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 (A β) peptides in the brain. Neuroinflammation occurs in the AD brain and plays a critical role in the neurodegenerative pathology. Particularly, A β evokes an inflammatory response that leads to synaptic dysfunction, neuronal death, and neurodegeneration. Apolipoprotein E (ApoE) proteins are involved in cholesterol transport, A β 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 A β -induced inflammatory response and in the neuroinflammation of AD. Our recent work showed that ApoE isoforms differentially regulate and modify the A β -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 A β , ApoE, and neuroinflammation in AD.

Keywords: ApoE; Alzheimer's disease; A β ; 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 (A β) peptides)^[1].

Alzheimer's Disease and Amyloid-beta

A β 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 $A\beta$ in either its 40- or 42-amino-acid form^[3] (Fig. 1). $A\beta_{1-40}$ is more abundant, while $A\beta_{1-42}$ is more closely associated with AD^[2]. Although the mechanism that determines the length of $A\beta$ produced is unknown, it is clear that an increase in the $A\beta_{1-42}/A\beta_{1-40}$ 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 $A\beta$ production. In this process, an α -secretase cleaves APP within the $A\beta$ domain, producing a truncated $A\beta$ 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 $A\beta$ 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, $A\beta$ 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 $A\beta$, 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 $A\beta$ 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 $A\beta$

Fig. 1. Pathways of APP processing. APP has two primary endogenous processing pathways. The first is non-amyloidogenic: α -secretase cleavage produces the soluble APPs α 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 APPs β and C99. C99 is then cleaved by γ -secretase, producing the $A\beta$ 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 $A\beta$ 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.

production, providing protection against AD and cognitive decline^[12].

Deposition of $A\beta$ peptides was first identified in the form of plaques, but the environment in the AD brain is diverse, with a variety of assemblages of $A\beta$. 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 $A\beta$ in AD^[13]. This was answered, to some degree, by the discovery of oligomeric species of $A\beta$, 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 A β build-up relative to disease progression is also important. A β 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 A β 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 A β -induced neuronal hyperexcitability, may be one of the factors responsible for the premature death of these mice^[19]. Studies with 3 $\times$ Tg-AD mice have shown that A β can induce tau pathology^[20]; A β pathology occurs first, followed by tau pathology in AD^[17]. Another study suggests that A β toxicity is tau-dependent^[21]. As an axonal protein, tau also has a dendritic function that confers A β 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 A β aggregation and plaque formation is the development of neuroinflammation. A β 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 A β . Groups have reported that blockade of TLR2 or TLR4 with specific antibodies decreases the degree of microglial activation upon challenge with A β peptides^[23, 24]. TLR2- and TLR4-knockout microglia also show a decreased inflammatory response to A β ^[25, 26]. A recent study showed that expression of TLR2 in HEK293 cells that do not endogenously express TLR2 triggers an inflammatory response to A β ^[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]. A β 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 A β synthesis by modulating APP processing and aid the oligomeric association of A β by upregulating pro-oligomeric pathways^[34]. This can lead to a self-amplifying cycle of inflammatory activation, with increased A β levels triggering further inflammation, cytokine release, and neuronal death.

The nature and extent of the link between neuroinflammation 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 A β ^[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 A β 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, ϵ 2, ϵ 3, and ϵ 4, among which the ϵ 3 allele is the most prevalent, with a frequency of 77% in the general population^[37]. The ϵ 2 allele is the least frequent (8%), and is associated with some degree of neuroprotection from AD and a form of hyperlipoproteinemia^[53, 54]. The ϵ 4 allele is a strong risk factor for AD: compared to an ϵ 3/ ϵ 3 individual, an ϵ 3/ ϵ 4 heterozygote has a 2- to 3-fold greater risk of developing AD, and an ϵ 4 homozygote has ~12-fold greater risk^[55]. It follows that the ϵ 4 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 ϵ 4 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 A β . Isoform-related differences have been characterized at several steps of A β processing and deposition in some model systems (Fig. 2). The conflicting findings on ApoE's function in A β deposition illustrates the uncertainty of its role in the pathogenesis of AD. *In vitro* studies have shown that human ApoE either promotes or inhibits A β aggregation and fibril formation depending on the conditions. Furthermore, different A β 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 A β deposition and an isoform-specific difference, with ApoE4 preferentially encouraging aggregation^[68-70]. In addition, the lipidation state of ApoE may significantly impact A β fibril formation: all three ApoE isoforms exhibit decreased inhibition of A β fibrillation upon lipidation in a cell-free model^[71].

Fig. 2. Interaction of ApoE isoforms with A β . ApoE isoforms differentially affect each of these processes, the end result being deposition, degradation, or clearance of A β . 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 A β 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 A β 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 A β , with $\epsilon 3/\epsilon 4$ carriers showing a lower A β burden than $\epsilon 4$ homozygotes^[78].

ApoE has also been suggested to modulate APP processing and thus mediate A β levels, although the evidence in the literature is conflicting. Some have demonstrated that ApoE has no effect on the production of A β ^[79, 80], while others have shown that exogenous ApoE stimulates A β production in cell culture models^[81, 82]. Further, others have suggested that ApoE discourages the formation of A β 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 A β production^[85, 87]. This may be due to the less efficient clearance of A β 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 A β 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 A β through its lipid-binding region^[90–92]. This interaction is dependent on the arrangement of A β into a β -sheet conformation, which promotes the formation and aggregation of A β fibers^[93, 94]. Unlike the other ApoE isoforms, ApoE4 has a diminished ability to bind A β in both plaques and neurotoxic oligomers, compared to the other ApoE isoforms^[95, 96]. On the other hand, A β binding also inhibits ApoE's ability to bind lipids, possibly due to the overlap between the A β -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-A β interaction leads to a decrease in fibrillogenesis and amyloid burden^[98, 99].

ApoE is also involved in the clearance of A β across the blood-brain barrier (BBB), a process mediated by both astrocytes and microglia through the interaction of A β -ApoE

complexes with receptors for ApoE, particularly LRP1^[100–103]. Other evidence suggests that ApoE promotes the retention of A β within the CNS, by hindering the peptide's clearance across the BBB. The switch between promoting clearance *versus* retention of A β 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-A β complexes use the VLDL receptor pathway, a slower method of clearance, while ApoE3- and ApoE2-A β complexes use LRP1^[105]. Additional work in transgenic mice has confirmed that ApoE4 is much less efficient in clearing A β 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 A β clearance by ApoE4. ApoE also reduces the ability of peripheral tissues to clear A β , though the impact of this, if any, on A β levels in the CNS is unknown^[107, 108].

The clearance of A β 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 A β degradation could affect the course of the disease^[112, 113]. ApoE has been implicated in modulating this response, in both microglial degradation of A β 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 A β than ApoE3 and ApoE4^[114]. In *ApoE*-knockout microglia incubated with human ApoE isoforms, ApoE2 shows the strongest effect of promoting A β degradation and ApoE4 the weakest^[47]. ApoE4 has also been demonstrated to down-regulate IDE expression, which could contribute to differential levels of A β 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 A β degradation^[47]. A recent study showed that lower microglial cholesterol levels, a result of ApoE activity, promote the degradation of A β in lysosomes^[116].

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

degradation pathway, where LDLRs interact directly with A β 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 A β 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 A β , 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 A β -induced expression of inducible nitric oxide synthase and cyclo-oxygenase-2 in rat glial cells. However, in the absence of A β , exogenous ApoE actually promotes the production of IL-1 β , 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 A β *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-1 β , 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 A β 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- κ B (NF- κ B) signaling cascade or by augmenting and prolonging the increase in cytokine levels (IL-1 β , IL-6, and TNF α) 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 ϵ 3 allele (ϵ 3/0) had a greater inflammatory response on LPS challenge than ϵ 3/ ϵ 3 mice, but a lower response than ϵ 4/ ϵ 4 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 A β induces the release of ApoE lipoproteins by a mechanism involving NF- κ B^[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* A β 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. A β is a key factor for triggering neuroinflammation. A number of studies have demonstrated that A β peptides evoke an inflammatory response in cultured microglial cells, astrocytes, and brain endothelial cells, and that activated microglia are associated with A β plaques in the AD brain^[136, 137]. Many factors may affect and modify the A β -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 A β -induced inflammation. Our study showed that A β 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 A β binding/clearance, ApoE isoforms may modify the A β -induced inflammatory response in cells. Our work confirmed that ApoE isoforms influence the A β -induced inflammatory response in astrocytes. Consistent with the *in vivo* observations, ApoE2 protects against the A β -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 A β peptides and may affect the states of A β aggregation, oligomer formation, and A β clearance. ApoE4 may promote the potency of A β as an inflammatory stimulus, while ApoE2 may destabilize A β peptides and enhance their cellular degradation or clearance. The state of ApoE lipidation may also affect the activity of ApoE isoforms in the A β -induced inflammatory

response. Furthermore, ApoE proteins may modify A β -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, A β 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, A β 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 A β -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, A β , and neuroinflammation in AD.

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REFERENCES

- [1] McKee AC, Kosik KS, Kowall NW. Neuritic pathology and dementia in Alzheimer's disease. *Ann Neurol* 1991, 30: 156–165.
- [2] Scheuner D, Eckman C, Jensen M, Song X, Citron M,

- Suzuki N, *et al.* Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat Med* 1996, 2: 864–870.
- [3] Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, *et al.* Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* 1999, 286: 735–741.
- [4] Pauwels K, Williams TL, Morris KL, Jonckheere W, Vandersteen A, Kelly G, *et al.* Structural basis for increased toxicity of pathological abeta42:abeta40 ratios in Alzheimer disease. *J Biol Chem* 2012, 287: 5650–5660.
- [5] Vella LJ, Cappai R. Identification of a novel amyloid precursor protein processing pathway that generates secreted N-terminal fragments. *FASEB J* 2012, 26: 2930–2940.
- [6] Guo Q, Wang Z, Li H, Wiese M, Zheng H. APP physiological and pathophysiological functions: insights from animal models. *Cell Res* 2012, 22: 78–89.
- [7] Morley JE, Farr SA, Banks WA, Johnson SN, Yamada KA, Xu L. A physiological role for amyloid-beta protein: enhancement of learning and memory. *J Alzheimers Dis* 2010, 19: 441–449.
- [8] Soscia SJ, Kirby JE, Washicosky KJ, Tucker SM, Ingelsson M, Hyman B, *et al.* The Alzheimer's disease-associated amyloid beta-protein is an antimicrobial peptide. *PLoS One* 2010, 5: e9505.
- [9] Li C, Zhao R, Gao K, Wei Z, Yin MY, Lau LT, *et al.* Astrocytes: implications for neuroinflammatory pathogenesis of Alzheimer's disease. *Curr Alzheimer Res* 2011, 8: 67–80.
- [10] Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 2002, 297: 353–356.
- [11] Irvine GB, El-Agnaf OM, Shankar GM, Walsh DM. Protein aggregation in the brain: the molecular basis for Alzheimer's and Parkinson's diseases. *Mol Med* 2008, 14: 451–464.
- [12] Jonsson T, Atwal JK, Steinberg S, Snaedal J, Jonsson PV, Bjornsson S, *et al.* A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature* 2012, 488: 96–99.
- [13] Aizenstein HJ, Nebes RD, Saxton JA, Price JC, Mathis CA, Tsopelas ND, *et al.* Frequent amyloid deposition without significant cognitive impairment among the elderly. *Arch Neurol* 2008, 65: 1509–1517.
- [14] Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, *et al.* Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation *in vivo*. *Nature* 2002, 416: 535–539.
- [15] McLean CA, Cherny RA, Fraser FW, Fuller SJ, Smith MJ, Beyreuther K, *et al.* Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann Neurol* 1999, 46: 860–866.
- [16] Gaspar RC, Villarreal SA, Bowles N, Hepler RW, Joyce JG, Shughrue PJ. Oligomers of beta-amyloid are sequestered into and seed new plaques in the brains of an AD mouse model. *Exp Neurol* 2010, 223: 394–400.
- [17] Jack CR Jr, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, *et al.* Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol* 2010, 9: 119–128.
- [18] Minkeviciene R, Rheims S, Dobszay MB, Zilberter M, Hartikainen J, Fulop L, *et al.* Amyloid beta-induced neuronal hyperexcitability triggers progressive epilepsy. *J Neurosci* 2009, 29: 3453–3462.
- [19] Xiong H, Callaghan D, Wodzinska J, Xu J, Premyslova M, Liu QY, *et al.* Biochemical and behavioral characterization of the double transgenic mouse model (APPswe/PS1dE9) of Alzheimer's disease. *Neurosci Bull* 2011, 27: 221–232.
- [20] Oddo S, Caccamo A, Tseng B, Cheng D, Vasilevko V, Cribbs DH, *et al.* Blocking Abeta42 accumulation delays the onset and progression of tau pathology via the C terminus of heat shock protein70-interacting protein: a mechanistic link between Abeta and tau pathology. *J Neurosci* 2008, 28: 12163–12175.
- [21] Ittner LM, Ke YD, Delerue F, Bi M, Gladbach A, van Eersel J, *et al.* Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. *Cell* 2010, 142: 387–397.
- [22] Kitazawa M, Yamasaki TR, LaFerla FM. Microglia as a potential bridge between the amyloid beta-peptide and tau. *Ann N Y Acad Sci* 2004, 1035: 85–103.
- [23] Jana M, Palencia CA, Pahan K. Fibrillar amyloid-beta peptides activate microglia via TLR2: implications for Alzheimer's disease. *J Immunol* 2008, 181: 7254–7262.
- [24] Udan ML, Ajit D, Crouse NR, Nichols MR. Toll-like receptors 2 and 4 mediate Abeta(1-42) activation of the innate immune response in a human monocytic cell line. *J Neurochem* 2008, 104: 524–533.
- [25] Reed-Geaghan EG, Savage JC, Hise AG, Landreth GE. CD14 and toll-like receptors 2 and 4 are required for fibrillar A{beta}-stimulated microglial activation. *J Neurosci* 2009, 29: 11982–11992.
- [26] Walter S, Letiembre M, Liu Y, Heine H, Penke B, Hao W, *et al.* Role of the toll-like receptor 4 in neuroinflammation in Alzheimer's disease. *Cell Physiol Biochem* 2007, 20: 947–956.
- [27] Liu S, Liu Y, Hao W, Wolf L, Kiliaan AJ, Penke B, *et al.* TLR2 is a primary receptor for Alzheimer's amyloid beta peptide to trigger neuroinflammatory activation. *J Immunol* 2012, 188: 1098–1107.

- [28] Reed-Geaghan EG, Reed QW, Cramer PE, Landreth GE. Deletion of CD14 attenuates Alzheimer's disease pathology by influencing the brain's inflammatory milieu. *J Neurosci* 2010, 30: 15369–15373.
- [29] Craft JM, Watterson DM, Van Eldik LJ. Human amyloid beta-induced neuroinflammation is an early event in neurodegeneration. *Glia* 2006, 53: 484–490.
- [30] Atamna H, Boyle K. Amyloid-beta peptide binds with heme to form a peroxidase: relationship to the cytopathologies of Alzheimer's disease. *Proc Natl Acad Sci U S A* 2006, 103: 3381–3386.
- [31] Jana A, Pahan K. Fibrillar amyloid-beta-activated human astroglia kill primary human neurons via neutral sphingomyelinase: implications for Alzheimer's disease. *J Neurosci* 2010, 30: 12676–12689.
- [32] Hu J, Ferreira A, Van Eldik LJ. S100beta induces neuronal cell death through nitric oxide release from astrocytes. *J Neurochem* 1997, 69: 2294–2301.
- [33] Garwood CJ, Pooler AM, Atherton J, Hanger DP, Noble W. Astrocytes are important mediators of Abeta-induced neurotoxicity and tau phosphorylation in primary culture. *Cell Death Dis* 2011, 2: e167.
- [34] Blasko I, Stampfer-Kountchev M, Robatscher P, Veerhuis R, Eikelenboom P, Grubeck-Lobenstein B. How chronic inflammation can affect the brain and support the development of Alzheimer's disease in old age: the role of microglia and astrocytes. *Aging Cell* 2004, 3: 169–176.
- [35] Schwab C, Klegeris A, McGeer PL. Inflammation in transgenic mouse models of neurodegenerative disorders. *Biochim Biophys Acta* 2010, 1802: 889–902.
- [36] Parachikova A, Agadjanyan MG, Cribbs DH, Blurton-Jones M, Perreau V, Rogers J, *et al.* Inflammatory changes parallel the early stages of Alzheimer disease. *Neurobiol Aging* 2007, 28: 1821–1833.
- [37] Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 1988, 240: 622–630.
- [38] Bu G. Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy. *Nat Rev Neurosci* 2009, 10: 333–344.
- [39] Hatters DM, Peters-Libeu CA, Weisgraber KH. Apolipoprotein E structure: insights into function. *Trends Biochem Sci* 2006, 31: 445–454.
- [40] Dieckmann M, Dietrich MF, Herz J. Lipoprotein receptors—an evolutionarily ancient multifunctional receptor family. *Biol Chem* 2010, 391: 1341–1363.
- [41] Qiu Z, Crutcher KA, Hyman BT, Rebeck GW. ApoE isoforms affect neuronal N-methyl-D-aspartate calcium responses and toxicity via receptor-mediated processes. *Neuroscience* 2003, 122: 291–303.
- [42] Herz J, Chen Y. Reelin, lipoprotein receptors and synaptic plasticity. *Nat Rev Neurosci* 2006, 7: 850–859.
- [43] DeMattos RB, Brendza RP, Heuser JE, Kierson M, Cirrito JR, Fryer J, *et al.* Purification and characterization of astrocyte-secreted apolipoprotein E and J-containing lipoproteins from wild-type and human apoE transgenic mice. *Neurochem Int* 2001, 39: 415–425.
- [44] Fagan AM, Holtzman DM, Munson G, Mathur T, Schneider D, Chang LK, *et al.* Unique lipoproteins secreted by primary astrocytes from wild type, apoE (-/-), and human apoE transgenic mice. *J Biol Chem* 1999, 274: 30001–30007.
- [45] Hauser PS, Narayanaswami V, Ryan RO. Apolipoprotein E: from lipid transport to neurobiology. *Prog Lipid Res* 2011, 50: 62–74.
- [46] Wahrle SE, Jiang H, Parsadanian M, Legleiter J, Han X, Fryer JD, *et al.* ABCA1 is required for normal central nervous system ApoE levels and for lipidation of astrocyte-secreted apoE. *J Biol Chem* 2004, 279: 40987–40993.
- [47] Jiang Q, Lee CY, Mandrekar S, Wilkinson B, Cramer P, Zelcer N, *et al.* ApoE promotes the proteolytic degradation of Abeta. *Neuron* 2008, 58: 681–693.
- [48] Garai K, Frieden C. The association-dissociation behavior of the ApoE proteins: kinetic and equilibrium studies. *Biochemistry* 2010, 49: 9533–9541.
- [49] Krimbou L, Denis M, Haidar B, Carrier M, Marcil M, Genest J, Jr. Molecular interactions between apoE and ABCA1: impact on apoE lipidation. *J Lipid Res* 2004, 45: 839–848.
- [50] Hirsch-Reinshagen V, Maia LF, Burgess BL, Blain JF, Naus KE, McIsaac SA, *et al.* The absence of ABCA1 decreases soluble ApoE levels but does not diminish amyloid deposition in two murine models of Alzheimer disease. *J Biol Chem* 2005, 280: 43243–43256.
- [51] Wahrle SE, Shah AR, Fagan AM, Smemo S, Kauwe JS, Grupe A, *et al.* Apolipoprotein E levels in cerebrospinal fluid and the effects of ABCA1 polymorphisms. *Mol Neurodegener* 2007, 2: 7.
- [52] Wahrle SE, Jiang H, Parsadanian M, Kim J, Li A, Knoten A, *et al.* Overexpression of ABCA1 reduces amyloid deposition in the PDAPP mouse model of Alzheimer disease. *J Clin Invest* 2008, 118: 671–682.
- [53] Corder EH, Saunders AM, Risch NJ, Strittmatter WJ, Schmechel DE, Gaskell PC, Jr., *et al.* Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. *Nat Genet* 1994, 7: 180–184.
- [54] Genin E, Hannequin D, Wallon D, Sleegers K, Hiltunen M, Combarros O, *et al.* APOE and Alzheimer disease: a major gene with semi-dominant inheritance. *Mol Psychiatry* 2011, 16: 903–907.
- [55] Roses AD. Apolipoprotein E alleles as risk factors in Alzheimer's disease. *Annu Rev Med* 1996, 47: 387–400.

- [56] Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, *et al.* Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 1993, 261: 921–923.
- [57] Chuang WL, Hsieh YC, Wang CY, Kuo HC, Huang CC. Association of apolipoproteins e4 and c1 with onset age and memory: a study of sporadic Alzheimer disease in Taiwan. *J Geriatr Psychiatry Neurol* 2010, 23: 42–48.
- [58] Patterson C, Feightner JW, Garcia A, Hsiung GY, MacKnight C, Sadovnick AD. Diagnosis and treatment of dementia: 1. Risk assessment and primary prevention of Alzheimer disease. *CMAJ* 2008, 178: 548–556.
- [59] Hanlon CS, Rubinsztein DC. Arginine residues at codons 112 and 158 in the apolipoprotein E gene correspond to the ancestral state in humans. *Atherosclerosis* 1995, 112: 85–90.
- [60] Morrow JA, Hatters DM, Lu B, Hochtl P, Oberg KA, Rupp B, *et al.* Apolipoprotein E4 forms a molten globule. A potential basis for its association with disease. *J Biol Chem* 2002, 277: 50380–50385.
- [61] Chen HK, Ji ZS, Dodson SE, Miranda RD, Rosenblum CI, Reynolds IJ, *et al.* Apolipoprotein E4 domain interaction mediates detrimental effects on mitochondria and is a potential therapeutic target for Alzheimer disease. *J Biol Chem* 2011, 286: 5215–5221.
- [62] Frieden C, Garai K. Structural differences between apoE3 and apoE4 may be useful in developing therapeutic agents for Alzheimer's disease. *Proc Natl Acad Sci U S A* 2012, 109: 8913–8918.
- [63] Mahley RW, Weisgraber KH, Huang Y. Apolipoprotein E: structure determines function, from atherosclerosis to Alzheimer's disease to AIDS. *J Lipid Res* 2009, 50 Suppl: S183–188.
- [64] Rapp A, Gmeiner B, Huttinger M. Implication of apoE isoforms in cholesterol metabolism by primary rat hippocampal neurons and astrocytes. *Biochimie* 2006, 88: 473–483.
- [65] Bandaru VV, Troncoso J, Wheeler D, Pletnikova O, Wang J, Conant K, *et al.* ApoE4 disrupts sterol and sphingolipid metabolism in Alzheimer's but not normal brain. *Neurobiol Aging* 2009, 30: 591–599.
- [66] Wood SJ, Chan W, Wetzel R. Seeding of A beta fibril formation is inhibited by all three isoforms of apolipoprotein E. *Biochemistry* 1996, 35: 12623–12628.
- [67] Naiki H, Hasegawa K, Yamaguchi I, Nakamura H, Gejyo F, Nakakuki K. Apolipoprotein E and antioxidants have different mechanisms of inhibiting Alzheimer's beta-amyloid fibril formation *in vitro*. *Biochemistry* 1998, 37: 17882–17889.
- [68] Ma J, Yee A, Brewer HB, Jr., Das S, Potter H. Amyloid-associated proteins alpha 1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer beta-protein into filaments. *Nature* 1994, 372: 92–94.
- [69] Wisniewski T, Castano EM, Golabek A, Vogel T, Frangione B. Acceleration of Alzheimer's fibril formation by apolipoprotein E *in vitro*. *Am J Pathol* 1994, 145: 1030–1035.
- [70] Dafnis I, Stratikos E, Tzinia A, Tsilibary EC, Zannis VI, Chroni A. An apolipoprotein E4 fragment can promote intracellular accumulation of amyloid peptide beta 42. *J Neurochem* 2010, 115: 873–884.
- [71] Beffert U, Poirier J. ApoE associated with lipid has a reduced capacity to inhibit beta-amyloid fibril formation. *Neuroreport* 1998, 9: 3321–3323.
- [72] Bales KR, Verina T, Cummins DJ, Du Y, Dodel RC, Saura J, *et al.* Apolipoprotein E is essential for amyloid deposition in the APP(V717F) transgenic mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A* 1999, 96: 15233–15238.
- [73] Bales KR, Verina T, Dodel RC, Du Y, Altstiel L, Bender M, *et al.* Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. *Nat Genet* 1997, 17: 263–264.
- [74] Bien-Ly N, Gillespie AK, Walker D, Yoon SY, Huang Y. Reducing human apolipoprotein E levels attenuates age-dependent Abeta accumulation in mutant human amyloid precursor protein transgenic mice. *J Neurosci* 2012, 32: 4803–4811.
- [75] Bales KR, Liu F, Wu S, Lin S, Koger D, DeLong C, *et al.* Human APOE isoform-dependent effects on brain beta-amyloid levels in PDAPP transgenic mice. *J Neurosci* 2009, 29: 6771–6779.
- [76] Castellano JM, Kim J, Stewart FR, Jiang H, DeMattos RB, Patterson BW, *et al.* Human apoE isoforms differentially regulate brain amyloid-beta peptide clearance. *Sci Transl Med* 2011, 3: 89ra57.
- [77] Fagan AM, Watson M, Parsadanian M, Bales KR, Paul SM, Holtzman DM. Human and murine ApoE markedly alters A beta metabolism before and after plaque formation in a mouse model of Alzheimer's disease. *Neurobiol Dis* 2002, 9: 305–318.
- [78] Reiman EM, Chen K, Liu X, Bandy D, Yu M, Lee W, *et al.* Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. *Proc Natl Acad Sci U S A* 2009, 106: 6820–6825.
- [79] Cedazo-Minguez A, Wiehager B, Winblad B, Huttinger M, Cowburn RF. Effects of apolipoprotein E (apoE) isoforms, beta-amyloid (Abeta) and apoE/Abeta complexes on protein kinase C-alpha (PKC-alpha) translocation and amyloid precursor protein (APP) processing in human SH-SY5Y neuroblastoma cells and fibroblasts. *Neurochem Int* 2001, 38: 615–625.
- [80] Biere AL, Ostaszewski B, Zhao H, Gillespie S, Younkin SG, Selkoe DJ. Co-expression of beta-amyloid precursor protein

- (betaAPP) and apolipoprotein E in cell culture: analysis of betaAPP processing. *Neurobiol Dis* 1995, 2: 177–187.
- [81] Ye S, Huang Y, Mullendorff K, Dong L, Giedt G, Meng EC, *et al.* Apolipoprotein (apo) E4 enhances amyloid beta peptide production in cultured neuronal cells: apoE structure as a potential therapeutic target. *Proc Natl Acad Sci U S A* 2005, 102: 18700–18705.
- [82] He X, Cooley K, Chung CH, Dashti N, Tang J. Apolipoprotein receptor 2 and X11 alpha/beta mediate apolipoprotein E-induced endocytosis of amyloid-beta precursor protein and beta-secretase, leading to amyloid-beta production. *J Neurosci* 2007, 27: 4052–4060.
- [83] Irizarry MC, Deng A, Lleo A, Berezovska O, Von Arnim CA, Martin-Rehrmann M, *et al.* Apolipoprotein E modulates gamma-secretase cleavage of the amyloid precursor protein. *J Neurochem* 2004, 90: 1132–1143.
- [84] Dodart JC, Bales KR, Johnstone EM, Little SP, Paul SM. Apolipoprotein E alters the processing of the beta-amyloid precursor protein in APP(V717F) transgenic mice. *Brain Res* 2002, 955: 191–199.
- [85] Hoe HS, Pocivavsek A, Dai H, Chakraborty G, Harris DC, Rebeck GW. Effects of apoE on neuronal signaling and APP processing in rodent brain. *Brain Res* 2006, 1112: 70–79.
- [86] Minami SS, Cordova A, Cirrito JR, Tesoriero JA, Babus LW, Davis GC, *et al.* ApoE mimetic peptide decreases Abeta production in vitro and in vivo. *Mol Neurodegener* 2010, 5: 16.
- [87] Huebbe P, Schaffer S, Jofre-Monseny L, Boesch-Saadatmandi C, Minihiene AM, Muller WE, *et al.* Apolipoprotein E genotype and alpha-tocopherol modulate amyloid precursor protein metabolism and cell cycle regulation. *Mol Nutr Food Res* 2007, 51: 1510–1517.
- [88] Cam JA, Bu G. Modulation of beta-amyloid precursor protein trafficking and processing by the low density lipoprotein receptor family. *Mol Neurodegener* 2006, 1: 8.
- [89] Hoe HS, Rebeck GW. Functional interactions of APP with the apoE receptor family. *J Neurochem* 2008, 106: 2263–2271.
- [90] Strittmatter WJ, Weisgraber KH, Huang DY, Dong LM, Salvesen GS, Pericak-Vance M, *et al.* Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease. *Proc Natl Acad Sci U S A* 1993, 90: 8098–8102.
- [91] Naslund J, Thyberg J, Tjernberg LO, Wernstedt C, Karlstrom AR, Bogdanovic N, *et al.* Characterization of stable complexes involving apolipoprotein E and the amyloid beta peptide in Alzheimer's disease brain. *Neuron* 1995, 15: 219–228.
- [92] Liu Q, Wu WH, Fang CL, Li RW, Liu P, Lei P, *et al.* Mapping ApoE/Abeta binding regions to guide inhibitor discovery. *Mol Biosyst* 2011, 7: 1693–1700.
- [93] Golabek AA, Soto C, Vogel T, Wisniewski T. The interaction between apolipoprotein E and Alzheimer's amyloid beta-peptide is dependent on beta-peptide conformation. *J Biol Chem* 1996, 271: 10602–10606.
- [94] Castano EM, Prelli F, Wisniewski T, Golabek A, Kumar RA, Soto C, *et al.* Fibrillogenesis in Alzheimer's disease of amyloid beta peptides and apolipoprotein E. *Biochem J* 1995, 306 (Pt 2): 599–604.
- [95] Tokuda T, Calero M, Matsubara E, Vidal R, Kumar A, Permanne B, *et al.* Lipidation of apolipoprotein E influences its isoform-specific interaction with Alzheimer's amyloid beta peptides. *Biochem J* 2000, 348 Pt 2: 359–365.
- [96] Petrova J, Hong HS, Bricarello DA, Harishchandra G, Lorigan GA, Jin LW, *et al.* A differential association of Apolipoprotein E isoforms with the amyloid-beta oligomer in solution. *Proteins* 2011, 79: 402–416.
- [97] Tamamizu-Kato S, Cohen JK, Drake CB, Kosaraju MG, Drury J, Narayanaswami V. Interaction with amyloid beta peptide compromises the lipid binding function of apolipoprotein E. *Biochemistry* 2008, 47: 5225–5234.
- [98] Hao J, Zhang W, Zhang P, Liu R, Liu L, Lei G, *et al.* Abeta20-29 peptide blocking apoE/Abeta interaction reduces full-length Abeta42/40 fibril formation and cytotoxicity *in vitro*. *Neuropeptides* 2010, 44: 305–313.
- [99] Yang J, Ji Y, Mehta P, Bates KA, Sun Y, Wisniewski T. Blocking the apolipoprotein E/amyloid-beta interaction reduces fibrillar vascular amyloid deposition and cerebral microhemorrhages in TgSwDI mice. *J Alzheimers Dis* 2011, 24: 269–285.
- [100] Lee CY, Landreth GE. The role of microglia in amyloid clearance from the AD brain. *J Neural Transm* 2010, 117: 949–960.
- [101] Thal DR. The role of astrocytes in amyloid beta-protein toxicity and clearance. *Exp Neurol* 2012, 236: 1–5.
- [102] Kang DE, Pietrzik CU, Baum L, Chevallier N, Merriam DE, Kounnas MZ, *et al.* Modulation of amyloid beta-protein clearance and Alzheimer's disease susceptibility by the LDL receptor-related protein pathway. *J Clin Invest* 2000, 106: 1159–1166.
- [103] Shibata M, Yamada S, Kumar SR, Calero M, Bading J, Frangione B, *et al.* Clearance of Alzheimer's amyloid-ss(1-40) peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. *J Clin Invest* 2000, 106: 1489–1499.
- [104] Bell RD, Sagare AP, Friedman AE, Bedi GS, Holtzman DM, Deane R, *et al.* Transport pathways for clearance of human Alzheimer's amyloid beta-peptide and apolipoproteins E and J in the mouse central nervous system. *J Cereb Blood Flow Metab* 2007, 27: 909–918.
- [105] Deane R, Sagare A, Hamm K, Parisi M, Lane S, Finn MB, *et*

- al.* apoE isoform-specific disruption of amyloid beta peptide clearance from mouse brain. *J Clin Invest* 2008, 118: 4002–4013.
- [106] Ji Y, Permanne B, Sigurdsson EM, Holtzman DM, Wisniewski T. Amyloid beta40/42 clearance across the blood-brain barrier following intra-ventricular injections in wild-type, apoE knock-out and human apoE3 or E4 expressing transgenic mice. *J Alzheimers Dis* 2001, 3: 23–30.
- [107] Hone E, Martins LJ, Fonte J, Martins RN. Apolipoprotein E influences amyloid-beta clearance from the murine periphery. *J Alzheimers Dis* 2003, 5: 1–8.
- [108] Sharman MJ, Morici M, Hone E, Berger T, Taddei K, Martins LJ, *et al.* APOE genotype results in differential effects on the peripheral clearance of amyloid-beta42 in APOE knock-in and knock-out mice. *J Alzheimers Dis* 2010, 21: 403–409.
- [109] Iwata N, Tsubuki S, Takaki Y, Watanabe K, Sekiguchi M, Hosoki E, *et al.* Identification of the major Abeta1-42-degrading catabolic pathway in brain parenchyma: suppression leads to biochemical and pathological deposition. *Nat Med* 2000, 6: 143–150.
- [110] Kurochkin IV, Goto S. Alzheimer's beta-amyloid peptide specifically interacts with and is degraded by insulin degrading enzyme. *FEBS Lett* 1994, 345: 33–37.
- [111] Koistinaho M, Lin S, Wu X, Esterman M, Koger D, Hanson J, *et al.* Apolipoprotein E promotes astrocyte colocalization and degradation of deposited amyloid-beta peptides. *Nat Med* 2004, 10: 719–726.
- [112] Mueller JC, Riemenschneider M, Schoepfer-Wendels A, Gohlke H, Konta L, Friedrich P, *et al.* Weak independent association signals between IDE polymorphisms, Alzheimer's disease and cognitive measures. *Neurobiol Aging* 2007, 28: 727–734.
- [113] Carrasquillo MM, Belbin O, Zou F, Allen M, Ertekin-Taner N, Ansari M, *et al.* Concordant association of insulin degrading enzyme gene (IDE) variants with IDE mRNA, Abeta, and Alzheimer's disease. *PLoS One* 2010, 5: e8764.
- [114] Zhao L, Lin S, Bales KR, Gelfanova V, Koger D, Delong C, *et al.* Macrophage-mediated degradation of beta-amyloid via an apolipoprotein E isoform-dependent mechanism. *J Neurosci* 2009, 29: 3603–3612.
- [115] Du J, Chang J, Guo S, Zhang Q, Wang Z. ApoE 4 reduces the expression of Abeta degrading enzyme IDE by activating the NMDA receptor in hippocampal neurons. *Neurosci Lett* 2009, 464: 140–145.
- [116] Lee CY, Tse W, Smith JD, Landreth GE. Apolipoprotein E promotes beta-amyloid trafficking and degradation by modulating microglial cholesterol levels. *J Biol Chem* 2012, 287: 2032–2044.
- [117] Basak JM, Verghese PB, Yoon H, Kim J, Holtzman DM. Low-density lipoprotein receptor represents an apolipoprotein E-independent pathway of Abeta uptake and degradation by astrocytes. *J Biol Chem* 2012, 287: 13959–13971.
- [118] Elliott DA, Tsoi K, Holinkova S, Chan SL, Kim WS, Halliday GM, *et al.* Isoform-specific proteolysis of apolipoprotein-E in the brain. *Neurobiol Aging* 2011, 32: 257–271.
- [119] Riddell DR, Zhou H, Atchison K, Warwick HK, Atkinson PJ, Jefferson J, *et al.* Impact of apolipoprotein E (ApoE) polymorphism on brain ApoE levels. *J Neurosci* 2008, 28: 11445–11453.
- [120] Guo L, LaDu MJ, Van Eldik LJ. A dual role for apolipoprotein e in neuroinflammation: anti- and pro-inflammatory activity. *J Mol Neurosci* 2004, 23: 205–212.
- [121] Laskowitz DT, Fillit H, Yeung N, Toku K, Vitek MP. Apolipoprotein E-derived peptides reduce CNS inflammation: implications for therapy of neurological disease. *Acta Neurol Scand Suppl* 2006, 185: 15–20.
- [122] Lynch JR, Tang W, Wang H, Vitek MP, Bennett ER, Sullivan PM, *et al.* APOE genotype and an ApoE-mimetic peptide modify the systemic and central nervous system inflammatory response. *J Biol Chem* 2003, 278: 48529–48533.
- [123] Baitsch D, Bock HH, Engel T, Telgmann R, Muller-Tidow C, Varga G, *et al.* Apolipoprotein E induces antiinflammatory phenotype in macrophages. *Arterioscler Thromb Vasc Biol* 2011, 31: 1160–1168.
- [124] Grainger DJ, Reckless J, McKilligin E. Apolipoprotein E modulates clearance of apoptotic bodies in vitro and in vivo, resulting in a systemic proinflammatory state in apolipoprotein E-deficient mice. *J Immunol* 2004, 173: 6366–6375.
- [125] LaDu MJ, Shah JA, Reardon CA, Getz GS, Bu G, Hu J, *et al.* Apolipoprotein E and apolipoprotein E receptors modulate A beta-induced glial neuroinflammatory responses. *Neurochem Int* 2001, 39: 427–434.
- [126] Colton CA, Needham LK, Brown C, Cook D, Rasheed K, Burke JR, *et al.* APOE genotype-specific differences in human and mouse macrophage nitric oxide production. *J Neuroimmunol* 2004, 147: 62–67.
- [127] Jofre-Monseny L, Loboda A, Wagner AE, Huebbe P, Boesch-Saadatmandi C, Jozkowicz A, *et al.* Effects of apoE genotype on macrophage inflammation and heme oxygenase-1 expression. *Biochem Biophys Res Commun* 2007, 357: 319–324.
- [128] Maezawa I, Zaja-Milatovic S, Milatovic D, Stephen C, Sokal I, Maeda N, *et al.* Apolipoprotein E isoform-dependent dendritic recovery of hippocampal neurons following activation of innate immunity. *J Neuroinflammation* 2006, 3: 21.
- [129] Zhang KJ, Zhang HL, Zhang XM, Zheng XY, Quezada HC, Zhang D, *et al.* Apolipoprotein E isoform-specific effects on

- cytokine and nitric oxide production from mouse Schwann cells after inflammatory stimulation. *Neurosci Lett* 2011, 499: 175–180.
- [130] McGeer PL, Walker DG, Pitas RE, Mahley RW, McGeer EG. Apolipoprotein E4 (ApoE4) but not ApoE3 or ApoE2 potentiates beta-amyloid protein activation of complement *in vitro*. *Brain Res* 1997, 749: 135–138.
- [131] Ophir G, Amariglio N, Jacob-Hirsch J, Elkon R, Rechavi G, Michaelson DM. Apolipoprotein E4 enhances brain inflammation by modulation of the NF-kappaB signaling cascade. *Neurobiol Dis* 2005, 20: 709–718.
- [132] Belinson H, Michaelson DM. ApoE4-dependent Abeta-mediated neurodegeneration is associated with inflammatory activation in the hippocampus but not the septum. *J Neural Transm* 2009, 116: 1427–1434.
- [133] Bales KR, Du Y, Holtzman D, Cordell B, Paul SM. Neuroinflammation and Alzheimer's disease: critical roles for cytokine/Abeta-induced glial activation, NF-kappaB, and apolipoprotein E. *Neurobiol Aging* 2000, 21: 427–432; discussion 451–423.
- [134] Gafencu AV, Robciuc MR, Fuior E, Zannis VI, Kardassis D, Simionescu M. Inflammatory signaling pathways regulating ApoE gene expression in macrophages. *J Biol Chem* 2007, 282: 21776–21785.
- [135] Aleong R, Blain JF, Poirier J. Pro-inflammatory cytokines modulate glial apolipoprotein E secretion. *Curr Alzheimer Res* 2008, 5: 33–37.
- [136] Vukic V CD, Liu H, Zhang W. Molecular mechanisms of neuroinflammation in Alzheimer's disease. In: Gemma C (Ed.). *Neuroinflammation: Pathogenesis, Mechanisms and Management*. New York: Nova Science Publishers, Inc., 2012: 47–64.
- [137] Vukic V, Callaghan D, Walker D, Lue LF, Liu QY, Couraud PO, *et al*. Expression of inflammatory genes induced by beta-amyloid peptides in human brain endothelial cells and in Alzheimer's brain is mediated by the JNK-AP1 signaling pathway. *Neurobiol Dis* 2009, 34: 95–106.
- [138] Bamji-Mirza M, Callaghan D, Najem D, Shen S, Hasim MS, Yang Z, *et al*. Stimulation of insulin signaling and inhibition of JNK-AP1 activation protect cells from amyloid-beta-induced signaling dysregulation and inflammatory response. *J Alzheimers Dis* 2013. Doi: 10.3233/JAD-131949