Alzheimer's disease: the cholesterol connection

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A hallmark of all forms of Alzheimer's disease (AD) is an abnormal accumulation of the β-amyloid protein (Aβ) in specific brain regions. Both the generation and clearance of Aβ are regulated by cholesterol. Elevated cholesterol levels increase Aβ in cellular and most animals models of AD, and drugs that inhibit cholesterol synthesis lower Aβ in these models. Recent studies show that not only the total amount, but also the distribution of cholesterol within neurons, impacts Aβ biogenesis. The identification of a variant of the apolipoprotein E (*APOE*) gene as a major genetic risk factor for AD is also consistent with a role for cholesterol in the pathogenesis of AD. Clinical trials have recently been initiated to test whether lowering plasma and/or neuronal cholesterol levels is a viable strategy for treating and preventing AD. In this review, we describe recent findings concerning the molecular mechanisms underlying the cholesterol–AD connection.

Alzheimer's disease (AD) is the most common form of dementia, affecting up to 15 million individuals worldwide. Because of the ongoing increase in life expectancy, by 2050 we can expect approximately 25% of people living in the Western hemisphere to be over 65 years of age, one third of whom are likely to develop AD. AD is a complex and genetically heterogeneous disease, characterized by progressive memory deficit, cognitive impairment and personality changes accompanied by specific structural abnormalities in the brain.

The main histological features of AD are extracellular protein deposits termed β-amyloid (or senile) plaques, β-amyloid deposits in blood vessels and intraneuronal neurofibrillary tangles. Loss of neurons and synapses in the neocortex, hippocampus and other subcortical regions of the brain are also common features. High numbers of amyloid plaques in limbic and association cortices serve as the basis for a definitive post-mortem diagnosis of AD. The chief component of the plaque core is the ~4 kDa amyloid β-peptide (Aβ) organized in 7–10 nm fibrils intermixed with non-fibrillar forms of the peptide.

ApoE is a 299 amino acid glycoprotein and the major protein component of very low-density lipoproteins (VLDL). ApoE is also the major apolipoprotein in the brain. The identification of the ε4 variant of *APOE* as the most common genetic risk factor for late-onset AD suggests that cholesterol may play a direct role in the pathogenesis of the disease. Epidemiological, molecular and biochemical evidence have further strengthened the hypothesis that cholesterol is a risk factor for AD. Although cholesterol homeostasis in the brain is largely unexplored, new findings strongly support the involvement of cholesterol in both the generation and deposition of Aβ.

Genetics and biology of AD

The genetics of AD is best explained by an age-dependent model involving either rare, early-onset (<60 years old) causative mutations or late-onset (>60 years old) genetic risk factors that increase the risk of developing the disease (for review, see ref. 1). Early-onset familial AD (FAD) has so far been linked to mutations in the genes for the β-amyloid precursor protein (*APP*) on chromosome 21, presenilin 1 (*PSEN1*) on chromosome 14, and presenilin 2 (*PSEN2*) on chromosome 1. Together, they are thought to account for ~40% of early-onset AD (for review, see refs. 1,2). Late-onset AD accounts for ~95% of AD cases. Late-onset AD genes implicated thus far harbor genetic polymorphisms that act as either risk factors and/or genetic modifiers. Although over three dozen putative genetic risk factors for AD have been reported in the literature, only the ε4 allele of the *APOE* gene on chromosome 19 has been consistently found associated with AD in several independent studies (for review, see ref. 1). Additional genetic loci for late-onset AD have been localized on chromosomes 9 and 10 (for review, see ref. 1), as well as other chromosomes[3].

The common pathogenic event that occurs in all forms of AD is the abnormal accumulation of Aβ in amyloid deposits and cerebral blood vessels. In most forms of early-onset FAD, this correlates with increased production of the 42-amino acid isoform of Aβ ($Aβ_{42}$), which promotes the aggregation of total Aβ into amyloid fibrils. These patients develop AD pathology by middle age. In contrast to early-onset FAD, the accumulation of Aβ in late-onset AD is the result of a complex interplay between genetic and environmental factors, affecting generation, clearance and aggregation of the peptide (for review, see ref. 4).

Several isoforms of the APP protein are expressed, APP_{695} being the most common form expressed in the brain, and originate from alternative splicing of a single gene, *APP*. Aβ is generated by an initial cleavage of APP at the N-terminus by a beta secretase (BACE, which stands for β-site APP cleaving enzyme), and then in the transmembrane domain by a γ-secretase, a multimeric protein complex that includes presenilin (**Fig. 1**). The two major sites of γ-cleavage are located at positions 40 and 42 of Aβ, generating $Aβ_{40}$ and $Aβ_{42}$, respectively.

The physiologic role of APP still remains elusive (for review, see ref. 5). Secreted APP isoforms containing the KPI domain

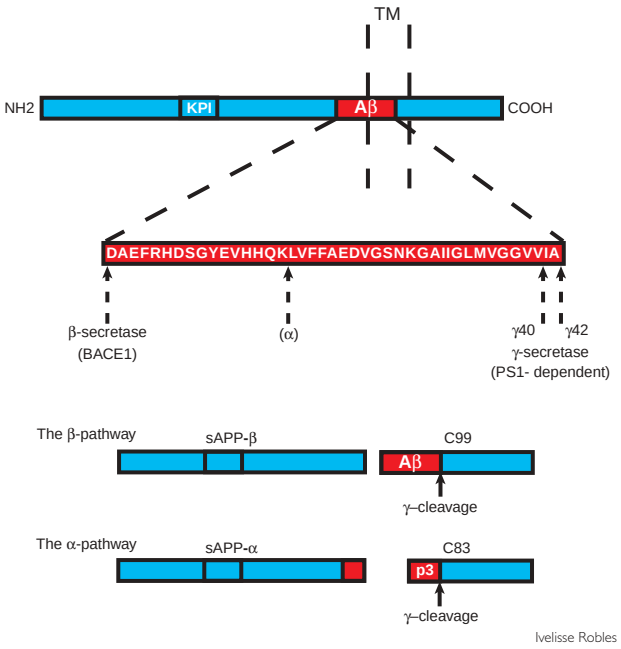

Fig. 1. Proteolytic processing of APP. APP is a type-I glycoprotein with its amino terminus on the lumenal/extracellular surface and a short C-terminal cytoplasmic tail. The major component of amyloid plaques, the Aβ peptide (showed in red), is produced by the β pathway, where APP is first cleaved at the N-terminus of Aβ (β-cleavage) and then in the transmembrane domain (γ-cleavage), either at position 40 or 42. In contrast, APP is more frequently cleaved at the α position, between amino acids 16 and 17 of the Aβ region, precluding the generation of Aβ. The APP C-terminal fragments (APP-CTFs) produced after α (α-APP-CTF) and β cleavage (β-APP-CTF) of APP are also respectively called C-83 and C-99, based on the number of amino acids.

(Kunitz-type serine protease inhibitors) may function in the coagulation pathway[5]. APP's cytoplasmic domain has been shown to interact with several cytosolic adaptor proteins and can function as a signaling molecule[6,7], but a putative extracellular ligand has not yet been identified.

Cholesterol and AD: apoE and epidemiology

ApoE is one of the major apolipoproteins in the plasma and the principal cholesterol carrier protein in the brain. In humans, there are three common alleles of the *APOE* gene: ε2, ε3 and ε4. The protein isoforms produced by these alleles differ in the amino acids at positions 112 and/or 158: E2 (Cys112, Cys158), E3 (Cys112, Arg158), which is the most common, and E4 (Arg112, Arg158), which is present in at least one copy in ~25% of the population. Numerous independent studies have consistently confirmed that the *APOE* ε4 allele is the most prevalent risk factor for AD (for review, see ref. 1). The risk for AD conferred by *APOE* ε4 increases in a dose-dependent manner; individuals that are homozygous for *APOE* ε4 alleles (~2% of the population) are eight times more likely to develop AD than are homozygotes for *APOE* ε3. However, *APOE* ε4 is neither necessary nor sufficient to cause AD; it only increases risk for the disease[1].

The association between AD and *APOE* ε4 remains largely undefined at the mechanistic level. Studies in a transgenic mouse model expressing human $APP^{V717F+/-}$ (PDAPP mice) suggest that apoE contributes to the deposition of Aβ. Disruption of the *APOE* gene in PDAPP mice inhibited the accumulation of Aβ in immunoreactive deposits[8]. Moreover, amyloid deposition was found to be strictly dependent on apoE expression levels in a dose-dependent manner: PDAPP mice hemizygous for apoE were found to have a ~60% reduction in amyloid deposition, whereas no nonfibrillar or fibrillar Aβ was detected in any brain region of homozygous knockout mice. The absence of apoE was shown to affect the extracellular accumulation, but not the synthesis, of Aβ[9]. Re-introduction of human *APOE* genes in the $APP^{V717F+/-}$, $APOE^{-/-}$ mice restored deposition of fibrillar Aβ[10]. Deposition of Aβ associated with severe neuritic dystrophy was more evident with the *APOE* ε4 isoform.

Although controversial, several *in vitro* and *in vivo* studies have shown that apoE can bind Aβ[11,12]. Methodological problems have made it difficult to draw firm conclusions on the role of lipid-free or lipid-associated apoE in Aβ aggregation, and the pathophysiological importance of this phenomenon is not clear. Nevertheless, some reports indicate that apoE-mediated binding of Aβ to the cell surface could be isoform-specific: E2>E3>E4 (refs. 12,13). ApoE may also mediate internalization of Aβ through its binding to the LDL receptor–related protein[11,14–17] (LRP; see **Box 1** and **Fig. 2**).

Recent studies also show that Aβ internalization is not necessarily followed by its degradation. Instead, Aβ aggregates in the endocytic compartment and can be secreted again in the fibrillar and more toxic form[14,15]. Thus, apoE may ultimately contribute to Aβ aggregation by mediating its internalization (**Fig. 2**, Model I). In addition, lipid-free apoE4 has been shown to more effectively bind Aβ[18] and promote Aβ fibril formation than the apoE3 isoform[19,20]. One possible scenario is that apoE could promote the aggregation of Aβ in the endosomal compartment subsequent to receptor-mediated internalization and enzymatic digestion of the lipid particle, which renders apoE lipid-free and able to interact with Aβ. This conclusion would be supported by the facilitation of Aβ fibril formation observed in AD transgenic mice expressing the apoE4 isoform[10,21,22].

In addition to directly facilitating Aβ internalization and aggregation, apoE4 may also alter brain cholesterol homeostasis by modifying lipoprotein-particle formation. In the plasma, apoE4 tends to associate with VLDL particles, which contain more cholesterol, whereas apoE3 prefers to associate with HDL. Subjects homozygous for the *APOE* ε4 allele have higher levels of cholesterol in the plasma[23,24] and 24S-hydroxycholesterol in the cerebrospinal fluid (CSF)[25]. 24S-hydroxycholesterol is a catabolic derivative of cholesterol and represents the major metabolic route for cholesterol clearance from the brain. The lipoproteins produced in the brain are very different in virtually all respects (composition, density and other properties) from the particles found in the plasma (for review, see ref. 21). As apoE4 shows differential preference for VLDL particles in the plasma, it is also conceivable that different apoE isoforms modify brain cholesterol homeostasis by preferentially associating with specific lipoprotein particles. The predominant nature of apoE in brain lipoproteins would accentuate small differences in lipoprotein affinity for apoE isoforms. Therefore, the role of apoE in maintaining cholesterol homeostasis in the brain may contribute to the increased risk for AD associated with *APOE* ε4 (Model II in **Fig. 2**).

A number of epidemiological studies suggest that high levels of cholesterol may contribute to the pathogenesis of AD. Individuals with elevated levels of plasma cholesterol have an increased susceptibility to AD, apparently influenced by the *APOE* ε4 genotype[26,27]. Moreover, AD patients have increased levels of total serum and low-density lipoprotein (LDL) cholesterol[26,27] along

Box 1. LRP

LRP is a multi-ligand receptor and a member of the LDL receptor (LDLr) family. With the LDLr it shares common structural motifs and high-affinity binding activity for LDL particles. LRP's main protein ligand is apoE, but it also binds other molecules such as α2-macroglobulin and secreted $APP_{751/770}$ containing the KPI domain (for review, see ref. 17). LRP may be linked to the degradation of secreted Aβ by facilitating the internalization of Aβ bound to apoE[14]. Recent studies, however, have shown that LRP-mediated internalization of Aβ is followed by its intracellular aggregation, rather than degradation, suggesting that LRP might be involved in the process of Aβ deposition[15] (**Fig. 2**). LRP not only mediates the internalization of Aβ, but also its production. The interaction of LRP with the KPI domain of $APP_{751/770}$ increases the generation of Aβ[16].

with reduced levels of apoA/high-density lipoprotein (HDL) in their plasma[27,28], as compared to age-matched controls. This is further strengthened by the fact that lecithin cholesterol acyltransferase (LCAT) activity is significantly decreased in AD patients[29]. LCAT is an enzyme found in plasma that catalyzes an acyltransferase reaction on lipoprotein-associated cholesterol and is a key step in reverse cholesterol transport in humans (the process that eliminates cholesterol from peripheral cells). This metabolic profile (high plasma cholesterol with high LDL-cholesterol and low HDL-cholesterol) is commonly found in patients with atherosclerosis. Also, atherosclerosis, intimately related to high blood cholesterol, has been shown to correlate with an increased risk of AD, with higher levels of risk being associated with advanced atherosclerosis[30]. As mentioned above, AD patients in early stages of dementia show increased levels of 24S-hydroxyxholesterol in the CSF[25]. Finally, cholesterol abnormally accumulates in the dense cores of amyloid plaques in the brain of AD patients[31]. Similar accumulation of cholesterol has also been found in amyloid plaques of transgenic mice expressing a mutant form of APP_{695} (Swedish mutation) associated with FAD[31].

Cholesterol in Aβ generation and deposition

Two initial reports indicated that statins, which reduce serum levels of cholesterol, also protect against AD[32,33]. Although both studies were retrospective (data obtained from past hospital records) in nature, the results already prompted the initiation of clinical trials for the use of statins in AD. In one of these initial studies[32], the protective effect of statins was independent of the presence or absence of untreated high lipid levels, suggesting that statins may protect against AD through an unknown mechanism

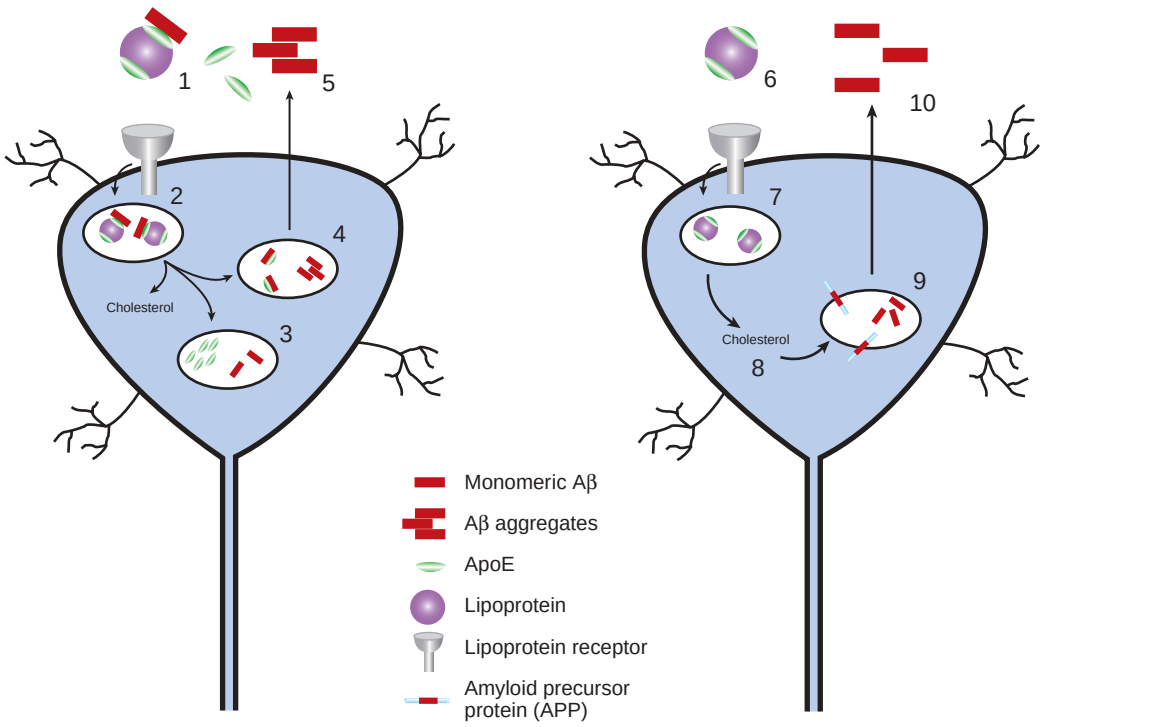

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Fig. 2. Two possible models for apoE's role in Aβ accumulation. **Model I**: Soluble Aβ interacts with apoE associated with a lipoparticle (1) and undergoes receptor-mediated endocytosis. Lipoproteins are then enzymatically digested in the lysosomal compartment (2), releasing cholesterol to the cell. In the lysosomes, a fraction of apoE and Aβ undergoes degradation (3), while the rest of apoE remains associated with Aβ and promotes its aggregation into amyloid fibrils (4), and is then secreted back into the extracellular milieu (5). Given that apoE4 has more affinity for Aβ than apoE2 and apoE3, it would be expected to accelerate this process. **Model II**: In addition to directly facilitating Aβ internalization and aggregation, apoE may also up-regulate the rate of Aβ generation by increasing cellular cholesterol. After receptor-mediated internalization (6) and enzymatic digestion of the lipoproteins (7), cholesterol is released to cellular membranes (8). ApoE4 lipoproteins tend to contain more cholesterol. The increased sterol content of intracellular membranes promotes the rate of Aβ generation (from its precursor APP) (9), resulting in increased secretion into the extracellular milieu (10).

not directly related to cholesterol. In the other[33], lovastatin and pravastatin reduced the risk of AD up to 73%. Despite the preliminary nature of these studies' conclusions, they readily corroborate pre-existing epidemiological data, and were subsequently confirmed by similar studies conducted in independent sets of patients[33–35]. Interestingly, recent results from the first 26-week randomized study in 44 AD patients with normal cholesterol showed that simvastatin was able to reduce $A\beta_{40}$ levels in the cerebrospinal fluid of patients with mild, but not severe, AD[36]. A slight improvement in cognitive function reported in this study was not confirmed by a second randomized controlled trial on a much larger scale (called PROSPER for "pravastatin in elderly individuals at risk of vascular disease")[37]. However, patients enrolled in the PROSPER trial were not screened for AD, and the trial included individuals with high cholesterol levels. Results from additional ongoing randomized controlled trials should soon be available and will allow for definitive conclusions on the possible use of statins against AD. Finally, it should be pointed out that the effects of statins on Aβ deposition in the brain may be independent of the changes in the CNS cholesterol metabolism[38], and may instead involve anti-inflammatory effects of statins[39] or alterations in the cycling of Aβ between the brain and plasma.

Studies using animal models of AD, including rabbits[40,41], transgenic mice[42–44] and guinea pigs[45], show a strong connection between plasma cholesterol levels and Aβ generation. A recent report shows that a high fat/high cholesterol diet raises cholesterol levels in plasma and CNS of transgenic mice expressing the FAD mutants $APP_{K670N,M671L}$ and $PS1_{M146V}$[42]. Because both β-APP C-terminal fragment (CTF) and Aβ levels were increased in the brain of these animals, the authors concluded that cholesterol levels can regulate APP processing and Aβ generation *in vivo*, possibly by a mechanism shown in **Fig. 2** (Model II). Neuropathological analysis showed that a high cholesterol diet also increased the deposition of amyloid plaques. Additionally, cholesterol-lowering agents reversed the effect of high fat/high cholesterol diet on Aβ accumulation and cholesterol levels in the plasma and CNS[43]. In a similar study on mice harboring a different APP FAD mutation (Swedish mutant), the high-cholesterol diet also elevated cholesterol levels in the plasma and CNS, but had an opposite effect on Aβ generation[44]. In this model, the levels of both $A\beta_{40}$ and $A\beta_{42}$ were reduced in the brain. The reason for the apparent discrepancy may reside in either the genetic background of the mice or, more likely, in the different transgene introduced. Species differences in mice may affect cholesterol generation, and therefore the choice of APP transgenic mouse model used becomes extremely important for the outcome of studies in which cholesterol levels are manipulated. A recent study in guinea pigs has strengthened the role of lipid-lowering drugs in the regulation of Aβ generation, as simvastatin reduced cholesterol and both $A\beta_{40}$ and $A\beta_{42}$ levels in guinea pig plasma[45].

In addition to the *in vivo* results from animal studies, strong biochemical evidence also supports a direct role of intracellular cholesterol in Aβ generation and deposition. First, both APP and Aβ are associated, at least in part, with cholesterol-rich domains (CRDs)[46,47], specialized membrane micro-domains characterized by the tight association of cholesterol, sphingomyelin and highly-charged galactosphingolipids. CRDs serve as clustering domains for several membrane-bound proteins, and they have also been proposed to regulate APP processing by favoring the clustering of APP and BACE[48]. Several studies have also reported a physical association of Aβ with GM1, the major galacto-sphingolipid in CRDs[49,50]. This association facilitates the aggregation of soluble Aβ in the form of amyloid fibrils[51]. CRDs may facilitate the aggregation of secreted Aβ, but their specific role in the processing of APP remains to be determined. Second, studies in primary neurons or peripheral cell lines in which total cholesterol content has been either reduced or increased indicate that cholesterol levels can actively regulate APP processing and Aβ generation. An increase in cholesterol up-regulates, whereas a decrease down-regulates, Aβ generation[45,52,53]. Corresponding changes in the β-cleavage of APP suggest a direct effect of cholesterol on APP processing. In other studies, brief exposure to water-solubilized cholesterol reduced only the α-cleavage of APP[54]. This was most likely due to transitory changes in cholesterol levels/distribution at the plasma membrane since the effect was independent of receptor-mediated internalization of cholesterol[55] and may involve regulated, but not necessarily constitutive, α-cleavage of APP[56]. Finally, drugs that arrest cholesterol trafficking along the endocytic compartment affect the subcellular localization of PS1[57], whereas those that induce the expression of the cholesterol efflux receptor ABCA1 increase the secretion of Aβ[58].

We have recently shown, through genetic, biochemical and metabolic approaches, that intracellular cholesterol distribution, rather than total cholesterol level, regulates APP processing and Aβ generation[59]. Cellular cholesterol is stored either as free cholesterol in the membrane or as cholesteryl-esters in the form of cytoplasmic droplets (**Fig. 3**). An endoplasmic reticulum (ER) resident enzyme, acyl-coenzyme A cholesterol acyltransferase (ACAT), catalyzes the formation of cholesteryl-esters from cholesterol and long-chain fatty acids (**Box 2**; reviewed in refs. 60,61). ACAT controls the dynamic equilibrium between these two forms of cellular cholesterol, ultimately affecting cholesterol homeostasis (for review, see ref. 62). Our results indicate that this dynamic equilibrium ultimately regulates the generation of Aβ. A selective increase in cholesteryl-esters is sufficient to up-regulate the generation of Aβ and increase the steady-state levels of β-APP CTFs. We also showed that, in several cell lines and in primary neurons, ACAT competitive inhibitors reduce both cholesteryl-ester and Aβ biosynthesis in a dose-dependent manner, while increasing free cholesterol. Similar results were obtained with agents that block delivery of free cholesterol to ACAT, using water-solubilized cholesterol intermixed with LDL to radio-label intracellular cholesterol[57]. Confirming evidence comes from *in vivo* studies, showing that a decrease in cholesteryl-ester levels in the brain of *Drosophila melanogaster* was associated with a vacuolar form of neurodegeneration and altered processing of fly APP[63]. The fact that *Drosophila* brains harbor even fewer cholesteryl-esters than mammalian brains strongly indicates that very small amounts of this lipid are sufficient for its regulatory function in APP processing.

How cholesterol distribution affects the processing of APP is not yet clear. Some key questions remain unresolved. First, what is the specific contribution of cholesteryl-esters and free cholesterol? Whereas our previous findings implicate cholesteryl-esters, free cholesterol in cellular membranes also participates in the regulatory process because altered cholesteryl-ester levels modulate the free cholesterol pool. Even undetectable changes in free cholesterol levels may affect specific compartments involved in APP processing. Second, what are the molecular mechanisms involved in ACAT-mediated regulation of APP processing? Our results show that ACAT activity regulates all three major cleavages of APP. Thus, altered cholesterol distribution affects either the activity of all three secretases (α, β and γ), APP itself, or an as-yet-unidentified protein that controls APP processing. The

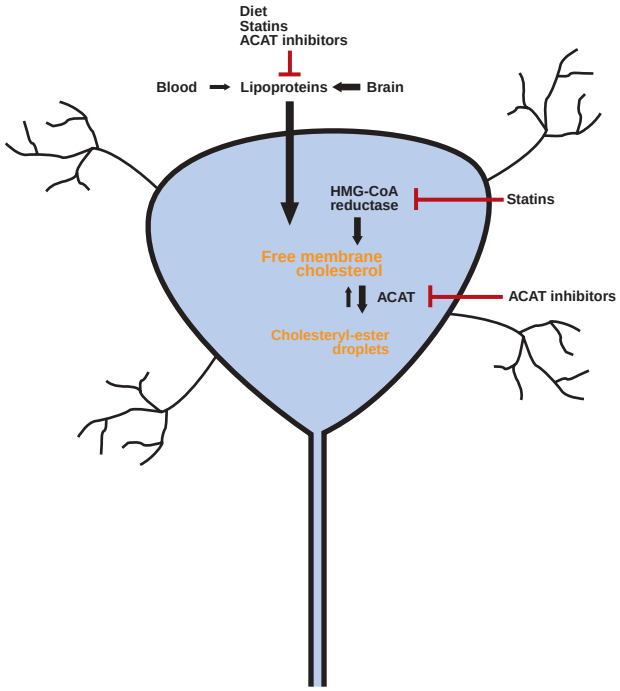

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Fig. 3. Schematic view of cholesterol homeostasis in neurons and cholesterol-related targets for therapeutic treatment of AD. Neurons maintain cholesterol homeostasis mainly through *de novo* biosynthesis in the endoplasmic reticulum and receptor-mediated internalization of lipoproteins. The latter are either generated in the brain or, possibly, obtained from the plasma. This pool of free cholesterol constitutes the substrate of ACAT, which generates cholesteryl-esters and regulates Aβ generation. Free cholesterol available in the cell allosterically regulates ACAT activity. Statins, which inhibit hydroxy-methyl-glutaryl-CoA (HMG-CoA) reductase (the rate-limiting enzyme in cholesterol biosynthesis), act at two different levels. They reduce the neo-synthesis of cholesterol in the CNS (after crossing the blood–brain barrier), as well as levels of lipoprotein-associated cholesterol in the plasma. Cholesterol levels in the plasma can also be regulated by dietary restriction. Reduction of sterol content in neurons will affect ACAT activity and therefore the generation of cholesteryl-esters. Studies with animals indicate that such an approach is effective in regulating Aβ generation in the brain. ACAT activity can be directly regulated through specific competitive and non-competitive inhibitors, and may constitute a novel and more direct approach for the treatment and prevention of AD.

Box 2. ACAT

ACAT is essential for the regulation of intracellular cholesterol homeostasis and distribution of cholesterol throughout the body. In the small intestine and liver it also regulates the secretion of chylomicrons and very large-density lipoproteins (VLDL), respectively. Mammals, including humans, express two different isoforms of ACAT, called ACAT-1 and ACAT-2. Whereas ACAT-1 is almost uniformly distributed among several tissues, including the brain, ACAT-2 is selectively expressed in the liver and intestine (for review, see refs. 60,61). Both ACAT-1 and ACAT-2 are ER resident enzymes, allosterically regulated by cholesterol available in the ER membrane. Elevated free cholesterol results in the activation of ACAT and production of cholesteryl-esters. A close relationship exists between ACAT, intracellular cholesterol trafficking, and the sterol regulatory element binding protein (SREBP) pathway, a complex group of membrane proteins that regulate cholesterol homeostasis[62]. ACAT keeps the levels of free cholesterol in the ER membrane under strict control. This pool of free cholesterol ultimately regulates the SREBP pathway, cholesterol trafficking and several molecular events in which cholesterol is directly involved, including gene regulation and expression, post-translational modifications and sorting of proteins, signal transduction and membrane trafficking.

above findings, from our and other groups, clearly indicate that changes in cholesterol homeostasis, distribution and compartmentation impact both the processing of APP and the biogenesis of Aβ. The relevance of these data to brain Aβ generation remains to be confirmed. High levels of free cholesterol immobilized in the myelin membranes have, in the past, made quantitation of 'mobile' free cholesterol and cholesteryl-esters in the brain very difficult (discussed further below). Cell culture and transgenic animal studies on the effect of cholesterol on Aβ generation must be carried out along with more basic research to seek a better understanding of the mechanisms that govern cholesterol balance in the CNS and cholesterol metabolism in neurons.

Cholesterol metabolism in the brain

Overall, the brain is the organ with the highest content of cholesterol in the body, containing ~20% of total body cholesterol, but accounting for only ~2% of body mass (for review, see ref. 64). Most brain cholesterol is unesterified (free) and is found within the specialized membranes of myelin[65]. Since myelin has a very slow turnover rate, myelin-associated cholesterol is virtually immobilized. The remaining brain cholesterol is found in neurons, glial cells and extracellular lipoproteins, and these pools of cholesterol participate in cholesterol homeostasis of the CNS. However, the large mass of cholesterol sequestered into myelin membranes makes the analysis of cholesterol distribution in the brain technically challenging. Direct analysis of brain cholesterol metabolism is further complicated by the separation of brain cholesterol from plasma cholesterol owing to the blood–brain barrier.

Direct quantification of plasma cholesterol in the brain, which is very difficult, has thus far yielded negative results (for review, see refs. 64,65). Therefore, it is commonly assumed that all brain cholesterol originates from *in situ* neo-synthesis. This conclusion is based mainly on studies tracking the incorporation of tritiated water into the pool of sterols contained in the brain. Additionally, only small amounts of apoB, the main apolipoprotein associated with LDL particles, can be detected in the brain, arguing against significant amounts of full-size LDL particles passing the blood–brain barrier (for review, see refs. 64,65). A few studies suggesting that at least small amounts of cholesterol from the plasma may enter the CNS are also worthy of mention. First, brain cholesterol content is increased in hypercholesterolemic transgenic mice[66], and second, a high-lipid diet not only increases cholesterol levels in the plasma, but also the influx of sterols into the CNS[42–44] (see also refs. 64 and 67 for review). The third line of evidence derives from observations obtained from patients affected by an inherited human disease, cerebrotendinuous xanthomatosis. This disorder is caused by a metabolic defect in the biosynthesis of bile acids, which leads to an abnormal hepatic production of cholestanol (a 5 *c*-dihydro derivative of cholesterol)[68]. Cholestanol abnormally accumulates in CNS of patients[69,70], indicating that LDL and/or VLDL can cross the adult blood–brain barrier into the CNS.

In summary, although conventional wisdom would predict that cholesterol homeostasis in the brain is not affected by plas-

ma cholesterol, there is growing evidence to the contrary. On the basis of these observations, cholesterol-lowering drugs as well as diet could become valid candidates for the therapeutic treatment and prevention of AD.

Conclusions

The past few years have witnessed the establishment of cholesterol as a *bona fide* risk factor in the pathogenesis of AD, supported by genetic, epidemiological and biochemical data. The application of this knowledge to the treatment and prevention of AD is already a major focus of current research. Drugs that lower cholesterol levels are currently being considered and tested as potential therapies for the treatment of AD (**Fig. 3**). Statins, which are relatively safe and have been used for a long time against hypercholesterolemia, are now being directly tested in clinical trials for efficacy against AD. Some of the potentially beneficial effects of statins might also represent improved cardiovascular health, resulting in a reduction in ischemic events that are also considered risk factors for AD. An effective therapy for patients whose cognitive function does not benefit from statin treatment may ultimately consist of a combination of lipid-regulating products, perhaps in combination with statins. Alternative products for cholesterol management so far include extended-release niacin, cholesterol absorption inhibitors, ACAT inhibitors and cholesteryl ester transfer protein (CETP) inhibitors. Results from *in vitro* studies suggest that ACAT inhibitors are good candidates for regulating Aβ biogenesis, but more research is needed to understand the exact molecular mechanisms underlying the AD–cholesterol connection.

Moreover, it is also necessary to gain an in-depth understanding of brain cholesterol metabolism. Although technical difficulties have been an obstacle for many studies, new technologies are rapidly emerging and should clarify the contribution of plasma cholesterol to brain cholesterol. These data will be invaluable in efforts to understand how plasma cholesterol levels may affect β-amyloid deposition in the brain. Additionally, several questions need to be answered to elucidate the molecular events underlying cholesterol's connection to APP processing. Does cholesterol interact directly with APP and/or the secretases? Does cholesterol require other unknown protein(s) to regulate APP processing? Finally, do cholesterol–Aβ interactions in plasma influence the overall accumulation of Aβ in the CNS? The coming years will surely witness new and exciting discoveries regarding the role of cholesterol in AD pathogenesis, thereby enabling new strategies for the prevention and treatment of this devastating neurodegenerative disease.

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