Cerebrovascular Effects of Apolipoprotein E

Implications for Alzheimer Disease

Berislav V. Zlokovic, MD, PhD

Human apolipoprotein E (apoE) has 3 isoforms: apoE2, apoE3, and apoE4. APOE4 is a major genetic risk factor for Alzheimer disease and is associated with dementia in Down syndrome and poor neurological outcome after traumatic brain injury, cerebral hemorrhage, and other neuropathological disorders. While apoE4 can induce neuropathology by participating in various cellular and molecular pathways, herein I review data supporting the hypothesis that apoE4 has direct toxic effects on the cerebrovascular system that in turn can lead to secondary neuronal dysfunction and degeneration as well as accumulation of neurotoxins in brain such as β-amyloid (Aβ) in Alzheimer disease. I review Aβ-independent cerebrovascular effects of apoE, particularly activation of a proinflammatory cyclophilin A–mediated pathway in brain vascular pericytes by apoE4 that has recently been shown to lead to a loss of cerebrovascular integrity and blood-brain barrier breakdown causing neuronal injury. I also review Aβ-dependent cerebrovascular effects of apoE such as faulty Aβ clearance from brain to circulation by apoE4. Finally, I discuss isoform-specific interactions of apoE with low-density lipoprotein receptor-related protein 1 on brain vascular cells (ie, endothelial cells, pericytes), which play an important role in Aβ-independent and Aβ-dependent effects of apoE on cerebral vasculature.


Apolipoprotein E (apoE) was discovered in the early 1970s as a protein associated with cholesterol-rich and triglyceride-rich plasma lipoproteins (for review, see the article by Mahley et al1). It is synthesized by the liver and secreted into the circulation as a protein incorporated into very low-density lipoproteins, chylomicron remnants, and a subclass of high-density lipoproteins. It regulates transport of cholesterol and lipids throughout the body and mediates clearance of plasma lipoproteins by the low-density lipoprotein receptor and other low-density lipoprotein receptor-related protein family members.1 In the brain, apoE is produced mainly by astrocytes.2 In the cerebrospinal fluid and interstitial fluid of the central nervous system (CNS), apoE circulates incorporated into small particles and disks resembling high-density lipoproteins. Throughout the body and within the CNS, apoE contributes to restorative processes mediating redistribution of lipids to cells that require cholesterol and phospholipids.

Human apoE has 3 alleles located on a single gene locus on chromosome 19q13 encoding 3 major apoE isoforms: apoE2, apoE3, and apoE4.3 These apoE isoforms differ by single–amino acid substitutions at 2 residues, which have a major effect on the structure and function of apoE at the molecular and cellular level and association with neuropathological conditions. In apoE2 there is a cysteine residue at position 158, whereas apoE3 and apoE4 each have arginine at that position. It is believed that this confers greater stability of apoE2 and is associated with its protec-

Author Affiliations: Center for Neurodegeneration and Regeneration, Zilkha Neurogenetic Institute, and Department of Physiology and Biophysics, University of Southern California, Los Angeles.

©2013 American Medical Association. All rights reserved.

Downloaded From: http://jamanetwork.com/ on 09/24/2016
tive effect against Alzheimer disease (AD). The ApoE4 isoform is a major genetic risk factor for AD and possesses an arginine at residue 112, whereas both apoE2 and apoE3 have a cysteine residue at that position. This single–amino acid difference is associated with relative instability of apoE4 and its multiple pathogenic effects on cells. The most common isoform, apoE3, has a cysteine residue at position 112 and an arginine at position 158, which provide greater protein stability compared with apoE4, and is associated with much lower risk for neuropathological disorders compared with APOE4 carriers.

Diverse neuropathological effects of apoE4 on cells within the CNS include but are not limited to the following: (1) direct toxic effects on neurons that could be mediated by impaired neurite outgrowth, cytoskeletal disruption, hyperphosphorylation of tau, mitochondrial dysfunction, and impaired synaptogenesis; (2) reduced β-amyloid (AB) clearance from brain in AD; and (3) direct toxic effects on the cerebrovascular system causing and/or contributing to neuronal dysfunction and neurodegenerative changes. Several recent reviews provide an excellent overview on apoE isoform-specific toxic effects on neurons and effects on neuropathological disorders. Therefore, the focus of the present review is on cerebrovascular effects of apoE. Particularly, I discuss apoE isoform-specific effects on cerebrovascular integrity and Aβ vascular clearance as well as how apoE4, acting through the cerebrovascular system, contributes to development of cognitive and neuropathological changes related to the onset and progression of AD.

APOE4 AND AD

In a recent review on AD genetics, Tanzi summarized multiple genome-wide association studies indicating that APOE4 is by far the strongest genetic risk factor for AD, increasing the risk of developing disease by 400% to 1500% for apoE4 carriers compared with apoE3 carriers. For comparison, the 10 other genes most frequently associated with AD by genome-wide association studies carry much smaller risk change for AD, typically ranging between 10% and 15% (eg, CD33, CLU [clusterin], or PICALM). Recent studies have suggested that heterozygous rare variants in TREM2, encoding the triggering receptor expressed on myeloid cells 2 protein, are associated with a significant increase in the risk for AD.

Approximately 25% of all individuals are carriers of the apoE4 allele, which makes the detrimental effects of APOE4 expression quite frequent. As illustrated in Table 1, the apoE4 allele is substantially enriched in individuals with AD, with 64% and 80% of all patients with sporadic or familial AD carrying at least 1 copy of the apoE4 allele, respectively. A recent study using more than 10,000 control subjects and more than 7000 AD cases has shown that the lifetime risk for developing AD in apoE4 homozygotes is close to that of BRCA1 for breast cancer, which is 57% at age 70 years. Table 2 illustrates that apoE4 homozygotes have lifetime risks for developing AD of approximately 30% at age 75 years and 60% at age 85 years. In contrast, the lifetime risk for AD in apoE3 homozygotes is 2% at age 75 years and 10% at age 85 years.

It has been reported that the apoE4 allele decreases the age at onset of AD by approximately 8 years and 15 years in apoE4 heterozygotes and homozygotes, respectively. In addition, APOE4 is associated with dementia in Down syndrome and poor neurological outcome after traumatic brain injury and hemorrhage. Some studies have suggested that APOE4 is associated with other neuropathologies such as multiple sclerosis, stroke, frontotemporal dementia, and Parkinson disease. However, the data for these associations are not as strong as for AD and have yet to be reproduced by multiple independent studies.

NEUROVASCULAR UNIT

The neurovascular unit comprises vascular cells including endothelial and mural cells such brain capillary pericytes and arterial and/or venous vascular smooth muscle cells, glial cells including astrocytes, microglia, and oligodendroglia, and neurons. The endothelial cells of brain capillaries are connected by the tight junctions forming a continuous cellular membrane that underlies the anatomical blood-brain barrier (BBB). The BBB limits the entry of potentially toxic plasma components, red blood cells, and leukocytes into the brain. The BBB also regulates the delivery of circulating energy metabolites such as glucose and essential nutrients (eg, amino acids, vitamins) into the CNS, which are required for proper neuronal and synaptic function. Larger molecules such as peptides and proteins normally do not cross the BBB unless they have specialized transport systems.
Recent studies have shown that pericytes regulate BBB permeability and play a major role in maintaining cerebrovascular integrity at the level of brain capillaries, which in turn prevents entry of various potentially neurotoxic and vasculotoxic macromolecules in the blood from entering the CNS. The BBB also plays a key role in removal of potentially neurotoxic molecules from brain such as glutamate or Aβ in AD. Neurodegenerative disorders including AD are associated with microvascular dysfunction and/or degeneration in the brain, neurovascular disintegration, defective BBB, and vascular risk factors. In addition, several genes identified by genome-wide association studies might directly affect the cerebral vascular system and/or vascular clearance of Aβ.

In AD, a compromised cerebrovascular pathology such as degeneration of brain capillary endothelium, reduced endothelial tight junction protein levels, thickening of the capillary basement membrane, or degeneration of small cerebral arteries contributes to reductions in the resting cerebral blood flow, dysregulation of cerebral blood flow responses to brain activation, and/or impaired BBB function. These microvascular changes typically diminish the brain's supply of oxygen, energy metabolites, and nutrients. Moreover, some studies have suggested that primary vascular dysfunction precedes neuronal dysfunction and neurodegenerative changes and might also contribute to accumulation of Aβ in the brain and the vessel wall, resulting in development of cerebral β-amyloidosis and cerebral amyloid angiopathy, both features of AD.

**APOE Aβ-INDEPENDENT EFFECTS ON CEREBROVASCULAR INTEGRITY**

As reviewed elsewhere, neurovascular dysfunction may be present in normal APOE4 carriers before cognitive decline and Aβ accumulation occur, and it is found in individuals with APOE4-associated neurological disorders including AD, stroke, and brain hemorrhage. A recent meta-analysis of BBB permeability based on imaging and biochemical cerebrospinal fluid studies indicated that patients with AD have a greater increase in BBB permeability compared with neurologically healthy human controls, which has also been confirmed by postmortem brain tissue studies (for review, see the articles by Zlokovic9 and Sengillo et al13). Importantly, postmortem analysis indicated that the BBB breakdown is more pronounced in individuals with AD who carry the apoE4 allele. It has been reported that BBB breakdown in patients with AD is associated with significant reductions in the pericyte populations in the cortex and hippocampus. However, whether disruption in cerebrovascular integrity precedes cognitive decline in patients with AD, particularly in the APOE4 carriers, remains elusive. In addition, the cellular and molecular mechanisms leading to BBB disruption in AD and particularly in AD cases with the apoE4 allele remain largely unknown.

A possible limitation of any study of human brain tissue is the postmortem sampling, with results reflecting an end-stage process. Moreover, longitudinal studies in individuals with mild cognitive impairment or patients with AD who have different APOE genotypes to compare changes in BBB permeability with a loss of executive functions, cognitive decline, disease onset, and/or progression do not currently exist. Therefore, studies in experimental models such as transgenic mice expressing different human apoE isoforms are needed to better characterize age-dependent effects of APOE genotype on brain microcirculation, to determine how changes in cerebrovascular integrity contribute to neuronal dysfunction and neurodegenerative changes, and to elucidate the underlying cellular and molecular mechanisms of BBB disruption.

Recently, we studied transgenic mice with targeted replacement of murine apoE with each human apoE isoform, mice lacking murine apoE (Apoε⁻⁻), mice expressing each human apoE isoform under control of the astrocyte-specific glial fibrillary acidic protein promoter on an apoE null background, and Apoe⁻⁻ and APOE4 transgenic mice with ablation and/or pharmacological inhibition of the proinflammatory cytokine cyclophilin A (CypA). In search of molecules that could mediate BBB dysfunction observed in Apoe⁻⁻ and APOE4 mice, we focused on CypA because CypA has been shown to have deleterious vascular effects in Apoe⁻⁻ mice including aortic aneurysms and atherosclerosis.

Using different humanized APOE transgenic mouse lines expressing apoE in brain, mainly in astrocytes, we showed that astrocyte-derived human apoE4, but not human apoE2 and apoE3, leads to an age-dependent progressive BBB breakdown by activating a proinflammatory CypA–nuclear factor κB–matrix metalloproteinase 9 (MMP-9) pathway in brain capillary pericytes (Figure 1). We next showed that activation of MMP-9 in APOE4 mice leads to enzymatic degradation of the BBB tight junction and basement membrane proteins, resulting in BBB breakdown followed by neuronal uptake of multiple blood-derived neurotoxic proteins (eg, thrombin, fibrin), perivascular deposition of erythrocyte-derived hemosiderin, and microvascular and cerebral blood flow reductions. Importantly, our data show that the vascular defects in APOE4-expressing mice precede neuronal dysfunction and can initiate neurodegenerative changes. In addition, this study showed that astrocyte-secreted apoE3 and apoE2, but not apoE4, suppress the CypA–nuclear factor κB–MMP-9 pathway in pericytes via low-density lipoprotein receptor-related protein 1 (LRP1). The study confirmed previous findings in cerebral vascular cells showing that apoE3 and apoE2 have a relatively high affinity for binding and/or interaction with LRP1; this is in contrast to apoE4, which shows weak interaction with LRP1 in brain endothelial cells and pericytes. In summary, these findings suggest that CypA is a key target for treating apoE4-mediated neurovascular injury and the resulting neuronal dysfunction and degeneration.
APOE Aβ-DEPENDENT EFFECTS ON CEREBROVASCULAR CLEARANCE

According to the 2-hit vascular hypothesis of AD, the Aβ peptide and its different forms (particularly the lower-molecular-weight oligomers) are thought to contribute to neuronal and synaptic dysfunction as a second hit in the disease process.9 It is well established that there are apoE isoform-specific effects in the Aβ pathway.2,3,7 For example, apoE4 expression is associated with a significant increase in amyloid plaques in brain at earlier ages compared with apoE3 or apoE2. Importantly, several experimental studies have shown that apoE4 impairs Aβ clearance from brain and across the BBB in animal models and patients at risk for developing AD,16,17 which in turn promotes Aβ retention in brain and contributes to formation and deposition of amyloid fibrils.

With respect to Aβ clearance across the BBB, studies in rodents have shown that Aβ binding to apoE4 redirects the rapid clearance of free Aβ40/42 from LRP1 to the very low-density lipoprotein receptor, which internalizes apoE4 and Aβ-apoE4 complexes at the BBB more slowly than LRP1 (Figure 2).16 In contrast, apoE2 and apoE3 as well as Aβ-apoE2 and Aβ-apoE3 complexes are cleared at the BBB via both very low-density lipoprotein receptor and LRP1 at a substantially faster rate than Aβ-apoE4 complexes. Astrocyte-secreted lipoprotein apoE2, lipoprotein apoE3, and lipoprotein apoE4 as well as their complexes with Aβ are cleared at the BBB by mechanisms similar to those of their respective lipid-poor isoforms but at 2- to 3-fold slower rates. Thus, apoE isoforms differentially regulate Aβ clearance from the brain, and this might contribute to the effects of APOE genotype on the disease process in both individuals with AD and animal models of AD.16 Interestingly, it has also been suggested that Aβ binding to clusterin (apoJ) leads to rapid clearance of Aβ across the BBB via LRP2 receptor (Figure 2).18

CONCLUSIONS

In summary, several studies have suggested that apoE4, in contrast to apoE2 and apoE3, has direct toxic effects on the cerebrovascular system and may affect neurovascular functions independent of Aβ pathology. In this review, I summarized some recent experimental studies demonstrating differential effects of apoE isoforms on neurovascular functions.

As illustrated in Figure 3A, I propose that astrocyte-secreted apoE3 and apoE2 bind to and interact well with LRP1 on pericytes, blocking the proinflammatory CypA–MMP-9 pathway and maintaining cerebrovascular integrity,13 and with LRP1 on brain endothelial cells, which results in clearance of Aβ across the BBB.16 On the other hand, I propose that poor interaction of apoE4 with LRP1 results in activation of the CypA–nuclear factor κB–MMP-9 pathway in pericytes, causing degradation of the BBB tight junction and basement membrane proteins.
causing neuronal injury. In endothelial cells, the weak interaction between apoE4 and LRP1 fails to proinflammatory CypA–NFκB pathway, a weak interaction of apoE4 with low-density lipoprotein receptor-related protein 1 (LRP1) on pericytes (P) and suppresses the system via regulation of the cyclophilin A (CypA)–nuclear factor κB (NFκB) signaling pathways. This results in BBB disruption with accumulation of different proteins. This results in BBB disruption with accumulation of different blood-derived potentially neurotoxic molecules (e.g., thrombin, fibrin, plasmin) and erythrocyte-derived hemosiderin in brain preceding neuronal injury and degenerative changes (Figure 3B). In the Aβ pathway, a weak interaction of apoE4 with LRP1 in brain endothelial cells diminishes Aβ clearance across the BBB, resulting in accumulation of Aβ in brain, which can injure neurons directly and/or potentiate neurodegenerative changes caused by BBB breakdown.

Future studies using transgenic mice with targeted replacement of human APOE and reduced or complete deletion of LRP1 from brain endothelium and pericytes and crossed with AD mice should, however, further validate the proposed clearance hypothesis (Figure 3) exploring whether apoE and LRP1 interaction in both endothelium and pericytes can regulate Aβ clearance from brain via efflux across the BBB to efficiently remove Aβ from brain, contributing to Aβ accumulation and Aβ-mediated neuronal injury. BM indicates basement membrane; TJ, tight junction.

Accepted for Publication: December 12, 2012.
Published Online: February 11, 2013. doi:10.1001/jamaneurol.2013.2152

Conflict of Interest Disclosures: None reported.
Funding/Support: This work was supported by grants AG039452, AG23084, and NS34467 from the National Institutes of Health.

Additional Contributions: Abhay Sagare, PhD, prepared the figures.

REFERENCES


Figure 3. Proposed role of apolipoprotein E (apoE) isoform-specific effects on the cerebrovascular system via regulation of the cyclophilin A (CypA)–nuclear factor-κB (NFκB)–matrix metalloproteinase 9 (MMP-9) pathway and β-amyloid (Aβ) clearance. A, Astrocyte (A)–secreted apoE2 or apoE3 interacts with low-density lipoprotein receptor-related protein 1 (LRP1) on pericytes (P) and suppresses the proinflammatory CypA–NFκB–MMP-9 pathway, which maintains blood-brain barrier (BBB) integrity. Additionally, apoE2 and apoE3 interact with LRP1 in the endothelial cells (E) to mediate Aβ clearance from brain to blood. B, Astrocyte (A)–secreted apoE4 interacts weakly with LRP1 in pericytes (P) and endothelial cells (E), and its binding to LRP1 on vascular cells is barely detectable. In pericytes, this weak interaction results in increased intracellular CypA; this activates the proinflammatory NFκB–MMP-9 pathway, leading to BBB disruption and accumulation of neurotoxic blood-derived molecules in brain and causing neuronal injury. In endothelial cells, the weak interaction between apoE4 and LRP1 fails to efficiently remove Aβ from brain, contributing to Aβ accumulation and Aβ-mediated neuronal injury. BM indicates basement membrane; TJ, tight junction.