced elevated mRNA expression of hippocampal C1qB and C4 [109]. Though the acute neuronal damage in these studies differs from the chronic, progressive neurodegenerative process that occurs in AD, these results demonstrated that the neuronal response to injury includes upregulation of native complement protein synthesis. The significance of this upregulation, i.e. whether it promotes neuroprotection or neurotoxicity, was not addressed.

Infusion of Aβ and C1q into rats

Frautschy et al. [56] examined the effects of infusion of human C1q and oral administration of rosmarinic acid on glial cell proliferation (microgliosis and astrocytosis), plaque load, and memory (Morris water maze) in Aβ-infused rats. Rosmarinic acid inhibits both the classical and the alternative complement cascades, by covalent binding to newly formed C3b [110]; it also possesses anti-inflammatory [111,112], anti-oxidative [113], and anti-amyloidogenic properties [114]. Gliosis was greater with C1q and Aβ infusion than with Aβ alone. Plaque density was decreased by C1q infusion (note: this result differs from the *in vitro* study of Webster et al. [57], in which C1q was found to inhibit microglial phagocytosis of Aβ, and also from the recent study of Fonseca et al. [99] in which C1q deficiency had no effect on plaque density in APP mice), but, curiously, performance in the water maze worsened. Treatment with rosmarinic acid had the opposite effect; though plaque load increased, memory was improved. These findings were interpreted as suggesting that C1q and/or complement activation may, by promoting microglial activation, worsen memory independent of the clearance of Aβ.

Additional animal models for studying AD-related complement activation

TAPP and 3xTg-AD mice

Mutations in the gene encoding for human tau protein have been linked to the development of frontotemporal dementia with parkinsonism [115]. By combining this mutation with the human APP and PS1 mutations associated with familial AD, animal models of AD have been produced in which NFTs as well as SPs are formed. Lewis et al. [116] crossed human APP_{swe} mice (Tg2576) with mice expressing the transgene for a human tau mutation (JNPL3 mice) to generate a double mutant tau/APP mouse (the "TAPP mouse"). These mice develop SPs similar to APP mice (high numbers of plaques are present in older [8.5–15 months of age] mice, in the olfactory cortex, cingulate gyrus, amygdala, entorhinal cortex, and hippocampus), and older TAPP mice have NFTs, in association with increased astrocyte proliferation, in limbic areas. The plaques contain both $Aβ_{40}$ and $Aβ_{42}$. Oddo et al. [117] injected the human transgenes for APP and mutated tau into embryos of PS1 "knock-in" mice, generating the "3xTg-AD" mouse which develops both SPs and NFTs in an age-related, region-specific manner. Aβ deposition in these animals precedes NFT formation, with

extracellular Aβ (primarily $A\beta_{42}$) detected in the frontal cortex by 6 months of age, and in other cortical regions and hippocampus by 12 months. Many of the extracellular Aβ deposits are thioflavin-S-positive and are associated with reactive astrocytes. Phosphorylated tau initially appears in the hippocampus and subsequently in cortical regions; it is detected within neurons by 12–15 months and within dystrophic neurites at 18 months. Though Aβ immunoreactivity precedes that of tau, these proteins colocalize to the same neurons. The presence of NFTs as well as SPs suggests that the 3xTg-AD and TAPP models may be more relevant than APP or APP/PS-1 mice for studying the significance of complement activation in the development of AD-type pathology. Potential drawbacks for using these models for complement-related studies include, as discussed earlier, functional deficiencies in activation of mouse complement [93], decreased complement levels in common laboratory mouse strains [94], and the decreased efficiency of binding of mouse C1q by the human Aβ within the SPs in these animals [95]. It is not known whether a similar decrease in the efficiency of activation of mouse complement occurs when mouse C1q binds to human, rather than murine, tau protein.

AD11 (anti-NGF) mouse

Ruberti et al. [118] developed a mouse transgenic model, the AD11 mouse, in which neutralizing antibody to nerve growth factor (NGF) is secreted by neurons and glial cells. NGF exerts trophic effects on basal forebrain cholinergic neurons and is widely distributed in these neurons [119]; the local secretion of anti-NGF antibody in these mice results in marked loss of basal forebrain cholinergic neurons. Aβ-containing plaques, tau hyperphosphorylation, and NFTs are present at 15–18 months of age. CNS production of anti-NGF antibody increases with age in these animals, therefore pathology develops only in adult mice. Extracellular deposition of APP is widespread in the brain, including the cortex and hippocampus. Phosphorylated tau immunoreactivity is present in neurons and glia in the cortex and hippocampus, and intracellular NFTs, extracellular neurofibrillary deposits, neuropil threads, and dystrophic neurites are observed in the cortex. Behavioral abnormalities, including impaired object recognition and spatial learning, are associated with this neuropathology [120]. The Aβ-containing plaques in the AD11 mouse are of murine, rather than human, origin, allowing the problem of the poor efficiency of activation of mouse complement by human Aβ [95] to be overcome. However, it is unclear whether plaques in these animals contain Aβ in the β-pleated sheet conformation, which is thought to be the most effective conformation for activating complement [71]. The distribution of SPs and NFTs in this model is less similar to AD than for 3xTg-AD and TAPP mice, because in addition to the cortex and hippocampus, large numbers of APP-reactive structures are present in the neostriatum (where, in AD, plaques are primarily diffuse [121]), and in other areas of the brain. Despite these concerns, the AD11 mouse is attractive as a potential model for studying the significance of AD-related complement activation.

Chlamydia pneumoniae*-infected mouse*

C. pneumoniae is an intracellular, gram-negative or gram-variable bacterium long identified as a respiratory pathogen. It has more recently been demonstrated to be a causative agent in reactive arthritis [122] and to be associated with autoimmune disorders including multiple sclerosis [123] and atherosclerosis [124]. Some laboratories have also reported an association of this agent with AD [125-127], although this has not been confirmed by others [128-131]. A recent study by Little et al. [132] examined the hypothesis that experimental *C. pneumoniae* infection in BALB/c mice could produce AD-like pathology. Intranasal inoculation with *C. pneumoniae* resulted in deposition of $A\beta_{1-42}$ in the hippocampus, amygdala, entorhinal cortex, perirhinal cortex, and thalamus by 3 months post-inoculation. The majority of these Aβ deposits appeared similar to diffuse plaques, though a small number of them were thioflavin-S-reactive. NFTs were not detected. The authors suggested that soluble factors such as lipopolysaccharides, which are present in the cell wall of all Chlamydiae [133], may have been responsible for the altered amyloid processing which resulted in Aβ deposition. Because the Aβ within the SPs in these animals is of endogenous origin, and because other chlamydial species have been shown to activate complement [134,135], the *C. pneumoniae*-infected mouse may offer a novel infectious model for studying the relationship of complement activation to the development of Aβ-containing plaques.

Aged dogs

Old dogs, in particular the beagle, have been extensively investigated as a model for CNS Aβ deposition and associated age-related cognitive dysfunction. Aβ deposits are detectable in the brains of most older dogs [136]. The regional distribution of Aβ in the dog brain resembles that in humans, found initially in the prefrontal cortex, subsequently in entorhinal and parietal cortices, and lastly in occipital cortex [137]. $A\beta_{42}$ is the predominant type of Aβ deposited in plaques [138]. Canine plaques are nonfibrillar and do not contain neuritic elements; thus, they resemble diffuse Aβ deposits in the human brain, but not the mature plaques predominating in AD. The neuropathological findings in old dogs also differ from AD in that activated glial cells are rarely associated with Aβ deposits, and NFTs are not detected [136,139]. Age-related cognitive impairment, termed "canine cognitive dysfunction syndrome," occurs in some older dogs and correlates with Aβ deposition in the hippocampus and frontal cortex [140,141]. The endogenous nature of the deposited Aβ in

old dog brain, and similarities between canine and human Aβ in their patterns of regional deposition, suggest that this model may be useful for studying the relationship between complement activation and plaque formation.

Non-human primates

Age-related formation of SPs has been reported in a variety of non-human primates including the cynomolgus monkey [142], rhesus monkey [143], chimpanzee [144], and marmoset [145]. Aβ within these plaques is predominantly $A\beta_{40}$ [146]. NFTs apparently do not form in the brains of most aged primates, with a few exceptions. The brain of the aged baboon contains phosphorylated tau protein [147,148], and an age-related accumulation of tau also occurs in the neocortex of the mouse lemur [149-151]. In this latter species, Aβ deposition occurs in the cerebral cortex and amygdala but is not age-dependent [151]. The mouse lemur appears to be the most promising primate species to date for studying the significance of AD-related complement activation because of the presence of NFTs as well as plaques.

Other animal species

Scattered reports of AD-type pathology in other species have also appeared. Adding trace amounts of copper to the water supply of cholesterol-fed rabbits results in Aβ deposition within SP-like structures in the hippocampus and temporal cortex, with associated learning deficits [152]. The neuropathology in the aged cat is similar to that in the old dog in that Aβ is deposited only as diffuse, $A\beta_{42}$-containing plaques, and NFTs are not detected [138]. A report of AD-type pathology in an aged wolverine [153] described neuritic as well as diffuse plaques in the cortex and hippocampus, and intracellular NFTs containing phosphorylated tau protein in cortical and hippocampal neurons. Finally, the aged polar bear brain also contains both diffuse plaques and NFTs [154]. While the neuropathological findings in the aged wolverine and polar bear resemble AD more closely than in most species examined to date, their inaccessibility to laboratory researchers limits the usefulness of these species for studies of AD-related complement activation.

Conclusions

1. Complement activation has been extensively studied in the AD brain. There is convincing evidence for activation of both the classical and alternative pathways, resulting in full activation as indicated by the presence of the MAC. Both aggregated Aβ (in SPs) and phosphorylated tau (in NFTs) are likely to be responsible for this activation.

2. Because complement activation generates both both neuroprotective and neurotoxic effects, the significance of increased complement activation in the development and progression of AD is unclear.

3. An optimal animal model for studying the significance of complement activation in the development of AD-type pathology would have complete activation of this process, with co-localization of complement activation proteins with SPs and with NFTs (if present). Other desirable features include early complement activation prior to the development of extensive neuropathology, increased CNS production of native complement proteins, and both classical and alternative pathway activation.

4. Surprisingly little is known about the extent of complement activation in animal models of AD. The postischemic hyperthermic rat [103] is the only animal model of AD in which full complement activation has been reported. The few studies with APP-transgenic mice have yielded conflicting results, with one investigation suggesting a neuroprotective role for complement activation [88], while another found that early complement activation (as indicated by C1q deposition) was associated with a loss of neuronal integrity [99]. Transgenic mouse models may be problematic for studies of AD-related complement activation because of inherent deficiencies in mouse complement activation and inefficient activation of mouse complement by the human Aβ present in the SPs in these animals. Other animal models in which SPs (and NFTs, if present) are of endogenous, rather than human, origin offer alternatives to transgenic mice for studying this issue.

5. The extent of complement activation and its association with neuropathology must be determined in animal models of AD to clarify the relevance of these models for investigating the significance of complement activation in the development of AD-type pathology.

Abbreviations used

Aβ, amyloid beta; AD, Alzheimer's disease; APP, amyloid precursor protein; CNS, central nervous system; MAC, membrane attack complex; mRNA, messenger ribonucleic acid; NFTs, neurofibrillary tangles; NGF, nerve growth factor; PS-1, presenilin-1; sCrry, soluble complement receptor-related protein y; SPs, senile plaque; TGF-β1, transforming growth factor beta1.

Competing interests

The author declares that he has no competing interests.

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