Evidence for impaired amyloid β clearance in Alzheimer’s disease

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Abstract
Alzheimer’s disease (AD) is a common neurodegenerative disease characterized by the accumulation of extracellular plaques and intracellular tangles. Recent studies support the hypothesis that the accumulation of amyloid beta (Aβ) peptide within the brain arises from an imbalance of the production and clearance of Aβ. In rare genetic forms of AD, this imbalance is often caused by increased production of Aβ. However, recent evidence indicates that, in the majority of cases of AD, Aβ clearance is impaired. Apolipoprotein E (ApoE), the dominant cholesterol and lipid carrier in the brain, is critical for Aβ catabolism. The isoform of ApoE and its degree of lipidation critically regulate the efficiency of Aβ clearance. Studies in preclinical models of AD have demonstrated that coordinately increasing levels of ApoE and its lipid transporter, ABCA1, increases the clearance of Aβ, suggesting that this pathway may be a potential therapeutic target for AD.

Introduction
Alzheimer’s disease (AD) is the most common form of dementia. It affects nearly 27 million people worldwide, and an estimated 4.6 million new cases were diagnosed this year. Nearly 60% of those afflicted live in the Western world and the majority of these individuals are over 65 [1]. The memory loss and cognitive decline that accompany AD impart a heavy burden both emotionally and financially on patients and their families. Pathologically, AD is characterized by the presence of extracellular plaques composed of aggregated amyloid beta (Aβ) and intraneuronal tangles composed of hyperphosphorylated tau. Aβ is a peptide formed by the sequential cleavage of amyloid precursor protein (APP) by β-secretase (BACE1) and γ-secretase. Evidence from genetic, biochemical, and animal model studies strongly supports the hypothesis that Aβ is a causative agent in the pathogenesis of AD [2]. There is growing evidence that impaired clearance of Aβ (specifically of the hydrophobic form, Aβ42) is responsible for the most common type of AD: sporadic or late-onset AD (LOAD). Age is the greatest overall risk factor for developing LOAD. However, the APOEε4 allele is the strongest genetic risk factor for LOAD as the ApoE4 isoform is less efficient than ApoE2 or ApoE3 at promoting Aβ clearance. In this review, in vivo evidence supporting the hypothesis that impaired clearance of Aβ contributes to the development of AD will be covered, along with the current understanding of the influence of apolipoprotein E (ApoE) and cholesterol metabolism on Aβ clearance in the central nervous system.

In vivo evidence for impaired clearance of amyloid beta in Alzheimer’s disease

In vivo microdialysis is a method used to measure levels of small diffusible proteins such as soluble Aβ in the extracellular interstitial fluid (ISF) of the brain. This technique allows direct monitoring of protein levels in ISF over time in an awake, behaving animal. Microdialysis probes are small enough to measure protein levels within specific cortical or subcortical brain regions such as the hippocampus, striatum, and amygdala. When coupled with a γ-secretase inhibitor to halt production of Aβ, microdialysis can determine the kinetics of Aβ clearance [3]. Combining microdialysis in genetic models of disease with pharmacological interventions has allowed insight into mechanisms of Aβ clearance. Aβ can be transported across the blood-brain barrier (BBB) by low-density lipoprotein receptor (LDLR) family members [4] or undergo proteolytic degradation intracellularly in microglia and astrocytes via nephrilysin and extracellularly via insulin-degrading enzyme (IDE) (for an in-depth review of Aβ-degrading enzymes, see [5]).

Microdialysis studies comparing young (3 months old) and old (12 to 15 months old) PDAPP mice found that the half-life of Aβ within the ISF is doubled in older animals, even when Aβ production was stopped by a γ-secretase inhibitor [3]. These data imply that the brain's
ability to clear Aβ diminishes with age. Hippocampal microdialysis revealed a strong correlation between the age-dependent decrease of Aβ42 in the ISF and increase of Aβ42 in the insoluble pool in APP transgenic mice [6]. Plaque growth is dependent upon high levels of Aβ in the ISF as APP/PS1 mice treated with a γ-secretase inhibitor demonstrated that even a modest decrease (~30%) of Aβ in ISF was enough to arrest plaque growth [7].

In vivo microdialysis studies determined that mice expressing the different human ApoE isoforms exhibit altered Aβ homeostasis in the ISF [8]. ApoE4 mice had higher ISF and hippocampal Aβ levels, beginning as early as 3 months of age. The half-life of Aβ was longest in ApoE4 mice (E4 > E3 > E2). Products of APP and rate of Aβ synthesis did not change between genotypes, strongly pointing to a difference in the clearance, rather than the production, of Aβ in the ApoE2, ApoE3, and ApoE4 mice.

One challenge of working with animal models based on the genetic forms of AD is determining how well pathologies correlate to the sporadic form of the human disease. An encouraging example supporting the translation of mouse models to humans is from in vivo stable isotope-labeling kinetic (SILK) experiments, which allow the determination of the rates of biosynthesis and subsequent clearance of Aβ peptides. These studies have demonstrated that the rates of synthesis and clearance are similar in normal subjects; thus, modest perturbations can result in accumulation of Aβ in the brain [9].

An important study, by Bateman and colleagues [10], demonstrated that clearance of Aβ is impaired by approximately 30% in patients with LOAD (5.6% per hour in AD versus 7.6% per hour in controls). Although the mechanism is still unknown, it is likely to reflect age-related impairment in Aβ clearance mechanisms which are influenced by APOE genotype.

Influence of apolipoprotein E genotype on amyloid clearance

Population studies have demonstrated that APOE genotype is the strongest risk factor for LOAD. Three common isoforms of ApoE, differing from each other at two amino acids, occur in humans: ApoE2 (cys112 and cys158), ApoE3 (cys112 and arg158), and ApoE4 (arg112 and arg158). Possession of one ε4 allele imparts a threefold increase in risk for LOAD and two alleles impart a 12-fold increased risk [11], whereas the ε2 allele decreases the likelihood of developing LOAD [12]. With a prevalence of about 15% in the population, the ε4 allele has been estimated to account for 50% of all AD cases [13]. The ε4 allele is also associated with an earlier age of onset [14,15] and increased Aβ deposition both in animal models of AD [8,16,17] and in human AD [18].

ApoE is the predominant apolipoprotein in the brain, where it is secreted primarily by astrocytes, but also by microglia, in high-density lipoprotein (HDL)-like particles (reviewed by Bu [19]). Lipidation of ApoE is mediated primarily by ATP-binding cassette A1 (ABCA1) and secondarily by ABCG1 [20,21], and the lipidation status of ApoE has been shown to regulate its Aβ-binding properties [22]. Direct evidence that ABCA1-mediated lipidation influences amyloid degradation has been demonstrated in multiple transgenic models of AD. Deletion or overexpression of ABCA1 results in increased or decreased Aβ deposition, respectively [23-25]. Both intracellular and extracellular degradation of Aβ is also dramatically enhanced by lipidated ApoE [26], ApoE4 is less stable [16,17] and a less effective lipid carrier under physiological conditions than ApoE3 or ApoE2 [27,28], and this probably contributes to its influence in AD pathogenesis. The effects of the various ApoE isoforms on Aβ clearance were further investigated in targeted-replacement mice expressing human ApoE isoforms at the murine locus. Aβ deposition and cognitive deficits are exacerbated in APP/ABCA1−/− targeted-replacement mice expressing ApoE4 but not ApoE3 [29].

It has been proposed that ApoE4 modulates amyloid pathology by enhancing Aβ deposition into plaques and reducing clearance of Aβ from the brain [17,30-33]. One of the first pieces of evidence linking ApoE to AD pathology was ApoE immunoreactivity in amyloid deposits and neurofibrillary tangles [34]. It has since been shown that ApoE forms complexes with Aβ, with ApoE2 and E3 binding Aβ more efficiently than E4 [35-37], and these complexes are thought to influence both seeding of fibrillar Aβ and transport of soluble Aβ. It has been shown that AD transgenic mice lacking ApoE have decreased plaque deposition and increased levels of soluble Aβ in the cerebrospinal fluid and ISF [32,38]. Crosses between AD transgenic mice and human ApoE targeted-replacement mice exhibit Aβ accumulation in an isoform-dependent manner, with greater Aβ deposition observed in ApoE4-expressing mice than those expressing E2 and E3 [8,16]. The cause of the accumulation is most likely due to the degree to which the isoforms impact Aβ clearance and deposition [8,39]. However, a recent study by Holtzman and colleagues [40] has provided new evidence that ApoE does not directly interact with ApoE to any significant extent. Instead, ApoE competes with Aβ in an isoform- and concentration-dependent manner for binding to lipoprotein receptor-related protein 1 (LRP1), and this could impact Aβ clearance by glia and across the BBB [40].

Apolipoprotein E facilitates amyloid beta clearance by proteolytic degradation

The expression of ApoE is transcriptionally regulated by ligand-activated nuclear receptors, which act broadly in
the brain to regulate lipid metabolism, inflammation, and neuroprotection. The principal type II nuclear receptors regulating ApoE expression are peroxisome proliferator-activated receptor gamma (PPARγ) and liver X receptors (LXRs) [41], which form an active transcription factor through dimerization with the retinoid X receptors (RXRs). LXR:RXR, upon binding of endogenous oxysterol ligands, promotes the expression of reverse cholesterol transport genes (ApoE and ABCA1) [21,42]. Astrocytes upregulate ApoE mRNA and protein expression in response to RXR, PPARγ, and LXR agonists, leading to the synthesis of ApoE-containing HDL particles [19,43]. There is strong evidence that the isoform of ApoE and its degree of lipiddation influence the ability of ApoE to promote Aβ proteolysis both extracellularly and intracellularly and to modulate γ-secretase activity [26,44,45].

Microglia, which play a prominent role in Aβ degradation, are influenced by ApoE. Terwel and colleagues [46] demonstrated that ApoE secreted in media from primary astrocytes treated with LXR agonists stimulated phagocytosis of Aβ in primary microglia; however, the mechanistic basis of this finding is unknown. This corroborates earlier work from Giunta and colleagues [47], who described increased microglial phagocytosis of aggregated Aβ with the addition of recombinant ApoE3. The degree of lipiddation and ApoE isoform impacts the efficiency of intracellular degradation of Aβ within microglia, and more highly lipiddated ApoE isoforms (E2 > E3 > E4) are most effective [26]. Lee and colleagues [48] recently established that the cholesterol efflux function of ApoE is responsible for accelerating the transport of Aβ to lysosomes in microglia, where it can be degraded by lysosomal proteases.

Many studies in mouse models of AD have demonstrated that treatment with LXR agonists increases levels of ApoE and ABCA1, and this is correlated with cognitive improvements and decreased Aβ deposition [26,46, 49-53]. Similarly, PPARγ activation can stimulate the degradation of Aβ [41,54]. In addition to its ability to increase ApoE and ABCA1 levels, PPARγ activation has been shown to induce the expression of the scavenger receptor CD36 on microglia, which increased the uptake of Aβ [55]. LXR agonists and PPARγ agonists have been valuable tools for elucidating the role of ApoE and mechanism of Aβ clearance in AD. Currently, therapeutic potential for LXR agonists has been limited by an unfavorable side-effect profile and inadequate BBB permeability. Therefore, bexarotene, a BBB-permeable US Food and Drug Administration-approved drug that stimulates both LXR and PPARγ pathways, has been used in AD mouse models. The RXR agonist bexarotene facilitates degradation of soluble Aβ42, in a PPARγ-, LXR-, and ApoE-dependent manner in both primary microglia and astrocytes [52]. Interestingly, the levels of IDE and nprilysin were unchanged with bexarotene treatment, suggesting that type II nuclear receptor activation may facilitate soluble Aβ42 degradation through other mechanisms. In vivo microdialysis revealed that bexarotene reduced the half-life of Aβ in APP/PS1 and C57BL/6 wild-type mice but had no effect on Aβ clearance in ApoE-null mice, and this clearly demonstrates that the bexarotene treatment increased Aβ clearance in an ApoE-dependent manner [52].

Brain to blood and peripheral clearance of amyloid beta
ApoE and ApoE receptors have also been implicated in the clearance of Aβ across the BBB. Dysfunction of the BBB is seen in both human and animal studies of AD and is linked to poor cerebral blood flow, hypoxia, and accumulation of neurotoxic molecules in the parenchyma (reviewed in [56]). The transport of Aβ across the BBB is of considerable interest because only very small, nonpolar molecules are able to passively diffuse at the BBB. Unlike in peripheral blood-organ interfaces, peptides such as Aβ along with other nutrients and large molecules must be actively transported. Therefore, the equilibrium between Aβ in the plasma and parenchymal ISF can be influenced by the ability of receptors at the BBB to transport Aβ. The existence of such an equilibrium is the basis of the ‘peripheral sink’ hypothesis of AD treatment, which emphasizes clearance of peripheral Aβ species in order to provide a vacuum or ‘sink’ which favors transport of Aβ out of the brain and into the plasma [57].

Receptor-mediated transport of Aβ from brain to periphery is mediated principally by the ApoE receptor, LRPs, and impairing LRPs function significantly decreases the clearance of Aβ from the brain [33,58]. Conversely, the receptor for advanced end glycation products (RAGE) transports Aβ in the reverse direction and contributes to Aβ accumulation at the BBB and in the parenchyma [59]. LRP1 and RAGE recognize and transport free Aβ, but the association of Aβ with ApoE influences receptor transport of Aβ. ApoE-bound Aβ is redirected from LRPs to other LDLR family members, reducing the speed of Aβ clearance at the BBB [39,60]. The isoform of ApoE further influences this process, as discussed above.

Conclusions
Growing evidence from mouse models of AD and in vivo SILK studies in humans indicates that impaired clearance of Aβ leads to the development of AD pathology. ApoE plays an important role in mediating Aβ clearance through multiple mechanisms, as depicted in Figure 1. The expression of ApoE and ABCA1 is regulated by the activation of type II nuclear hormone receptors (LXR, PPARγ, and RXR). ApoE is lipiddated predominantly by ABCA1. Lipiddated ApoE promotes the intracellular...
degradation of Aβ by enzymes like neprilysin through its cholesterol efflux function. Extracellular degradation of Aβ by IDE is more efficient in the presence of highly lipidated ApoE. Aβ can also directly bind to ApoE receptors and cross the BBB. ApoE4 is less effective than ApoE3 and ApoE2 at stimulating Aβ clearance, and this may explain, at least in part, why it is such a strong risk factor for AD. Targeting the type II nuclear receptors, such as RXRs, has shown promising therapeutic benefit in mouse models of AD. Treatment with LXR, PPARγ, and RXR agonists decreased Aβ pathology and improved cognition in various studies, supporting the hypothesis that increasing the level of lipidated ApoE may be a strong therapeutic strategy for AD.

**Figure 1. Mechanisms of amyloid beta (Aβ) clearance are mediated by apolipoprotein E (ApoE) and ATP-binding cassette A1 (ABCA1).**

Activation of nuclear hormone receptors – liver X receptor (LXR), peroxisome proliferator-activated receptor gamma (PPARγ), and retinoid X receptor (RXR) – induces the expression of ApoE and ABCA1. The lipidation of ApoE by ABCA1 stimulates the degradation of Aβ through multiple pathways: extracellular degradation by insulin-degrading enzyme (IDE) or uptake by microglial cells and subsequent lysosomal degradation. Aβ can also be cleared from the central nervous system by binding to ApoE receptors such as low-density lipoprotein receptor (LDLR) or LDLR-related protein 1 (LRP1) that mediate transport across the blood-brain barrier.

**Abbreviations**

Aβ, amyloid beta; ABCA1, ATP-binding cassette A1; AD, Alzheimer’s disease; ApoE, apolipoprotein E; APP, amyloid precursor protein; BBB, blood-brain barrier; HDL, high-density lipoprotein; IDE, insulin-degrading enzyme; ISF, interstitial fluid; LOAD, late-onset Alzheimer’s disease; LRP1, lipoprotein receptor-related protein 1; LXR, liver X receptor; PPARγ, peroxisome proliferator-activated receptor gamma; RAGE, receptor for advanced end glycation products; RXR, retinoid X receptor; SILK, stable isotope-labeling kinetics.
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