thematic review

Membrane dynamics, cholesterol homeostasis, and Alzheimer's disease

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Abstract **Alzheimer's disease (AD) is characterized by the deposition of β-amyloid (Aβ) plaques derived from the amyloidogenic processing; of a transmembrane protein called β-amyloid precursor protein (APP). In addition to the known genetic/sporadic factors that promote the formation of Aβ, the composition and structural dynamics of the membrane are also thought to play a significant role in the amyloidogenic processing of APP that promotes seeding of Aβ. This minireview reinforces the roles played by membrane dynamics, membrane microdomains, and cholesterol homeostasis in relation to amyloidogenesis, and reviews current strategies of lowering cholesterol in treating AD.**—N. B. Chauhan. **Membrane dynamics, cholesterol homeostasis, and Alzheimer's disease.** ***J. Lipid Res.*** **2003.** 44: **2019–2029.**

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STRUCTURE OF CELL MEMBRANE

Biological membranes play a critical role in carrying out almost all physiological functions of the cell. The molecular organization of the cell membrane forms an infrastructural basis for all specialized function(s) carried out by the cell membrane. Many model membranes of varying molecular organization have been hypothesized and proposed to justify membrane functions (1–4). Among these, a dynamic fluid mosaic model (5) that evolved after several hypothetical modifications (6–8) has become a widely accepted model applicable to most biological membranes.

Fluid mosaic model of cell membrane

According to this classic model, membrane is composed of a bulk of phospholipid organized as a bilayer, with globular integral proteins embedded within the phospholipid texture. Phospholipids are arranged with their ionic and polar head groups in direct contact with the aqueous phase at the exterior and interior surfaces of the bilayer (**Fig. 1**, black gradient-filled circles), thereby maximizing hydrophilic interactions at the aqueous surfaces, while the nonpolar saturated fatty acid chains interdigitate within the interior of the membrane bilayer, forming a matrix (5). Voids and spaces between these chain aggregates are filled with lipids, of which cholesterol constitutes a major component (9, 10). The lipid matrix of the model membrane exists in three different phases, i.e., gel, liquid-ordered, and liquid-disordered states, in order of increasing fluidity (11). In the gel state, lipids are semifrozen; in the liquid-ordered state, lipids are viscous; and in the liquid-disordered state, lipids exist as fluids. This liquid-disordered fluid phase is essential for protein functions (12). The liquid-disordered fluid phase can be transformed to the liquid-ordered or gel state by tight packing of phospholipids and hydrocarbon chains with cholesterol intercalations in order of increasing rigidity (9). Thus, the amount of cholesterol present in the membrane determines the fluid/rigid state of the membrane.

Integral membrane proteins [membrane anchor proteins, i.e., glycophosphatidylinositol (GPI), *Src*-family proteins, receptor ligands, signaling molecules, transmembrane proteins] are embedded within the phospholipid bilayer with their ionic and highly polar groups protruding to the exterior surface, and nonpolar groups largely buried in the hydrophobic interior of the membrane (Fig. 1, purple gradient-filled objects) (5). These integral proteins are involved in cellular signaling.

Membrane microdomains

The fluid mosaic model of Singer and Nicolson (5) hypothesized the existence of small membrane microdomains. A decade later, the presence of membrane microdomains was confirmed (13). Membrane microdomains were conceived of as part of a cellular mechanism for the intracellular trafficking of lipids and lipid-anchored proteins (13, 14).

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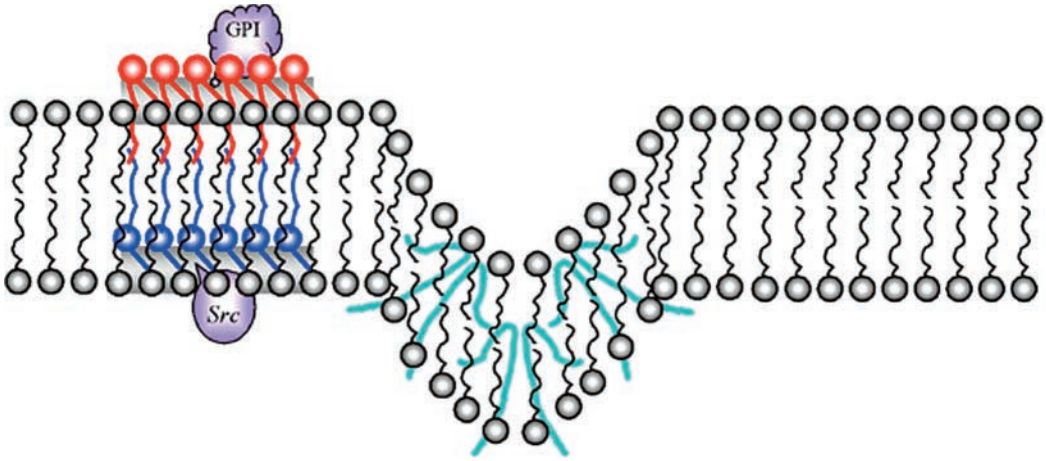

Fig. 1. Fluid mosaic model structure of the cell membrane including membrane-microdomains: black gradient-filled circles, phospholipid polar heads of lipid bilayer; black wavy lines, nonpolar fatty acid tails from phospholipid polar heads; red gradient-filled circles, lateral assemblies of sphingolipids forming exoplasmic leaflet of the lipid raft; red lines, saturated hydrocarbon chains of sphingolipid heads within the exoplasmic leaflet; blue gradient-filled circles, lateral assemblies of glycerolipids (phosphatidylserines/phosphatidylethanolamines) forming cytoplasmic leaflet of the lipid raft; blue lines, saturated hydrocarbon chains of glycerolipid heads within the cytoplasmic leaflet; gray fill, cholesterol intercalations filling the voids between sphingolipid/phospholipid heads in the exoplasmic leaflet and glycerolipid/phospholipid heads in the cytoplasmic leaflet of a representative lipid raft; purple gradient-filled objects, globular integral proteins within the raft leaflets [glycophosphatidylinositol (GPI)-anchored proteins embedded in the exoplasmic leaflet (GPI) and *Src*-family proteins embedded in the cytoplasmic leaflet (*Src*) of the raft]; teal lines, hairpin-shaped caveolin molecules within the bilayer structure of caveolae.

These membrane microdomains are now considered to be essential components of various physiological functions. (15). There are two types of lipid microdomains, namely rafts, also called noncaveolar lipid microdomains (16), and caveolae, also called modified rafts (17). Both rafts and caveolae are sphingolipid-cholesterol-rich structures that form the "docking sites" for cellular signaling (10).

Rafts. Lipid rafts are dynamic assemblies of cholesterol and sphingolipids in the lateral leaflet of the membrane bilayer that behave as "moving platforms" (10). The lipid bilayer in rafts is asymmetric, with sphingolipids and glycosphingolipids enriched in the exoplasmic leaflet and glycerolipids (phosphatidylserine/phosphatidylethanolamine) in the cytoplasmic leaflet (10). The sphingolipids associate laterally with one another with their carbohydrate head groups, and are connected to the lipid bilayer by their saturated hydrocarbon tails. These hydrocarbon tails are interconnected by cholesterol intercalations. In addition, all spaces and voids between associating sphingolipid/glycerolipid-phospholipid head groups and between hydrocarbon chains are filled with cholesterol molecules. Thus, cholesterol behaves as a spacer in the formation of lipid rafts.

SYNTHESIS, TRAFFICKING, AND DISTRIBUTION. Cholesterol is synthesized in the endoplasmic reticulum (ER) (18). Sphingolipid synthesis and head-group modifications are completed largely in the Golgi (19). Cholesterol-sphingolipid rafts are first assembled in the Golgi (11), and are then transported to the plasma membrane (20). Trafficking of lipid rafts does not end with surface delivery; they are continuously endocytosed and recycled back to the cell membrane in response to different stimuli (21), one of which is increased membrane cholesterol (22). Rafts are roughly ~50 nm in size, corresponding to ~3,500 molecules of sphingolipids (10). Individual rafts can coalesce to form bigger structures in response to specific physiological stimuli (23). The distribution of lipid rafts over the cell membrane depends upon the cell type. In neurons, lipid rafts accumulate on somal and axonal membranes more frequently than on somatodendritic membranes. Lipid rafts are also found to be present in the postsynaptic sites of neurons (16).

SIGNALING. Rafts are involved in cellular signaling, cell adhesion, and molecular sorting (23). As mentioned earlier, rafts are stable lipid-ordered membrane microdomains that provide a platform for membrane signaling and trafficking events (10). Rafts harbor globular integral proteins within the exoplasmic (e.g., GPI, FcR) and cytoplasmic (e.g., *Src* family) leaflets. The target protein binds to these integral membrane proteins through receptors or ligands and activates downstream signaling (24, 25). Many signaling proteins are transferred in and out of the lipid rafts during the signal transduction process (26). These include epidermal growth factor receptor, insulin growth factor receptor, tyrosine kinases, G-proteins, Ras, adenylyl cyclase, PI3 kinase, protein kinase C (PKC) isoenzymes, Fas ligands, and tumor necrosis factor-α (TNFα) (24). Lipid rafts serve as docking sites for extracellular ligands (11). In addition, the components of exocytotic machinery, including Syntaxin, SNAP-25, and VAMP-2, are closely associated with rafts, suggesting the involvement of rafts in exocytosis (27). Although the size of steady-state lipid rafts is smaller, ranging between 10 and 100 nm in diameter (17, 28), they can

coalesce to form larger domain(s), which are necessary for carrying out complex signaling (23, 28, 29). The coalition of smaller rafts into a larger domain is mediated by ceramide, a specific sphingolipid that constitutes a hydrophobic backbone for all complex sphingolipids (26).

Caveolae. Caveolae ("little caves") are morphologically defined cell surface invaginations made up of a characteristic protein called caveolin that is found to be present only in caveolae and not in rafts (25). Caveolae are modified from rafts by polymerization of caveolin, a hairpin-like, palmitylated integral membrane protein that tightly binds with cholesterol within the lipid bilayer (Fig. 1, teal-colored, hairpin-like structures) (24). Caveolae serve as a scaffold for signaling molecules (30).

SYNTHESIS, TRAFFICKING, AND DISTRIBUTION. Caveolins are synthesized in the ER, and caveolin-cholesterol molecules are assembled in the trans-Golgi network (TGN) (24). Caveolae are localized in the TGN, exocytotic vesicles, ER, and plasma membrane (31, 32). Some studies have suggested that caveolin is internalized and recycled via a microtubule-independent pathway back to the ER/Golgi (16). Caveolin exists in three distinct isoforms, i.e., Cav-1, Cav-2, and Cav-3. Cav-1 and Cav-2 are expressed in most cell types, including neurons (29). It has been shown that Cav-1 is required for the formation of caveolae, because caveolae are not formed in Cav-1 knock-out mice (33, 34). Cav-1 oligomerizes with cholesterol and triggers the formation of caveolae (35, 36). The Cav-1 isoform of caveolin interacts with lipid-anchored integral membrane proteins and soluble signaling proteins, including *Src*-family tyrosine kinases, G-proteins, endothelial nitric oxide (NO) synthase, PKC, Ca^{+} pumps, and inositol 1,4,5-triphosphate receptor (30). This interaction is reported to take place via a highly conserved 20-amino acid domain termed the caveolin-scaffolding domain (26).

SIGNALING. In comparison to rafts, the role of caveolae in signaling is poorly defined (37). Although caveolae are implicated in cellular signaling (24, 25, 38), they may not be absolutely necessary, because some cell types, such as lymphocytes and neurons, either lack or have very few caveolae (25). Clusters of GPI-anchored proteins and glycosphingolipids are enriched in caveolae, suggesting that proteins and lipids associated with sphingolipid-cholesterol rafts might become trapped in caveolae (37, 39). It appears that protein associating with rafts get fixed in caveolae as a result of caveolin-induced invaginations of sphingolipid-cholesterol rafts (10). Thus, caveolae either may serve as stores of inactive signaling molecules or may function as active signaling depots, depending upon the physiological need.

CHOLESTEROL HOMEOSTASIS AND MEMBRANE FUNCTIONS

Although the brain is the organ richest in cholesterol (18), most of it is derived from neosynthesis in the brain itself (40). Dietary cholesterol is transported to the liver in the form of chylomicrons by receptor-mediated endocytosis. The liver releases cholesterol in the form of LDL or VLDL, with apolipoproteins as their carrier-coat proteins (40). Most of the peripheral cells obtain cholesterol via LDL or VLDL receptor uptake (41). After uptake, lipoproteins are degraded and cholesterol is released within the cell, where it is stored in the form of free or esterified cholesterol. Extra amounts of cholesterol are removed by HDLs (42, 43). Thus, intracellular cholesterol levels are tightly regulated by LDL/VLDL→free/esterified cholesterol→HDL recycling.

Because the brain is located behind the blood-brain barrier (BBB), it does not compete with circulating lipoproteins for cholesterol to the extent that peripheral tissues do (40). Brain cells, including neurons and non-neuronal cells, meet their demand for cholesterol by de novo synthesis (44), and very little cholesterol is taken up from plasma (45). The brain apolipoproteins are not involved in the transport of cholesterol to and from the brain but are involved in the redistribution of cholesterol within the brain during axonal/synaptic remodeling (46). Removal of excess brain cholesterol occurs mainly via the conversion of cholesterol to 24-hydroxycholesterol that can pass the BBB (47). Cellular cholesterol levels are tightly regulated, because membrane cholesterol affects membrane functions by regulating the physico-chemical properties of the cell membrane (40). Therefore, all membrane-associated proteins, including β-amyloid (Aβ) precursor protein (APP), are likely to be affected by the lipid composition of the membrane to which they are anchored. Cholesterol may alter the activity of proteins embedded within the membrane by modulating membrane fluidity (48), or may perturb membrane protein function by a mechanism independent of its membrane-ordering effect (49).

Cholesterol and APP processing

APP is a glycosylated transmembrane protein with a short intracellular C-terminal segment, a long extracellular N-terminal segment, and an intermediate Aβ segment shared between the intracellular and extracellular segments (50). It is cleaved by α-secretase immediately before or after reaching the cell surface, releasing the nonamyloidogenic secreted form of the N-terminal soluble APP fragment (sAPPα) (51). APP molecules that escape α-secretase cleavage are internalized and subjected first to β-site cleavage by β-secretase, leaving behind a membrane-bound C-terminal stub (52). This C-terminal stub is the substrate for γ-secretase that cleaves the molecule at a γ site(s) to release 40aa/42aa/43aa long Aβ peptides (53). Processing of APP by β- and γ-secretases also occurs under normal physiological conditions, indicating that all fragments of APP, including Aβ peptide, are part of normal physiology (53, 54). In neurons, ~95% of APP is cleaved by α-secretase, while the remaining 5% is subjected to β cleavage (40). Both sAPPα and Aβ produced at normal levels have their own physiological functions. sAPPα is synaptotrophic (55), while Aβ at normal physiological concentrations potentiates tyrosine phosphorylation, increases the activity of phosphoinositol 3-kinase and extracellular PKC (56), and functions as a vascular sealant (57). It is the excessive

genesis of Aβ that produces detrimental effects, due to subsequent oligomerization of Aβ and fibrillation.

Under normal circumstances, excess amyloidogenic Aβ peptides are cleared. However, under abnormal genetic [e.g., overexpressed normal APP (Down's syndrome), mutated APP (Swedish/Flemish/London mutations)] or nongenetic/physiological (e.g., high cholesterol, aging, other unknown factors) circumstances, clearance of excess Aβ is not achieved to the fullest extent (40). In the case of overexpressed normal or mutated APP, biochemical equilibrium between substrate, enzyme, and the end product is disturbed. Available α-secretase may fail to cope with abundantly increased substrate (APP), thus rendering extra APP to β- and γ-cleavage. In the case of increased cholesterol, a change in membrane fluidity/dynamics and other effects due to increased membrane cholesterol may lead to increased production of Aβ.

Membrane association and colocalization of enzyme and substrate (APP) are critical for the activity of α-secretase (58, 59). α-Secretase cleavage requires that APP be inserted into a membrane, and it cleaves APP at a fixed distance from the membrane rather than at a specific amino acid sequence (60). A change in the lipid ordering of the membrane perturbs the association/insertion of APP within the membrane and is likely to inhibit α-secretase cleavage. Increased membrane rigidity due to cholesterol loading seems to inhibit the required contact between the enzyme and the target protein (61).

In addition to the effect of cholesterol on membrane lipid ordering, subcellular distribution of cholesterol also has been shown to regulate proteolytic processing of APP (62). Runz and coworkers (63) supported this finding by demonstrating a dose-dependent reduction in both secreted and cellular Aβ species in neurons and neuroblastoma cells exposed to a cholesterol transport-inhibiting agent. They hypothesized that the inhibition of cholesterol transport from the endocytic compartment to the ER may affect reinternalization of surface APP during Aβ generation and thus reduce Aβ. On the other hand, retention of cholesterol in the endosomal/lysosomal compartment induced accumulation of γ-secretases in the vesicular organelles involved in cholesterol sorting (63).

Mounting evidence indicates a role for cholesterol in the metabolism of APP in vitro and in vivo. Racchi et al. (48) have demonstrated that membrane cholesterol enrichment in COS cells with increasing concentrations of nonesterified cholesterol caused a dose-dependent inhibition in sAPPα release, while selective loss of cellular cholesterol increased sAPPα release. Membrane cholesterol modulation of sAPPα secretion appeared to be specific, because another steroid, progesterone, did not affect sAPPα secretion in COS cells. Similar modulation of sAPPα secretion was reported by Bodovitz and Klein (61). They showed significant inhibition of sAPPα secretion from HEK 293 cells overexpressing APP when exposed to high cholesterol concentrations ranging between 0.4 and 2.4 mg/ml. Cholesterol-mediated modulation of APP processing has been demonstrated by a dramatic reduction of Aβ production after exposing cultured hippocampal neurons to the cholesterol-extracting agent methyl-β-cyclodextrin (64), and after exposing mixed cortical neurons and primary hippocampal neurons to the cholesterol-lowering drug simvastatin (65). Cholesterol depletion also resulted in marked reduction of the C-terminal β-stub, indicating a role of cholesterol in β-cleavage (64). Increased levels of cholesterol inhibited the activity of the α-secretase ADAM 10, while reductions in cholesterol and treatment with the cholesterol-lowering drug lovastatin stimulated α-secretase activity and expression, which were overcome by supplying exogenous cholesterol but not by supplying mevalonic acid (66). When APP-transfected human HEK cells were treated with the cholesterol-lowering drug lovastatin, the β-secretase cleavage of newly synthesized APP was markedly reduced, while addition of cholesterol to the medium increased β-cleavage 4-fold (67).

The dependence of APP processing on cholesterol has not been as well explored in vivo as it has been in vitro. Available data show that suppression of cholesterol neosynthesis drastically reduced cerebral Aβ levels in guinea pigs treated with the cholesterol-lowering drug simvastatin (65). In transgenic mice overexpressing APP, a high-cholesterol diet led to increases in Aβ and neuritic plaques and decreases in sAPPα (68).

Because cholesterol elevation is synergistic with Aβ production as well as with raft formation, it is logical to postulate that the raft microdomain has a role in the amyloidogenic processing of APP. In fact, the amyloidogenic processing of APP is known to occur in the raft microdomain of the membrane, while α-cleavage takes place outside the rafts (40, 64, 66, 69). Therefore, conditions that elevate brain cholesterol would facilitate raft formation and amyloidogenic processing, while lowering cholesterol not only would reduce Aβ formation but also would enhance production of synaptotrophic sAPPα by facilitating α-cleavage, as suggested by Anderson et al. (59) and Sisodia (60).

Recent research supports this hypothesis that lipid raft may be a site for proteolytic processing of APP. Bouillot and coworkers reported that ~5% of the total normal APP is associated with detergent-insoluble-glycolipid (DIG)-enriched fraction that is predominantly present in rafts (70). Another study reported that a significant portion of total cellular APP is associated with DIGs isolated from APP-transfected rat hippocampal neurons (64), and from rat brain cortical gray matter (69). It has been shown that a large proportion of Aβ along with presenilins are present in DIGs isolated from brain tissues or neuronal cell cultures (69, 71, 72).

Aβ within rafts seems to promote fibrillogenesis of soluble Aβ. A recent report indicated that Aβ associated with cholesterol-rich membranes adopts a different conformation, acting as a seed for fibrillation (73, 74). The presence of a ganglioside, GM1, within the raft is known to bind Aβ and is proposed to change the conformation of Aβ (75, 76).

Apolipoprotein E polymorphism and APP processing

Apolipoprotein E (apoE) molecules are lipid carriers involved in the transport and distribution of cholesterol

and lipids from the liver to all extrahepatic tissues via an LDL receptor-mediated mechanism (77). ApoE is not involved in the transport of cholesterol to and from the brain, but instead is involved in the redistribution of cholesterol in the brain to different cellular and subcellular sites of neurons (46) needed for membrane remodeling during regeneration of neurites, axons, and synapses (78–81). ApoE is known to maintain the synaptic integrity of the synapto-dendritic apparatus of neurons by stabilizing the neuronal cytoskeleton, regulating intracellular calcium, and regulating interaction between neurons and the extracellular matrix, in addition to supplying esterified cholesterol as a building block to the regenerating neurons (82). ApoE also is known to be an Aβ-scavenging molecule that regulates extracellular Aβ concentration through apoE receptor internalization via the endosomal/lysosomal mechanism (83).

Apo E is a 34 kDa protein (46) encoded by a polymorphic gene located in chromosome 19 (84). The three different isoforms of ApoE (ApoE2, ApoE3, ApoE4) are respectively coded by three separate alleles (e2, e3, e4) that are inherited in a codominant fashion at a single genetic locus (85). These isoforms differ in amino acids at positions 112 and 158 (46). The most common isoform ApoE3 has cysteine at position 112 and arginine at position 158, ApoE2 has cysteine at both positions, whereas ApoE4 lacks cysteine at both positions and contains arginine instead (86). Therefore, ApoE4 cannot undergo intramolecular or intermolecular disulfide cross-linking (87).

ApoE mRNA is most abundant in the liver and brain; however, substantial amounts are also found in other peripheral tissues (88, 89). Within the central nervous system (CNS), apoE is primarily synthesized and secreted by astrocytes (90, 91) and macrophages (92). Glial-derived apoE is taken up by neurons and concentrated at the synaptic terminal and neuromuscular junction (93), where it may play a role in plasticity (42, 94). While the peripheral nervous system and other tissues produce apoE, apoD, apoA-I, and apoA-IV (95), the CNS mainly synthesizes apoE (79). This suggests that apoE might be important in CNS development, maintenance during aging, and neuroregeneration after injury (76, 79).

There has been considerable interest in identifying the role of apoE in the development, maturation, and aging of the CNS. Studies in in vitro models have shown that apoE promotes neuritic extension in an isoform-dependent manner (apoE-2 > apoE-3 > apoE-4), indicating that apoE-2 plays a significant role in the maturation of the nervous system (80). While studies in young apoE-deficient homozygous mice have not shown significant CNS alterations, aged apoE-deficient homozygous mice (C57BL/6J) showed significant synaptic and dendritic alterations in the neocortex and limbic system when compared with age-matched and littermate controls (81). ApoE-deficient homozygous mice showed an age-dependent 15–40% decrease in synaptophysin and microtubule-associated protein 2 immunoreactivity in the hippocampus and neocortex, and displayed increased reactivity of astrocytic and microglial markers, when compared with age-matched controls (81). Consistent with this are studies showing significant learning deficits in apoE-deficient mice, as evaluated by Morris water maze performance (96–98). Furthermore, infusion of recombinant apoE into the lateral ventricles of apoE-deficient mice resulted in a reversal of the behavioral and neuronal alterations, indicating that this molecule might have neurotrophic capabilities (82, 96).

In addition to the neurotrophic and synaptotrophic capabilities of apoE, an amyloid-scavanging role for apoE has also been proposed. Beffert and Poirier (99) presented evidence supporting the role of apoE as an amyloid-scavanging molecule that regulates extracellular Aβ through apoE receptor-mediated internalization via the endosomal/lysosomal path. This concept is supported by the finding that breeding PDAPP mice with apoE knockout mice completely abolished amyloid deposition in the resulting hybrids without affecting steady-state levels of cerebral Aβ40/42 (100). Another study showed that crossbreeding PDAPP mice with apoE-3 or apoE-4 knock-in mice produced a marked reduction of amyloid deposition (101). It has also been suggested that apoE may form a complex, not only with fibrillar Aβ but also with soluble Aβ, as has been shown in human brain (102), indicating that apoE-Aβ interactions prior to Aβ deposition are likely to regulate Aβ clearance. ApoE-containing lipoproteins in the brain may sequester Aβ and facilitate its cellular uptake and degradation locally by cells or its removal from the brain into the systemic circulation. ApoE/Aβ complexes are transported from the brain extracellular space into the systemic circulation through bulk cerebrospinal fluid flow or through specific apoE receptors at the BBB (103). These findings strengthen the biological role of apoE in clearing extracellular amyloid.

The physiological effects of apoE are isoform specific, possibly because of the lack of cysteine at positions 112 and 158 (87). The neurotrophic and synaptotrophic effects of apoE-2 and apoE-3 are more pronounced than those of apoE-4 (80). Similarly, the Aβ-scavenging effects of apoE also seem to be isoform specific (103). The isoform-specific effects of apoE on Aβ levels and neuritic plaque formation in PDAPP mice with different human apoE isoforms are shown to be in accordance with those found in Alzheimer's disease (AD) (E4 > E3 > E2) (104, 105).

The role of apoE-4 in AD was first discovered by Strittmatter and coworkers (106), who showed a correlation with the genetic linkage site on chromosome 19 in individuals affected with AD. This observation was supported by the finding that the apoE-4 allele was present in ~65% of the cases with late-onset familial autosomal dominant AD and 50% of the sporadic AD cases with onset between 65 and 80 years of age (107). Recent studies have shown that ~64% of AD cases are associated with the presence of allele e4 of apoE (108, 109), suggesting that an abnormally functioning isoform of apoE (apoE-4) may be a potentiating factor in AD pathogenesis, in addition to amyloid deposition and tangle formation (110, 111). However, the precise mechanisms by which abnormal

functioning of apoE-4 might lead to AD are not yet fully understood. While some studies support a role of apoE in amyloid clearance (112), others suggest a role in protection against excessive tau phosphorylation (111) and abnormal intracellular calcium influx (113). Fagan and coworkers (105) have reported that cerebral levels of soluble Aβ40/42 were increased in AD subjects carrying apoE-4, compared with non-apoE-4 AD subjects. This finding was supported by the observed association between apoE-4 dose and increased Aβ levels in the hippocampus and cortex of AD subjects (99). Another observation showed that AD cases with the APOE e4 allele had a more-severe dementia when compared with AD cases without the APOE e4 allele (114).

ApoE-4 is postulated to affect Aβ fibrillation. Strittmatter and colleagues (106) have demonstrated that purified human apoE binds to Aβ fragments in vitro. They proposed that the apoE/amyloid complex triggers a cascade of molecular events that could lead to plaque formation. Further, Strittmatter et al. (106) demonstrated that purified apoE-4 has a higher affinity for Aβ than do apoE-3 and apoE-2, in a decreasing order.

Taken together, the findings appear to show a strong correlation between apoE-4, cholesterol homeostasis, and APP processing. Normal apoE isoforms (apoE-2 or apoE-3) are Aβ-scavenging molecules that regulate extracellular Aβ concentration through apoE receptor internalization via the endosomal/lysosomal path (83). Because apoE-4 cannot undergo intramolecular or intermolecular disulfide cross-linking (87), this particular isoform may fail to internalize extracellular Aβ to endosomes/lysosomes and hence may not clear extracellular Aβ efficiently.

Furthermore, the biochemical difference in the apoE-4 molecule (lack of cysteine) may lead to reduced uptake and transport of cholesterol/lipids from the liver to extrahepatic tissues, and to reduced distribution and relocation of cholesterol within the brain. Therefore, expression of apoE-4 may lead to increased steady-state levels of serum/plasma cholesterol (115, 116), while in the brain, it may lead to asymmetrical distribution of cholesterol within the different subcellular compartments or microdomains of neurons. In fact, the expression of apoE-4 is reported to be involved in cholesterol loading of the exoplasmic leaflets that are associated with synaptic membranes (117), which may promote the formation of rafts (10). Raft microdomains provide a suitable environment for amyloidogenic processing of APP (64, 69, 72).

Thus, lipid/cholesterol homeostasis, lipid/cholesterol carrier proteins (apoE), cholesterol, and AD are linked. Therefore, cholesterol-lowering strategies have acquired therapeutic importance in treating AD.

3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE INHIBITORS

Cholesterol synthesis in neurons is regulated by 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. The enzyme HMG-CoA reductase catalyzes a rate-limiting step in cholesterol biosynthesis and is markedly inhibited by statins (HMG-CoA reductase-inhibitors) (118).

Statins

Epidemiologic studies demonstrate that hypercholesterolemia is a risk factor for AD (119) and that reduced prevalence of AD is observed in patients using statins (120, 121). Although some AD clinical trials favor the use of statins (122, 123), a few inconclusive clinical reports have questioned the efficacy of statins in preventing the progression of AD (124, 125). This discrepancy may be due to differential metabolism, immunomodulatory effects, and the permeability of the BBB by lipophilic and hydrophilic statins.

Lipophilic versus hydrophilic statins. Pharmacologically, statins are categorized as lipophilic or hydrophilic, depending upon their solubility in lipid solvents or water (126). Lipophilic statins readily cross the BBB, while hydrophilic statins cross the BBB very slowly (127).

SIMILARITIES. Both types of statins inhibit HMG-CoA reductase and reduce the formation of mevalonate. This not only blocks the sterol-mevalonate path, leading to the reduction of squalene and cholesterol, but also inhibits the nonsterol-mevalonate path, resulting in the reduction of isoprenoids and G-proteins (Rho, Rab, Rap), thus leading to the subsequent inactivation of nuclear factor κB (NFκB) (128). Despite the fact that the mevalonate blockade remains identical for both types of statins, lipophilic statins produce adverse systemic effects, possibly due to the following differences.

DIFFERENCES. Lipophilic statins differ from hydrophilic statins in that lipophilic statins possess a methyl moiety, rather than the hydroxyl moiety present in hydrophilic statins at position 6. When orally administered, both types of drugs are metabolized in the liver before entering systemic circulation. Lipophilic statins are metabolized by the cytochrome P-450 3A (CYP3A) enzyme, while hydrophilic statins are broken down to their active metabolites in a non-CYP3A-dependent manner (129). This difference is expected to have a clinically significant effect. It is interesting to note that skeletal muscle toxicity was observed when lovastatin (a lipophilic statin) was administered, even when coadministered with a CYP3A-inhibitor, whereas no such toxicity was reported when pravastatin (a hydrophilic statin) was administered with or without CYP3A inhibitors (129). The reason for the toxicity may be higher lipophilicity and accelerated penetration across the cell membrane. Lipophilic statins, and not hydrophilic statins, are reported to be associated with gastrointestinal and skeletal abnormalities (129). Lipophilic statins readily cross the BBB and hence are more likely to affect CNS functions, whereas hydrophilic statins cross the BBB very slowly and hence have less potential to cause CNS side effects (126, 127). Therefore, although both lipophilic and hydrophilic statins inhibit the formation of mevalonate, resulting in reduced cholesterol levels and inflammation, differences in their lipophilicity, membrane penetration, processing by CYP-450 3A enzyme in the liver, and other as yet uncharacterized and unidentified

factors associated with lipophilic statins seem to abolish their anti-inflammatory effect and tend to produce skeletal muscle abnormalities.

Statins and APP processing. Evidence for regulation of APP processing and Aβ formation by either cholesterol or an intermediate product is mounting. Of greater significance is the evidence that statin exposure not only reduces Aβ formation but also shifts the balance of APP processing from the toxic β-secretase to the nontoxic α-secretase pathway. In transgenic mice overexpressing APP, a high-cholesterol diet led to increases in Aβ and neuritic plaques and decreases in sAPPα, thus indicating changes in APP processing (68). It was found that elevated cholesterol levels inhibited the activity of the α-secretase ADAM 10, while reductions in cholesterol and incubations with lovastatin stimulated α-secretase activity and expression, which were overcome by supplying exogenous cholesterol but not overcome by supplying mevalonic acid (66). Depletion of cholesterol resulted in decreased APP endocytosis, thus possibly exposing APP to a greater degree of α-secretase activity on the surface of cells. It has been found that exposure of neural cells to cholesterol reduces glycosylation of APP (48,) which might shift the balance between the β- and α-secretase pathways. Additionally, cholesterol increases seeding of Aβ and fibrillation in epithelial cells (73). Cholesterol facilitates binding of Aβ to GM1 ganglioside clusters in lipid bilayers, and the increased membrane-bound Aβ triggers a conformational transition to β-sheet structure (74). Thus, there are several pathways by which either cholesterol itself, an intermediate, or a direct effect of statin on α-secretase expression may influence APP processing and Aβ formation. There has been only one reported study of statin effects on brain APP and Aβ in vivo (65). In this seminal study in guinea pigs, it was found that very high doses of simvastatin fed to guinea pigs (0.5% of diet) for 3 weeks resulted in 40–50% reductions in Aβ in the CSF and brain. While total brain cholesterol levels were not reduced, levels of the precursor lathosterol were reduced ~50%, thus indicating reduced de novo cholesterol synthesis in the brain. Because the total brain cholesterol did not decline in this experiment, the effect of simvastatin may have been via a precursor of cholesterol. The most clinically significant result in this experiment is the decrease in the toxic Aβ peptide produced by dietary statin, which corroborates the reported clinical studies indicating the inverse relationship of statin therapy with AD (120, 121).

Statins and AD. There is increasing evidence that cholesterol metabolism, trafficking among cellular compartments, or binding to receptors in the brain is related to pathogenesis of AD. Cortical cholesterol was found to be elevated in AD brain compared with controls (130). The rate of formation of Aβ in hippocampal neurons is inversely correlated with the content of cholesterol (64). ApoE is a cholesterol transport protein that binds to lipoprotein receptor-related protein (LRP), which is also involved in cellular uptake of cholesterol and Aβ. The e4 isoform of apoE is one of the strongest known risk factors for AD (131). Alpha-2 microglobulin, a putative risk factor for AD, also binds to LRP and affects endocytosis of Aβ (132). This information suggests that the clinical use of statins to reduce endogenous synthesis of cholesterol might reduce the progression of AD. In the first reported clinical studies, strong inverse relationships were found between AD (121) or dementia and treatment with statins (120). In a longitudinal study of a Finnish population, elevated serum cholesterol concentrations (>6.5 mmol/l) during midlife were found to be a significant risk factor for development of AD in later life (133).

Aged garlic extract

Extract of fresh garlic that is aged over a prolonged period of time is known as aged garlic extract (AGE). AGE contains multipotent water- and lipid-soluble phytochemicals (134). These include water-soluble organosulfur compounds [*S*-allyl-cysteine (SAC), *S*-allyl-mercaptocysteine, allixin, selenium] and lipid-soluble organosulfur compounds [diallyl sulfide (DAS), diallyl disulfide (DADS)]. All of these organosulphur components of AGE have been demonstrated to impart multiple beneficial effects in various in vivo and in vitro systems.

Antiamyloidogenic effects of AGE. Although statins constitute one of the investigational drugs by virtue of being hypocholesterolemic agents that are expected to reduce the formation of lipid rafts, change membrane fluidity, reduce amyloidogenic processing, and promote secretory processing, all of which are demonstrated to reduce amyloid burden (119), lipophilic statins are proinflammatory and may produce adverse effects (118). AGE (SAC, DAS, and DADS) on the other hand is a naturally tolerable alternative that inhibits HMG-CoA reductase (135, 136) and other lipogenic enzymes, such as glucose-6-phosphatase and fatty acid synthase (137). Current data from our laboratory show that AGE treatment significantly reduces, by 15–22%, a cerebral amyloid load that was originally elevated ~21-fold by Swedish transgene in Tg2576 (138).

Anti-inflammatory effects of AGE. Plaque-initiated neuroinflammation is recognized as a secondary prominent feature in AD pathology, and hence nonsteroidal anti-inflammatory drugs (NSAIDs) have acquired therapeutic importance. However, due to their adverse systemic effects (gastrointestinal bleeding, hepatic and renal toxicity), their use is limited. AGE that targets the inflammatory cascade at multiple steps seems to be a good alternative to synthetic single-effect NSAID(s), due to its proven NSAID-like effect and lack of adverse side effects.

Aβ is known to induce inflammation and stimulate the activation and transcription of NFκB, subsequently inducing inducible nitric oxide synthase (iNOS) and NO production occurring through an NFκB-dependent mechanism (139). AGE has been shown to attenuate the inflammatory cascade. Geng and coworkers (140) demonstrated that SAC dose-dependently inhibited the NFκB activation induced by TNFα in Jurkat cells. Kim and coworkers (141) supported this finding by examining the effect of AGE on NFκB activation, NO production, and free radical formation in LPS-stimulated RAW264.7 cells. The results show that AGE inhibited NFκB activation and NO production

via downregulation of iNOS mRNA. Additionally, AGE suppressed formation of free radicals, confirming its antioxidant activity. Another study showed that AGE induces enhancement of the free radical scavenging enzymes superoxide dismutase, glutathione peroxidase, and catalase, and inhibits lipid peroxidation (142). AGE protects DNA against free radical damage (134).

Antiapoptotic effects of AGE. Jackson and coworkers (143) have shown that AGE treatment resulted in the inhibition of the activity of caspase-3, a key enzyme that mediates apoptosis, in a dose-dependent manner. This finding was strengthened by the observation that AGE attenuated Aβ-induced apoptosis in PC12 cells (144).

Finally, AGE may have a role in protecting against loss of brain function, as suggested by its ability to increase cognitive functions, memory, and longevity in the senescence-accelerated mouse model (145, 146). Other beneficial effects of AGE include neurotrophic activity on cultured rat hippocampal neurons that not only enhanced the survival but also promoted the branching of hippocampal neurons. Numagami and Ohnishi reported neurotrophic effects of AGE in attenuating ischemic damage in rat brain (142).

In summary, AGE is a natural HMG-CoA reductase inhibitor, NSAID, antioxidant, and antiapoptotic agent. Due to its known dietary consumption for centuries, it will undoubtedly be well tolerated and have virtually no adverse side effects.

Effects of acute cholesterol depletion

Cholesterol homeostasis is critically important to cellular functioning, and hence the levels of cholesterol are tightly maintained. Both an excess of cholesterol and an acute depletion of cholesterol are harmful to the cell. Acute cholesterol depletion disrupts the clusters of soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors required for exocytosis (147), blocks the formation of clathrin-coated endocytic vesicles (148, 149), and most importantly, delocalizes the plasma membrane signaling phospholipid, phosphatidylinositol (4,5)bisphosphate [PIP(4,5)P2], from the plasma membrane (150). PIP(4,5)P2 is a major regulator of the actin cytoskeleton (151–153), and is intimately involved in endocytosis (154). Hence, acute cholesterol depletion that disperses PIP(4,5)P2 from sites of functional reaction can disrupt many cell functions. If the threshold of plasma membrane cholesterol falls below 40 mol%, membrane microdomains cannot be formed (36).

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