Cholesterol modulation as an emerging strategy for the treatment of Alzheimer's disease

Todd E. Golde and Christopher B. Eckman

The basis for therapeutic strategies targeting the amyloid-β protein (Aβ) has come from studies showing that accumulation and aggregation of the Aβ within the brain is likely to cause Alzheimer's disease (AD). Along with an ever-increasing understanding of Aβ metabolism, many potential therapeutic strategies aimed at altering Aβ metabolism have emerged. Among the more intriguing targets for therapy are enzymes involved in cholesterol homeostasis, because it has been found that altering cholesterol can influence Aβ metabolism in experimental model systems, and that cholesterol-lowering agents, specifically HMG-CoA reductase inhibitors, could reduce the incidence of AD. It is likely that cholesterol influences Aβ metabolism in several ways, including altering Aβ production and perhaps altering Aβ deposition and clearance. Thus, pharmacological modulation of cholesterol levels could provide a relatively safe means to reduce Aβ accumulation in the brain, and thereby prevent or slow the development of AD.

***Todd E. Golde and Christopher B. Eckman**
Mayo Clinic Jacksonville
Department of Neuroscience
4500 San Pablo Road
Jacksonville
FA 32224, USA
tel: +1 904 953 2538
fax: +1 904 953 7370
e-mail: tgolde@mayo.edu

▼ Amyloid-β protein (Aβ) deposition is one of the pathological hallmarks of Alzheimer's disease (AD). In the AD brain the ~4 kDa Aβ is deposited within senile plaques and often in walls of cerebral blood vessels. Much of the Aβ that accumulates in the AD brain is deposited as amyloid, which is, by definition, a highly insoluble, proteinaceous material with a β-pleated sheet conformation. There is now compelling biochemical, pathological and genetic evidence that accumulation and aggregation of Aβ plays a causal role in the development of AD (Ref. 1). Thus, it appears that AD is analogous to atherosclerotic disease, in that these are both age-dependent diseases in which abnormal accumulation of a normal metabolite leads to disease. When contemplating therapeutic strategies aimed at prevention or treatment of AD, it is possible that lessons learned from the study of atherosclerotic disease might be applicable to AD. In atherosclerotic disease, cholesterol deposition precedes clinical symptoms by many years. It is also likely that Aβ deposition precedes the clinical symptoms of AD by many years, and perhaps even decades. Because of this, it is likely that the best treatment for AD will not be therapeutic intervention at the time symptoms appear, but rather primary prevention. Of course, there are currently numerous difficulties with primary prevention studies in AD, including the lack of ability to predict who will get the disease, a poor understanding of when would be the best time for preventative therapy to be initiated, and a lack of safe drugs with which to attempt early intervention studies. In the development of therapeutic strategies for atherosclerotic diseases, similar issues were faced and largely overcome. Finally, just as debate over the role of Aβ in AD continues, and will persist until therapeutic strategies targeting Aβ are fully evaluated, so similar debates over the relevance of cholesterol levels to atherosclerotic disease persisted until evidence emerged that cholesterol lowering did actually reduce the risk of ischemic events.

Aβ is generated from the APP by proteolytic processing

Because decreasing Aβ production is likely to be therapeutic, the proteases that produce Aβ, as well as the factors that regulate their activity, are major targets for drug discovery. Aβ is produced from the amyloid-β precursor protein (APP) through two sequential proteolytic

1359-6446/01/$ – see front matter PII: S1359-6446(01)01965-1

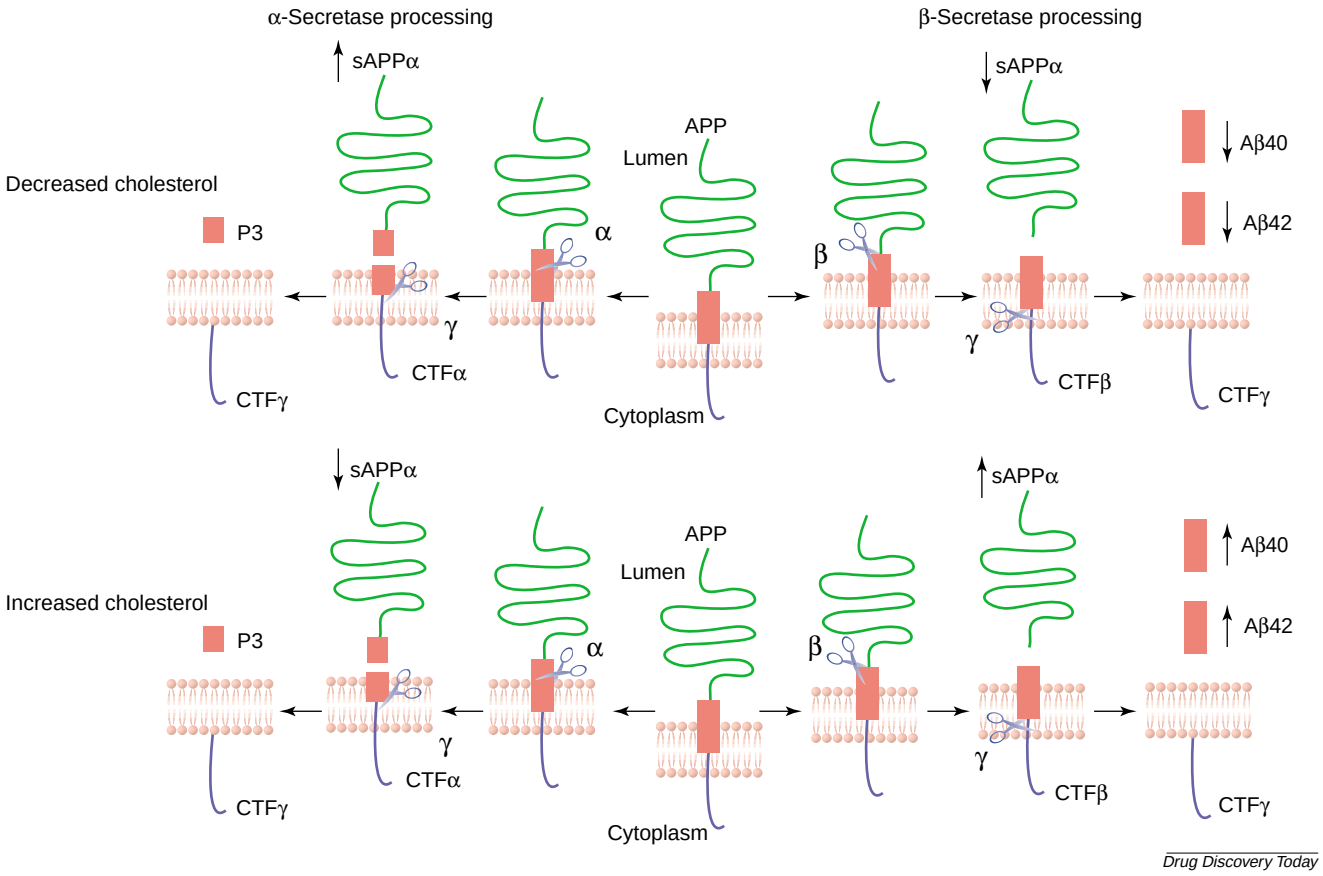

Figure 1. The effect of cholesterol on APP-processing pathways. The amyloid protein precursor (APP) is a type I transmembrane protein that is processed in several different pathways. Generation of the amyloid-β protein (Aβ) in the β-secretase pathway requires two proteolytic events: a proteolytic cleavage at the amino terminus of the Aβ sequence, referred to as β-secretase cleavage, and a cleavage at the carboxyl terminus, known as γ-secretase cleavage, which results in another carboxyl terminal fragment (CTFγ). In the α-secretase pathway, the APP is cleaved within Aβ to generate large, secreted derivative, referred to as sAPPα, and a membrane-associated CTFα. Like CTFβ, CTFα can be cleaved by γ-secretase to produce CTFγ and a small peptide referred to as P3. Elevations in cholesterol decrease α-secretase activity, reduces sAPPα, and increases sAPPβ and Aβ. Reductions in cholesterol levels lead to an increase in α-secretase, increased sAPPα and reduced sAPPβ and Aβ.

cleavages[1]. APP is first cleaved by a membrane-bound aspartyl protease, referred to as β-secretase, at the amino terminus of Aβ to generate a large secreted derivative (sAPPβ) and a membrane-bound APP carboxyl terminal fragment (CTFβ) (Fig. 1). Subsequent cleavage of CTFβ by γ-secretase results in the production of Aβ peptides of varying length, with the two species of most interest being a 40 aa Aβ peptide (Aβ40) and a 42 aa Aβ peptide (Aβ42). Concurrently, a cognate CTFγ is produced. Two homologous polytopic membrane proteases, referred to as presenilin 1 (PS1) and 2 (PS2), are probably γ-secretases[2]. If not γ-secretases, presenilins (PSs) are at least essential cofactors for this cleavage. In an alternative, presumably non-pathogenic pathway, APP is cleaved within the Aβ sequence by α-secretase, which generates another large secreted derivative (sAPPα) and CTFα. Like CTFβ, CTFα can be cleaved by γ-secretase to generate CTFγ. Two metalloprotease disintegrins, ADAM10 and ADAM17 (also known as TNF-α converting enzyme; TACE) have been implicated as α-secretases, although it is likely that additional α-secretases exist that have yet to be identified.

Because drugs targeting proteases have been successfully developed for the treatment of other diseases, targeting β- and γ-secretase activities has been a major focus of drug discovery efforts, to date. Inhibition of either of these proteolytic activities will decrease Aβ production. However, there are additional ways to lower Aβ production that do not involve direct inhibition of the proteases that generate Aβ. Under certain circumstances the α- and β-secretase pathways are competitive, and stimulation of one pathway reduces the amount of APP processed by the other pathway. Indeed, activation of the α-secretase pathway using protein kinase C (PKC) activators can lead to a decrease in Aβ production[3–5]. Finally, it is clear that other compounds

such as wortmannin can influence the APP processing pathways through unknown mechanisms to reduce Aβ production[6].

Regulation of APP-secretase activity by cholesterol

Enzymes regulating cholesterol have emerged as targets for the treatment of AD, because it has been shown that decreasing cholesterol decreases Aβ production, and that increasing cholesterol increases Aβ production (Fig. 1)[7–11]. Although the exact mechanisms by which cholesterol regulation alters Aβ production are not known, it is apparent that cholesterol can have numerous effects on the APP secretases. Cholesterol has been reported to negatively regulate α-secretase[12–14], whereas β- and γ-secretase activities are positively regulated by cholesterol[7,15]. The effect of cholesterol on β- and α-secretase appears to be indirect. Some evidence indicates that cholesterol appears to influence trafficking of secretase or enzyme[12–14]. Alternatively, cholesterol could influence the conformation of either substrate or enzyme, thereby altering the site or rate of cleavage of the substrate[13]. However, additional studies are needed to better understand the effect of cholesterol on these enzymatic activities that cleave the APP ectodomain. By contrast, the amount of cholesterol in the membrane appears to directly affect γ-secretase activity (Golde, T., unpublished observation). Thus the net effect of decreasing cholesterol is to increase α-secretase cleavage of APP and decrease both β- and γ-secretase cleavage, resulting in a net decrease in Aβ production. Conversely, increasing cholesterol appears to have the opposite effect on these combined activities, resulting in increased Aβ production.

More recent evidence suggests that it might not be total cholesterol that regulates these secretase activities, but rather the ratio of free cholesterol (FC) to cholesterol esters (CE) (Ref. 16). In cells devoid of acetyl co-enzyme A:cholesterol acyltransferase (ACAT), the enzyme that converts FC to CE, Aβ production is decreased. These cells have decreased levels of CE and increased FC. In cells with increased CE, Aβ production is increased, whereas ACAT inhibitors decrease Aβ production from a variety of cells. These effects, however, were only observed when cells contained physiological or supra-physiological levels of FC, and not when cells had low FC levels. In contrast to treatments such as methyl-β-cyclodextrin, which alter total cholesterol and reduce β- and γ-secretase activity and increase α-secretase activity, decreases in CE levels were accompanied by an apparent decrease in all three secretase activities. Of particular interest was the finding that ACAT inhibitors reduced levels of the putative secretase, PS1, suggesting that CE could modulate the expression levels of at least one of the secretases.

Other effects of cholesterol on Aβ metabolism

In addition to effects on Aβ production mediated by the APP secretases, cholesterol might also play a role in Aβ clearance and in Aβ aggregation. *In vitro* studies demonstrate that cholesterol can accelerate the formation of Aβ aggregates. These effects have been observed when Aβ is aggregated in aqueous solutions[17], and when Aβ is applied to membranes that vary in cholesterol content[18]. In addition, studies in Niemann Pick type C (NPC1) deficient cells that exhibit defects in cholesterol transport demonstrate that Aβ accumulates as cholesterol accumulates in late endosomes[19]. Significantly, accumulation of Aβ was also noted *in vivo* in brains of mice with Niemann Pick disease.

Lipid rafts, cholesterol and Aβ production

Lipid rafts are membrane microdomains rich in cholesterol, select gangliosides, and glycosylphosphatidyl-inositol (GPI)-anchored proteins that are thought to play a role in cell signalling[20]. Previous studies have shown that PS1, a probable γ-secretase, and APP CTFs, γ-secretase substrates, reside in lipid rafts[21]. We have recently shown that the majority of γ-secretase activity localizes to lipid-raft microdomains, indicating that these domains are the major site where Aβ is produced in cells, and that γ-secretase cleavage in these membranes is cholesterol-dependent (Golde, T., unpublished). Lipid rafts have also been implicated as a site for cellular accumulation of Aβ in the brain[22,23]. Two raft components that are likely to mediate this accumulation are GM-1 ganglioside and cholesterol. Like cholesterol, GM-1 ganglioside appears to bind to Aβ and possibly promotes fibril formation[24]. Based on these observations, it is possible to speculate that raft integrity and lipid composition create an optimal environment for amyloidogenic processing of APP. Because rafts could cluster enzyme and substrate in an optimal configuration for amyloidogenic cleavage, it is possible that modifying raft components could alter the configuration of either secretase or substrate within the raft. Therefore, factors that would increase the stability of existing raft structures, increase the number of rafts in a given membrane structure, or alter the recruitment of additional protein factors to existing rafts, might tend to increase Aβ production, whereas disrupting rafts would inhibit cleavage. Extracellular, soluble Aβ is typically present at low nanomolar concentrations that are unlikely to cause aggregation without additional fibril-promoting factors; therefore, it is also possible that Aβ deposition in the AD brain begins in lipid rafts, where there is local production and concentration of Aβ in an environment that might be conducive to aggregation.

Because cholesterol is an essential component of lipid-raft domains, and removal of cholesterol from membranes

disrupts raft function, it is possible that the effect of cholesterol on secretase activity can largely be explained by APP-processing events that are associated with lipid rafts. However, additional studies will be needed to determine whether altering cholesterol levels can influence APP distribution in raft domains. To date, there have been no studies exploring whether modulation of cholesterol levels *in vivo* can alter raft function and composition.

ApoE4, atherosclerotic disease and AD

Genetic studies of late-onset AD provided one of the first clues that cholesterol might play a role in AD pathology, when it was discovered that the E4 isoform of apolipoprotein E (APOE) is a risk factor for AD (Ref. 25). APOE is a plasma lipoprotein, but is also the major lipoprotein expressed in the brain. In the periphery, APOE plays a basic role in the degradation of particles rich in cholesterol and triglycerides. It can bind to low density lipoprotein (LDL) receptors, but also to receptors for chylomicron remnants. There are three major APOE isoforms, E2, E3, and E4. Their role in lipoprotein metabolism is related to their affinity for receptors. The E3 allele is the predominant form and E3 affects metabolism of lipoproteins in a standard way. When compared with the E3 allele, the E2 allele is associated with lower LDL levels, whereas E4 is associated with higher LDL levels. This has some impact on the progression of arteriosclerosis, and is likely to be responsible for the modest increase in risk of cardiac events in patients with the E4 allele, and the slight protective effect of the E2 allele[26]. Similarly APOE2 appears to be associated with a decreased risk for AD (Ref. 27).

Although the parallels between the risk for atherosclerotic disease and AD and APOE isoforms is tantalizing, it is not clear whether the increased risk of AD associated with the APOE4 allele is attributable to alterations in plasma cholesterol levels; if so, then small differences in plasma Aβ associated with APOE4 can increase the risk of AD. In fact, APOE4 only increases plasma cholesterol on average by 10–20 mg dl^{-1}, and >85% of the variability in plasma cholesterol is independent of the APOE genotype[26]. Furthermore, there is no evidence available that would support the notion that the risk for AD could be attributable to changes in brain cholesterol. APOE knockout mice show no changes in total brain-cholesterol levels, despite the fact that plasma cholesterol is elevated, suggesting that the APOE genotype is unlikely to markedly influence total brain cholesterol, although it is possible that more subtle alterations in cholesterol metabolism do exist, such as alteration in sterol recycling (Holtzman, D., pers. commun.). Studies on transgenic and knockout mice have revealed that APOE is a necessary factor for fibrillar amyloid deposition; in its absence, Aβ is deposited, but only in non-fibrillar forms[28]. When human APOE3 or APOE4 are expressed under the control of brain-specific promoters in an APOE-knockout background, and these mice are then crossed to mice overexpressing APP, fibrillar Aβ deposits do form. Moreover, in mice expressing APOE4 these Aβ fibrils form faster than in mice expressing APOE3 (Refs 29–31). Together with *in vitro* data suggesting that APOE might promote Aβ fibrillization[32], these studies suggest that APOE plays a direct role in Aβ aggregation. Thus, although it has not been excluded that the increased risk for AD associated with APOE is attributable to alterations in cholesterol levels, it appears that the effect of APOE4 is to directly alter Aβ aggregation.

Cholesterol and Aβ: animal model studies

Evidence that increasing cholesterol can increase amyloid deposition in animal models first came from studies showing that Aβ immunoreactivity was increased in the brain of rabbits fed a high-cholesterol diet[33]. Subsequent studies in APP transgenic mouse models also showed that hypercholesteremia increased Aβ deposition and senile-plaque formation in the brain. More recently, two studies have shown cholesterol-lowering agents can alter Aβ levels in animal models. In one study, guinea pigs receiving high doses of the hydroxymethyl co-enzyme A (HMG-CoA)-reductase inhibitor, simvastatin, for several weeks showed reduced levels of Aβ in the brain and cerebrospinal fluid (CSF) (Ref. 15). This compound and other HMG-CoA-reductase inhibitors (statins) decrease cholesterol levels through inhibition of *de novo* synthesis by blocking the conversion of HMG-CoA to mevalonate. In the simvastatin-treated animals, serum cholesterol was reduced by 80%, and although brain cholesterol was not decreased, there was a twofold decrease in the cholesterol precursor lanosterol. In another study, APP/PS1 co-transgenic mice were chronically treated with the cholesterol inhibitor AY9944, a 7-dehydrocholesterol-reductase inhibitor that lowers cholesterol by inhibiting the conversion of lanosterol to cholesterol. The results of this study showed a significant reduction in the Aβ levels and plaque load of animals receiving the drug (Refolo, L., pers. commun.). In this case a small, but significant, 12% reduction in total brain cholesterol was seen in the treated animals. These studies provide evidence that compounds targeting enzymes that regulate cholesterol levels can have an impact on Aβ accumulation in the brain. Additional studies, however, are needed to determine whether this reduction results in any significant behavioural improvements in the mouse models, and also to better understand the mechanisms responsible for these *in vivo* alterations in Aβ

production and deposition, especially as changes in total cholesterol are either absent or minimal in these two studies.

Human studies

Evidence from epidemiological studies suggests that cholesterol might influence the development of AD. There is good agreement between these studies, with all of them demonstrating that high serum-cholesterol levels increase the risk for AD (Refs 34–37). In addition, two recent studies indicate that the use of statins is also associated with a decreased risk of dementia and AD (Refs 38,39). Together, these observations provide a provocative, but preliminary link in humans between cholesterol and the development of AD-type dementia that would correlate with the data from cells and animal studies. Lowering cholesterol reduces amyloid production, slows deposition, and reduces the risk for AD, whereas increasing cholesterol increases Aβ production, increases deposition, and increases the risk for AD. However, some caution in interpreting these studies in this manner is warranted. With one exception, all of these studies are retrospective incidence studies. The exception being a recent prospective population-based study that examined a variety of mid-life vascular risk factors, and then followed the patients for subsequent development of AD (Ref. 37). In addition, all of these studies rely on clinical diagnoses of AD, and not autopsy-proven AD. Also, these studies do not establish that alterations in Aβ account for the increased risk associated with increased cholesterol and decreased risk associated with the use of statins. This issue becomes even more complex when data demonstrating that co-incident stroke can precipitate cognitive changes is considered[40]. Because the link between cholesterol and ischemic cerebrovascular events is well-established[41], it is possible that the beneficial effect of statins and the risk associated with increased cholesterol are attributable to the fact that a CNS ischemic event can convert preclinical AD to clinical diagnosable dementia[40]. In this regard, it is worth noting that in the prospective population-based study, high systolic blood pressure was associated with a higher relative risk for AD than serum cholesterol alone[37]. In any case and regardless of mechanism, treatment with statins could have a significant clinical benefit for the prevention of AD.

Cholesterol synthesis inhibitors, cholesterol metabolism in the brain, and Aβ lowering

Until recently, the study of brain cholesterol metabolism had been relatively ignored compared with the study of peripheral cholesterol metabolism. The recent links between cholesterol and AD provide strong impetus for further study of CNS cholesterol metabolism. Currently, it is known that there are many differences between cholesterol metabolism in the brain and periphery, but many aspects of cholesterol metabolism have yet to be explored in the brain. One of the most striking differences is that the turnover of cholesterol in the brain is 1% as rapid as the turnover of cholesterol in the periphery. Another is that the input in the CNS comes almost exclusively from *de novo* synthesis and not from transfer from the periphery[42]. However, most drugs that have been developed to lower cholesterol have largely been evaluated for their effects on peripheral cholesterol levels and not on CNS cholesterol levels. Although the animal model studies strongly suggest that known inhibitors of cholesterol synthesis can influence Aβ production and possibly its deposition, it is still not clear that this is caused by changes in cholesterol levels. Inhibition of cholesterol synthesis results in a marked alteration in cellular physiology, and the brain appears to respond by trying to maintain normal levels of cholesterol[42]. Thus, even when peripheral cholesterol is markedly reduced, CNS cholesterol balance is maintained. Indeed, marked cellular changes can occur in response to cholesterol synthesis inhibitors, in an apparent effort by the cell to maintain physiological levels of cholesterol.

One of the difficulties in studying statins in animal models is that statin treatment in many small animals, including mice and guinea pigs, results in massive upregulation of HMG-CoA reductase[43]. Therefore, to achieve effects on cholesterol levels, extremely high doses of these drugs are needed. In addition, it is documented that statins can have additional effects that might be independent of their effects on cholesterol levels. In fact, studies of statins in stroke and myocardial infarction show that they have beneficial effects beyond the measured effect on cholesterol levels[41].

Future directions

The link between cholesterol and Aβ is certainly provocative. Cell-culture studies indicate that cholesterol can alter Aβ production and there is circumstantial evidence suggesting that cholesterol can alter Aβ deposition. However, there is no strong evidence demonstrating that cholesterol levels directly influence Aβ production, deposition, or both in humans. As mentioned previously, it is entirely possible that cholesterol-lowering agents decrease the risk for AD through mechanisms that are independent of Aβ. Furthermore, another interpretation of the data on cholesterol-lowering agents in the animal studies is that it is the response of the cell to the cholesterol-lowering agent, and not the reduced cholesterol itself, that mediates the decrease in production and subsequent decrease in Aβ deposition. Alternatively, as suggested by the recent work on

CE, Aβ production and deposition could be regulated by other cholesterol metabolites or other lipids that could potentially be altered by statins and other cholesterol synthesis inhibitors. In any case, future studies will be needed to further evaluate the link between cholesterol, cholesterol metabolites and Aβ production and deposition, because such studies might identify novel therapeutic targets (e.g. ACAT) and perhaps potential risk-factors for AD.

Despite the uncertainties regarding the precise mechanism, it would seem that further study of statins as potential AD therapeutics is warranted. Indeed, Pfizer (New York, NY, USA) and the Institute for the Study of Aging (ISOA; New York, NY, USA) are co-sponsoring a Phase II placebo-controlled therapeutic trial on the effect of Lipitor® (Atorvastatin) on cognition in AD patients, and the National Institutes of Health (NIH, Bethesda, MD, USA) is currently planning a therapeutic trial of lovastatin. It is also likely that pharmaceutical companies with other FDA-approved statins will consider therapeutic and or preventative trials to study the effect of statins in the treatment or prevention of AD. One issue regarding statins and clinical trials for AD is which statin to use. Although some differences in brain penetration might exist, all FDA-approved statins block cholesterol synthesis in the brain; thus, there is no *a priori* data to suggest that one statin will be superior to another. Indeed, treatment with multiple statins showed a reduced risk for AD in the epidemiological studies.

As with any potential AD therapy, it is possible that therapeutic trials could fail, even though the therapy might be effective if it was implemented as primary prevention. For this reason it will be extremely important to determine whether statins lower CSF Aβ and plasma Aβ in any therapeutic trial, even if they do not result in cognitive improvements. Because statins are extremely safe drugs that are well tolerated with chronic use, they are more likely to be considered for primary prevention trials than emerging drugs, such as secretase inhibitors, that have unknown long-term safety profiles. Given the recently issued NIH guidelines, which have decreased the threshold for intervention in patients with elevated LDL-cholesterol levels, it is likely that such studies could be readily undertaken because an increasing numbers of individuals are likely to be placed on statins because of these recent recommendations. Indeed, if statins do reduce the risk for AD and these NIH guidelines are implemented, a sufficient number of people could be placed on statins in the next few years to see an appreciable decline in the incidence of AD.

References

1 Golde, T.E. *et al.* (2000) Biochemical detection of Aβ isoforms: implications for pathogenesis, diagnosis, and treatment of Alzheimer's disease. *Biochim. Biophys. Acta* 1502, 172–187

2 Golde, T.E. and Younkin, S.G. (2001) Presenilins as therapeutic targets for the treatment of Alzheimer's disease. *Trends Mol. Med.* 7, 264–269

3 Buxbaum, J.D. *et al.* (1993) Protein phosphorylation inhibits production of Alzheimer amyloid-β/A4 peptide. *Proc. Natl. Acad. Sci. U. S. A.* 90, 9195–9198

4 Nitsch, R.M. *et al.* (1992) Release of Alzheimer amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptors. *Science* 258, 304–307

5 Skovronsky, D.M. *et al.* (2000) Protein kinase C-dependent α-secretase competes with β-secretase for cleavage of amyloid-β precursor protein in the trans-Golgi network. *J. Biol. Chem.* 275, 2568–2575

6 Haugabook, S.J. *et al.* (2001) Reduction of Aβ accumulation in the Tg2576 animal model of Alzheimer's disease after oral administration of the phosphatidylinositol kinase inhibitor wortmannin. *FASEB J.* 15, 16–18

7 Simons, M. *et al.* (1998) Cholesterol depletion inhibits the generation of β-amyloid in hippocampal neurons. *Proc. Natl. Acad. Sci. U. S. A.* 95, 6460–6464

8 Frears, E.R. *et al.* (1999) The role of cholesterol in the biosynthesis of β-amyloid. *NeuroReport* 10, 1699–1705

9 Howland, D.S. *et al.* (1998) Modulation of secreted β-amyloid precursor protein and amyloid β-peptide in brain by cholesterol. *J. Biol. Chem.* 273, 16576–16582

10 Mizuno, T. *et al.* (1998) Cholesterol-dependent generation of a unique amyloid β-protein from apically missorted amyloid precursor protein in MDCK cells. *Biochim. Biophys. Acta* 1373, 119–130

11 Mizuno, T. *et al.* (1999) Cholesterol-dependent generation of a seeding amyloid β-protein in cell culture. *J. Biol. Chem.* 274, 15110–15114

12 Bodovitz, S. and Klein, W.L. (1996) Cholesterol modulates α-secretase cleavage of amyloid precursor protein. *J. Biol. Chem.* 271, 4436–4440

13 Kojro, E. *et al.* (2001) Low cholesterol stimulates the non-amyloidogenic pathway by its effect on the α-secretase ADAM 10. *Proc. Natl. Acad. Sci. U. S. A.* 98, 5815–5820

14 Racchi, M. *et al.* (1997) Secretory processing of amyloid precursor protein is inhibited by increase in cellular cholesterol content (Part 3). *Biochem. J.* 322, 893–898

15 Fassbender, K. *et al.* (2001) Simvastatin strongly reduces levels of Alzheimer's disease β-amyloid peptides Aβ 42 and Aβ 40 *in vitro* and *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.* 98, 5856–5861

16 Puglielli, L. *et al.* Acyl-coenzyme A: cholesterol acyltransferase (ACAT) modulates the generation of the amyloid β peptide. *Nat. Cell Biol.* (in press)

17 Avdulov, N.A. *et al.* (1997) Lipid binding to amyloid β-peptide aggregates: preferential binding of cholesterol as compared with phosphatidylcholine and fatty acids. *J. Neurochem.* 69, 1746–1752

18 Kakio, A. *et al.* (2001) Cholesterol-dependent formation of GM1 ganglioside-bound amyloid β-protein, an endogenous seed for Alzheimer amyloid. *J. Biol. Chem.* 276, 24985–24990

19 Yamazaki, T. *et al.* (2001) Accumulation and aggregation of amyloid-β-protein in late endosomes of Niemann–Pick type C cells. *J. Biol. Chem.* 276, 4454–4460

20 Simons, K. and Ikonen, E. (1997) Functional rafts in cell membranes. *Nature* 387, 569–572

21 Lee, S.J. *et al.* (1998) A detergent-insoluble membrane compartment contains Aβ *in vivo*. *Nat. Med.* 4, 730–734

22 Yanagisawa, K. *et al.* (1995) GM1 ganglioside-bound amyloid beta-protein (Aβ): a possible form of preamyloid in Alzheimer's disease. *Nat. Med.* 1, 1062–1066

23 Yanagisawa, K. *et al.* (1997) Amyloid β-protein (Aβ) associated with lipid molecules: immunoreactivity distinct from that of soluble Aβ. *FEBS Lett.* 420, 43–46

24 Choo-Smith, L.P. and Surewicz, W.K. (1997) The interaction between Alzheimer amyloid-β(1-40) peptide and ganglioside GM1-containing membranes. *FEBS Lett.* 402, 95–98

25 Corder, E.H. *et al.* (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 261, 921–923

26 Breslow, J.L. (2000) Genetics of lipoprotein abnormalities associated with coronary artery disease susceptibility. *Annu. Rev. Genet.* 34, 233–254

27 Roses, A.D. (1997) Apolipoprotein E, a gene with complex biological interactions in the aging brain. *Neurobiol. Dis.* 4, 170–185

28 Bales, K. *et al.* (1997) Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. *Nat. Genet.* 17, 263–264

29 Holtzman, D.M. *et al.* (1999) Expression of human apolipoprotein E reduces amyloid-β deposition in a mouse model of Alzheimer's disease. *J. Clin. Invest.* 103, R15–R21

30 Holtzman, D.M. *et al.* (2000) Apolipoprotein E facilitates neuritic and cerebrovascular plaque formation in an Alzheimer's disease model. *Ann. Neurol.* 47, 739–747

31 Holtzman, D.M. *et al.* (2000) Apolipoprotein E isoform-dependent amyloid deposition and neuritic degeneration in a mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U. S. A.* 97, 2892–2897

32 Castano, E.M. *et al.* (1995) Fibrillogenesis in Alzheimer's disease of amyloid beta peptides and apolipoprotein E (Part 2). *Biochem. J.* 306, 599–604

33 Sparks, D.L. *et al.* (1994) Induction of Alzheimer-like β-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. *Exp. Neurol.* 126, 88–94

34 Jarvik, G.P. *et al.* (1995) Interactions of apolipoprotein E genotype, total cholesterol level, age, and sex in prediction of Alzheimer's disease: a case-control study. *Neurology* 45, 1092–1096

35 Notkola, I.L. *et al.* (1998) Serum total cholesterol, apolipoprotein E epsilon 4 allele, and Alzheimer's disease. *Neuroepidemiology* 17, 14–20

36 Hofman, A. *et al.* (1997) Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer's disease in the Rotterdam Study. *Lancet* 349, 151–154

37 Kivipelto, M. *et al.* (2001) Midlife vascular risk factors and Alzheimer's disease in later life: longitudinal, population based study. *Br. Med. J.* 322, 1447–1451

38 Wolozin, B. *et al.* (2000) Decreased prevalence of Alzheimer's disease associated with 3-hydroxy-3-methyglutaryl coenzyme A reductase inhibitors. *Arch. Neurol.* 57, 1439–1443

39 Jick, H. *et al.* (2000) Statins and the risk of dementia. *Lancet* 356, 1627–1631

40 Snowdon, D.A. *et al.* (1997) Brain infarction and the clinical expression of Alzheimer's disease. The Nun Study. *J. Am. Med. Assoc.* 277, 813–817

41 Hess, D.C. *et al.* (2000) HMG-CoA reductase inhibitors (statins): a promising approach to stroke prevention. *Neurology* 54, 790–796

42 Dietschy, J.M. and Turley, S.D. (2001) Cholesterol metabolism in the brain. *Curr. Opin. Lipidology* 12, 105–112

43 Pitman, W.A. *et al.* (1998) The effects of diet and lovastatin on regression of fatty streak lesions and on hepatic and intestinal mRNA levels for the LDL receptor and HMG-CoA-reductase in F1B hamsters. *Atherosclerosis* 138, 43–52