Apolipoprotein E, Amyloid-β, and Blood-Brain Barrier Permeability in Alzheimer Disease

John E. Donahue, MD and Conrad E. Johanson, PhD

Abstract
There is increasing evidence for blood-brain barrier (BBB) compromise in Alzheimer disease (AD). The presence of the ε4 allele of the apolipoprotein E (apoE) gene is a risk factor for sporadic AD. Apolipoprotein E is essential both for maintenance of BBB integrity and for the deposition of fibrillar amyloid-β (Aβ) that leads to the development of Aβ plaques in AD and to cerebral amyloid angiopathy. This review investigates the relationships between apoE, Aβ, and the BBB in AD. Alterations in the expression and distribution of the BBB Aβ transporters receptor for advanced glycation end-products and low-density lipoprotein receptor-related protein 1 in AD and the potential roles of apoE4 expression in adversely influencing Aβ burden and BBB permeability are also examined. Because both apoE and Aβ are ligands for low-density lipoprotein receptor-related protein 1, all 3 molecules are present in AD plaques, and most AD plaques are located close to the cerebral microvasculature. The interactions of these molecules at the BBB likely influence metabolism and clearance of Aβ and contribute to AD pathogenesis. Therapeutic alternatives targeting apoE/Aβ and sealing a compromised BBB are under development for the treatment of AD.

Key Words: Alzheimer disease, Amyloid angiopathy, Amyloid-β, Apolipoprotein E, Blood-brain barrier, LRP-1, RAGE

INTRODUCTION
This review addresses the interrelationships among apolipoprotein E (apoE), amyloid-β (Aβ), and the blood-brain barrier (BBB) both under physiologic conditions and in Alzheimer disease (AD). Blood-brain barrier anatomy, including its tight junction proteins and neurovascular unit, will be discussed. Evidence for increased BBB permeability and alteration of the expression and distribution of the BBB Aβ transporters and tight junction proteins in AD will be presented. Hypotheses suggesting that the apoE genotype plays an important role in brain Aβ burden and BBB alterations will be evaluated in light of recent studies. Finally, potential therapeutic alternatives for sealing a compromised BBB and reversing Aβ deposition in AD will be analyzed.

Apolipoprotein E
Apolipoprotein E is a plasma cholesterol transport molecule; the gene for apoE (apoE) is located on chromosome 19q13.2. The concentration of apoE mRNA in the central nervous system (CNS) is second only to that in the liver. However, CNS apoE is not derived from liver because it is synthesized mainly by astrocytes, to some extent by microglia, and a small amount by neurons (1). Apolipoprotein E has been implicated as a neurotrophic factor in the growth and repair of the CNS during development or after injury as an anti-oxidant and as an immune response mediator (2). CNS apoE is endocytosed by receptors such as the low-density lipoprotein receptor and the low-density lipoprotein receptor-related protein (LRP) 1; this endocytosis regulates the amount of apoE in the CNS (1).

Apolipoprotein E exists as 3 major alleles (i.e. ε2, ε3, and ε4) that translate into 3 isoforms of the protein. The isoforms differ only at amino acid residues 112 and 158; apoE3 (60%–70% frequency in the general population) has a cysteine at residue 112 and arginine at residue 158; apoE4 (15%–20%) has an arginine at both sites; apoE2 (5%–10%) has cysteine at both sites (3). The arginine at residue 112 in apoE4 reduces stability of the protein and likely leads to apoE4-induced pathology (3). Persons who are homozygous for the ε4 allele are more likely to develop the sporadic form of AD because this allele has been shown to be an independent risk factor for sporadic AD (4). Apolipoprotein E4 is thought to play a role in approximately 50% of AD cases and to be second only to aging in pathogenetic importance (5). Apolipoprotein E4 has also been shown to be a risk factor for atherosclerosis (6), stroke (7, 8), prolonged recovery from closed head injury (9), and increased mortality from intracerebral hemorrhage (8). These correlations suggest that apoE4 has deleterious effects on the cerebral microvasculature, and, conversely, that vascular disease is an important component of AD pathogenesis.

Amyloid-β
Amyloid-β is a 40- or 42-amino acid protein that is cleaved from the much larger amyloid precursor protein (APP), the gene for which is on chromosome 21. To form the free Aβ peptide, the enzyme β-secretase cleaves APP to generate the N-terminus of the Aβ peptide; the major neuronal β-secretase is called β-site APP cleaving enzyme.
Cleavage is followed by liberation of the C-terminus via APP cleavage by the γ-secretase complex that includes presenilin (PS), either PS-1 or PS-2 (10). This γ-secretase cleavage can occur at either position 40 or 42 of Aβ, resulting in Aβ1-40 and Aβ1-42 peptides, respectively. Amyloid-β1-40 is more soluble than Aβ1-42, which is more prone to fibrillation. Under normal conditions, most secreted Aβ is in the Aβ1-40 form, with minimal Aβ1-42 secreted. However, certain APP mutations near the γ-secretase cleavage site favor formation of the Aβ1-42 form and lead to familial AD (10); apoE genotype may influence the ratio of secreted Aβ1-40–Aβ1-42 (11) (see succeeding paragraphs).

In AD, Aβ accumulates in the brain neuropil in the form of homogeneous deposits of fibrillar material without surrounding dystrophic neurites (i.e. diffuse plaques) and in concentrated, dense, central cores of Aβ surrounded by dystrophic neurites (i.e. neuritic or senile plaques). Amyloid-β can also be deposited within the walls of cerebral blood vessels, leading to the condition known as cerebral amyloid angiopathy (CAA). In CAA, the Aβ deposits weaken the vessel walls, thereby increasing the risk of intracerebral hemorrhage. Figure 1 demonstrates Aβ accumulation in both a cerebral blood vessel and surrounding neuropil in a case of severe AD. As illustrated, Aβ plaques are frequently present in the vicinity of the cerebral microvasculature, reinforcing the concept that vascular disease is often a significant component of AD pathology. Therefore, it is not surprising that there is increasing evidence for BBB compromise in AD (12–20).

Blood-Brain Barrier

The BBB was originally observed in 1885 by Paul Ehrlich, who noted that water-soluble dyes injected into the systemic circulation did not stain the brain and spinal cord. Subsequently, in 1913, a student of Ehrlich, Edwin Goldmann, injected trypan blue dye directly into the cerebrospinal fluid (CSF) and discovered that the dye stained the CNS but did not penetrate into the periphery (21). The concept of the BBB was debated for decades, and its anatomic substrate was not defined at the ultrastructural level until the late 1960s (21). The major morphologic determinants of the BBB are the tight junctions between and lack of fenestrations in the capillary endothelial cells of the cerebral microvasculature.

Occludin and the claudins are integral membrane proteins that are exclusively localized to cerebral microvascular endothelial cell tight junctions (22). The claudins are thought to function as the primary seal of the tight junction, whereas occludin acts as an additional support structure and increases electric resistance across the barrier (21). Two claudins, claudins 1 and 5, are primarily expressed in BBB endothelial cells (22). In addition, there are tight junction-associated proteins, which include 2 members of the membrane-associated guanylate kinase family, namely, zonula occludens (ZO) 1 and ZO-2 (21, 22). The ZO proteins link the integral membrane proteins of the tight junction to the underlying cytoskeleton and bind to signaling molecules such as transcription factors during cellular states of stress and proliferation; they also act as signaling molecules that communicate the condition of the tight junction to the interior of the cell (21). Under normal conditions, all of these tight junction proteins and accessory proteins are localized along the endothelial cell plasma membrane at cell-to-cell contacts (22).

The interaction of the cerebral microvasculature with the surrounding CNS tissue has given rise to the concept of the “neurovascular unit” (21). This unit consists of astrocytes and pericytes, the processes of which somehow help to maintain BBB morphology, and neurons, whose innervation may help to regulate BBB function. In addition, the extracellular matrix (ECM) of the basal lamina can also be considered part of the neurovascular unit because the ECM interacts with the microvascular endothelial cells (21), and disruption of the ECM can lead to increased BBB permeability in disease states such as bacterial infection (23) and malignant glioma (24). Extracellular matrix proteins are also likely involved in BBB/tight junction maintenance (21).

INTERRELATIONSHIPS AMONG THE BBB, Aβ, AND ApoE in AD

There is growing evidence for BBB compromise in human AD (13–20) particularly in regions surrounded by Aβ plaques or involved in CAA. In a recent study of 13 patients who came to autopsy and fulfilled diagnostic criteria for AD, 4 had physiologic evidence of BBB impairment, as determined by CSF-albumin index, although none of these patients had any significant cerebrovascular disease (18). Therefore, CAA is insufficient as the sole explanation for BBB impairment in AD. Another recent study demonstrated perivascular leakage of Aβ, complement, and immunoglobulins in human AD brains (20). This enhanced permeability can potentially lead to increased deposition of Aβ within AD brains, thereby further worsening the disease (25). Studies of transgenic mouse models of AD similarly reveal BBB dysfunction. For example, a compromised BBB has recently been demonstrated in a transgenic mouse model of AD even

![FIGURE 1. Parietal cortex of a 55-year-old woman with severe Alzheimer disease. Amyloid-β (Aβ) is present within the full thickness of the wall of an arteriole (black arrow), and there are numerous deposits within neuritic plaques (white arrows) in the same field. Aβ immunostain, 600×.](http://jnen.oxfordjournals.org/)

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prior to the deposition of Aβ peptide (26), and a preliminary experiment using mass spectrometry in a 55-week-old, doubly transgenic mouse with the (APP[Swe]/PS-1[del exon 9] line 85) mutation (27) revealed an Evans blue peak within the brain tissue that indicated BBB leakage (unpublished observations). Because the apoE4 genotype is a known risk factor for AD, we hypothesize that the apoE4 protein might enhance brain Aβ burden or adversely affect the BBB in AD. Understanding the mechanisms of BBB failure or alteration in AD requires evaluating the 2 pathways by which molecules can cross the BBB: transport (i.e. via BBB receptors or transporters) and permeability (i.e. simple diffusion). Figure 2A depicts the possible interrelationships among the BBB, Aβ, and apoE diagrammatically, and Figure 2B is a schematic representation of the BBB and its interrelationships with Aβ and apoE, both at the BBB and

FIGURE 2. (A) Schematic diagram depicting the interrelationships among apolipoprotein E (apoE), amyloid-β (Aβ), and the blood-brain barrier (BBB). See text for details. (B) Schematic diagram depicting the BBB and adjacent brain neuropil. Extracellular matrix molecules such as agrin are important in the formation and maintenance of the BBB and its tight junctions (TJs). Astrocytic processes are part of the BBB “neurovascular unit” and also help to maintain BBB morphology. Aβ is transported into the brain by the receptor for advanced glycation end-products (RAGE) and out of the brain by the low-density lipoprotein receptor-related protein (LRP) 1. In the neuropil, apoE is required for fibrillation of Aβ, and LRP-1 colocalizes with apoE in the Aβ plaques of Alzheimer disease.
Aβ and the BBB

The source of the Aβ burden in AD brain is not entirely clear, with neuronal (28-30), glial (31), and systemic (25, 32) origins proposed. Although the neuronal contribution to the Aβ pool is likely greater than the glial contribution within the CNS, increased levels of Aβ on plasma lipoproteins and proteins in AD suggest that circulating Aβ can be a precursor of brain Aβ (33) and thus contribute significantly to the Aβ burden in AD. Although some systemic Aβ may enter the brain passively via a leaky BBB (14), active transport of Aβ across the BBB also occurs (34), using a number of Aβ receptors (35, 36). Two of the better-characterized receptors are the receptor for advanced glycation end-products (RAGE) (37) and the low-density LRP-1 (38). Even more recently, the multidrug resistance transporter P-glycoprotein (P-gp) has been shown to have potential importance in BBB Aβ transport (39, 40).

Receptor for Advanced Glycation End-Products

Receptor for advanced glycation end-products is a multiligand receptor in the immunoglobulin superfamily of cell surface molecules. It binds a wide range of molecules, including the products of nonenzymatic glycoxidation (advanced glycation end-products), Aβ, proinflammatory cytokine-like mediators in the S-100/calgranulin family, and a DNA-binding protein, amphoterin (41). Interactions between RAGE and advanced glycation end-products are thought to result in an enhanced inflammatory response and vascular injury associated with certain disease states such as diabetes mellitus and renal failure (42). Receptor for advanced glycation end-products has been postulated to be a signaling receptor that regulates certain cellular functions upon the binding of its ligands (42); RAGE expression is upregulated with increasing ligand concentration, including Aβ (42, 43). In AD brains, RAGE is found in neurons, astrocytes, and microglia particularly in proximity to Aβ plaques and neurofibrillar tangles. Binding of Aβ to RAGE in nanomolar concentrations initiates a cascade of cellular reactions, resulting in increased secretion of inflammatory cytokines, potentially exacerbating AD pathology (42). Moreover, RAGE is expressed in both endothelial and smooth muscle cells of blood vessels, and it has been shown to be a major transporter of systemic Aβ across the BBB and into the brain (43). Binding of Aβ to vascular RAGE also suppresses cerebral blood flow due to enhanced production of the vasoconstrictor endothelin 1 (37), which can worsen ischemia and further propagate cytotoxic damage in AD.

Low Density Lipoprotein Receptor-Related Protein 1

Low density lipoprotein receptor-related protein 1 is a member of the low-density lipoprotein receptor family. It functions as a multifunctional scavenger and signaling receptor and as a transporter and metabolizer of cholesterol and apoE-containing lipoproteins (44). Low density lipoprotein receptor-related protein 1 binds a wide range of ligands, including apoE, α2-macroglobulin, APP, and Aβ (33). The LRP-1/apoE interaction on neurons promotes neurite outgrowth in vitro, which is important in early neuronal differentiation and development (45), and, as previously noted, endocytosis of the LRP-1/apoE complex helps to regulate CNS apoE concentration (1). The interaction of LRP-1 with APP at the neuronal surface may enhance APP endocytosis and Aβ production (45). Low density lipoprotein receptor-related protein 1 is also expressed in the endothelial cells of the cerebral microvasculature and is a major transporter of Aβ out of the brain and into the systemic circulation (38).

P-Glycoprotein

P-Glycoprotein is a 170-kD plasma membrane glycoprotein that belongs to the superfamily of adenosine triphosphate-binding cassette transporters (39). It is a product of the multidrug resistance 1 gene that was first discovered in 1976 in multidrug-resistant cancer cells; when overexpressed in these cells, transport of cytotoxic agents out of the cells takes place (39). P-Glycoprotein has been shown to transport Aβ out of the brain (46), and crossing P-gp knockout (KO) mice with transgenic Tg2576 mice that overexpress APP results in enhanced Aβ deposition within the CNS (39). However, these P-gp KO × APP mice also have reduced vascular expression of LRP-1 (39), and another study suggests that the contribution of P-gp to Aβ extrusion from the brain may be minimal (47). Then again, both LRP-1 and P-gp are downregulated in both aging and AD (43). A recent study demonstrated adenosine triphosphate-dependent, P-gp-mediated transport of Aβ out of cells and into the apical extracellular space (40). This study used porcine-derived renal tubular epithelial cells transfected with human P-gp cDNA and growing in a polarized cell monolayer as an in vitro model of the BBB. The role of P-gp in CNS efflux of Aβ and its contribution to neurodegenerative diseases such as AD need further elucidation.

Transport: Alteration of BBB Aβ Receptors in AD

The net flux of Aβ into or out of the brain is the algebraic sum of the inward (e.g. via RAGE) and outward flux (e.g. via LRP-1) and presumably depends upon the density and activity of these 2 receptors. Recently, we showed that AD is associated with alterations in the relative distributions of both RAGE and LRP-1 receptors at the BBB in human hippocampus (48). We found a robust RAGE presence within the neurons of age-matched control hippocampi, whereas RAGE within the microvasculature was barely detectable. In severe AD cases there was significant upregulation of RAGE within the microvasculature and reduction of RAGE within neurons. The opposite was true for LRP-1. The amount of LRP-1 was greater within the microvasculature of control cases than in neurons, whereas in severe AD, there was downregulation of LRP-1 within the vessels and an increased presence within neurons. In the Aβ plaques seen in AD, LRP-1, but not RAGE, colocalized with Aβ in the plaques. With the concurrent upregulation of RAGE within AD vessels, there would presumably be an increase in the influx and decrease in the efflux of Aβ in the brain, resulting in a net increase in the amount of systemic Aβ transported into the brain. Thus, Aβ from the systemic
circulation may be an important contributor to the overall brain Aβ burden in AD. In addition, the upregulation of LRP-1 on neurons in AD may mediate clearance of Aβ through its binding with LRP-1. This clearance and subsequent increase in intraneuronal Aβ is enhanced by overexpression of the LRP-1 ligand transforming growth factor-β (49). Signaling interactions between the LRP-1 and transforming growth factor-β pathways in early AD alter both Aβ clearance and neuronal function and, combined with enhanced toxicity from the increased amounts of intraneuronal Aβ, results in neuronal death and may be one of the early pathogenic events in AD.

Permeability: Effect of Aβ on Endothelial Cell Tight Junctions

An intriguing recent study demonstrated long-term restoration of a compromised BBB in the Tg2576 AD mouse model by immunizing with Aβ peptide (50). This, coupled with another study demonstrating that infusions of soluble Aβ compromised the BBB with ensuing cortical perivascular gliosis (51), suggests that Aβ somehow has a detrimental effect on the endothelial cells and/or tight junctions at the BBB. Proposed mechanisms include increased microgliosis with its associated inflammatory mediators and cytokines, and alterations of the tight junctions induced by proapoptotic and proangiogenic responses in the endothelial cells mediated by Aβ (50). The latter hypothesis was evaluated in another study (22), which revealed that Aβ1-42 caused alterations in the expression and distribution of certain tight junction proteins in isolated rat brain microvascular endothelial cell cultures without associated astrocytic coculture. There was disruption of claudin 5 distribution at the cell-to-cell contact sites with a shift to the cytoplasm and discontinuous distribution of ZO-2 at the cell borders of Aβ1-42-treated endothelial cells compared with control cells (22). There was also increased expression of ZO-2 at 1 day in Aβ1-42-treated cells with decreased expression at 3 days, significantly reduced expression of occludin at 1 day followed by a return to control levels, and significantly increased expression of claudin 1 at all times (22). Thus, Aβ-induced alterations of tight junction protein expression and distribution may have adverse effects on BBB integrity in vivo.

Aβ and ApoE

Apolipoprotein E has been shown to bind in Aβ plaques and neurofibrillary tangles in AD brains and has also been detected in blood vessels (2). The association of apoE with Aβ in plasma, CSF, and brain homogenates has led to the hypothesis that apoE is a chaperone for Aβ, and that it regulates its conversion from a mixed random coil/α-helix to a β-sheet amyloid conformation (1). Indeed, apoE is necessary for the fibrillation of Aβ because apoE KO mice accumulate Aβ but are protected from the formation of fibrillar Aβ and from its deposition within cerebral blood vessels (52).

Aβ Deposition and ApoE

Studies have shown that apoE4 results in increased deposition of Aβ1-40 within the brain parenchyma (53), an increased ratio of Aβ1-40−Aβ1-42 in brain extracellular pools (11), and enhanced vascular sequestration and brain uptake of soluble Aβ1-40 complexed with apoE4 (as compared with apoE2 and apoE3) (54). Although Aβ1-40 tends to be more soluble than Aβ1-42, in the presence of apoE4, it may be more prone to fibrillation, resulting in enhanced Aβ aggregation and plaque formation (53). Moreover, the presence of apoE4 and increased ratio of Aβ1-40−Aβ1-42 may favor the preferential development of CAA more than Aβ plaques (11, 55).

Aβ Clearance and ApoE

Apolipoprotein E KO mice have higher levels of soluble Aβ and increased Aβ half-life in brain interstitial fluid, which suggests facilitation and clearance of soluble Aβ by apoE (1). This clearance is likely mediated by LRP-1 because 1 study showed that Aβ clearance was reduced by 30% in 2-month-old apoE KO mice compared with age-matched controls, and that the clearance rate was lowered further as these mice aged (40% at age 9 months), and expression of vascular LRP-1 was diminished (38). This suggests that receptor-mediated clearance of apoE may play some role in removing Aβ from the brain and regulating CNS Aβ metabolism (1). However, apoE genotype does not seem to play a role in LRP-1-mediated clearance of Aβ because 1 study showed that LRP-1 binds to immobilized apoE isoforms in vitro similarly. In solution, LRP-1 does not recognize lipid-free apoE2 and apoE3 and only weakly binds to lipid-free apoE4 (56). Another study demonstrated that, although clearance of exogenous, intraventricularly injected Aβ1-42 from the CSF into the systemic circulation was much slower than clearance of injected Aβ1-40 (and may depend more on catabolic pathways than transport across the BBB), the elimination rate of both Aβ1-40 and Aβ1-42 was not apoE genotype specific, and the clearance was similar even in apoE KO mice (56). However, this does not rule out an apoE isoform-specific difference in the clearance of endogenous, parenchymal Aβ.

Aβ Production and ApoE

Apolipoprotein E4 has been shown to enhance Aβ production in rat neuroblastoma cells transfected with human APP at twice the rate of apoE3; in addition, apoE4 significantly stimulated recycling of APP (3). This Aβ production and enhanced APP recycling are likely mediated by LRP-1 because the effect is repressed by pretreatment with LRP-1’s receptor-associated protein or LRP-1 small interfering RNA, blocking the LRP-1 pathway (3).

BBB and ApoE

In apoE KO mice, the BBB has been shown to be severely compromised (58). This study showed that Evans blue-albumin, with a molecular weight of approximately 69 kD and which does not cross an intact BBB, crossed the BBB easily in these mice, and that the brains, particularly the cerebellum, stained light blue to the naked eye. Moreover, this BBB permeability increases as these apoE KO mice age (59). The reasons why loss of apoE leads to BBB dysfunction
basement membranes secondary to loss of apoE from the basement membrane (60, 61), and, because apoE inhibits leukocyte proliferation and suppresses microglial responses, a hyperactive immune response that damages the BBB (58, 59). Therefore, apoE is necessary both to maintain BBB integrity and to form the classic fibrillar Aβ plaques and deposits that occur in AD and CAA.

Agrin in BBB Development and AD

As previously stated, the ECM is an important component of the BBB neurovascular unit, and ECM proteins are likely involved in BBB/tight junction maintenance (21). One such ECM protein is agrin. Agrin is a multidomain heparan sulfate proteoglycan that was initially discovered to be crucial for the normal development of the neuromuscular junction and peripheral synaptogenesis because it causes acetylcholine receptors to aggregate at the postsynaptic membrane, hence the name (62). Although agrin may play a role in CNS synaptogenesis, its role has not been as clearly defined as it has in the periphery. Agrin is an integral component of the developing BBB and is ubiquitously found within the cerebral microvasculature where it binds with the ECM protein laminin (63). Immunoreactivity for agrin is present throughout the cerebral microvasculature in both AD and age-matched control brains; however, closer examination of the capillaries in AD brains reveals ragged and irregular outer walls and attenuated diameters (Fig. 3A) in contrast to controls (Fig. 3B). This suggests thinning and fragmentation of agrin within the AD microvasculature (64). Because agrin is involved in development (63) and maintenance (21) of the BBB, fragmentation of blood vessel walls in AD indicates possible compromise of the BBB within these vessels. In addition, agrin immunoreactivity was also concentrated within both diffuse and neuritic plaques in all AD brains, including “puncta” of reactivity around microvessels and around and within the plaques (Fig. 3A) (64). A subsequent study showed that immunoreactivity for laminin in AD is virtually identical to that of agrin (65). These agrin-immunoreactive puncta are likely fragments of the damaged microvascular basement membrane.

Agrin, ApoE Genotype, and Microvascular Surface Area

Another study demonstrated the relationship between apoE genotype and capillary basement membrane surface area, as measured by anti-agrin immunoreactivity (66). There was a statistically significant reduction in capillary basement membrane surface area in brains with moderate to severe AD that had an apoE4/4 genotype compared with those with an apoE3/3 genotype. (Those with an apoE3/4 genotype had a modest reduction in capillary basement membrane surface area compared with apoE3/3, but the difference was not statistically significant.) Because apoE is present within the vascular basement membrane and likely interacts with agrin and laminin, it is possible that apoE genotype may affect the nature of these interactions (60, 61). Moreover, apoE might alter BBB permeability by altering transport mechanisms at the level of the endothelial cell (58).
ApoE4 AND Aβ: RELATIONSHIP TO BBB ALTERATIONS IN AD?

It is well known that the presence of the e4 allele of apoE is an independent risk factor for the development of AD (4, 5), but the exact mechanism by which apoE4 exacerbates the pathology of AD is not clear. Proposed hypotheses include promoting Aβ fibrillation and deposition (67), destabilization of microtubules leading to intracellular neurofibrillary tangles (68), lack of apoE4 stimulation of neurite outgrowth in the presence of lipid (3), and apoE4-containing lipoproteins secreted by glia being less protective of CNS neuronal apoptosis by a mechanism that requires LRP-1 compared with apoE3-containing lipoproteins (69).

Two transgenic mouse studies demonstrated substantially more fibrillar Aβ deposits in transgenic mice with mutated APP that expressed apoE4 compared with apoE3 (70, 71). Apolipoprotein E and Aβ are ligands for LRP-1 (33), and LRP-1 is a major transporter of Aβ out of the brain (whereas RAGE is a major transporter of Aβ into the brain; Fig. 2B) (38). Moreover, apoE, LRP-1, and Aβ colocalize within AD senile plaques (48), and LRP-1 is downregulated on the cerebral microvasculature in severe AD cases, reducing Aβ transport out of the brain (48). This suggests that the interaction among these molecules likely regulates metabolism and clearance of Aβ and is somehow involved in the pathogenesis of AD. Most Aβ AD plaques are located in the vicinity of, or are directly in contact with, cerebral capillaries (50, 64), consistent with BBB damage in AD (13–20).

Although the presence of apoE is necessary for normal BBB development and deposition of fibrillar Aβ in AD, it is intriguing to speculate that the presence of the e4 allele somehow results in loss of BBB integrity compared with the other apoE alleles. Given the evidence previously discussed, if this hypothesis is correct, then a plausible explanation would be an enhanced Aβ burden in apoE4 brains. Although the contribution of apoE genotype to Aβ clearance from the brain is debatable (57), apoE4 does promote increased Aβ production systemically (3), and this might have a toxic effect on CNS endothelial cell tight junctions with resulting BBB compromise (22, 50, 51) even in the absence of overt CAA. In addition, the presence of apoE4 enhances cerebral parenchymal and vascular deposition of Aβ1–40 (11), which may be more prone to fibrillation in the presence of apoE4 (53). This would lead to toxic effects due to increased amounts of Aβ in the brain, with exacerbation of AD pathology and loss of BBB integrity secondary to CAA and endothelial cell tight junction dysfunction due to Aβ.

Apolipoprotein E might also alter BBB permeability by altering transport mechanisms at the level of the endothelial cell (58). Indeed, in a recently completed preliminary experiment, we found that 52-week-old transgenic human apoE4/4 knockin mice expressed an approximately 7-fold increase in the amount of RAGE mRNA and a 3-fold increase in the amount of LRP-1 mRNA compared with 26-week-old apoE4/4 mice and both 26- and 52-week-old apoE3/3 mice (unpublished observations). This suggests that in addition to enhanced Aβ production and deposition with BBB tight junction dysfunction with or without CAA, apoE4 might also influence the expression of Aβ transporters at the BBB with aging. As previously stated, evaluating the mechanisms of BBB failure or alteration in AD would require studying the 2 mechanisms by which molecules can cross the BBB, namely, permeability and transport. Apolipoprotein E4 would seem to exert an influence on both of these mechanisms.

Aβ-, ApoE-, AND BBB-SPECIFIC THERAPEUTIC ALTERNATIVES

Aβ Vaccine

The restoration of the BBB after Aβ immunization in the Tg2576 mouse model of AD (50) is potentially exciting with respect to possibly therapy in AD, with the potential of sealing a compromised BBB and removing parenchymal Aβ. However, the initial human clinical trial of an intramuscular Aβ vaccine in AD patients had to be halted when some patients developed an aseptic meningencephalitis, and at least 1 had multiple cortical hemorrhages (72). The etiology of the meningencephalitis was thought to be enhancement of the T-cell immune response to an abnormal antigenic fragment of Aβ after Aβ immunization and processing; the relationship between immunization and hemorrhage is unclear (72). Recently, a transcutaneous vaccine was tested in both wild-type mice and the doubly transgenic mouse with the (APP[Swe]/PS1[del exon 9] line 85) mutation, and no evidence of brain inflammation or cerebral hemorrhage was evident (73). The transcutaneous route may be safer because of the presence of anti-inflammatory cytokines secreted by Langerhans cell precursors and skin keratinocytes that reduce T-cell stimulatory function (73). Thus, Aβ vaccination may be a promising therapeutic option in the future.

Gene Delivery of Human ApoE

A recent study demonstrated that gene delivery of human apoE2 via a lentivirus vector plasmid directly into the hippocampus of another transgenic AD mouse model expressing endogenous mouse apoE significantly reduced hippocampal levels of insoluble Aβ1–42 and overall hippocampal Aβ burden (74). The same study revealed that gene delivery of human apoE4 into the hippocampus of the same transgenic mouse model whose endogenous mouse apoE had been knocked out greatly increased the hippocampal Aβ burden. In the future, when gene delivery to treat human diseases becomes perfected, this may become another viable therapeutic option in AD.

Blocking the ApoE/Aβ Interaction

Another recent study showed that competitive inhibition of the Aβ binding site on apoE by a nontoxic, BBB-permeable, synthetic Aβ peptide (Aβ12-28P) resulted in a significant reduction of Aβ plaques and CAA and reduction of the total Aβ burden in 2 AD transgenic mouse models, likely as a result of increasing Aβ clearance via LRP-1 (75). This is another interesting potential therapeutic approach. However, it has been shown that expression of LRP-1 is downregulated on the cerebral microvasculature in human
AD (48). Therefore, if this approach is dependent on Aβ clearance via LRP-1, its use would likely decrease with worsening AD. In addition, the synthetic Aβ compound has the potential to reversibly raise serum cholesterol (75), which can adversely affect the cerebral microvasculature.

**Injection of Soluble LRP-1**

A recent study revealed that native soluble LRP-1 is a major binding protein for Aβ in human plasma, and plasma levels of soluble LRP-1 are 30% lower in AD patients than in nondemented controls (76). Furthermore, injections of soluble LRP-1 into mice heterozygous for the sw mutation of APP significantly reduced the Aβ burden in both the hippocampus and cortex. Thus, soluble LRP-1 replacement therapy can be a future therapeutic option in AD.

**Intraventricular Infusion of Fibroblast Growth Factor 2**

As previously noted, the heparan sulfate proteoglycan agrin is found in the basement membrane of the cerebral microvasculature, indicating its significant role in BBB formation and function (63) and maintenance (21). Agrin contributes to the establishment of axon pathways by modulating the function of neurite-promoting molecules such as fibroblast growth factor (FGF) 2 or basic FGF (77). Thus, agrin and FGF-2 have a proven interaction within the CNS. Astrocyte-secreted FGF-2 also plays a role in maintaining the BBB. Astrocyte-secreted FGF-2 and FGF-5 are released at the abluminal membrane, which expresses another heparan sulfate proteoglycan, perlecan. Perlecan facilitates internalization of FGF-2 and thus stabilizes the BBB (78). Fibroblast growth factor 2 KO mice have reduced levels of intermediate filaments in perivascular astroglial end-feet accompanied by enhanced permeability of the BBB (79). The same study demonstrated that exogenous FGF-2 applied to astroglial cell cultures of FGF-2/FGF-5 double-KO mice returned immunohistochemical staining for glial fibrillary acidic protein to normal. This suggests that treatment of FGF-2 KO mice with an exogenous intraventricular infusion of FGF-2 might repair a compromised BBB, and that a similar approach might be a potential therapeutic alternative in human AD (78). However, this approach needs to be regarded with caution because 1 study showed that chronic infusions of FGF-2 consistently caused ventricular enlargement in rats (80). There may be a narrow therapeutic window between dose infusions of FGF-2 that would repair a compromised BBB or lead to hydrocephalus, and a delicate infusion balance would have to be achieved. Alternatively, the hydrocephalus problem might be circumvented by bypassing “unregulatable” astroglial/endothelial cell complex using an endothelial cell-specific promoter to drive FGF-2 expression locally at the BBB.

**SUMMARY, CONCLUSIONS, AND FUTURE DIRECTIONS**

This review has examined the phenomenon of increased BBB permeability in AD and its potential relationship to apoE genotype and brain Aβ burden. We hypothesize that patients with the e4 allelic isoform of apoE have higher brain Aβ levels and leakier BBBS, and that this can be a reason why the apoE4/4 genotype predisposes to the development of sporadic AD. Whereas apoE predisposes to fibrillation of Aβ deposits (52), which progress to become neuritic plaques in AD, the presence of apoE is required for the maintenance of BBB integrity (58). Hypotheses include loss of apoE from BBB astrocytic end-feet, causing an abnormal signaling cascade for tight junction establishment (58); loss of apoE from the vascular basement membrane, resulting in weakening of the membrane (60, 61); and a hyperactive immune inflammatory response and microgliosis, damaging the BBB (58, 59). One study measuring the surface vascular basement membrane surface area using anti-agrin immunoreactivity showed reduction in vascular basement membrane surface area in AD cases with an apoE4/4 genotype (66). This suggests that the basement membrane might be weaker in the presence of apoE4 and thus less able to maintain the BBB. Because agrin is an important component of the BBB (63), studying the effects of agrin loss on the permeability and transport of Aβ across the BBB (using transgenic agrin-deficient mice) would likely provide considerable insight. Other future studies should examine the expression and distribution of tight junction integral membrane and accessory proteins and the presence and concentration of inflammatory mediators and cytokines in apoE4/4 brains to determine if there are differences from apoE3/3 brains, and if these differences can result in enhanced BBB permeability and direct diffusion of Aβ across the BBB.

Apolipoprotein E4/4 genotype also results in enhanced parenchymal, perivascular, and vascular Aβ deposition, leading to CAA and resulting weakness of the vascular wall (11, 53–55), and increased Aβ production (3), which provides more Aβ substrate both for deposition as previously noted and a direct toxic effect of Aβ on BBB endothelial cells (50, 57). The latter would lead to alterations in the expression and distribution of tight junction proteins (22), potentially leading to increased BBB permeability. A preliminary investigation also suggests that apoE4/4 genotype may alter the expression of both LRP-1 and RAGE at the BBB with advancing age, potentially influencing Aβ transport across the BBB.

A number of therapeutic alternatives to seal a compromised BBB and eliminate Aβ deposits have been tested in transgenic AD mouse models, including Aβ vaccination without significant inflammation or hemorrhage (73), gene delivery of human apoE2 (74), blocking the apoE/Aβ interaction with a synthetic Aβ peptide (75), and injection of soluble LRP-1. The application of FGF-2 to FGF-2 KO glial cell cultures restored glial fibrillary acidic protein immunoreactivity (79), suggesting that an FGF-2 infusion may also restore a compromised BBB. In addition, developing a treatment to seal a compromised BBB may lead to insights on how to finely regulate the BBB to allow therapeutic agents (e.g. antibiotics, chemotherapy) into the CNS.

Although these animal and cell culture studies are both exciting and important, one must remember that positive
results in animal and/or culture experiments do not necessarily translate into successful treatments at the bedside. This was certainly evident in the initial Aβ vaccination clinical trial that had to be discontinued prematurely due to a cohort of patients developing meningencephalitis and intracerebral hemorrhage after vaccination (72). Transgenic AD mouse models may simulate some aspects of AD pathology, but they are not an exact substitute for the actual disease. Therefore, research using banked human AD and control brains and CSF remains absolutely vital and must be supported and encouraged if neurodegenerative diseases such as AD are to be conquered.

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