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·Review·

Apolipoprotein E, amyloid-beta, and neuroinflammation in Alzheimer's disease

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Alzheimer's disease (AD) is characterized by the accumulation and deposition of amyloid-beta (Aβ) peptides in the brain. Neuroinflammation occurs in the AD brain and plays a critical role in the neurodegenerative pathology. Particularly, Aβ evokes an inflammatory response that leads to synaptic dysfunction, neuronal death, and neurodegeneration. Apolipoprotein E (ApoE) proteins are involved in cholesterol transport, Aβ binding and clearance, and synaptic functions in the brain. The ApoE4 isoform is a key risk factor for AD, while the ApoE2 isoform has a neuroprotective effect. However, studies have reached different conclusions about the roles of the isoforms; some show that both ApoE3 and ApoE4 have anti-inflammatory effects, while others show that ApoE4 causes a predisposition to inflammation or promotes an inflammatory response following lipopolysaccharide treatment. These discrepancies may result from the differences in models, cell types, experimental conditions, and inflammatory stimuli used. Further, little was known about the role of ApoE isoforms in the Aβ-induced inflammatory response and in the neuroinflammation of AD. Our recent work showed that ApoE isoforms differentially regulate and modify the Aβ-induced inflammatory response in neural cells, with ApoE2 suppressing and ApoE4 promoting the response. In this article, we review the roles, mechanisms, and interrelations among Aβ, ApoE, and neuroinflammation in AD.

Keywords: ApoE; Alzheimer's disease; Aβ; neuroinflammation

Introduction

Alzheimer's disease (AD) is the leading cause of dementia in the elderly, and is a substantial burden on health-care systems worldwide. It is a neurodegenerative disease, characterized by progressive synaptic loss and neuronal death, and manifests over time as memory loss and cognitive decline. The severity increases with disease progression until the patient can no longer recognize family members or perform basic daily activities. In general, they eventually die from complications resulting from advanced debilitation. The majority of AD patients suffer from the sporadic late-onset form. Familial and early-onset forms exist as well, but their prevalence is much lower (<5%). AD was initially described in 1906 by Alois Alzheimer upon examining the brain of a 51-year-old woman who had died from early-onset dementia. His examination revealed two important features that still form the basis for pathological diagnosis: the build-up of intracellular neurofibrillary tangles (aggregates of hyperphosphorylated tau protein) and the formation of extracellular amyloid plaques (abnormal aggregates consisting principally of amyloid-beta (Aβ) peptides)[1].

Alzheimer's Disease and Amyloid-beta

Aβ is a short peptide generated from β-amyloid precursor protein (APP) through two-step cleavage. The first step is

mediated by β-secretase or beta-site APP cleaving enzyme 1 (BACE1), which produces a large soluble protein and a 99-amino-acid membrane-bound C-terminal stub (C99). The C99 fragment is further processed by a γ-secretase to produce Aβ in either its 40- or 42-amino-acid form[3] (Fig. 1). $Aβ_{1-40}$ is more abundant, while $Aβ_{1-42}$ is more closely associated with AD[2]. Although the mechanism that determines the length of Aβ produced is unknown, it is clear that an increase in the $Aβ_{1-42}/Aβ_{1-40}$ ratio is associated with AD, as well as neurotoxicity and memory deficits in animal models[4].

The primary APP processing pathway does not result in Aβ production. In this process, an α-secretase cleaves APP within the Aβ domain, producing a truncated Aβ variant (p3), which is not associated with AD, and a soluble protein that has been suggested to be neuroprotective[5] (Fig. 1). The factors driving APP processing down a particular pathway are unclear. Under physiological conditions, α-secretase activity is predominant. However, β-secretase is also active, providing evidence that Aβ production is not purely a function of disease-state. The physiological roles of APP and its products are not yet known, but they have been suggested to be involved in synaptogenesis[6], to mediate cellular responses to ischemic conditions[7], and to act as anti-microbial peptides[8]. Within the central nervous system (CNS), APP is primarily expressed in neurons, but is also produced and processed in astrocytes and microglia[9].

Beyond being a diagnostic hallmark, Aβ is also a critical component in the progression of AD. Its overproduction and aberrant clearance from the brain are essential for the development of neurodegeneration. Initial evidence for this came from the APP gene on chromosome 21. Individuals with Down's syndrome (trisomy 21) almost universally develop dementia, and familial and early-onset forms of AD are linked to mutations in the APP gene. Hence, it follows that aberrant processing of APP, which results in the production of Aβ, plays a critical role in the pathology of AD. Amyloid plaques are proposed as the primary causative factor in AD[10]. Further evidence shows that some mutations in APP processing pathways are sufficient to cause AD, and transgenic mice expressing mutant human APP genes show Aβ pathology and AD-like memory and behavioral deficits[11]. More recently, a particular APP mutation found in Icelanders has been shown to cause decreased APP processing and Aβ production, providing protection against AD and cognitive decline[12].

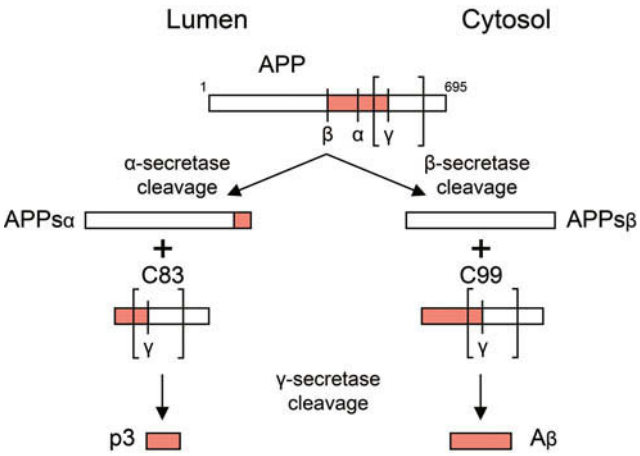

Fig. 1. Pathways of APP processing. APP has two primary endogenous processing pathways. The first is non-amyloidogenic: α-secretase cleavage produces the soluble APPsα fragment and membrane-bound C83. C83 is then cleaved by γ-secretase, producing a non-pathogenic p3 peptide. The alternate pathway is implicated in AD, with the first cleavage mediated by β-secretase BACE1, producing soluble APPsβ and C99. C99 is then cleaved by γ-secretase, producing the Aβ peptide. The mechanisms controlling the interaction of these pathways, and which is active at a given time or in a given tissue, are still unknown. The orange segment represents the area of APP corresponding to the Aβ peptide sequence. This segment can vary in length, but is most commonly 40 or 42 amino-acids long. The blue brackets represent the lipid membrane, with the majority of the APP peptide located on the luminal side.

Deposition of Aβ peptides was first identified in the form of plaques, but the environment in the AD brain is diverse, with a variety of assemblages of Aβ. It is not well understood how these combine and interact to contribute to AD pathology. One early criticism of the amyloid hypothesis was that plaque density does not correlate with disease progression, calling into question the causative role of Aβ in AD[13]. This was answered, to some degree, by the discovery of oligomeric species of Aβ, which self-associate from monomers and demonstrate significant neurotoxicity and a clear association with cognitive decline[14, 15]. Nevertheless, plaque aggregation still appears to play a significant role in AD development. Evidence suggests that the neurotoxic oligomers are strongly associated with the plaques and that the plaques themselves may be necessary

for the seeding and development of new plaques[16]. The time-frame of Aβ build-up relative to disease progression is also important. Aβ levels may increase in the brain decades before any cognitive deficit or plaque deposition is observed, which would make it difficult to accurately time anti-amyloid therapy[17]. A recent study reported that fibrillar Aβ peptides induce hyperexcitability in pyramidal cells in the APPsw/PS1dE9 double-transgenic mouse model of AD, leading to epilepsy[18]. We have also observed frequent seizures in APPsw/PS1dE9 mice[19]. The seizures, which result from Aβ-induced neuronal hyperexcitability, may be one of the factors responsible for the premature death of these mice[19]. Studies with 3×Tg-AD mice have shown that Aβ can induce tau pathology[20]; Aβ pathology occurs first, followed by tau pathology in AD[17]. Another study suggests that Aβ toxicity is tau-dependent[21]. As an axonal protein, tau also has a dendritic function that confers Aβ toxicity, as expression of truncated tau or tau deficiency has been shown to disrupt the postsynaptic targeting of Fyn (which uses the N-methyl-*D*-aspartate (NMDA) receptor as one of its substrates) and thus prevent memory deficits in an AD mouse model[21].

Amyloid-beta as an Inducer of Neuroinflammation and Neurodegeneration

One of the main pathological consequences of Aβ aggregation and plaque formation is the development of neuroinflammation. Aβ peptides are associated with the activation of microglia and astrocytes surrounding the amyloid plaques, and mediate the release of pro-inflammatory signals[22]. Many studies have implicated members of the toll-like receptor (TLR) family and CD14, membrane receptors responsible for activating the immune system, in mediating the inflammatory activation of astrocytes and microglia in response to Aβ. Groups have reported that blockade of TLR2 or TLR4 with specific antibodies decreases the degree of microglial activation upon challenge with Aβ peptides[23, 24]. TLR2- and TLR4-knockout microglia also show a decreased inflammatory response to Aβ[25, 26]. A recent study showed that expression of TLR2 in HEK293 cells that do not endogenously express TLR2 triggers an inflammatory response to Aβ[27]. Knockout of CD14, a co-receptor of TLRs 2 and 4, also increases inflammatory signaling, in keeping with its role as a repressor of TLR signaling[28].

Classically, astrocytes were primarily viewed as regulatory cells, delivering necessary nutrients to neurons, regulating the balance of ions, pH, and neurotransmitters, and maintaining homeostasis of the extracellular milieu of the brain. Astrocytes are now understood to play an active role in mediating the responses of the brain to acute injury. When left unchecked in chronic forms of injury, activated astrocytes ultimately have a detrimental effect and contribute to neurodegeneration. The mechanisms controlling the balance between the protective role of astrocytes and the long-term development of self-induced inflammatory damage are critical to understanding their role in neurodegenerative diseases.

Activated astrocytes and microglia produce a number of neurotoxic molecules, including reactive oxygen species[29, 30]. Aβ has been shown to induce neuronal death through the activation of astrocytes, with a potential mechanism involving the release of nitric oxide (NO)[31, 32]. Astrocytes also release a wide range of cytokines and chemokines, including interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and growth-related oncogene. These cytokines serve to further promote the activation of astroglia and caspases, which contribute to cell death[33]. Other evidence suggests that the cytokines can stimulate Aβ synthesis by modulating APP processing and aid the oligomeric association of Aβ by upregulating pro-oligomeric pathways[34]. This can lead to a self-amplifying cycle of inflammatory activation, with increased Aβ levels triggering further inflammation, cytokine release, and neuronal death.

The nature and extent of the link between neuro-inflammation and cognitive impairment in AD patients are not fully understood. However, multiple studies have shown an association of inflammatory markers with a decline in cognitive function, both in transgenic mouse models[35] and in patients[36], marking neuroinflammation as an attractive potential therapeutic target in AD patients.

Apolipoprotein E as a Risk Factor for Alzheimer's Disease

Apolipoprotein E (ApoE) is one of the major human apolipoproteins. It regulates lipid uptake into cells and intracellular cholesterol levels through interaction with the low-density lipoprotein (LDL) receptor[37]. ApoE is highly

expressed in both the brain and the liver, and is the major protein in the CNS mediating lipid transport and distribution. Peripherally, ApoE combines with other apolipoproteins, phospholipids, and cholesterol in very low density lipoprotein (VLDL) particles. In the CNS where VLDL is not present, ApoE forms high-density lipoprotein-like lipid particles[38]. The three-dimensional structure of ApoE consists of two separately folded domains, separated by a 'hinge' region: the C-terminal domain that is responsible for protein-lipid binding, and the N-terminal region that mediates binding to various ApoE receptors. Currently, there is still no accepted single model to describe the arrangement of protein and lipid in ApoE particles. Studies have suggested both the formation of a 'belt' of protein wrapped around a discoid lipid bilayer, and a spheroidal hydrophobic lipid core with surface ApoE proteins wrapped around polar lipid head groups [39].

ApoE-containing lipoproteins bind to a class of metabolic receptors known as LDL receptors (LDLRs), primarily LDLR itself and LDLR-related protein 1 (LRP1). LDLRs comprise a highly conserved family of transmembrane receptors that are responsible for the uptake and clearance of lipoproteins in plasma and cerebrospinal fluid, regulating lipid metabolism, and mediating a wide range of cell signaling pathways[40]. Some of these pathways have been shown to prevent neuronal death, including NMDA receptor signaling, which regulates intracellular calcium and cAMP response element-binding[41], and reelin signaling, which promotes synaptic plasticity and function, mainly through the actions of LRP1[42].

Lipoprotein particles containing ApoE secreted by astrocytes contain approximately equal amounts of ApoE and cholesterol, and make up virtually all of the cholesterol secreted by astrocytes[43, 44]. Poorly-lipidated ApoE particles have an altered conformation[45], decreased stability in the CNS[46], and altered interactions with Aβ[47]. For normal interactions with its receptors, ApoE must be appropriately lipidated; the lipidation state also affects its self-association: the lipid-free form exists primarily in a tetramer, while the lipid-bound forms are variably associated based on the amount and type of lipid[48].

ApoE lipidation is facilitated by a cholesterol-efflux protein on the cell membrane called ATP-binding cassette A1 (ABCA1). ABCA1 is necessary for the proper lipidation of ApoE: in knockout models, ABCA1 deficiency leads to a decrease in the overall levels of ApoE in the CNS[46, 49]. It is unclear whether ABCA1 activity plays any role in AD. Knockout of ABCA1 has no effect on Aβ levels in mouse models[50], and ABCA1 polymorphisms in human populations do not correlate with the prevalence of AD[51]. Nevertheless, one study demonstrated that over-expression of ABCA1 provides protection against amyloid deposition[52].

Differential Risks for Alzheimer's Disease among Apolipoprotein E Isoforms

ApoE has three common alleles, ε2, ε3, and ε4, among which the ε3 allele is the most prevalent, with a frequency of 77% in the general population[37]. The ε2 allele is the least frequent (8%), and is associated with some degree of neuroprotection from AD and a form of hyperlipoproteinemia[53, 54]. The ε4 allele is a strong risk factor for AD: compared to an ε3/ε3 individual, an ε3/ε4 heterozygote has a 2- to 3-fold greater risk of developing AD, and an ε4 homozygote has ~12-fold greater risk[55]. It follows that the ε4 allele is more common in AD populations, with a prevalence of 30–40% or greater in AD patients and ~15% in the general population[56, 57]. Despite its strong genetic association with AD, the ε4 allele is neither necessary nor sufficient for the development of AD[58]. Comparison of these human alleles with other species suggests that ApoE4 is an 'ancestral' form, similar to that found in mouse and rat models and in closer primate relatives, with ApoE3 developing in human populations relatively recently in evolutionary terms[59].

Structural and Functional Differences among Apolipoprotein E Isoforms

The three main human isoforms ApoE2–4 result from polymorphisms at two single nucleotides; ApoE3 features Cys112 and Arg158 residues, while ApoE2 has Cys112 and Cys158, and ApoE4 has Arg112 and Arg158. The exact consequent structural differences in these isoforms are not fully understood, especially those in the lipid-bound conformation of ApoE. One model derived from x-ray crystallography of lipid-free ApoE isoforms suggests that the Arg112 in ApoE4 causes a profound structural shift, with the side-chain of Arg112 interacting with Glu255[39]. As a result, a 'salt bridge' forms between the C-terminal and N-terminal domains. This domain interaction is believed

to play a critical role in the functional differences between ApoE4 and the other isoforms[60]. In one mitochondrial model of AD, relief of this domain interaction, through either mutation or treatment with a small molecule, restores normal function, suggesting that this interaction contributes to the deleterious effects of ApoE4[61]. Another recent model, based on nuclear magnetic resonance analysis of modified versions of human ApoE isoforms, offers a different view. It suggests that this domain interaction does not represent a critical difference between isoforms, but Arg112 in ApoE4 causes a shift in the region adjacent to the lipid-binding domain through interaction with a nearby histidine residue[62].

The functional change in ApoE2 is primarily due to conformational shifts at the LDLR-binding region, where loss of the arginine residue at position 158 reduces the positive charge at the binding site. This explains why ApoE2 has a significantly lower ability to interact with LDLR than the other isoforms, and this is believed to contribute to hyperlipidemia[63]. These differences among isoforms also result in altered cholesterol metabolism: one study showed higher uptake of ApoE2- and ApoE3-bound cholesterol than ApoE4-bound cholesterol by neurons and astrocytes[64]. Similar work has shown that ApoE4-expressing individuals suffering from cognitive decline have an altered lipid distribution within the brain, compared to ApoE3- or ApoE2-expressing individuals with similar cognitive levels[65].

Apolipoprotein E and Amyloid-beta

While the proposition that ApoE4 is a risk factor for AD is well established, the underlying mechanism remains an open question. Much work has focused on the interaction of ApoE with Aβ. Isoform-related differences have been characterized at several steps of Aβ processing and deposition in some model systems (Fig. 2). The conflicting findings on ApoE's function in Aβ deposition illustrates the uncertainty of its role in the pathogenesis of AD. *In vitro* studies have shown that human ApoE either promotes or inhibits Aβ aggregation and fibril formation depending on the conditions. Furthermore, different Aβ preparations have distinct patterns of aggregation and deposition, and they are likely to respond differentially to ApoE. Studies have shown that recombinant ApoE isoforms produced in *Escherichia coli* are capable of inhibiting fibrillar formation regardless of the isoform[66, 67], while others found an increase in Aβ deposition and an isoform-specific difference, with ApoE4 preferentially encouraging aggregation[68-70]. In addition, the lipidation state of ApoE may significantly impact Aβ fibril formation: all three ApoE isoforms exhibit decreased inhibition of Aβ fibrillation upon lipidation in a cell-free model[71].

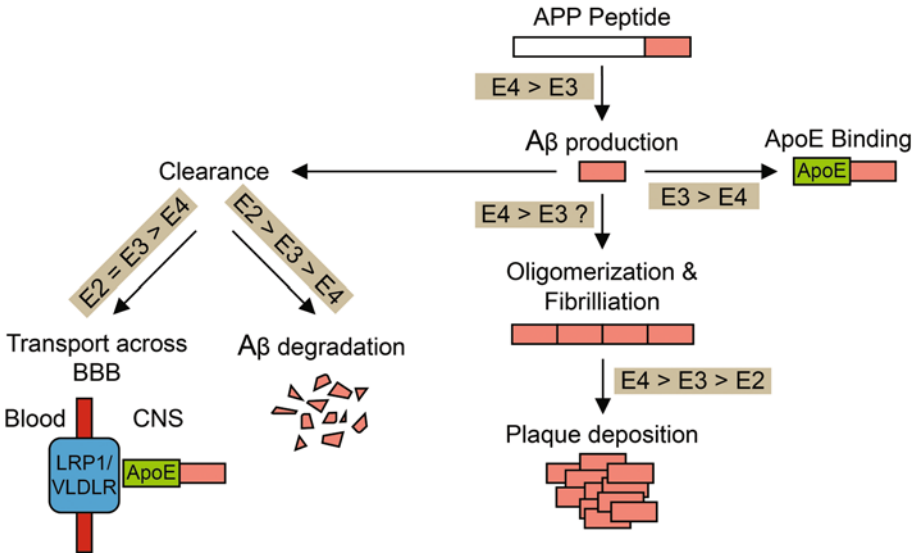

Fig. 2. Interaction of ApoE isoforms with Aβ. ApoE isoforms differentially affect each of these processes, the end result being deposition, degradation, or clearance of Aβ. The specific roles of the isoforms in these processes are believed to contribute to the peptide's overall role in AD.

Knockout of *Apoe* in mouse models over-expressing human APP showed that ApoE promotes Aβ accumulation and plaque deposition[72, 73]. Studies in both mice and AD patients have shown an isoform-specific effect: ApoE4 expression leads to higher levels of Aβ and more advanced and larger amyloid plaques than ApoE3, while ApoE2 expression leads to lower levels, corresponding to its protective role[74-77]. Moreover, an increased ApoE4 gene dosage contributes to higher levels of Aβ, with ε3/ε4 carriers showing a lower Aβ burden than ε4 homozygotes[78].

ApoE has also been suggested to modulate APP processing and thus mediate Aβ levels, although the evidence in the literature is conflicting. Some have demonstrated that ApoE has no effect on the production of Aβ[79, 80], while others have shown that exogenous ApoE stimulates Aβ production in cell culture models[81, 82]. Further, others have suggested that ApoE discourages the formation of Aβ both *in vitro* and in transgenic mouse models[83-86]. Studies comparing ApoE isoforms have generally found isoform-specific differences, with ApoE4 associated with higher levels of Aβ production[85, 87]. This may be due to the less efficient clearance of Aβ by ApoE4. It has also been suggested that ApoE receptors, rather than ApoE itself, may be responsible for the modulation of APP processing[88, 89].

The role of ApoE in mediating Aβ aggregation may directly relate to its ability to bind the peptide, a characteristic that has been well documented. ApoE is associated with amyloid plaques and forms complexes with Aβ through its lipid-binding region[90-92]. This interaction is dependent on the arrangement of Aβ into a β-sheet conformation, which promotes the formation and aggregation of Aβ fibers[93, 94]. Unlike the other ApoE isoforms, ApoE4 has a diminished ability to bind Aβ in both plaques and neurotoxic oligomers, compared to the other ApoE isoforms[95, 96]. On the other hand, Aβ binding also inhibits ApoE's ability to bind lipids, possibly due to the overlap between the Aβ-binding site and the lipid-binding domain, which may contribute to the general dysfunction of ApoE[97]. Both *in vitro* and transgenic mouse models have suggested that disruption of the ApoE-Aβ interaction leads to a decrease in fibrillogenesis and amyloid burden[98, 99].

ApoE is also involved in the clearance of Aβ across the blood-brain barrier (BBB), a process mediated by both astrocytes and microglia through the interaction of Aβ-ApoE complexes with receptors for ApoE, particularly LRP1[100-103]. Other evidence suggests that ApoE promotes the retention of Aβ within the CNS, by hindering the peptide's clearance across the BBB. The switch between promoting clearance *versus* retention of Aβ is mediated by the lipidation state of ApoE (lipid-binding slows transport across the BBB), and is further affected by the specific ApoE isoforms[104]. One study suggested that ApoE4-Aβ complexes use the VLDL receptor pathway, a slower method of clearance, while ApoE3- and ApoE2-Aβ complexes use LRP1[105]. Additional work in transgenic mice has confirmed that ApoE4 is much less efficient in clearing Aβ from the brain than the other isoforms, although the mechanism is not clear[76, 106]. The greater plaque density in the presence of the ApoE4 allele may be due to, at least in part, a reduction in the amount and speed of Aβ clearance by ApoE4. ApoE also reduces the ability of peripheral tissues to clear Aβ, though the impact of this, if any, on Aβ levels in the CNS is unknown[107, 108].

The clearance of Aβ from the CNS also occurs through degradation of the peptide, both extracellularly by insulin degrading enzyme (IDE) and within microglia and astrocytes by neprilysin[109-111]. Genetic association studies have indicated that IDE variants may be associated with increased risk of AD, suggesting that altering the degree of Aβ degradation could affect the course of the disease[112, 113]. ApoE has been implicated in modulating this response, in both microglial degradation of Aβ and macrophage-mediated proteolysis[47, 114]. An isoform-specific effect has also been demonstrated in mouse cells expressing human ApoE isoforms, with ApoE2 showing more robust extracellular degradation of exogenous Aβ than ApoE3 and ApoE4[114]. In *Apoe*-knockout microglia incubated with human ApoE isoforms, ApoE2 shows the strongest effect of promoting Aβ degradation and ApoE4 the weakest[47]. ApoE4 has also been demonstrated to down-regulate IDE expression, which could contribute to differential levels of Aβ degradation[115]. However, the overall importance of this down-regulation in terms of disease risk is unknown. Lipids are involved in the process as well: ABCA1 knockout astrocytes have a reduced ability to facilitate Aβ degradation[47]. A recent study showed that lower microglial cholesterol levels, a result of ApoE activity, promote the degradation of Aβ in lysosomes[116].

The importance of ApoE in the Aβ degradation process is unclear, as there is evidence for an ApoE-independent

degradation pathway, where LDLRs interact directly with Aβ peptides[117]. Another important functional difference is the decreased stability and the increased proteolysis of ApoE4 relative to other ApoE isoforms[60, 118]. This difference is thought to contribute to the lower levels of ApoE protein in the CNS of ApoE4 transgenic mice[119]. Given ApoE's role in Aβ clearance and degradation, the decreased stability of ApoE4 may predispose individuals to AD.

The Role of Apolipoprotein E in Neuroinflammation

ApoE is involved in the neuroinflammatory response, even in the absence of Aβ, suggesting its native role in the inflammatory pathway. ApoE differentially affects the inflammatory response, depending on the experimental conditions and the gene examined. One study found that exogenous ApoE3 and ApoE4 repress the oligomeric Aβ-induced expression of inducible nitric oxide synthase and cyclo-oxygenase-2 in rat glial cells. However, in the absence of Aβ, exogenous ApoE actually promotes the production of IL-1β, a pro-inflammatory cytokine (ApoE4 more so than ApoE3)[120]. The overall consensus is that ApoE has an anti-inflammatory effect, since addition of exogenous ApoE and its mimetics to cultured cells down-regulates the activation of microglia and peripheral macrophages[121-123]. Further confirmation stems from the findings that *Apoe*-knockout mice show greater systemic activation of macrophages and increased circulating inflammatory markers[124], and that *Apoe*-knockout glial cells show a greater inflammatory response to Aβ *in vitro*[125].

Isoform-specific differences also exist in the ApoE-mediated inflammatory response. As expected, the ApoE4 isoform is associated with increased inflammation and the secretion of inflammatory factors. This has been shown in some cell types and in response to a variety of inflammatory triggers. Chen and colleagues[126] showed that ApoE4 stimulates the secretion of prostaglandin E2 and IL-1β, while ApoE3 does not. In addition, macrophages transfected with human ApoE4 respond more strongly to lipopolysaccharide (LPS), a potent inflammatory activator, than ApoE3-expressing cells[127]. ApoE4-expressing astrocytes also show a significantly impaired ability to promote neuronal recovery after an inflammatory insult[128]. A similar study with Schwann cells demonstrated that expression of ApoE3 resulted in decreased inflammatory markers relative to the expression of both ApoE4 and ApoE2, suggesting that despite its protective role, ApoE2 may contribute to inflammatory dysfunction in some cell types[129]. Furthermore, ApoE4 preferentially increases NO release in human-derived macrophages after LPS stimulation[126] and increases oxidative stress in neuronal cultures[87]. It also triggers activation of the pro-inflammatory complement system upon Aβ challenge, but this does not occur with the ApoE3 or ApoE2 isoform[130].

Transgenic mouse models also provide evidence for the differential effects of ApoE isoforms. ApoE4 mice show impaired activation of astrocytes following LPS treatment compared to ApoE3 mice[131]. Following intracerebroventricular injection of LPS, ApoE4 enhances brain inflammation by dysregulating the nuclear factor-κB (NF-kB) signaling cascade or by augmenting and prolonging the increase in cytokine levels (IL-1β, IL-6, and TNFα) relative to ApoE2 and ApoE3[131]. ApoE4 also increased inflammatory activation in hippocampal areas in transgenic mice, which are critical to memory and in AD neurodegeneration[132]. Interestingly, Vitek and colleagues found that mice expressing only one human ε3 allele (ε3/0) had a greater inflammatory response on LPS challenge than ε3/ε3 mice, but a lower response than ε4/ε4 mice, suggesting that ApoE4 either predisposes to inflammation or actively promotes a pro-inflammatory response[131]. An inverse relationship also exists: inflammatory activation has been shown to mediate ApoE expression. Treatment of astrocytes with Aβ induces the release of ApoE lipoproteins by a mechanism involving NF-κB[133]. However, other studies have shown that ApoE gene expression is decreased after inflammatory activation in macrophages[134], and that the inflammatory cytokines IL-1 and TNF-α reduce astrocytic and glial release of ApoE, suggesting that these cytokines act to suppress the basal anti-inflammatory activity of ApoE[135].

Discrepancies among the findings on the roles of ApoE in the inflammatory response are evident in the above studies. These may arise from the different models employed (*in vitro* and *in vivo* models), different cell types with transient ApoE expression or ApoE knockin, knockdown or knockout, different ApoE preparations (recombinant *versus* native proteins; lipid-poor *versus* lipidated ApoE proteins), different experimental conditions and approaches, and different inflammatory stimuli (LPS *versus* Aβ and others), all of which may account for the discrepancies. Nevertheless, these studies show that ApoE

does play a role, and the different isoforms play different roles in the inflammatory response.

Neuroinflammation occurs in the AD brain and plays a critical role in neurodegenerative pathology. Aβ is a key factor for triggering neuroinflammation. A number of studies have demonstrated that Aβ peptides evoke an inflammatory response in cultured microglial cells, astrocytes, and brain endothelial cells, and that activated microglia are associated with Aβ plaques in the AD brain[136, 137]. Many factors may affect and modify the Aβ-induced inflammatory response in cultured cells and in the AD brain. Genome-wide association studies have revealed that genetic variations such as single-nucleotide polymorphisms in genes involved in the immune/inflammatory response represent an important category of risk factors for late-onset AD. However, little is known about the role of specific ApoE isoforms in AD neuroinflammation and Aβ-induced inflammation. Our study showed that Aβ peptides induce an inflammatory response in cultured brain endothelial cells[137, 138] and astrocytes (Dorey and Zhang, unpublished data). However, the recombinant ApoE isoform proteins added to cultured astrocytes do not change the cellular expression of inflammatory genes. This suggests that ApoE isoform proteins themselves do not evoke an inflammatory response, but may alter the cellular response state (weaker or stronger) to inflammatory stimulators. As described above, ApoE isoforms can modify the inflammatory response induced by stimuli such as LPS. Thus, in addition to their roles in cholesterol transport and Aβ binding/clearance, ApoE isoforms may modify the Aβ-induced inflammatory response in cells. Our work confirmed that ApoE isoforms influence the Aβ-induced inflammatory response in astrocytes. Consistent with the *in vivo* observations, ApoE2 protects against the Aβ-induced inflammatory response, while ApoE4 enhances it (Dorey and Zhang, unpublished data).

The mechanisms underlying the different effects of isoforms are not clear. But it is known that ApoE isoforms have different capacities to bind Aβ peptides and may affect the states of Aβ aggregation, oligomer formation, and Aβ clearance. ApoE4 may promote the potency of Aβ as an inflammatory stimulus, while ApoE2 may destabilize Aβ peptides and enhance their cellular degradation or clearance. The state of ApoE lipidation may also affect the activity of ApoE isoforms in the Aβ-induced inflammatory response. Furthermore, ApoE proteins may modify Aβ-induced signaling, since the isoforms bind to ApoE receptors with different affinities and may trigger different magnitudes of cell signaling responses. Different models (*in vitro* and *in vivo*), cell types, experimental conditions, and the inflammatory stimuli used in different studies may lead to different findings and conclusions about the roles of ApoE isoforms in the inflammatory response, but ApoE isoforms do play a role in the inflammatory response in different biological scenarios.

Conclusion

In summary, Aβ plays a key role in evoking the inflammatory response that contributes to neurodegeneration in the AD brain. ApoE4 is a well-recognized risk factor for AD. ApoE proteins are key players in cholesterol transport, Aβ binding and clearance, and synaptic function in the brain. ApoE4 may predispose cells or animals to inflammation or promote an inflammatory response following exposure to a stimulus. Our work showed that the different isoforms of ApoE can modify the Aβ-induced inflammatory response in neural cells: ApoE2 has an inhibitory effect, while ApoE4 has a pro-inflammatory effect. Further *in vivo* studies in our laboratory will attempt to confirm these findings. Despite the considerable work already done in the field, further research is needed to better understand the roles, mechanisms, and interrelations of ApoE, Aβ, and neuroinflammation in AD.

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