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The Role of Cholesterol in Pathogenesis of Alzheimer's Disease

Dual Metabolic Interaction between Amyloid β-Protein and Cholesterol

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Abstract

The implication that cholesterol plays an essential role in the pathogenesis of Alzheimer's disease (AD) is based on the 1993 finding that the presence of apolipoprotein E (apoE) allele ε4 is a strong risk factor for developing AD. Since apoE is a regulator of lipid metabolism, it is reasonable to assume that lipids such as cholesterol are involved in the pathogenesis of AD. Recent epidemiological and biochemical studies have strengthened this assumption by demonstrating the association between cholesterol and AD, and by proving that the cellular cholesterol level regulates synthesis of amyloid β-protein (Aβ). Yet several studies have demonstrated that oligomeric Aβ affects the cellular cholesterol level, which in turn has a variety of effects on AD-related pathologies, including modulation of tau phosphorylation, synapse formation and maintenance of its function, and the neurodegenerative process. All these findings suggest that the involvement of cholesterol in the pathogenesis of AD is dualistic—it is involved in Aβ generation and in the amyloid cascade, leading to disruption of synaptic plasticity, promotion of tau phosphorylation, and eventual neurodegeneration. This review article describes recent findings that may lead to the development of a strategy for AD prevention by decreasing the cellular cholesterol level, and also focuses on the impact of Aβ on cholesterol metabolism in AD and mild cognitive impairment (MCI), which may result in promotion of the amyloid cascade at later stages of the AD process.

Index Entries: Alzheimer's disease; cholesterol; amyloid β-protein; tau phosphorylation; statin; HMG-CoA reductase inhibitor; raft.

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Introduction

The brain is the most cholesterol-rich organ in the human body. However, cholesterol metabolism in the central nervous system (CNS) is not fully understood. Because the CNS is segregated from the systemic circulation by the blood-brain barrier, lipids transported by the systemic circulation are not generally available to the CNS. Moreover, the CNS contains high-density-lipoprotein (HDL)-like particles, but does not contain low-density lipoprotein (LDL) or very low-density lipoprotein (VLDL) *(1),* and it contains fewer types of apolipoproteins than the systemic circulation. Apolipoproteins identified in cerebrospinal fluid (CSF) are mainly apolipoprotein E (apoE) and apoA-I associated with small HDL *(2–4).* These lines of evidence indicate that there is a distinct system for cholesterol metabolism in the CNS that is independent of that in the systemic circulation. ApoE is one of the major apolipoproteins that regulates cholesterol metabolism in the CNS by promoting the release of cellular cholesterol to generate HDL-like particles and by the uptake of these HDL particles via apoE receptors *(1,3–7).*

The discovery that the presence of apoE allele ε4 is a strong risk factor for the development of Alzheimer's disease (AD) *(8–11)* suggests the involvement of apoE and its metabolite, cholesterol, in AD pathogenesis. AD is a slowly progressive neurodegenerative disease, pathologically characterized by the extracellular accumulation of senile plaques—the major component of which is amyloid β-protein (Aβ)—and the intracellular formation of neurofibrillary tangles (NFTs), which are composed of hyperphosphorylated tau *(12).* Biochemical and morphological analyses of AD suggest the involvement of early synaptic dysfunction followed by its subsequent progression, including increased synaptic loss, neurite dystrophy, formation of NFTs, and eventual neuronal death *(13,14).* The mechanism underlying this progression is widely believed to be initiated and promoted by an aggregated Aβ, which is known as the amyloid cascade hypothesis *(15,16).* Thus, it is reasonable to determine the roles of apoE and lipids—including cholesterol, whose metabolism is regulated by apoE—in the pathogenesis of AD from the viewpoints of their effect on Aβ generation and AD-related pathologies such as synaptic damage, tau phosphorylation, and neuronal death. A growing body of evidence suggests the association and underlying mechanism(s) of these two factors. However, some conflicting issues must be resolved regarding the putative association between the two. In this article, recent studies that describe the association between cholesterol and AD pathophysiology are categorized into several groups according to the research aspect and assumption; a high or low cholesterol level promotes or prevents AD pathogenesis, respectively.

Association of Elevated Cholesterol Level in Serum or Brains with a Risk of Developing AD

Several epidemiological studies have shown that an increased serum cholesterol level during the long-preclinical phase is correlated with the development of AD *(17–19)* and mild cognitive impairment (MCI) *(20).* Based on these findings, it is assumed that a reduction in the serum cholesterol level could reduce the prevalence of AD. In support of this theory, recent studies have shown that the prevalence of AD in patients taking statins, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, is significantly reduced compared with that in a total patient population or patients taking other medications *(21),* and that the current use of statins reduced the risk of dementia *(22).* These findings suggest that a high level of serum cholesterol could elevate the CSF cholesterol level, which may lead to the development of AD. However, a previous study indicates that there is no correlation between the level of serum total cholesterol and that of CSF total cholesterol *(23),* suggesting that the association is complex, and that

further studies are needed to determine this. In the case of statins, their inhibitory effect on AD may not be attributable only to the decrease in the CSF cholesterol level following the decrease in the serum cholesterol level, but also to the decrease in the cellular cholesterol level. In support of this theory, a recent study showed that statins directly affect cholesterol metabolism in the human brain *(24).* It was demonstrated that the levels of 24S-hydroxycholesterol—for which conversion occurs mainly in the brain—in the plasma are reduced in patients using high-dosage simvastatin *(24).* Eckert et al. presented more direct evidence that the brain cholesterol level significantly decreased following lovastatin treatment in mice *(25).* These findings suggest that statins could be directly involved in the prevention of AD development by decreasing the cellular cholesterol level in the brain. However, statins have various biological effects in addition to the inhibition of cholesterol synthesis. These include protection against nitric oxide (NO) as well as oxidative stress, and anti-inflammatory and anti-platelet effects *(26),* prompting treatment alternatives to reducing cholesterol levels, which could explain the reduction in the prevalence of AD among those taking statins. This idea may raise criticism based on the interpretation of findings that focus on a causal relationship between statins and AD or cholesterol and AD by highlighting the inconsistency in effects of different statins with similar brain-blood-barrier permeabilities and similar inhibitory effects on cholesterol synthesis *(21).* However, as the authors have suggested, the duration of treatment with statins rather than any other factors may have caused this inconsistency, because other studies have shown a difference in risk of AD based on a long preclinical history of elevated serum cholesterol levels, but not on current cholesterol levels *(17,18).*

Several biochemical studies have explored the molecular mechanism(s) by which AD development may be prevented, revealing that cellular cholesterol levels regulate the metabolism of amyloid precursor protein (APP) as well as Aβ synthesis and its secretion both in vitro and in vivo. Previously, cholesterol was shown to modulate processing of APP in cultured neurons *(27–29).* The groups of Bodovitz and Klein *(27),* and Racchi et al. *(28)* showed that cholesterol modulates α-secretase cleavage of APP, and the level of cellular cholesterol is inversely correlated with the amount of soluble APP, an N-terminal fragment of APP cleaved by α-cleavage *(27).* Another recent study showed that a decreased cellular cholesterol level promotes the nonamyloidogenic pathway (α-secretase activity) *(30).* Other studies demonstrated that when the cellular cholesterol level in neurons decreases following treatment with an 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor with or without additional treatment with methyl-β-cyclodextrin, the amount of Aβ released into the culture media markedly decreases *(29–31).* It was shown that a decreased cellular cholesterol level inhibits the amyloidogenic pathway (β-and g-secretase activity) *(29)* and promotes the nonamyloidogenic pathway (α-secretase activity) *(27,28,30).* In particular, Fassbender's study suggests that the mechanism by which statins reduce the risk of AD is associated with the reduced production of Aβ in the brain *(31).* The detailed mechanism underlying the putative association between cholesterol and Aβ generation has not yet been elucidated, but one possible explanation for this association is that the cholesterol-rich domain, known as the lipid raft, is one of the key domains generating Aβ. Since it was shown that APP in neurons is associated with lipid rafts *(32,33),* that cholesterol depletion decreases APP association with these rafts *(29),* and that the sites of γ-secretase activity and Aβ generation are associated with cholesterol-rich microdomains *(34),* it may be possible that Aβ generation requires raft integrity and a lipid component as optimal conditions. Therefore, an alteration in raft components could change the configuration of either the enzymes or the substrate associated with the rafts, leading to an alteration in Aβ generation. Since cholesterol is an essential component for

generating lipid rafts, cholesterol depletion in cells induces disruption of the structure and function of lipid rafts in which amyloidogenic processing of APP occurs to generate Aβ, and also alters the processing of APP. However, one may note a discrepancy between studies using cultured cells and those using animals. The significant reduction in the generation and secretion of Aβ was demonstrated in cultures, in which at least 50% cholesterol was depleted *(29,30),* and although the amount of secreted Aβ in the CSF was reduced, the total brain cholesterol levels were not significantly reduced in statin-treated guinea pigs *(31).* These findings suggest another possibility—that a reduction of Aβ secretion or reduction in the prevalence of AD resulting from statin treatment may not be associated with cholesterol, but may involve nonsterol mechanisms. Further studies are needed to clarify these issues.

Association of Decreased Level of Serum, CSF, or Cellular Cholesterol with a Risk of Developing AD

Several studies have revealed conflicting findings: cholesterol levels in serum, cell membranes of the brain, and CSF decreased in AD patients compared to those in controls *(35–39),* and increased dietary cholesterol levels reduced Aβ secretion *(40).* However, there are no definitive data across studies to indicate that the level of total brain cholesterol differs between AD patients and normal individuals. A few studies showed that cholesterol in the plasma membrane acts as a modulator of the Aβ effect on brain membranes *(41)* and Aβ neurotoxicity by modulating the membrane insertion of Aβ. At a low cholesterol level, Aβ remains on the membrane surface mainly in a β-sheet structure, leading to exhibition Aβ neurotoxicity *(42);* and a high cholesterol level in the plasma membrane results in a decreased level of Aβ-cell-surface binding and subsequent cell death *(42,43).* The increased cholesterol level in the membrane was reported to attenuate the disordering effect of Aβ on brain membranes *(44–46).* It has also been suggested that apoE4, one of the strongest risk factors for AD, may contribute to disturbances in lipid metabolism that finally lead to a low cholesterol level in the AD brain *(47).* These lines of evidence, together with the results described in the Introduction to this article, indicate that the relationship between the alteration in cholesterol metabolism and AD pathogenesis still remains controversial. These conflicting results may direct attention to our target: which cholesterol level is altered, that of the physiological fluid (serum or CSF), an organ (brain), cells (neurons or glial cells), organelles (including the plasma membrane, endoplasmic reticulum [ER], Golgi), or microdomains (lipid rafts or others). Depending on the subject, the role of cholesterol, and thus the effect of the alteration of its level, should be different. For example, cholesterol accumulation is observed in Niemann-Pick disease, type C, and the total cholesterol level in cells—particularly in late endosomes and lysosomes—is elevated. However, the total cholesterol level in specific cell compartments such as caveolae *(48)* and detergent-insoluble, low-density membrane fraction (our unpublished data) is reduced. Another example is a study showing cholesterol accumulation in mature senile plaques of AD brains and in transgenic APPsw mouse brains *(49).* This study supports the theory that an elevated level of cholesterol is associated with AD pathogenesis. However, another possible interpretation is that cholesterol accumulation in senile plaques may induce cholesterol deficiency in specific domains as a result of repartitioning of cholesterol from areas in which it plays a normal physiologic role in brain regions. This theory is supported by recent findings demonstrating that oligomeric Aβ promotes cholesterol release resulting in the generation of HDL-like particles that cannot be internalized by neurons *(50),* eventually leading to a reduction in the cholesterol level

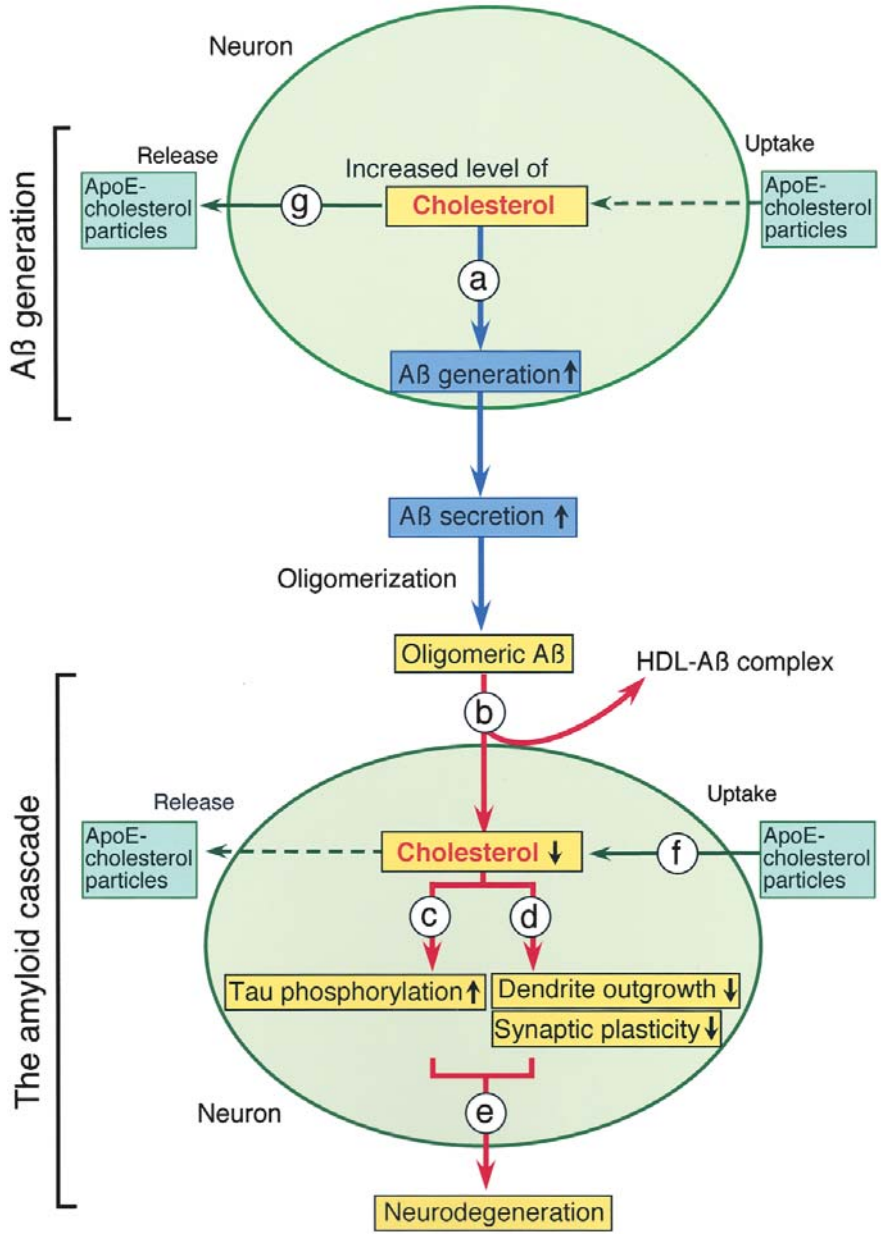
in neurons *(51).* These studies suggest that cholesterol associated with oligomeric Aβ may be accumulated extracellularly, while the intracellular cholesterol level decreases. Thus, further studies are needed to elucidate the association between cholesterol and the mechanisms promoting AD pathology.

The Critical Role of Cholesterol in the Amyloid Cascade

The previously described studies and those that follow in this section have defined the role of cholesterol in the amyloid cascade in a hypothesis explaining the mechanism underlying the amyloid cascade theory—Aβ accumulation leads to AD-related pathologies including tau phosphorylation, synaptic loss, and neurodegeneration. Fig. 1 summarizes the findings that focus on the involvement of cholesterol in the pathogenesis of AD and Aβ generation. As mentioned in the second section of this article, the cellular level of cholesterol modulates Aβ synthesis and secretion (Fig. 1a). When the concentration of monomeric Aβ increases, Aβ is assumed to form oligomers under physiological conditions, particularly in the case of Aβ1-42 *(52).* Oligomeric Aβ was shown to promote lipid release from neurons, resulting in the generation of HDL-like particles and inhibition of cholesterol synthesis, which eventually led to a reduction in the cellular cholesterol level (Fig. 1b).

The Role of Cholesterol in Synapse Formation

This Aβ-mediated alteration in cellular cholesterol homeostasis presumably leads to synaptic dysfunction, because cholesterol from glial cells as apoE-containing lipoproteins has been shown to play a critical role in the formation of mature synapses *(53).* Cholesterol as apoE-lipid complex generated by astrocytes was shown to be the limiting factor that regulates synapse formation and its functions. The dependence of synapse formation on astrocyte-derived cholesterol is also supported by a previous study showing that most synapses in the developing brain are formed coincidentally with the development of astrocytes *(54).* The importance of cholesterol in maintaining synapse formation has been investigated by modulating cellular cholesterol levels. Cholesterol was suggested to contribute to modulation of cellular kinases and phosphatases in neurons, and a decrease in the level of cholesterol results in a dendrite-specific inhibition of neurite outgrowth *(55,56).* In the CNS, apoE is one of the major apolipoproteins regulating cholesterol metabolism and is mainly synthesized and secreted as apoE-lipid particles from astrocytes; the ability of astrocytes as a cholesterol supplier may be partially dependent on apoE *(2,4).* Thus, it is reasonable and also important to study the isoform-dependent contribution of apoE to AD development from the perspective of the isoform-dependent function of apoE in the regulation of cholesterol. Cellular cholesterol metabolism is regulated by endogenous cholesterol biosynthesis, uptake of apoE-containing lipoproteins via apoE receptors, and cholesterol release by lipid acceptors such as apoE. Recently, we discovered an apoE-isoform dependence for one of these functions; that is the ability of apoE3 to promote cholesterol release is greater than that of apoE4 *(6)* (Fig. 1g). Although the role of apoE in AD pathogenesis remains unclear, this isoform-specific cholesterol release from neurons may result in a higher cholesterol level in CNS neurons of apoE4 carriers than in those of apoE3 carriers, leading to increased generation of Aβ in the CNS of apoE4 carriers (Fig. 1g). However, we also found that apoE3-expressing astrocytes generate more HDL-like particles than apoE4-expressing astrocytes with similar amount of apoE molecule *(7),* implying that apoE3-expressing astrocytes can supply more cholesterol to neurons (Fig. 1f) for use in regeneration and for synaptic plasticity in neurons. Further studies are required to address these issues.



The Role of Cholesterol in Tau Phosphorylation

The effect of Aβ on cellular cholesterol metabolism has also been investigated. Liu et al. *(57)* showed that Aβ alters intracellular vesicle trafficking and cholesterol homeostasis. Our recent studies showed that oligomeric—but not monomeric—Aβ affects cholesterol metabolism, and that oligomeric Aβ promotes cholesterol release, resulting in generation of HDL-Aβ particles that cannot be

Fig. 1. Putative roles of cholesterol in AD pathogenesis. The involvement of cholesterol in AD pathogenesis may be dualistic; it is involved in Aβ generation (blue lines) and in the amyloid cascade (red lines). ApoE-cholesterol complexes serve to maintain the homeostasis of cellular cholesterol metabolism by the uptake and release of cholesterol in an isoform-specific manner (green lines). For the effect of cellular cholesterol level on Aβ generation, the increased levels of cellular cholesterol promote the generation and subsequent secretion of Aβ, and the decreased levels of cellular cholesterol following statin treatment attenuate them **(a).** For the role of cholesterol in the amyloid cascade, an increasing amount of extracellular Aβ leads to the formation of oligomers by a still undetermined mechanism, which in turn reduces the cellular cholesterol level by promoting cholesterol release and inhibiting cholesterol synthesis **(b).** Cholesterol deficiency was shown to induce tau phosphorylation and inhibit synapse formation, which may lead to neurodegeneration (**c, d,** and **e**). ApoE contributes to the maintenance of cholesterol homeostasis in neurons by two mechanisms: cholesterol release from neurons **(g)** and its uptake into neurons **(f).** The mechanism by which the apoE isoform specifically contributes to AD development remains undetermined. However, the fact that extracellular apoE3 has a stronger ability as a cholesterol acceptor than apoE4 *(6)* **(g)** suggests that apoE may be involved in the isoform-dependent, increased level of cholesterol, which may affect Aβ generation. We also found that endogenous apoE3 synthesized in astrocytes can generate more HDL-like particles with less apoE than apoE4 *(7)* **(f),** implying that apoE3-expressing astrocytes can supply more cholesterol to neurons than apoE4-expressing astrocytes, and thereby supporting neuronal plasticity and promoting neurodegeneration. Under these conditions, cholesterol demand of neurons markedly increased.

internalized by neurons *(50),* and subsequently reducing cellular cholesterol levels *(51).* In addition, recent studies demonstrated the relationship between tau phosphorylation and cholesterol. When the cellular cholesterol level decreases following treatment with HMG-CoA reductase inhibitors or β-cyclodextrin, tau phosphorylation is enhanced in cultured neurons *(58)* and in hippocampal-slice cultures *(56).* The link between alteration in cholesterol metabolism and the promotion of tau phosphorylation was also supported by the finding that tau is hyperphosphorylated in the brains of the NPC and NPC model mouse, in which cholesterol metabolism is altered because of the lack of the NPC1 protein *(59).* The promotion of tau phosphorylation in NPC was suggested to be the result of a cholesterol deficiency in a specific compartment in the plasma membrane *(59,60)*, despite an elevated total cellular cholesterol level. In support of these findings, it was shown that in NPC-deficient cells, the cholesterol levels in the detergent-insoluble, low-density membrane fractions—also called lipid rafts, caveolae, or detergent-insoluble glycosphingolipid-rich domains (DIGs)—decrease *(48).* These findings suggest that the extracellular accumulation of Aβ and subsequent formation of oligomeric Aβ affect cholesterol metabolism in neurons, leading to reduced cholesterol levels in the plasma membrane, particularly in lipid rafts, a critical domain for signal pathways *(61,62),* which in turn affects raft function and leads to tau phosphorylation.

Considering all the findings described in the first three sections of the article, it is possible that the involvement of cholesterol in AD pathogenesis is dualistic: The elevated levels of cellular cholesterol contribute to AD development by elevating Aβ secretion; however, the increased amount of oligomerized Aβ reduces cellular cholesterol levels, which in turn may promote AD progression.

Another Role of Cholesterol in Aβ Aggregation

Several groups have proposed another putative role for cholesterol in AD pathogenesis, claiming that cholesterol is one of the key molecules in the fibril formation of Aβ. Because the oligomeric and aggregated Aβ are assumed to play a critical role in the amyloid cascade, the conversion of soluble, nontoxic

Aβ to oligomeric and aggregated Aβ is the critical step in AD development. A recent paper showed that increased cholesterol levels in the lipid bilayers facilitate the binding of Aβ to the membranes, and an increase in the membrane-bound Aβ concentration triggers the promotion of conformational change from a helix-rich to a β-sheet-rich structure, becoming an endogenous seed for amyloid formation *(63).* The cholesterol-dependent generation of Aβ seeds was demonstrated, and an increased level of cholesterol synthesis increase was shown to the amount of Aβ seeds in a conditioned medium for Madin-Darby canine kidney (MDCK) cells *(64).* A previous study also suggested the critical role of cholesterol in Aβ fibril formation by demonstrating that the generation of GM1 ganglioside-bound Aβ (GM1/Aβ) is enhanced by the combination of cholesterol and sphingomyelin in membranes in proportions similar to those in the lipid rafts *(65).* Since GM1/Aβ was reported to accelerate amyloid fibril formation *(66,67),* these findings suggest that the cellular cholesterol level—particularly in the cholesterol-rich domain such as the rafts—affects the interaction between GM1 and Aβ, and that an elevated cholesterol level in these domains could enhance Aβ aggregation at physiological concentrations. In support of this theory, Aβ was reported to be present in lipid rafts of mouse brains. These findings also explain the presence of GM1/Aβ in the brains of patients with AD who exhibit early pathological changes at the molecular level *(68).* As mentioned previously, alterations in the cholesterol level in lipid domains, in contrast to alterations in the total cholesterol level, are suggested as potential causes of AD. Recent studies have also shown that the alterations in transbilayer cholesterol distribution—but not those in the total cholesterol level—are similar in synaptic plasma membranes of aged mice and mice that express human apoE4, as compared to those in the same membranes of younger mice and mice that express human apoE3 *(69,70).* They showed that the largest changes occurred in the exofacial leaflet, where rafts as well as GM1 are believed to be located, suggesting that apoE is involved in the regulation of cholesterol distribution in rafts.

Concluding Remarks

As mentioned in the Introduction, many scientists agree that the cellular cholesterol level is involved in Aβ generation and that the prevalence of AD can be reduced by the treatment of patients with statins, which reduce cholesterol levels in serum and probably in CNS cells. The detailed mechanism underlying cholesterol-dependent modulation of Aβ synthesis and AD development is the next issue to be addressed. Determination of the association between statin treatment and inhibition of AD development and the establishment of statin therapy for AD and MCI are also important issues to be addressed.

Another perspective on the role of cholesterol in the formation of Aβ seeds is presented here. Although the concentration of Aβ in the extracellular space has not been determined, it is widely believed that Aβ concentrations in CSF are too low to form aggregates. Thus, it is important to elucidate the mechanism by which Aβ forms oligomers and amyloid. In this regard, it is feasible to theorize that Aβ seeds promote oligomerization and amyloid formation. Recent studies have demonstrated that cholesterol in the membrane is essential for formation of seeding Aβ and GM1 bound Aβ.

In addition to these important perspectives, a novel view of the putative role of cholesterol in the amyloid cascade is also proposed in this article. The findings in support of this theory show that oligomeric Aβ affects cellular cholesterol metabolism, leading to reduction of the cellular cholesterol level, which may induce AD-related pathologies. Based on these findings, it is possible that the role of cholesterol in AD pathogenesis is dualistic. Thus, a decreased level of cellular cholesterol may prevent AD development, yet may enhance AD pathologies when AD and MCI have already developed.

However, since these results are derived from basic research and no animal data or clinical data supporting this notion are currently available, further studies are required to determine whether decreased levels of cellular cholesterol promote AD pathologies in vivo.

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