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Review

Molecular pathogenesis of apolipoprotein E-mediated amyloidosis in late-onset Alzheimer's disease

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Abstract. Apolipoprotein E (apoE) $\varepsilon 4$ allele is a genetic risk factor for late-onset familial and sporadic Alzheimer's disease (AD). In the central nervous system, apoE is secreted mainly by astrocytes as a constituent of high-density lipoproteins. A recent study using apoE knockout mice provided strong evidence that apoE promotes cerebral deposition of amyloid β protein (Aβ). However, no clear explanation of the pathogenesis of apoE-induced AD has been provided. Here we discuss two possible mechanisms by which apoE might enhance Aβ deposition. One is the intracellular pathway in which apoE is internalized by neurons and induces lysosomal accumulation of Aβ and amyloidogenic APP (amyloid precursor protein) fragments, leading to neuronal death. The other is the extracellular pathway in which apoE-containing lipoproteins are trapped by Aβ1–42 deposits mobilizing soluble Aβ peptides and consequently enlarge amyloid plaques. These two mechanisms may operate at different stages of AD pathogenesis and suggest a chaperone-like function for the apoE molecule

Key words. Apolipoprotein E (apoE); Alzheimer's disease (AD); amyloid β protein (Aβ); apoE receptor; high-density lipoprotein (HDL).

ApoE genotype is a risk factor for late-onset AD

Alzheimer's disease (AD) is a progressive, neurodegenerative disorder with the principal clinical symptom of dementia. It is characterized by three major pathological changes in the brain, particularly in cerebral cortex and hippocampus: neuronal loss, senile plaques and neurofibrillary tangles. Senile plaques consist of extracellular amyloid deposits surrounded by dystrophic neurites, activated microglia and activated astrocytes. The principal component of this amyloid is insoluble fibrils of a 40–42/43 amino acid peptide called amyloid β protein (Aβ). Neurofibrillary tangles, which accumulate in the cytoplasm of degenerating neurons, are composed of insoluble twisted filaments of abnormally phosphoryrated tau protein. Senile plaque formation is observed only in AD, Down's syndrome (trisomy 21) and normal aging, whereas neurofibrillary tangles occur widely in numerous neurodegenerative disorders that are not accompanied by amyloid deposition. In Down's syndrome, which features neuropathology indistinguishable from that of AD in younger ages, amyloid deposition has been shown to precede the appearance of neurofibrillary tangles and dementia. Thus, amyloid deposition has been thought more likely to be a cause of AD than neurofibrillary tangles.

In 1993, a striking study of apolipoprotein E (apoE) was reported by Allen Roses and his colleagues [1]. They proposed that apoE type 4 allele ($\varepsilon 4$) is a genetic risk factor for late-onset familial AD. There are three

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common alleles of the apoE gene ($\varepsilon 2$, $\varepsilon 3$ and $\varepsilon 4$) in humans. They examined apoE genes in 234 individuals from 42 families afflicted with late-onset AD and found that the proportion of affected individuals increased with the number of $\varepsilon 4$ alleles: from 20% of subjects with no copy of $\varepsilon 4$, to 47% of subjects with one copy, to 91% of subjects with two copies [1]. Furthermore, the mean age of AD onset became younger as the gene dose of $\varepsilon 4$ alleles increased: from 84.3 years in subjects with no copy of $\varepsilon 4$, to 75.5 years in subjects with one copy, to 68.4 years in subjects with two copies [1]. In another study, they showed that the $\varepsilon 4$ allele is associated with not only familial but also sporadic late-onset AD [2]. Interestingly, the apoE $\varepsilon 2$ allele, but not the $\varepsilon 4$ allele, protected against late-onset AD, as demonstrated by the same group [3]. It is known that women have a higher incidence of late-onset AD than do men. A recent study suggests that this may in part be due to the gender difference in apoE-associated risk for AD [4]. In women, $\varepsilon 4$ heterozygotes had higher risk than those without $\varepsilon 4$, whereas there was no significant difference between $\varepsilon 4$ heterozygotes and $\varepsilon 4$ homozygotes. In men, on the other hand, $\varepsilon 4$ heterozygotes had lower risk than $\varepsilon 4$ homozygotes, and there was no significant difference between $\varepsilon 4$ heterozygotes and those without $\varepsilon 4$. A direct comparison of $\varepsilon 4$ heterozygous men and women revealed a significant twofold increased risk in women.

The neuropathological relationship between apoE and AD was first demonstrated by Namba and colleagues [5]. They found that apoE immunoreactivity was associated with amyloid in both senile plaques and cerebral vessels and with neurofibrillary tangles. Wisniewski and Frangione [6] also found apoE immunoreactivity in all types of cerebral and systemic amyloid. They proposed the hypothesis that apoE, as well as amyloid P component and glucosaminoglycans, acts as a pathological molecular chaperone which induces β-pleated conformation in amyloidogenic polypeptides [6]. However, the importance of the apoE allele in the pathogenesis of AD was not recognized at that time. The apoE genotype is presently the most important genetic risk factor for late-onset AD (see [7] for a review of the role of apoE polymorphisms in late-onset AD). The apoE gene is likely responsible for the locus on chromosome 19 previously linked to late-onset familial AD [8]. A recent study revealed that apoE $\varepsilon 4$ allele also influences the pathogenesis of dementia and peripheral neuropathy in human immunodeficiency virus (HIV) infection, which elicits the inflammatory responses in the central nervous system (CNS) similar to those in AD [9]. Thus there have been many in vivo and in vitro studies of the relationship of apoE to dementia, particularly AD. Nevertheless, the mechanism by which apoE affects AD onset in an allele-specific manner is not completely known. The purpose of this review is to address the role of apoE in AD pathogenesis from the viewpoint of the 'amyloid cascade hypothesis'. For background information, a brief review of the basic role of apoE in lipid transport is initially provided.

What is the role of apoE in plasma?

ApoE is one of the apolipoproteins which are associated with lipoprotein particles and involved in lipid transport among various cells and tissues (for a review of the role of apoE in cholesterol transport, see [10]). Plasma lipoproteins are usually divided into five groups by density: chylomicrons ($d < 0.96$); very-low-density lipoproteins, VLDL ($0.96 < d < 1.006$); intermediate-density lipoproteins, IDL ($1.006 < d < 1.019$); low-density lipoproteins, LDL ($1.019 < d < 1.063$); and high-density lipoproteins, HDL ($1.063 < d < 1.21$). Chylomicrons, the largest lipoproteins synthesized in the small intestine, transport dietary cholesterol and triglyceride to the liver and peripheral tissues. A large portion of the triglycerides in chylomicrons are hydrolyzed by lipoprotein lipase in peripheral tissues. The chylomicron remnant that contains the remaining triglyceride and cholesterol binds to apoE receptor(s) in the liver and is taken up by receptor-mediated endocytosis. VLDLs, the second largest lipoproteins, are synthesized in the liver and secreted to distribute triglycerides to peripheral tissues. VLDLs are also metabolized by lipoprotein lipase into the remnant lipoprotein, IDL. The biological role of IDL is unclear. Most IDLs are further metabolized by lipase and produce the smaller cholesterol-rich lipoproteins, LDLs. LDLs, in turn, transport liver-synthesized cholesterol to peripheral tissues. In peripheral tissues, as well as in the liver, LDL binds to the LDL receptor and is taken up by endocytosis. HDLs, the smallest lipoproteins synthesized in the liver and the small intestine and also produced in the metabolic pathway including chylomicrons and VLDLs, participate in reverse cholesterol transport from peripheral tissues to the liver. Each lipoprotein contains several specific apolipoproteins, such as apoA (AI, AII and AIV), apoB, apoC (CI, CII and CIII) and apoE. ApoE is a constituent of chylomicrons, VLDLs, IDLs and a subclass of HDLs, but not LDLs. It functions as a ligand for several lipoprotein receptors, as described below.

ApoE is a 299-amino acid protein with a molecular weight of 34,000 Da (fig. 1). ApoE has multiple amphipathic α helices, enabling it to interact with hydrophobic lipid particles and provide them with the hydrophilicity required for their transport in the blood (see [11, 12] for reviews of structure-function relationships of apoE). The C terminus of apoE is predicted to be a major lipid binding region [11, 12]. Due to its

hydrophobicity, the recombinant C-terminal fragment of apoE tends to aggregate into amyloid-like fibrils [13]. However, apoE does not exist as a free form in biological fluids.

As described above, there are three common isoforms of apoE, apoE2, E3 and E4, arising from respective alleles $\varepsilon 2$, $\varepsilon 3$ and $\varepsilon 4$ at a single gene locus on chromosome 19 (19q13.2). The differences between the three isoforms are in amino acid residues at positions 112 and 158: apoE2 has cysteine residues at both positions, apoE3 has a cysteine at position 112 and an arginine at position 158, whereas apoE4 has arginine residues at both positions. The region of apoE involved in receptor interaction is in the vicinity of residues 136–160, which is rich in basic amino acids, with 10 residues being either lysine or arginine (or histidine) [11]. The amino acid substitutions in apoE isoforms affect their binding to receptors. ApoE4 has binding affinity comparable to that of apoE3, whereas apoE2 exhibits much less binding affinity than other isoforms [11]. The defective binding of apoE2 to receptors is associated with type III hyperlipoproteinemia [11]. The genotypic frequencies for Caucasian samples are approximately 0.5% for $\varepsilon 2/2$, 11% for $\varepsilon 3/2$, 59% for $\varepsilon 3/3$, 2% for $\varepsilon 4/2$, 25% for $\varepsilon 4/3$ and 3% for $\varepsilon 4/4$ [7]. For Japanese, the corresponding frequencies are 0.3%, 6.1%, 71.9%, 0.7%, 19.3% and 1.7% [14]. Although there is a significant heterogeneity among various populations in genotypic frequencies [15, 16], the most common isoform is apoE3, whereas the frequency of apoE2 is low.

HDL is the carrier for apoE in the CNS

Not all lipoproteins found in plasma participate in lipid transport in the CNS. HDL is the sole lipoprotein known to be present in cerebrospinal fluid (CSF) [17]. Brain HDL, similar to plasma HDL, contains apoE and apoAI, although these two apolipoproteins exist largely on different lipoprotein particles [17]. Recently, novel large lipoprotein particles with a density of 1.006–1.060 g/ml, which corresponds to plasma LDL and HDL subfraction 1, were identified in CSF [18]. Unlike plasma LDL, these lipoproteins contained apoE but not apoAI or apoB [18]. Since there is no evidence of lipoprotein passage through the blood-brain barrier, the lipoproteins present in the brain must be synthesized in situ in the CNS. Most apolipoproteins other than apoB have been shown to be present in CSF, although only a few of them have been proven to be synthesized in the brain (see [19] for a review of apolipoproteins in the CNS). ApoE has been shown to be synthesized and secreted by glial cells, particularly astrocytes, but not by neurons [20]. In addition, primary cultures of rat astrocytes have been demonstrated to secrete discoidal lipoprotein particles similar to nascent HDL in plasma [21]. These findings imply that apoE-containing HDL in the brain is produced mainly by astrocytes and serves as a principal cholesterol transporter in the CNS.

To date, five major receptors for apoE have been identified: the LDL receptor, the LDL receptor-related protein (LRP), the VLDL receptor, apoE receptor 2 (apoER2) and gp330/megalin (see [19] for a review of

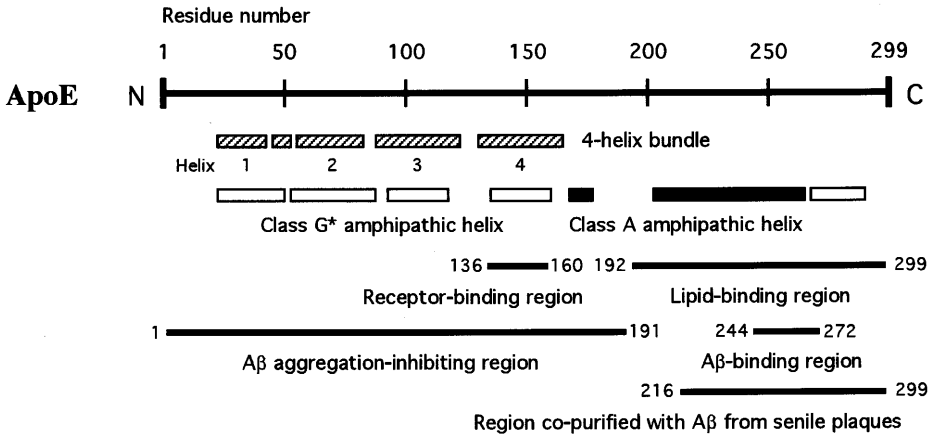

Figure 1. Functional domains of apoE. The structures of class A and class G* amphipathic helices, defined by Segrest et al. [12], are characteristic of apolipoproteins and globular proteins, respectively. Four of the five helices (helices 1–4) in the N-terminal half are arranged in an antiparallel four-helix bundle in solution [11]. In the presence of lipid, the bundle opens without disrupting α helices, exposing the hydrophobic core of the bundle and making it available to interact with lipid [11]. The regions involved in receptor binding (residues 136–160) [11], lipid binding (residues 192–299) [11, 12], Aβ binding (residues 244–272) [66], Aβ aggregation inhibition (residues 1–191) [71] and the region copurified with Aβ from senile plaques (residues 216–299) [13] are represented. Modified from Segrest et al. [12].

lipoprotein receptors in the CNS). The LDL receptor was initially identified as the apoB receptor. It is expressed in various tissues including the brain [22], where it is located primarily in astrocytes, but to only a small extent in neurons [23]. LRP is the α_2-macroglobulin receptor, and is thought to be the remnant receptor in liver [24]. In the brain, LRP is located in neurons and reactive astrocytes [23]. The VLDL receptor and apoER2 are apoE-specific receptors. The VLDL receptor is expressed in heart, muscle, adipose tissue and brain, but not in liver [25]. It is found in neurons and microglia in the brain [26]. ApoER2 is predominantly expressed in the brain, whereas its cellular localization is not yet known [27]. Gp330/megalin has been suggested to be an apoJ receptor [28], and has been shown to be expressed in a restricted group of epithelial cells [29]. Its expression in neurons was also demonstrated [30]. All of these receptors are members of the so-called 'LDL receptor family', which exhibit structural and functional similarities.

The cholesterol transport by apoE has been thought to be important for neuronal repair and neurite outgrowth. In the peripheral nervous system, pronounced production and accumulation of apoE are observed in response to nerve injury [31, 32]. ApoE is thought to be synthesized by nonneuronal cells, such as phagocytic cells, for the purpose of mobilization and reutilization of lipids in the repair, growth and maintenance of myelin and axonal membranes [31, 32]. In apoE-deficient mice, however, peripheral nerve regeneration following sciatic nerve crush occurred as well as in control animals, indicating that apoE is effective in but not essential for peripheral nerve repair [33]. In the CNS, on the other hand, the ability of synapses to regenerate after entorhinal cortex lesions was retarded in apoE knockout mice [34]. Intracerebral injection of apoE partially restored synaptic repair in these mice [35]. These findings imply that apoE plays an important role in the repair and maintenance of synapses in the CNS (see [36] for a review of the role of apoE in the CNS response to injury). In mixed cultures of fetal rabbit dorsal root ganglion cells, incubation with β-migrating VLDL (β-VLDL), which is rich in apoE and cholesterol, increased neurite outgrowth and branching [37]. Unesterified cholesterol alone had a similar, but less pronounced, effect [37]. Taken together with the finding that these cells expressed several apoE receptors [37], these results suggest that apoE stimulates neurite outgrowth and branching by receptor-mediated delivery of cholesterol.

ApoE and Aβ in the pathogenesis of AD

Aβ is produced from its larger precursor, termed amyloid precursor protein (APP) (for a review of the cell biology of APP, see [38]). APP is a glycoprotein with a single transmembrane domain and is expressed in both neural and nonneural tissues. The physiological role of APP is not well understood. Aβ is constitutively secreted as a soluble form during normal cellular metabolism and is detected in CSF and plasma of normal individuals as well as AD patients. There are two general pathways for APP processing. In the α pathway, APP is cleaved within the Aβ sequence by unknown enzyme(s) designated 'α-secretase'. In the β pathway, APP is sequentially cleaved at different sites in the extracellular and transmembrane domains by the uncharacterized 'β-secretase' and 'γ-secretase', respectively. Aβ is generated in the β pathway, but not in the α pathway. The predominant form of Aβ in normal metabolism is the 40-amino acid peptide (Aβ1–40).

The 'amyloid cascade hypothesis' is at present a dominant hypothesis concerning the pathogenesis of AD. This hypothesis is primarily supported by the finding by Yankner and colleagues [39] that exogenous addition of Aβ peptides has neurotoxic effects on cultured neurons. A number of studies using synthetic peptides have shown that the neurotoxicity of Aβ is dependent on its aggregation state (see [40, 41] for reviews of Aβ neurotoxicity). 'Aged' (i.e. previously incubated in solution), insoluble and aggregated types of Aβ are neurotoxic, whereas freshly prepared, soluble and monomeric types of Aβ are not neurotoxic (e.g. see [42]). More precisely, Aβ neurotoxicity is thought probably to require the assembly of Aβ into amyloid fibrils [43] that are closely associated with β-sheet conformation.

The length of the hydrophobic C terminus of Aβ is critical in determining the rate of aggregation. For example, Aβ1–42 aggregates more rapidly than Aβ1–40. Jarret and Lansbury [44] showed that the process of Aβ aggregation is a nucleation-dependent polymerization which can be accelerated by adding preformed aggregates as a seed. They proposed the so-called seeding hypothesis that Aβ1–42, which may be an abnormal species produced in AD, acts as an in vivo seed for amyloid formation by Aβ1–40 [44]. This hypothesis was supported by the finding that the Aβ species deposited in AD brain was predominantly the peptide ending at residue 42 (Aβ1–42), normally a minor product [45]. Furthermore, recent studies of familial AD also support this hypothesis; all mutations in the genes of APP (chromosome 21), presenilin 1 (chromosome 14) and presenilin 2 (chromosome 1) causing early-onset familial AD have been shown to alter APP processing to produce more Aβ1–42 (see [46] for a review of the effects of genetic mutations of APP and presenilins on Aβ production).

Aggregation of Aβ has been shown to be enhanced by oxidation of Aβ [47] and the presence of metal ions, such as Zn^{2+} [48]. It was also demonstrated that Aβ

spontaneously fragments and generates free radical peptides, which may react with one another to form aggregates and may attack nerve cell membranes [49]. Aggregated Aβ induces intracellular accumulation of reactive oxygen species [50] and disruption of cellular Ca^{2+} homeostasis [51] in cultured neurons. The former effect is thought to cause oxidative damage of neurons probably via hydroxyl radical generation [50]. In addition, Aβ activates microglia [52], which are mobilized in pathological lesions and may play a central role in inflammatory processes frequently associated with amyloid plaques. Microglia have been shown to internalize microaggregates of Aβ via their scavenger receptors [53, 54]. Thus Aβ, particularly Aβ1–42, is believed to lead to the cascade of numerous pathological changes, including amyloid deposition, neuronal degeneration and cerebral inflammation in AD. This is the amyloid cascade hypothesis.

By analogy with the genetic mutations in early-onset AD, it seems reasonable to suppose that the apoE ε4 allele affects APP processing, resulting in increased production of Aβ1–42. The effects of all three apoE alleles on the processing of APP and secretion of Aβ were therefore examined in cell systems that constitutively secrete both apoE and Aβ at concentrations similar to those in human CSF [55]. However, no consistent allele-specific effects of apoE on APP processing were detected in either neural (HS683 human glioma) or nonneural (Chinese hamster ovary) cells [55]. Using immunohistochemistry with antibodies specific to Aβ1–40 or Aβ1–42, Gearing and colleagues [56] found that the increased plaque frequency observed with ε4 genotypes is largely due to an increase in Aβ1–40-positive plaques but not to Aβ1–42-positive plaques. This finding was further confirmed by another histochemical study [57]. Ishii and colleagues [58] also found using enzyme-linked immunosorbent assay (ELISA) that cortical levels of Aβ1–40 were significantly correlated with dosage of ε4 allele, whereas levels of Aβ1–42 exhibited no significant association with apoE genotype (fig. 2). These findings indicate that the apoE ε4 allele does not affect Aβ1–42 production, unlike APP and presenilins 1 and 2, but may contribute to AD pathogenesis by facilitating Aβ1–40 deposition onto Aβ1–42-seeded plaques.

Recently, both purified and native apoE were shown to protect cultured cells from cytotoxicity induced by hydrogen peroxide and Aβ25–35 peptide, in the order E2 > E3 > E4 [59]. In addition, all apoE isoforms equivalently bound to metal ions, including Cu^{2+}, Fe^{2+}, Fe^{3+} and Zn^{2+}, but not Al^{3+} [59]. This antioxidant activity of apoE may contribute to the protection of Aβ peptides from oxidation-induced and metal-catalyzed aggregation and of the cells from aggregated Aβ-induced oxidative damage. Due to its lower antioxidant activity, apoE4 may be less effective in protecting neurons than apoE2 or apoE3, and thereby play a role in AD pathogenesis.

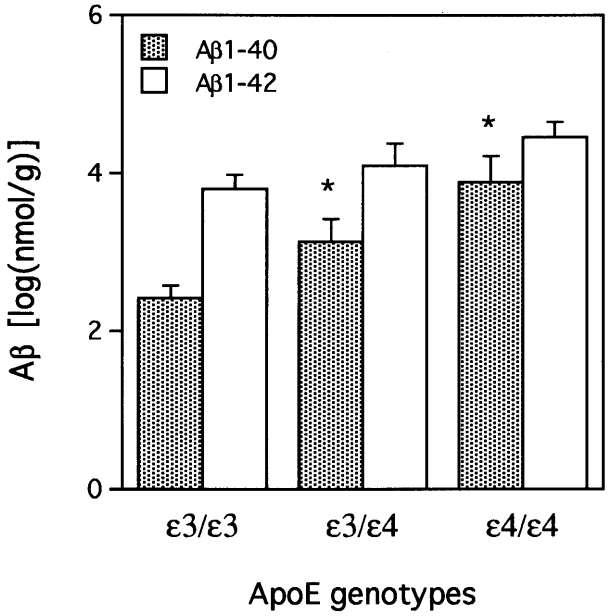

Figure 2. Effect of apoE ε4 allele on the amounts of Aβ1–40 and Aβ1–42 in cerebral cortex in sporadic AD. The cortical levels of each Aβ species were determined by ELISA in 36 cases of sporadic AD. Values represent the mean $\pm$ SE in logarithmic scale; $n = 20$ for $\varepsilon3/\varepsilon3$, $n = 12$ for $\varepsilon3/\varepsilon4$ and $n = 4$ for $\varepsilon4/\varepsilon4$. $^*P < 0.05$ versus $\varepsilon3/\varepsilon3$. Modified from Ishii et al. [58].

As described, apoE is involved in neuronal repair and neurite outgrowth. Nathan et al. [60] demonstrated that human apoE3 and apoE4 have different effects on neurite outgrowth of rabbit dorsal root ganglion neurons. Human apoE3 with rabbit β-VLDL increased neurite extension and decreased neurite branching, whereas human apoE4 with β-VLDL reduced both neurite extension and branching. Neither apoE3 nor apoE4 affected neurite branching or extension in the absence of β-VLDL. Similar findings were obtained using HDL particles derived from bovine plasma and CSF [61]. In cultures of a brain-derived mouse neuronal cell line, apoE3-enriched HDL significantly enhanced neurite outgrowth, compared with apoE4-enriched HDL [61]. The isoform-specific effect of apoE on neurite outgrowth was confirmed in animal experiments. Transgenic mice were generated in which human apoE3 and apoE4 expression is under control of the astrocyte-specific glial fibrillary acidic protein promoter and were then bred back to apoE knockout mice [62]. When astrocytes derived from these mice were used as feeder cells, primary hippocampal neurons exhibited more neurite outgrowth in the presence of apoE3-secreting astrocytes than apoE4-secreting or apoE knockout as-

trocytes [62]. In another study using the neuron-specific enolase promoter to express human apoE3 or apoE4 in neurons of transgenic mice lacking endogenous mouse apoE, apoE4 mice exhibited impairments in learning a water maze task and in vertical exploratory behavior, compared with apoE3 mice [63]. These defects induced by apoE4 were increased with age and were observed primarily in females [63]. Since neuronal degeneration progressively occurs in AD brain, the impairment by apoE4 of neuronal repair and neurite outgrowth may result in accelerated neuronal loss in AD patients with the $\varepsilon 4$ allele. In fact, neuronal reorganization was shown to be severely affected in AD patients carrying the apoE $\varepsilon 4$ allele [64].

Does apoE participate in Aβ clearance or deposition?

Since soluble Aβ was found to be constitutively produced in normal individuals as well as AD patients, apoE appears to be one of the molecular chaperones changing normal soluble Aβ to pathological insoluble Aβ [6]. Strittmatter and colleagues [65] reported that native, nonpurified apoE in CSF bound immobilized synthetic Aβ1–40 peptide. Furthermore, when purified apoE was incubated with soluble Aβ, a complex was found to be formed in an SDS-stable manner [66]. ApoE4 complexed with Aβ more rapidly than apoE3 [66]. This binding required residues 12–28 of Aβ peptide and residues 244–272 of apoE [66]. Moreover, subjects with $\varepsilon 4$ alleles exhibited greater Aβ staining in their brains at autopsy than other AD patients [67]. Following these studies, several groups reported that purified apoE accelerated Aβ fibril formation in vitro [68–70]. ApoE4 was more efficient than apoE3 in promoting assembly of Aβ1–40 or Aβ1–42 peptide into fibrils [68–70]. However, the effect of apoE on Aβ fibril formation has been controversial [71–73]. For example, Evans and colleagues [71] found that both purified apoE3 and apoE4 inhibited Aβ nucleation and subsequent fibril formation. This inhibition was attributed to the N-terminal 191 amino acids [71]. As a monomer, apoE3 was equipotent or slightly more potent than apoE4. On the other hand, apoE3 dimer was significantly more potent than apoE4 monomer, which is unable to form a disulfide dimer [71].

Notably, most of the above experiments used purified, nonnative forms of apoE. LaDu and colleagues [74] reported that unpurified apoE3 from tissue culture medium bound Aβ1–40 peptide with greater avidity than apoE4. The preferential binding of Aβ to apoE3 was attenuated with purification [75]. ApoE2/Aβ complex formation was comparable to apoE3/Aβ complex formation in both native and purified preparations of apoE [76]. In primary cultures of rat hippocampal pyramidal neurons, native apoE3 produced by transfected cells prevented the toxicity induced by Aβ1–40 or Aβ25–35, whereas apoE4 alone was toxic in cultures of these neurons [77]. Analysis of culture medium revealed that SDS-stable apoE3/Aβ complex was present in greater abundance than apoE4/Aβ complex [77].

In vitro formation of Aβ/apoE complex implies that Aβ is associated with HDL particles in the brain, because apoE exists as a constituent of HDL in CSF. Indeed, in human CSF, endogenous soluble Aβ was shown to be bound to HDL fraction [78]. The main species of Aβ associated with HDL was Aβ1–40 [78]. In an in vitro experiment with labeled Aβ1–40 or Aβ1–42 peptide, the majority (about 89%) of Aβ peptide added to human plasma was bound to serum albumin in a somewhat nonspecific manner, whereas up to 5% of Aβ peptide was bound to VLDL, LDL and HDL particles [79]. Very little Aβ peptide exists in a free form in serum and CSF [79]. The Aβ distribution in plasma is not significantly influenced by apoE genotype [79]. These observations suggest that Aβ normally exists in a bound form in biological fluids and that apoE (and potentially albumin) may function as a sequestering protein for secreted Aβ.

Thus, Rebeck and colleagues [23] proposed a mechanism of Aβ clearance mediated by apoE. They hypothesized that apoE produced from astrocytes would bind to soluble or already deposited Aβ and that the apoE/Aβ complexes would be taken up by neurons and activated astrocytes through LRP, one of the apoE receptors [23]. This clearance mechanism may play a role in the protection of cells from toxicity induced by extracellular Aβ. If that is the case, the disturbance of interaction between apoE and its receptors may lead to Aβ cytotoxicity. Native preparations of apoE3 added to primary cultures of rat hippocampal pyramidal neurons prevented the toxicity induced by Aβ1–40 or Aβ25–35, and this protective effect of apoE3 was abolished in the presence of receptor-associated protein (RAP), an inhibitor of apoE receptors [77]. The concentration of apoE in brains of AD patients with $\varepsilon 4$ allele was shown to be lower than that in AD patients with $\varepsilon 3$ allele [80]. Rebeck and colleagues [23] postulated that apoE4 may have decreased ability to clear Aβ from the neuropil, due to altered apoE-Aβ interaction or lower levels of apoE. ApoE-mediated Aβ clearance was also observed in smooth muscle cells [81]. Labeled Aβ1–40 and Aβ1–42 peptides added to primary cultures of human and canine smooth muscle cells were found to be rapidly internalized and appeared within endosomal and lysosomal vesicles [81]. The internalization of Aβ was inhibited by cycloheximide, brefeldin A, pretreatment with trypsin, lipoprotein-deficient serum or RAP, suggesting that this internalization occurs by lipoprotein receptor-mediated endocytosis [81]. This was

confirmed by colocalization of cell surface-bound or internalized Aβ with apoE and LRP [81].

If apoE functions in clearance of Aβ, the lack of apoE should result in increased extracellular Aβ deposition. Bales and colleagues [82] crossed apoE knockout mice with transgenic mice overexpressing a human mutant APP gene (V717F; PDAPP). At 6 months of age, PDAPP$+/+$; apoE$+/+$ mice had numerous Aβ deposits as visualized immunohistochemically. In dramatic contrast to these transgenic mice, bigenic PDAPP$+/+$; apoE$-/-$ mice exhibited only sparse Aβ deposits. These deposits had the characteristics of diffuse plaques. PDAPP$+/+$; apoE$+/-$ mice had reduced, essentially intermediate levels of Aβ deposits. Neither clear decrease in expression of the PDAPP transgene nor reduced total Aβ or Aβ1–42 level in hippocampus or cerebral cortex was observed in these mice. These findings strongly suggest that apoE acts as a pathological chaperone in Aβ deposition in vivo.

Hypothetical mechanisms of apoE-mediated Aβ deposition

The above findings for apoE knockout mice do not, however, rule out the possibility that apoE is involved in Aβ clearance. Internalized HDL particles containing apoE/Aβ complexes are thought to be delipidated in the endosomal/lysosomal pathway. ApoE and Aβ are then degraded in lysosomes. However, it was found that the internalized Aβ was not completely degraded. In an experiment using cultured human neuroblastoma cells, most Aβ peptides were degraded after uptake, but small amounts of peptide were accumulated in insoluble fractions of the cells and remained stable for several days [83]. This may occur by the formation of apoE/Aβ complexes. Purified apoE was shown to bind to Aβ with its hydrophobic C-terminal region (residues 244–272) in vitro [66]. Furthermore, a C-terminal fragment of apoE (residues 216–299) was shown to aggregate into amyloid-like fibrils in vitro and was copurified with Aβ from senile plaques [13]. These findings led us to speculate that apoE/Aβ complexes are somewhat resistant to lysosomal enzymes, resulting in intracellular aggregation/accumulation of Aβ peptides and C-terminal fragments of apoE. Since it was shown that purified apoE4 bound to Aβ more avidly than apoE3 [66], delipidated apoE4 may be more effective than apoE3 in forming protease-resistant complexes with Aβ. In support of this hypothesis, apoE was shown to enhance accumulation of Aβ in cultured vascular smooth muscle cells [84]. ApoE3 induced accumulation of Aβ in a higher percentage of cells than did apoE4, but the deposits induced by apoE4 were more stable [84]. Only cells containing Aβ deposits induced by apoE4 exhibited decreased cellular redox activity [84]. In AD brain, intracellular accumulation of apoE was correlated with intracellular Aβ immunoreactivity within the same cytoplasmic granules and with high expression of gp330/megalin, one of the apoE receptors [29]. DNA fragmentation was restricted to cells with intracellular Aβ immunoreactivity, but was not associated with extracellular Aβ deposition [30].

It is likely that the presence of Aβ1–42 facilitates intracellular aggregation/accumulation of Aβ and apoE fragments, resulting in seeding for subsequent intracellular Aβ1–40 accumulation. In cultures of cells stably expressing APP, internalized Aβ1–42 aggregates induced intracellular accumulation of amyloidogenic C-terminal fragments of APP [85]. This abnormal accumulation of amyloidogenic C-terminal fragments of APP has been claimed to cause neuronal degeneration. These observations suggest the presence of a novel cascade of Aβ deposition. That is, internalized apoE induces intracellular accumulation of Aβ by inhibiting lysosomal enzymes and thereby alters APP metabolism, causing neuronal degeneration. Once neurons die, intracellular Aβ aggregates associated with apoE become extracellular deposits (tentatively referred to as the 'lysosomal accumulation pathway' of apoE-mediated Aβ deposition; see fig. 3A).

Another possible pathway of apoE-mediated Aβ deposition is also considered. It is known that the V717F mutation of APP leads to production of more Aβ1–42 and causes early-onset AD [46]. The diffuse plaques in bigenic PDAPP$+/+$; apoE$-/-$ mice are composed of Aβ1–42 and appear to be ready to act as seeds for further Aβ deposition. Purified apoE was shown to avidly bind to Aβ in senile plaques in sections of AD brain [86]. ApoE-containing HDL should also bind to Aβ1–42 deposits to clear them. However, because Aβ1–42 has a strong tendency to aggregate, HDL will be trapped by the plaques instead of removing Aβ peptides. The trapped HDL probably mobilizes soluble Aβ1–40 and Aβ1–42 peptides to the plaques through apoE-Aβ interaction. The mobilized Aβ peptides are utilized for the growth of the plaques. Cholesterol within the trapped HDL may be removed by other, probably nascent HDL particles that contain little core lipid [21]. Aβ deposition is thus accelerated by the presence of apoE-containing HDL, and apoE is incorporated into amyloid fibrils (tentatively referred to as the 'plaque-trapping pathway' of apoE-mediated Aβ deposition; see fig. 3B). The finding that amyloid-associated apoE purified from AD brain exists not as free molecules but as stable complexes with Aβ fibrils [87] appears to be consistent with this hypothesis. On the other hand, immunoreactivity to apoAI, another constituent of HDL, was observed only occasionally in senile plaques [88]. This finding is not inconsistent with

our hypothesis, since apoE and apoAI have been shown to be contained primarily in separate populations of lipoproteins in CSF [17].

There are two possible explanations for how apoE genotype affects Aβ deposition in this pathway. The growth of amyloid plaques by deposition of Aβ pep-

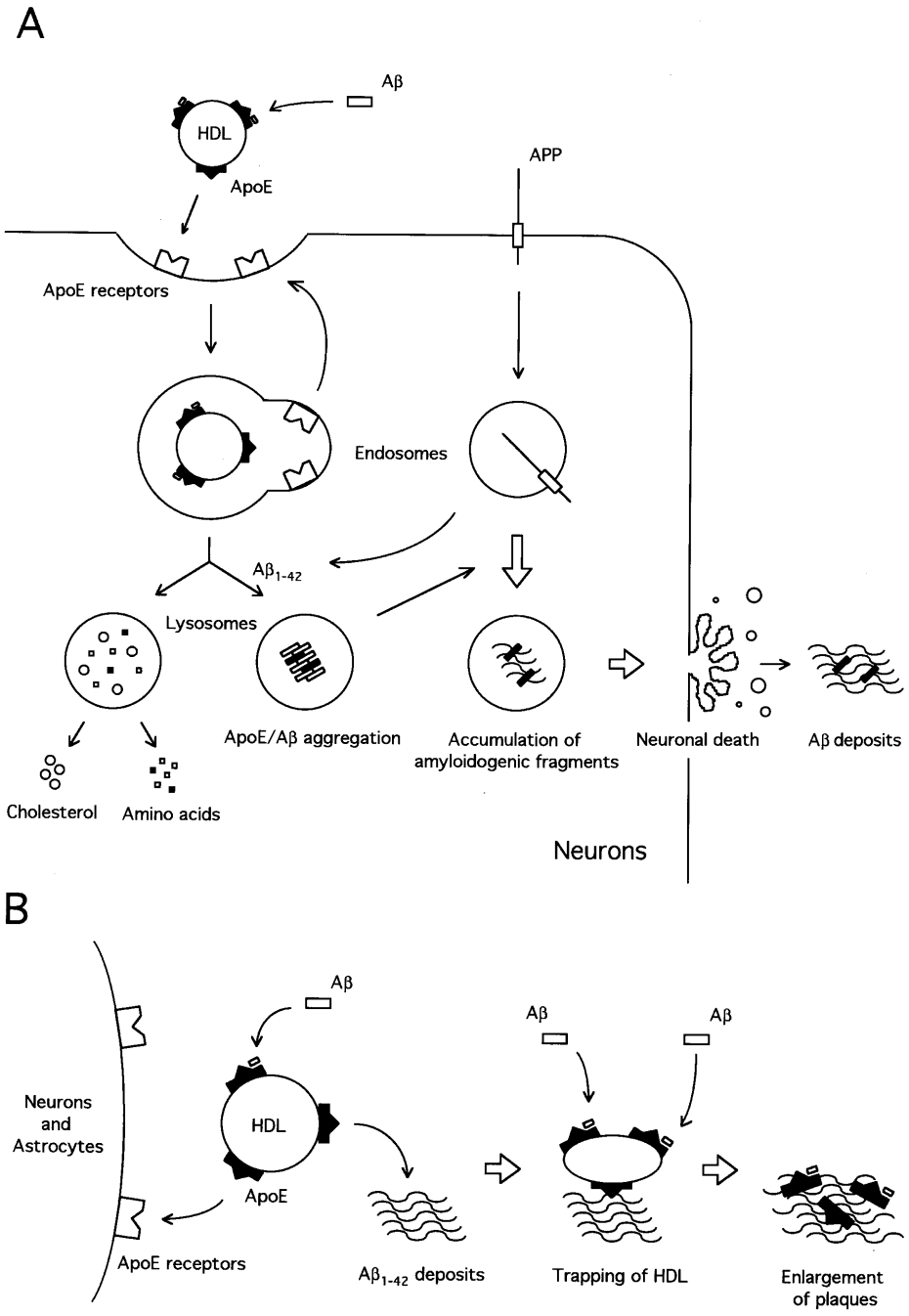

Figure 3. Models of apoE-mediated Aβ deposition. (*A*) The lysosomal accumulation pathway. ApoE-containing HDL is internalized by neurons by receptor-mediated endocytosis. ApoE/Aβ complexes may be somewhat resistant to lysosomal enzymes and thereby aggregate and accumulate intracellularly. This accumulation causes altered APP metabolism and subsequent neuronal degeneration. Once neurons die, intracellular Aβ aggregates become extracellular deposits. (*B*) The plaque-trapping pathway. ApoE-containing HDL binds to extracellular Aβ1–42 deposits to clear them, but is instead trapped by the plaques. The trapped HDL mobilizes soluble Aβ peptides (mainly Aβ1–40) to the plaques via apoE/Aβ interaction, resulting in enhanced deposition of Aβ.

tides has been shown to be reversible in vitro [89]. The avid binding of apoE to Aβ in plaques may shift an equilibrium between Aβ in plaques and in solution to the direction of enlargement of plaques. Since the avidity of purified apoE4 for Aβ was shown to be higher than that of apoE3 [66], plaques containing delipidated apoE4 may grow faster than those containing apoE3. Alternatively, the difference between apoE isoforms in their antioxidant activity may cause their different effect on Aβ deposition. As described previously, oxidation of Aβ has been shown to promote Aβ aggregation [47]. The growth of plaques by deposition of mobilized Aβ may also be enhanced by oxidation, which may occur more easily in the presence of apoE4 than in the presence of apoE3, because the antioxidant activity of apoE4 was shown to be lower than that of apoE3 [59].

In our hypothesis, even if a certain amount of Aβ1–42 deposits as a seed, soluble Aβ will not follow these Aβ1–42 peptides in the absence of apoE, resulting in virtually no enlargement of amyloid deposition. This accounts for the marked reduction of Aβ deposits in apoE knockout mice. Our hypothesis appears to agree with the finding that the effect of apoE genotype is mainly on the deposition of Aβ1–40 rather than that of Aβ1–42 [56–58], because the main species of soluble Aβ is Aβ1–40. The promotion of Aβ deposition by apoE should not be displayed in the absence of seed formation by Aβ1–42. Seed formation should be retarded with a low level of Aβ1–42 in individuals who have no genetic mutations, which may explain why apoE genotype is associated with the late-onset of AD.

These two mechanisms appear to be mutually exclusive, but both are consistent with previous findings, as described above. The lysosomal accumulation and plaque-trapping pathways may play roles in the early and late stages of AD pathogenesis, respectively.

Finally, the isoform-specific interactions of apoE with some specific proteins other than Aβ, such as tau [90] and α_2-macroglobulin [91], may also be involved in the pathogenesis of AD. Furthermore, the findings of receptor-mediated endocytosis of Aβ complexed with apoJ [92] or α_2-macroglobulin [93] suggest another pathway of Aβ clearance and possible involvement of the latter two proteins in AD pathogenesis. In fact, a recent study suggests a possible association of a polymorphism of α_2-macroglobulin gene with late-onset AD [94]. Of five major apoE receptors in the brain, LRP and gp330/megalin have been shown to be involved in Aβ metabolism, as described above. In addition, an association between a polymorphism of LRP gene and late-onset AD has been observed [95]. The VLDL receptor was also reported to be genetically associated with sporadic AD in Japanese samples [96], whereas no clear association with AD has been detected for the VLDL receptor and the LDL receptor in Caucasian samples [97]. It is not clear how these polymorphisms influence the pathogenesis of AD. Recently it was reported that one of the polymorphisms in the regulatory region of apoE gene was associated with AD and that this association was independent of ε4 allele [98]. In vitro studies suggest that this polymorphism may increase risk for AD by altering the level of apoE expression [98]. The possibility of altered expression of apoE receptors in AD brain should be further evaluated, in addition to the two mechanisms of apoE-mediated Aβ deposition discussed in this review.

Note added in proof. After this review was submitted, it was reported by Holtzman et al. [99] that in contrast to effects of mouse apoE, human apoE3 and apoE4 markedly suppressed early Aβ deposition in $APP^{V717F+/-}$ transgenic mice, even when compared with mice lacking apoE. Their findings suggest that human apoE isoforms have higher activity than mouse apoE in prevention of Aβ aggregation or in Aβ clearance. In AD brain, there may be some factor(s) to facilitate the formation of seed deposits of Aβ even in the presence of apoE.

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