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Review

APOE mediated neuroinflammation and neurodegeneration in Alzheimer's disease

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A B S T R A C T

Neuroinflammation is a central mechanism involved in neurodegeneration as observed in Alzheimer's disease (AD), the most prevalent form of neurodegenerative disease. Apolipoprotein E4 (APOE4), the strongest genetic risk factor for AD, directly influences disease onset and progression by interacting with the major pathological hallmarks of AD including amyloid-β plaques, neurofibrillary tau tangles, as well as neuroinflammation. Microglia and astrocytes, the two major immune cells in the brain, exist in an immune-vigilant state providing immunological defense as well as housekeeping functions that promote neuronal well-being. It is becoming increasingly evident that under disease conditions, these immune cells become progressively dysfunctional in regulating metabolic and immunoregulatory pathways, thereby promoting chronic inflammation-induced neurodegeneration. Here, we review and discuss how APOE and specifically APOE4 directly influences amyloid-β and tau pathology, and disrupts microglial as well as astroglial immunomodulating functions leading to chronic inflammation that contributes to neurodegeneration in AD.

1. Introduction

Inflammation is an important factor that can drive neurodegeneration, both in triggering neurodegeneration and in providing promising therapeutic avenues to limit neurodegeneration. In fact, genome-wide association studies (GWAS) have identified several innate immune related genes linked to increased risk of developing neurodegenerative disorders, suggesting that immune cells play a key role in the pathogenesis of neurodegeneration. One of the genes that has disease-associated variants is apolipoprotein E (*APOE*). The apolipoprotein E4 (*APOE4*) allele, is a major shared risk factor for several neurodegenerative diseases including Alzheimer's disease (AD) and the *APOE2* allele decreases risk for AD [1,2]. *APOE4* is also the strongest genetic risk factor for late onset AD, likely in part due to its role in lipid metabolism and related inflammation [3]. AD is the most common cause of dementia and is pathologically characterized by the presence of proteopathic aggregates in the brain including extracellular amyloid-β (Aβ)-containing plaques as well as intracellular neurofibrillary tangles containing hyperphosphorylated, aggregated tau. *APOE4* carriers in AD show earlier Aβ deposition and clinical disease onset as well as faster disease progression, heavier Aβ plaque burden and increased brain atrophy compared to non-*APOE4* carriers, highlighting a prominent role of

APOE4 in AD pathogenesis [4,5]. *APOE2* carriers have later Aβ deposition, clinical onset and increased longevity relative to non-*APOE2* carriers [6]. To date, there are clues to the cause(s) of AD, but there is still a lot that is not yet well understood. Early onset familial AD, also called autosomal dominant AD, which accounts for less than 1 % of AD cases, is primarily caused by overproduction of longer forms of Aβ relative to other forms resulting from mutations in the amyloid precursor protein (*APP*) gene or genes encoding presenilin 1 (*PSEN1*) or 2 (*PSEN*) [7]. Less common forms are caused by mutations in *APP* in the coding sequence of Aβ that do not influence Aβ production but result in a more amyloidogenic form of Aβ that leads to earlier seeding. The majority of AD cases occur later in life over the age of 65 years and is therefore commonly referred to as late onset AD. Accumulating genetic and functional evidence strongly indicates an active role of the brain's innate immunity in AD pathogenesis and progression [8]. Until recently, inflammatory processes driven by microglia and astrocytes were considered merely pathological bystanders in AD. However, an abundance of evidence now suggests that these immune cells adapt a dynamic disease-associated inflammatory profile in response to pathological aggregates [9,10]. This immune response may serve as a defense mechanism that initially protects the brain by promoting tissue repair and removing both cellular debris and Aβ aggregates. Under other conditions, it may contribute to

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disease progression by sustained chronic inflammation that provokes synapse loss and neuronal cell death, as observed in AD (Fig. 1). In this review, we highlight and discuss the significance of APOE in modulating immune responses that contribute to neurodegeneration in AD.

2. APOE in the central nervous system

APOE is considered the primary apolipoprotein lipid and cholesterol transporter in the central nervous system (CNS) [11,12]. Native APOE is primarily lipidated by adenosine triphosphate-binding cassette transporter A1 (ABCA1) in the brain [13,14]. Additionally, APOE facilitates transport of lipids among different cell types by serving as a ligand for the low-density lipoprotein receptor (LDLR) and lipoprotein receptor-related protein (LRP), the two major metabolic receptors for ApoE [15]. Compared to mice that express only a single type of ApoE protein, there are three major isoforms for human *APOE* encoded by E2, E3 and E4 alleles. Human APOE includes two separate N- and C-terminal domains joined by a flexible hinge. The N-terminus includes the receptor binding regions, which differs in the APOE isoform residues (APOE4, arginine 112; APOE3 and APOE2, cysteine 112). The C-terminal domain contains the lipid-binding region (residues 244–272) [16]. The cysteine residue at 112 found in both APOE2 and APOE3 may form disulfide-linked dimers. Additionally, while APOE3 and APOE4 that have arginine at residue 158 display normal LDLR binding activity, APOE2 with cysteine at residue 158 shows impaired LDLR binding abilities and is associated with the recessive form of type III hyper-lipoproteinemia [17–20]. This suggests that the differences in one or two amino acids in the N- and C-terminal domains of APOE significantly alter their interaction and preference to bind lipid and receptor proteins involved in cholesterol uptake [21] as well as Aβ peptides [22,23]. In contrast to *APOE4*, *APOE2* confers protection against AD. Rare variants in *APOE3* with mutations in the lipid binding region [24] or lipoprotein receptor binding region [25] decrease the risk of AD to a similar extent as *APOE2*. To date, it is not clear how the function of APOE as a lipid redistributor versus other effects it has is mechanistically related to AD. Based on different literature, some of the ways that the E4 allele disease risk association with AD and other diseases may be occurring are likely via Aβ aggregation and clearance as well as by influencing neuro-inflammation, impairments in blood brain barrier integrity, synaptic plasticity, and tau hyperphosphorylation [21,26,27].

3. APOE and non-glial AD pathology

3.1. APOE and aβ pathology

In addition to being a constituent of amyloid plaques, ApoE is involved in several aspect of Aβ pathology including Aβ seeding/fibrillization, and clearance [23,28–30]. Extensive imaging and cerebrospinal fluid (CSF) biomarker studies have consistently linked *APOE4* with greater Aβ deposition, not only in AD cases, but also in individuals with mild cognitive impairment and cognitively healthy elderly [31–33]. *APOE4* dose-dependently leads to earlier onset of Aβ deposition the brain of humans and animal models and *APOE2* leads to later onset of Aβ deposition than *APOE3* [30]. A large body of evidence suggests that ApoE influences Aβ aggregation in an isoform-dependent manner, with ApoE4 most actively promoting Aβ fibril formation compared to ApoE3 and ApoE2, respectively [29,34–37]. Interestingly, the Aβ:ApoE ratio was found to reflect that found in Aβ plaques in the AD brain [36].
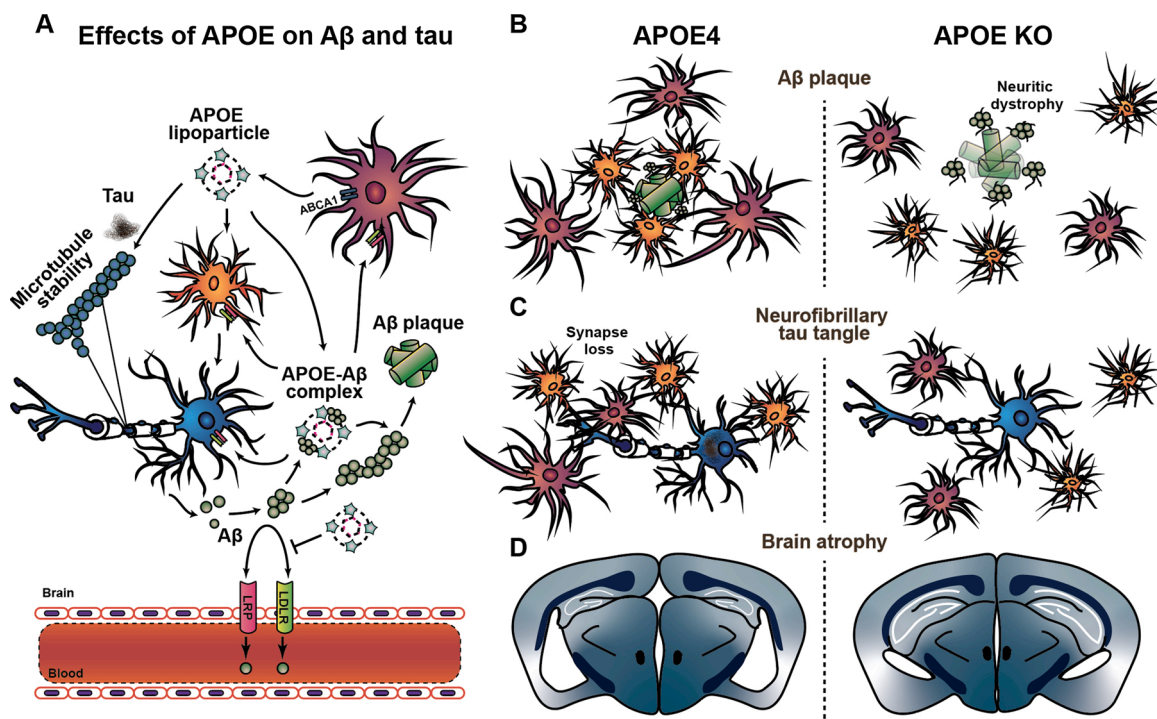

Fig. 1. ApoE4 as a risk factor for AD. A. Under physiological conditions, APOE is mainly produced and lipidated by ABCA1, and thereafter secreted into the interstitial fluid by glia. LDLR and LRP clear Aβ into neurons and glia and facilitate transport across the BBB, which is impaired by APOE4. Amyloidogenic APP processing leads to Aβ production and release from neurons into the interstitial fluid (ISF). APOE likely binds to smaller oligomeric soluble Aβ species to facilitate its fibrilization into Aβ plaques in an isoform-dependent manner. Similarly, APOE, especially E4, enhances tau pathogenesis, but whether this interaction influences tau aggregation, microtubule instability, or both, is unclear. **B.** APOE4 promotes a disease-associated microglial and astroglial reactivity to dense core Aβ plaques, where these glial cells are commonly observed clustering around plaques in attempt to phagocytose them. The absence of APOE leads to diffuse plaques with reduced peri-plaque microglial clustering as well as increased dystrophic neurites with enhanced plaque-associated tau pathology. **C.** APOE4 increases tau pathogenesis and leads to increased astroglial and microglial-mediated inflammation, with enhanced synaptic engulfment, whereas the absence of APOE in APOE KO mice blocks this glial-mediated inflammation **D.** A progressively persistent inflammatory response over time leads to significant neurodegeneration in the presence of APOE4, as marked by brain atrophy and ventricular enlargement. The absence of APOE blocks both the inflammation and the brain atrophy.

However, evidence suggests that the affinity for ApoE to bind to Aβ highly depends on ApoE lipidation state, Aβ species, as well as pH levels of the *in vitro* model system utilized [22,38–40]. Additionally, ApoE4 appears to greatly influence oligomeric Aβ stabilization [41,42], which may be essential in accelerating early seeding of Aβ pathology [43]. This is supported by *in vivo* findings where APOE isoforms were linked with an increased rate of longitudinal Aβ accumulation (E4 > E3 > E2) in amyloid-negative cognitively healthy individuals compared to amyloid-positive cases [44]. Interestingly, compared to *APOE3* carriers, cases with the rare *APOE3* V236E Jacksonville variant (APOE3-Jac) showed reduced fibrillar Aβ plaques [24] as well as a reduced risk of developing AD. Consequently, APOE3-Jac was far less prone to aggregate suggesting that changes in APOE aggregation propensity may directly influence Aβ pathology. Moreover, fibrillar amyloid deposits are observed nearly 20 years earlier in cognitively normal *APOE4* carriers, compared to *APOE4* non-carriers [45]. These studies further support a pivotal role of APOE in increasing AD risk by altering the early phase of amyloid deposition and metabolism (Fig. 1).

The effects of ApoE on Aβ deposition and aggregation *in vivo* have been widely reported using amyloid-depositing transgenic mice lacking *ApoE* expression or additionally expressing human *APOE* isoforms. Ablating murine *ApoE* expression in PDAPP or Tg2576 mouse models of cerebral amyloidosis dramatically reduces Aβ deposits compared with mice endogenously expressing *ApoE* [46–48]. This observed reduction in Aβ plaques was shown to be *ApoE* gene dose-dependent, further strengthening the relationship between *ApoE* expression and Aβ deposition. Lack of *ApoE* expression in amyloid mouse models also appears to specifically and robustly abolish the presence of fibrillar Aβ plaques in both brain parenchyma and in the form of cerebral amyloid angiopathy (CAA), suggesting that the ApoE co-deposited with Aβ plaques in mouse models as well as AD brain, may actively promote Aβ aggregation [49]. *APOE4* expression in APP transgenic mice also leads to a robust increase in fibrillar plaque burden (Fig. 1) compared to mice expressing *APOE3* or *APOE2*, respectively [28,50–52]. This increase has been confirmed in *APOE4* carriers that show an increase in both vascular and parenchymal Aβ plaques [53–56]. The exact mechanism through which APOE4 promotes Aβ deposition is not fully understood. One possible interpretation of the data presented above is an increased affinity for APOE4 to bind some form of aggregated Aβ such as oligomers may result in a shift that facilitates soluble Aβ pools to fibrillar Aβ plaques. This may in turn cause earlier disease onset and increase the rate of Aβ plaque formation by stabilizing progressively accumulating oligomeric Aβ species to enhance their neurotoxic effects. However, APOE also binds the diffuse plaques observed in Down's syndrome brains [57,58], which suggests that APOE may first bind to nascent diffuse plaques and help convert bound Aβ to β-sheet structures observed in dense cored plaques.

An alternative mechanism through which APOE influences Aβ pathology is through clearance, particularly of soluble Aβ monomers and oligomers (Fig. 1) that may otherwise further promote early stages of Aβ seeding [28,29]. APP transgenic mice lacking endogenous *ApoE* but expressing *APOE3* or *APOE4* isoform deposit less Aβ plaques compared to those expressing murine *ApoE*, suggesting that human *APOE* isoforms influence clearance [28]. It has also been shown that lipidated APOE has minimal to no binding to cell produced monomeric Aβ and that lipidated APOE competes with Aβ for the cellular update of Aβ via receptors that could slow monomeric Aβ clearance [40]. It is not yet known exactly how APOE influences Aβ clearance, though several mechanisms have been proposed including, lysosomal and enzymatic degradation [59], cellular uptake [60–62], interaction with receptors and transporters on cell surface [30,40], transport across the blood brain barrier [63], and interstitial fluid (ISF) flow [29,64].

While ApoE is understood to be largely lipidated in the brain, altering the amount of lipidated extracellular ApoE in the brain has a profound impact on Aβ fibrillization and related inflammation. For example, LDLR-deficient mice that show increased ApoE levels in the brain and cerebrospinal fluid (CSF) [51], display more thioflavine

S-positive fibrillar plaque burden in APP transgenic mice compared to non-transgenic controls [65,66]. In line with this, overexpression of LDLR in the brain of mice that develop amyloidosis not only dramatically reduced the amount of soluble ApoE by 90 percent due to enhanced LDLR-mediated degradation [67], but also inhibited plaque deposition and increased Aβ clearance [15]. Furthermore, an increase in the amount of lipidation per ApoE protein through *ABCA1* overexpression reduced Aβ deposition in PDAPP mice [68], with an opposite effect on plaque burden in *ABCA1*-deficient APP mice [14,69]. The protective effects of APOE3-Jac on reducing AD risk are also attributed to the variant's effects on enhanced APOE lipidation and lipid-loading properties [24]. Viral-mediated brain expression of APOE3-Jac decreased fibrillar Aβ deposition, plaque-associated ApoE as well as neuritic dystrophy compared to APOE3-expressing APP mice. Interestingly, introducing the V236E substitution in APOE4-expressing cells was sufficient to reduce APOE4 aggregation and increase cholesterol efflux [24], highlighting the importance of APOE structure in the effects of APOE on critical disease-related processes. Altogether, these studies suggest that APOE exhibits effects on both Aβ aggregation as well as on clearance to modulate AD pathogenesis (Fig. 1).

In contrast to APOE4-mediated effects on enhancing Aβ burden, reducing ApoE levels in the brain or increasing its lipidation state dramatically decreases plaque burden, further supporting a direct interaction between ApoE and Aβ plaque pathology in AD. Besides genetic ablation of ApoE in APP transgenic mice, immunotherapy studies using anti-ApoE antibody have shown to reduce parenchymal plaques [70–72] as well as CAA [73] burden in an antibody dose-dependent manner. Interestingly, the most effective antibody only binds to non-lipidated ApoE present in amyloid plaques and CAA and the effects require microglial-mediated phagocytosis of Aβ [71,73]. From a therapeutic perspective, it is of note that the anti-ApoE antibody did not cause an amyloid-associated microhemorrhages as occurred with an anti-Aβ antibody [73]. Similarly, knocking down ApoE levels by ~50 % in the brain with anti-sense oligonucleotides (ASO) from just after birth decreased Aβ plaque pathology [74]. However, the effect was lost if the treatment started after onset of plaque formation, affirming that ApoE plays an important role in modifying early stages of Aβ aggregation and accumulation. Of note, knocking down ApoE after plaque onset resulted in decreased neuritic dystrophy around plaques, suggesting that even though knocking down ApoE did not affect Aβ plaque accumulation, modulating ApoE after plaque onset may still influence the plaque-associated damage response in the brain. In line with this, homozygous APOE3 R136S Christchurch (APOE3ch) variant appeared to confer strong protection against developing cognitive decline in a patient with an autosomal dominant AD mutation (PSEN1-E280 carrier) in spite of high amyloid burden in the brain [25]. This particular individual only developed mild cognitive impairment three decades after the expected age of clinical onset, with limited changes to tau pathology, glucose metabolism and hippocampal atrophy compared to PSEN1-E280 carriers of a similar age. Compared to APOE3, APOE3ch impaired APOE binding to heparan sulfate proteoglycans, an interaction that is known to promote neuronal uptake of extracellular tau. Thus, while the Aβ-overproducing effects of PSEN1-E280 was not dampened or prevented by APOE3ch, the variant may potentially protect against Aβ-associated cognitive decline, as well as tau spreading and tau-mediated neurodegeneration. Whether this variant may additionally alter Aβ or tau-associated inflammatory response remains unknown.

3.2. APOE and tau pathology

A plethora of studies have thus far reported the interaction between ApoE and Aβ in AD, although until recently there has been a lack of evidence in understanding how ApoE isoforms may interact with tau or tau-mediated neurodegeneration. *APOE4* is associated with an increase in tau pathology particularly when amyloid pathology is also present [75]. However, *APOE4*-positive AD cases were recently found to have a

greater baseline Aβ and tau burden, with a higher tau accumulation rate compared to E4-negative cases [76], suggesting an Aβ-independent effect of *APOE4* on vulnerability to progressive tau accumulation in AD. A growing body of research provide novel evidence that *APOE4* is associated with increased tau pathology in the medial temporal lobe independently of age, clinical status, sex and Aβ [53,77–79]. Interestingly, *APOE* mRNA expression is highest in the medial temporal lobes [80]. Given the topographical concordance between tau pathology and neurodegeneration [81–83], these findings point to tau pathology as a possible culprit responsible for the medial temporal neurodegeneration observed in *APOE4* carriers. *APOE4* has also been closely linked to increased CSF tau levels in AD, particularly in women compared to men [84], suggesting a sex-dependent effect in *APOE4*-mediated toxicity.

Transgenic mouse models of tauopathy further support an interaction between *APOE* and tau pathology [85–89]. Using the P301S mouse model of tauopathy expressing either no ApoE or each of the human isoforms, Shi et al. reported that APOE exacerbates tau burden and the subsequent pathogenic cascade underlying neurodegeneration in an isoform-dependent fashion, independently of Aβ [85]. The presence of *APOE4* resulted in massive atrophy in the hippocampus, entorhinal cortex, piriform cortex and amygdala, with concomitant enlargement of the ventricular system (Fig. 1). In contrast, little to no injury was observed in the absence of APOE, despite the fact that the P301S mice lacking APOE still accumulated some p-tau and insoluble tau in the brain. Additionally, *APOE4* expression exacerbated tau accumulation, redistribution to neuronal cell bodies, as well as neuroinflammation, suggesting that APOE plays a pivotal role in neurodegeneration from soluble tau accumulation to brain atrophy. In support of these findings, *LDLR* overexpression in P301S tauopathy mice, which dramatically reduces ApoE levels in the brain, halved the normally observed phosphorylated tau levels, synaptic loss, and hippocampal atrophy in P301S mice [87]. Furthermore, knocking down ApoE levels similarly protected these mice from damage induced by tau in P301S mice, irrespective of Aβ. A 50 % reduction of APOE4 protein using an ASO directed against the human *APOE4* gene in P301S mice, similarly protected against tau pathology associated neurodegeneration, synaptic loss as well as neuroinflammation [88], supporting the conclusion that *APOE4* directly influences neurodegeneration via gain of toxic functions in tauopathy. A similar effect of *APOE4* on increased tau pathology, synaptic loss as well as neurodegeneration was recently reported in cerebral organoid models using induced pluripotent stem cells (iPSC) from AD patients [90]. Intriguingly, the *APOE4*-related phenotypes observed in AD patient-derived organoids were largely reversed through genome editing to *APOE3*. The mechanism through which APOE interacts with tau directly or indirectly and whether the ApoE lipidation status may influence said interaction remains to be clarified.

4. Systemic inflammation and glia-dependent effects of APOE on AD pathology

In addition to clearance or response to misfolded proteins, such as Aβ and tau, an overwhelming body of evidence demonstrate a critical role for APOE at the interface of inflammation and neurodegeneration via glial-mediated mechanisms. While ApoE is mainly produced by astrocytes and disease-associated microglia (DAM) in the CNS, in the periphery ApoE is predominantly expressed by leukocytes and hepatocytes, particularly a type of resident hepatic macrophage called Kuppfer cells [91]. Though the role of peripheral ApoE in AD is not as thoroughly investigated as in the CNS, studies suggest that the two sources and metabolism of each pool of ApoE function independently from one another [92,93]. Genetic restoration of peripheral ApoE expression in mice lacking *ApoE* expression in the brain rescues learning and memory deficits observed in global ApoE KO mice [94], suggesting that peripheral ApoE may have an effect on CNS functions through the vasculature [95,96]. Similarly to the CNS, *APOE4* is also associated with an increased inflammatory response in the periphery as evidenced by

APOE4 carriers that have increased proinflammatory IL-8 and TNFα cytokine levels after cardiopulmonary bypass surgery [97]. Furthermore, peripheral chronic low-grade inflammation in *APOE4* carriers was associated with increased risk of AD with earlier disease onset [98]. Immune-challenged *APOE4* mice peripherally injected with lipopolysaccharide (LPS) produce higher levels of proinflammatory cytokines such as TNFα, IL-6, as well as damage-inducing nitric oxide [99,100]. *APOE4* polymorphism has additionally been associated with inflammation-related metabolic disorders such as diet-induced adipose tissue inflammation [101], and more recently disease-associated changes in the gut microbiome [102]. These studies highlight the diverse effects of *APOE4* in systemic inflammation in general as well as in AD, and suggest the *APOE4* allele may contribute to AD pathology through an altered inflammatory state. An important remaining question is how APOE4 synergizes with immune cell function leading to AD-related neurodegeneration.

4.1. APOE and astrocytes

Astrocytes are required for neuronal survival and the loss of physiological astrocyte function can be a primary contributor to neurodegeneration [89]. This is not only observed in AD but also, Alexander disease and hepatic encephalopathy, where changes in expression of key astrocytic proteins such as the glutamate transporter 1 (GLT-1), aquaporin 4 (Aqp4), the glucose transporter GLUT1, and glial fibrillary acidic protein (GFAP) render astrocytes dysfunctional and unable to maintain CNS homeostasis, leading to chronic neuroinflammation and neurodegeneration [103]. Astrocytes constitute approximately 20 % of the glial cells in the brain, and as a highly heterogenous population that regulate a wide range of functions including maintenance of the BBB, synaptic function, as well as lipid and glucose metabolism. Given that astrocytes express the majority of ApoE in the brain, *APOE4* expression may influence these abilities required to support energy demanding neurons with aging.

In AD, reactive GFAP-positive astroglia are commonly found surrounding Aβ plaques as well as in brain regions with increased accumulation of aggregated, hyper-phosphorylated tau (Fig. 1). Astrocytes show dynamic plastic phenotypes such as migratory activity in response to injury as well as phagocytic and proteolytic degradation of Aβ. Adult mouse astrocytes expressing endogenous ApoE internalize and degrade human Aβ peptides effectively *in vitro* [104]. In contrast, cultured astrocytes from ApoE KO mice were unable to digest Aβ deposits, suggesting that ApoE plays an important role in Aβ clearance under these conditions [105]. In fact, *APOE* modulates Aβ burden in an isoform-dependent manner that is linked to impaired clearance of soluble Aβ in the brain ISF, with *APOE4* leading to greatest impairment compared to other *APOE* isoforms [29]. Interesting, the authors also showed that expressing human *APOE* isoforms did not affect the rates of Aβ synthesis in PDAPP mice, but rather modulated the onset of Aβ accumulation that was linked to differential regulation of Aβ clearance. These findings suggest that *APOE4* likely increases the risk for AD by both, increasing Aβ aggregation as well as impairing its clearance [106, 107]. The mechanism through which *APOE4* impairs clearance is not known, yet it is conceivable that multiple astrocytic functions may be involved. For example, astrocytic clearance of ApoE and ApoE-containing lipoproteins is regulated by LDLR and LRP1 [108], both of which facilitate Aβ uptake and clearance across the BBB (Fig. 1) [15]. Astrocytes are involved in the clearance of Aβ through the BBB via the AQP4 channels expressed in their end feet [96,104,109]. Furthermore, dysfunctional astroglia may affect levels of neprilysin and insulin-degrading enzyme levels, both of which are known to help degrade and clear Aβ [110].

The idea of astrocyte reactivity is currently under great discussion as recent evidence suggests that the all-or-none phenotype of activation may greatly vary depending on disease and disease stage-specific manner. To this end, several groups have independently identified the

existence of molecularly diverse subtypes of reactive astrocytes in response to injury, Aβ or tau pathology [85,89,111–113]. Using two different models of injury, focal cerebral ischaemia and systemic LPS injection, Zamanian et al. found that astrogliosis not only consisted of a rapid change in astrocytic function-specific genes, but also this phenotype varied according to the type of insult [111]. Interestingly, while ischaemia induced a reactive astrocyte phenotype that was considered more protective, LPS stimulation induced astroglial genes that were neurotoxic. Such differences in astroglial reactivity are now referred to different states of reactive astrogliosis [114]. In certain states of reactive astrocytes, the cells can have strongly upregulated genes related to classical complement activation pathway, such as C1q and C3, IL-1α, and TNFα, all of which detrimental to synapses. In contrast, in other states of reactive astrocytes, there is upregulation of anti-inflammatory genes that support neuronal survival and growth, and are therefore considered protective. The major damaging state of reactive astrocytes have been shown to generate neurotoxic factors that were found to mediate death of axotomized neurons [115]. A follow up of this work has shown that upon optic nerve crush in mice, long chain saturated lipids carried by ApoE and ApoJ containing lipoproteins mediate neurotoxicity through lipoapoptotic pathways that facilitate cell death [116]. This type of reactive astrocyte change appears to be found in post-mortem tissue of several neurodegenerative diseases, including AD, supporting a common mechanism through which these type of reactive astrocyte changes may promote neurodegeneration. It is not known whether an AD-specific reactive astrogliosis signature is possible given the astroglial diversity and dynamic pathologies involved in AD. Nonetheless, it opens important novel avenues into understanding astrocyte reactivity in the context of persistent inflammation that may lead to neurodegeneration. Further research is required to understand whether a more neurotoxic reactive astroglial state forms in an attempt to remove irreversibly damaged neurons, or whether a more damaging reactive astrocyte state malevolently removes healthy functioning neurons by inappropriately targeting them, similarly to that observed in chronic inflammation.

APOE4 expression in P301S tauopathy mice induces a strong activation of a more damaging related reactive astroglial signature compared to P301S mice expressing *APOE3* or no ApoE [85]. Of note, the increase in genes such as *Serping1*, *Gfap*, and *Ggta1* highly correlated with brain atrophy, suggesting these reactive astrocytes play a detrimental role in facilitating neurodegeneration. The *in vivo* findings were further supported by P301S expressing neurons co-cultured with E4 glia that displayed similar neuronal damage, compared to the protective effects seen in the absence of ApoE, highlighting that *APOE4* from astrocytes may not only increase neurodegeneration through its effect on phosphorylated tau, but also through enhancing neuroinflammation that together with tau further exacerbates neurodegeneration. As such, P301S mice lacking ApoE show minimal gliosis with little to no brain atrophy [85,89]. Similarly, reducing APOE4 levels in the brain via ASOs markedly reduces gliosis as well as tau-mediated neurodegeneration in P301S/APOE4 mice [88].

Astrocytes express very high levels of ApoE in the normal brain with little expression by microglia. However, reactive microglia express similar levels of ApoE as do astrocytes [89]. Under the *in vitro* conditions studied, it has been found that astrocyte-derived ApoE particles are significantly larger compared with ApoE-containing lipoproteins secreted by microglia, likely due to differences in lipidation [117]. This points to differential roles of ApoE based on glial origin. To distinguish the effects of astrocytic ApoE on tau-mediated neurodegeneration, Wang et al. used P301S mice and combined genetic as well as tamoxifen-based strategy to selectively modulate astrocytic APOE and found that a reduction in astrocyte-derived APOE4 over a period of 4 months, rescued the brain atrophy typically observed in P301S/APOE4 at 9.5 months of age [89]. A corresponding reduction in aggregated tau pathology was observed, specifically in females, suggesting not only a sex-dependent effect as seen in AD [84], but also an immune modulating

effect of astroglial APOE4 on neurodegeneration. Using single cell nuclei sequencing, the authors found that in the setting of tauopathy, *APOE4* expression has drastic consequences for astrocytes, as well as affects gene expression in non-astrocytic cells, such as an *APOE4*-dependent increase in Rorb-positive neurons, a distinct neuronal subpopulation that show selective vulnerability in AD [118]. Intriguingly, astrocytic ApoE removal decreased the disease-associated gene signature in not only astrocytes, but also neurons, oligodendrocytes, and microglia, strongly supporting crosstalk between glia and neurons to modulate key neurodegenerative pathways in the brain. In addition to *APOE4*-dependent protective effects on neurons, removal of astrocytic APOE4 reduced microglial engulfment PSD95-positive synapses, another key feature that contributes to AD neurodegeneration. These findings highlight that astrocyte-derived APOE4 is a major regulator of tauopathy-mediated neurodegeneration and represents a toxic gain of function.

Given the important role of APOE in glial lipid metabolism, it is conceivable that the lipidation status of APOE4 or APOE4′s effect on glial lipid metabolism is a key mediator of both, inter- and intracellular effects that contribute to neurodegeneration. In fact, lipid accumulation in glia is a pathologically defining feature of AD [119], which has been closely linked to reactive oxygen species (ROS)-induced inflammation and neurotoxicity. Recent studies using iPSC-derived astrocytes generated from *APOE4* or *APOE3* carriers have demonstrated that compared to astrocytes from *APOE3* carriers, *APOE4* promotes both, an increase in lipid droplets as well as an accumulation of unsaturated fatty acids [120]. An *APOE4*-dependent accumulation of lipid droplet further renders astrocytes incapable of supporting metabolic and synaptic functions for neurons [121]. Moreover, *APOE4* iPSC-derived glia accumulate unesterified cholesterol after excessive uptake of extracellular lipids, which triggers secretion of inflammatory chemokines and cytokines, suggesting that *APOE4* results in dysregulated cholesterol metabolism that further promotes a proinflammatory transcriptome similar to that observed in AD [122,123]. Interestingly, lack of *APOE* expression also leads to excess lipid droplet formation in a cerebral organoid model [90], and increased cholesterol ester accumulation in microglia [124]. These findings suggest that APOE-dependent cholesterol dyshomeostasis result in widespread proinflammatory responses that drive both astrocytes and microglia toward a neurodegenerative state. Future studies exploring the role of altered APOE lipidation and how APOE influences lipid metabolism in astrocytes, microglia, and oligodendrocytes may provide invaluable insights into neurodegenerative pathways.

4.2. APOE and microglia

Microglia constitute approximately 10 % of the total glial population in the healthy adult brain. Together with meningeal, choroid plexus, and perivascular macrophages, microglia compromise the brain myeloid cell population that plays a pivotal role in the CNS immune response and homeostasis. As the main innate immune cells of the CNS, microglia represent the first line of immunological defense by constantly surveying their microenvironment for signs of damage or debris allowing them to respond rapidly to focal injury or invading pathogens. Comprehensive single cell RNA sequencing analysis of AD and aging mouse models show that microglia are present in a continuum of distinct phenotypes that are highly dependent on their spatiotemporal context [9,89,112,113]. Microgliosis is commonly observed across several neurodegenerative diseases, including AD and in mouse models with AD-like pathology, where microglia are often found surrounding Aβ plaques as well as in brain regions with high tau accumulation and neurodegeneration (Fig. 1) [87,89,125–127]. These subsets of microglia upregulate specific genes during disease conditions such as *Apoe*, *Trem2*, *Clec7a*, *Cd68* and *Cst3*, and are called disease-associated microglia (DAM) [9] or microglia of neurodegenerative phenotype (MGnD) [10]. Compared to microglia in non-disease conditions, DAM downregulate homeostatic genes such as *P2ry12*, *Tmem119*, and *Cx3cr1*. Such transitioning changes in

microglial gene expression orchestrate their diverse functions involving axon growth and synaptogenesis, synaptic pruning, recognizing, and responding to abnormal events including focal injury or neuropathological insults such as proteopathic aggregates, as well as phagocytosis and apoptosis [128]. These functions are central to disease progression and modulation because chronic glial activation is a prominent feature of aging and often goes hand-in-hand with pathological protein accumulation as well as cell death, both of which constitute central characteristics of neurodegeneration.

Given that ApoE is upregulated in DAM, *APOE* isoform-dependent effects on plaque-associated microglia have yielded conflicting results [129–131], which may partially be owed to differences in biochemical composition of fibrillar plaques across different APP transgenic mouse models as an additional confounding variable in microglial response to plaques. In human iPSC-derived microglia, isogenic conversion of *APOE3* to *APOE4* transformed the microglial transcriptome phenotype to DAM, with significant overlap of microglial gene expression profile observed in human AD brain [123]. This suggests that *APOE4* may promote a DAM phenotype in different situations such as in AD. Ulrich et al. showed that lack of ApoE expression in APP/PS1 transgenic mice reduced expression of *Clec7a*, *Cst7* and *Itgax* among other microglial genes that are important for migration and chemotaxis, with a concomitant reduction in number of fibrillar plaque-associated microglia [62]. Although the removal of ApoE in this model resulted in a marked reduction of fibrillar plaques, the plaques that did form in ApoE KO mice showed increased neuritic dystrophy surrounding them. This suggests that while ApoE facilitates microglial functions such as migration to plaques to potentially phagocytose them, this microglia subset may additionally have a protective role in decreasing plaque-induced neuronal process damage. Furthermore, microglial depletion in APP transgenic mice reduces plaque-associated ApoE, with or without changes in plaque burden [126,132,133]. This discrepancy may be explained by differences in methods used to deplete microglia, the duration of microglial depletion and repopulation, as well as microglial removal before or after plaque onset. Of note, microglia depletion before plaque onset resulted in dramatically decreased parenchymal plaque burden; however, this resulted in increased CAA in the 5xFAD Tg6799 amyloid mouse model where CAA is not typically observed [132]. This suggests that ApoE-expressing microglia may be critical in early plaque deposition and may also exhibit mechanistic heterogeneity in amyloid fibril formation. Interestingly, a reduction in plaque-associated ApoE, either by genetic ablation or microglial depletion, consistently increases dystrophic neurites around plaques, illustrating an innate immune-based role of ApoE in modulating fibrillar plaques to become less neurotoxic, despite its role in promoting plaque fibrillogenesis. This may largely be due to DAM clustering around plaques essentially forming a barrier that protects against further damage [134]. However, genetic removal of murine *ApoE* from microglial using a CSF1R-Cre promoter neither altered plaque pathology, plaque-associated microgliosis nor the typical DAM signature associated with heightened inflammation [135]. Additionally, selective removal of microglial ApoE via this approach reduced neuritic dystrophy around plaques, contrary to that observed in global ApoE KO or microglia depletion. It is conceivable that murine ApoE, though to some extent comparable to APOE4, may have differential effects compared to human *APOE* isoforms. Furthermore, given the heterogeneity of microglial subset-specific functions, microglial ApoE may display CSF1R-independent interactions that may otherwise be recapitulated in a Cre-inducible model similar to that shown in astrocytes [89]. Altogether, these findings suggest that DAM may interact with other cellular sources of ApoE, likely astroglial-derived, through either uptake of secreted ApoE, or phagocytosis of plaques, which may result in changes that contribute to neuronal damage in AD.

Although removing microglia-derived ApoE did not alter changes in GFAP-positive astroglial reactivity [135], selective removal of astrocytic APOE4 in P301S tau transgenic mice considerably changed the DAM profile, in support of the notion that APOE may influence crosstalk among immune cells and neurons contributing to neurodegeneration [89]. While DAM genes were significantly upregulated in the presence of astroglial *APOE4* expression, genetic deletion of astrocytic *APOE4* decreased DAM and simultaneously attenuated microglial phagocytosis of PSD-95-positive synapses, demonstrating an important role of astrocyte-derived ApoE4 that contributes to microglia-dependent synaptic loss in tauopathy. Whether a crosstalk between cell-dependent sources of ApoE influence Aβ pathology remains to be explored.

Microglia drive APOE-dependent neurodegeneration in the presence of tau pathology [85], [86,88,89,136]. While pathological tau is directly linked to neurodegeneration, degenerating neurons as well as tau also further exacerbate microgliosis leading to a vicious cycle promoting chronic neuroinflammation. For example, *APOE4* expression in P301S mouse model of tauopathy not only increased pathological tau burden and tau-mediated neurodegeneration, but also upregulated a set of microglial genes reminiscent of DAM [85,86]. Consequently, *APOE4* expression downregulated microglial genes involved in homeostatic cell function once tau pathology was present. Of note, lack of *ApoE* expression in age-matched P301S mice rescued tau-mediated neurodegeneration and corresponding induction of DAM (Fig. 1). Furthermore, depleting microglia through selectively inhibiting colony stimulating factor 1 receptor (CSF1R), a marker crucial for microglial survival, completely blocked neurodegeneration in P301S/APOE4 mice [86] as well as blocking tau-mediated neurodegeneration in another P301S model [136], strongly supporting a role of microglia, specifically DAM, in regulating tau-mediated neurodegeneration [86,136]. Sustained inhibition of CSF1R signaling depletes a majority of microglia in the brain, though not all [132,137,138]. The remaining CSF1R-resistant microglia show a DAM-like phenotype [139], which have recently been reported to contribute to plaque-associated neuritic dystrophy [133]. Interestingly, P301S/ApoE KO mice showed increased microglial resistance to CSF1R inhibition, wherein a large proportion of the surviving microglia were CD68-negative, indicative of their homeostatic status [86]. The enhanced survival of non-activated microglia in the absence of ApoE suggests that *ApoE*-expressing DAM may be more susceptible to CSF1R signaling-mediated apoptosis. Consequently, it is tempting to speculate that a higher baseline of microglial activation contributing to DAM maybe more burned-out owing to accelerated disease progression in P301S/APOE4 mice, and as a result more vulnerable to apoptosis due to CSF1R inhibition. This suggests a bidirectional effect of tau pathology and microgliosis on neurodegeneration. A reduction in ApoE by *LDLR* overexpression [87] or ApoE4-targeting ASOs [88] show a similar decrease in microgliosis, which concurrently prevents tau-mediated neurodegeneration, supporting a role for *ApoE*-expressing activated microglia in facilitating neuronal damage. Additionally, reducing ApoE by *LDLR* overexpression does not affect tau pathology prior to DAM onset, suggesting that its protective effect on tau pathology likely occurs at a later stage by modulating or decreasing microglial reactivity under neurodegeneration. Altogether these findings indicate that microglia-derived ApoE may shift microglia to a neurodegenerative transcriptional state. However, it may be difficult to distinguish whether the observed changes on neurodegeneration is selectively due to microglial ApoE, rather than a combination of CSF1R-dependent DAM functions that contribute to neuronal dysfunction, or a reduction in total ApoE levels coming from other cells such as astrocytes that underlie these findings.

5. ApoE and other immune risk genes

Given the heterogenous nature of immune cells, it is highly likely that ApoE interacts with several other immune genes to regulate their diverse set of physiological functions, both in the presence and absence of AD pathogenesis. Recent GWAS have identified more than 30 genetic loci involved in increasing AD risk, half of which are related to immune response and microglia, affirming that the innate immune cells in the

brain, play an important role in the overall response during AD [8]. One of these genes, the triggering receptor expressed on myeloid cells 2 (*TREM2*), has shown to interact with APOE and alter inflammatory functions involving response to injury and microglial phagocytic ability [9,10]. Both *TREM2* and *APOE* directly influence DAM, Aβ and tau pathology, as well as tau-induced neurodegeneration [140]. This supports the hypothesis that the difference in AD and healthy aging may be the failure of microglia to appropriately respond to pathogenic insults contributing to neuronal injury. Other risk genes include *SPI1/PU.1*, which was recently reported as a master regulator of several AD risk genes that affect microglial development and homeostasis, including *ABCA7*, *CD33*, *MS4A4A*, *MS4A6A, CLU*, as well as *TREM2* and *APOE* [141]. Most of these genes have shown to interact with APOE to functionally modify DAM, or in regulating the homeostasis of phospholipids and cholesterol [9,142]. For example, several studies support a role for *ABCA7* and *CLU* in regulation of cholesterol and Aβ metabolism in AD pathogenesis [143–147]. Furthermore, the presence of *APOE4* in cases with either *CLU* or *ABCA7* variants enhances the risk for AD [148]. Clarifying the interactions between *APOE* and other immune risk genes will likely unravel pathomechanisms underlying AD as well as provide novel insights into refined therapeutic strategies.

6. Future directions and considerations

The role of *APOE4* in mediating AD risk is multifactorial and complex. The pleiotropic effects of the *APOE* involving a diverse array of cell types as well as cell type-specific functions must be considered for an attempt to therapeutically modulate *APOE* or related microglial and astroglial functions. In that case, is APOE a good therapeutic target and can targeting APOE mitigate AD pathology? A wide variety of therapeutic strategies reducing APOE4 in animal models with AD-like pathology appear to be beneficial in moderating parenchymal and cerebrovascular amyloid plaque and tau pathology, associated inflammation as well as tau-mediated neurodegeneration, highlighting a protective role of reducing APOE to lessen AD pathology. In support of this, the AD-associated APOE3 Jac and Christchurch variants demonstrate that functional APOE is required for full pathological and clinical development of AD. However, while reducing APOE may protect from sustained inflammation that contributes to tau-induced neurodegeneration, APOE also regulates multiple microglial and astroglial functions involving response to Aβ and tau, migration, clearance and phagocytosis, all of which play a crucial role in modulating AD pathogenesis (Fig. 1). Additionally, how different interventions will alter peripheral lipid homeostasis and vascular function would need to be determined.

The pre-clinical stage of AD begins decades before clinical symptoms, also referred to as the cellular phase of AD pathology [149]. During this phase, extensive changes occur in glial cells and vasculature, which may orchestrate subsequent neuronal deficits. Recent advances in transcriptomics have deepened our understanding of the plasticity of microglia and astrocytes, which allow them to rapidly react based on what is required of them from their immediate vicinity, and release soluble factors such as chemokines and cytokines, as a means of communication inter- and intracellularly to amplify or dampen their responses. Keren-Shaul et al. demonstrated that microglia alter their gene expression in a linear manner based on age and presence or absence of amyloid pathology, wherein APOE is required as an initial trigger [9]. A similar phenomenon is also observed with astrocytes across disease models [86,87,89,115]. This strongly suggests that microglia and astrocytes are not binary, altering their gene expression in an "on" or "off" switch, but rather co-exist in dynamic, assorted populations that simultaneously regulate varying degrees of both DAM and homeostatic genes. This likely allows the immune cells to ramp up and modulate their functions corresponding to the level of threat in the brain. Such plasticity also argues for a threshold that allows microglia and astrocytes to carry out specific functions based on the predominant set of genes

expressed. Moreover, an early stimulation of the immune system, both innate and adaptive, may induce long-lasting changes in glial response, which may dictate how microglia and astrocytes respond to stressors later with aging. Wendeln et al. demonstrated that systemic immune challenges essentially create an innate immunological memory that have lasting effects on microglial identity and function in response to amyloid pathology [150]. This study highlights that microglia in the brain are not only able to respond to emerging threats, but are also able to learn and modify their behavior and be primed in preparation for future immunological challenges, similar to adaptive immune responses.

It is conceivable that healthy glial cells are capable of dynamically altering their gene expression that allow these cells to transition between phenotypes. This may potentially prolong their life span and turnover rate as shown in the absence of disease [151,152]. However, the long-lived populations of microglia may also be primed and therefore inherently better equipped to face future challenges to mitigate AD pathology [150]. With accumulating proteopathic insults and heightened inflammation, the cellular energy demand may render these immune cells dysfunctional and unable to metabolically cope with additional stressors that eventually lead to neurodegeneration. Interestingly, microglial turnover occurs more rapidly in the hippocampus and cerebellum in mice, compared to cortex [153]. This brain region-dependent selectivity in microglia turnover correlates to higher immune surveillance and bioenergetics reported for glia residing in these compartments [154]. Given the role of *APOE* in facilitating microglial response to AD pathology, supporting cholesterol and lipid metabolism, as well as in aiding astrocytes in regulating energy homeostasis, it is tempting to speculate that *APOE* isoforms may be key to maintaining said threshold-dependent states, which may or may not be reversible based on the extent of damage. Therefore, APOE-dependent inflammation, irrespective of cellular source, is more complex than simply protective or detrimental, but rather may depend on a number of factors including, age, sex, underlying pathology as well as disease stage; a combination of which may drive chronic inflammation-induced neurodegeneration.

Declaration of Competing Interest

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