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REVIEW ARTICLE

Alzheimer's Disease and Cholesterol: The Fat Connection

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Abstract Since the discovery of the significance of the cholesterol-carrying apolipoprotein E and cholesterolaemia as major risk factors for Alzheimer's Disease (AD) there has been a mounting interest in the role of this lipid as a possible pathogenic agent. In this review we analyse the current evidence linking cholesterol metabolism and regulation in the CNS with the known mechanisms underlying the development of Alzheimer's Disease. Cholesterol is known to affect amyloid-β generation and toxicity, although it must be considered that the results studies using the statin class of drugs to lower plasma cholesterol may be affected by other effects associated with these drugs. Finally, we report some of our results pointing at the interplay between neurons and astrocytes and NADPH oxidase activation as a new candidate mechanism linking cholesterol and AD pathology.

Keywords Alzheimer's Disease, Amyloid-beta, Cholesterol, Statins, ApoE, NADPH oxidase

Special issue dedicated to John P. Blass.

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Introduction

Alzheimer's Disease (AD) is the most common form of dementia. It is characterized by deposition in the brain of neuritic plaques, mainly constituted of masses of fibrillary amyloid β (Aβ) peptide, and surrounded by dystrophic neurites (rich in phosphorylated tau), activated astrocytes and microglia. Apart from the small percentage of cases where there is a clear familial component due to a mutation in the genes either for the amyloid precursor protein (APP) or presenilins, for the vast majority of sporadic cases the primary causes of the disease remains elusive. Aβ is thought to be associated with neurodegeneration, and is neurotoxic in vitro and in vivo, but its exact role in the development of the disease is not fully understood. It has been linked with an increase in oxidative stress, dysregulation of calcium dynamics and inhibition of the activity of some enzymes, possibly through interaction with cellular membrane structures [1–3].

The cholesterol connection

Apart from mutations in the proteins involved in Aβ generation (APP, presenilins), the strongest known risk factor influencing the incidence of sporadic AD is the genotype for apolipoprotein E (ApoE), the major carrier of cholesterol in the CNS. Individuals carrying one or two copies of the ApoE ε4 allele have a higher risk of developing the disease [4], compared to those carrying the ε3 (the most common) or ε2 (which appears to be protective) forms. The ''Rotterdam study'' found an association between atherosclerosis and dementia, which was particularly strong in those with the apoE ε4 genotype [5]. In addition, it has been

observed that patients with cardiovascular disease undergoing cholesterol lowering therapy with cholesterol synthesis inhibitors (statins) have a lower risk of developing AD [6, 7]. This has led to the hypothesis, now supported by further evidence, that cholesterol is somehow involved in AD pathogenesis, although the mechanism is at present not clear.

In this review we will focus on the growing evidence of an involvement of brain cholesterol in the pathogenesis of AD with particular reference to its interactions with Aβ.

Cholesterol metabolism

Cholesterol is a crucial component of mammalian membranes, as well as being an important precursor of steroid hormones and bile acids. The complex structure of cholesterol confers rigidity to lipid bilayers, affecting the fluidity, permeability and thickness of cell membranes. It is fundamental for the functionality of the membranes and many membrane-associated proteins (reviewed in [8]). Cholesterol is mainly concentrated in the detergent-resistant dynamic liquid-ordered domains in the plasma membrane called lipid rafts, which are also rich in sphingolipids and saturated phospholipids [9]. Cholesterol is also present in caveolae, membrane invaginations rich in the protein caveolin [10]. A number of proteins involved in signal transduction, cell adhesion and other functions are associated with the rafts, including the amyloid precursor protein and γ-secretase, one of the enzymes involved in APP cleavage to generate Aβ. Membranes of intracellular organelles such as the ER and mitochondria typically have low levels of cholesterol [11].

Cholesterol in the body originates partly from the diet, and partly from de novo synthesis, mainly in the liver and intestine. The biosynthetic pathway also generates intermediates used in the synthesis of ubiquinone and in the prenylation of proteins. The first steps of cholesterol synthesis (Fig. 1) are the conversion of three molecules of acetyl-CoA into one 3-hydroxy-3-methyglutaryl-CoA (HMG-CoA) in the cytoplasm. Then HMG-CoA is converted to mevalonate by the HMG-CoA reductase (HMGR), associated with the ER. This is the rate-limiting step, and is subject to complex regulation. High levels of cholesterol exert control by feedback inhibition and by stimulating HMGR ubiquitination and degradation by the proteasome. When levels are low, cleavage of the ER-bound sterol regulated element binding protein (SREBP) induces the generation of transcription factors which bind to the sterol regulatory element (SRE-1) which in turn controls the transcription of HMGR and other genes involved in the metabolism and transport of cholesterol and other lipids (Fig. 2).

Cholesterol is transported in the serum mainly in esterified form, bound to apolipoproteins ApoB and ApoE, in low-density lipoproteins (LDLs). These deliver the lipid to cells by desorption or receptor-mediated internalization. Once in the cells it passes through the endosome system via a mechanism involving NPC1, the protein responsible for Niemann-Pick type C disease (NPC), a disorder which shares several features with AD (see below). Internalised cholesterol can then be cycled to the plasma membrane via vesicular transport [12].

Excess cholesterol is esterified in the ER by the Acyl-CoA acyltransferase (ACAT) and stored as lipid droplets, or released via a mechanism involving the ATP-binding cassette transporter ABCA-1. Cholesterol released in the serum is transported in high density lipoproteins (HDLs), and taken up by the liver which excretes it in the form of bile salts.

Cholesterol in the CNS

Cholesterol has a fundamental role in brain development and function. The CNS is particularly rich in cholesterol, containing a quarter of the total body content of this lipid, despite representing only 2% of body weight.

Brain cholesterol appears to be largely independent and unaffected by the serum levels [13], being impermeable to the blood brain barrier. Accordingly, cholesterol introduced with the diet has little or no impact on brain cholesterol, which is largely synthesised de novo within the organ.

Cholesterol turnover in the brain is slow, the half-life being estimated around 4–6 months in rodents [14] and 5 years in humans [15]. Release from the brain occurs only after oxidation to 24(S)-hydroxycholesterol (24-OHC) by the cholesterol 24-hydroxylase (also known as CYP46, a cytochrome P450 family member), a brain-specific, neuronal enzyme [16]. 24-OHC can

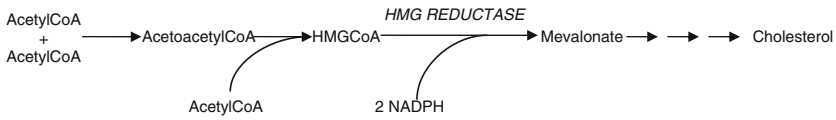

Fig. 1 Schematic diagram of the first steps of cholesterol biosynthesis

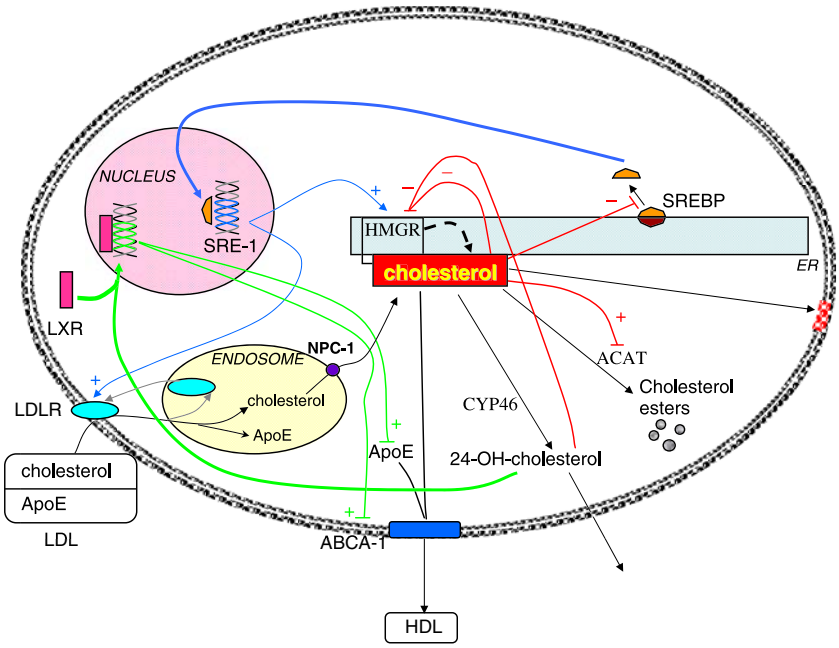

Fig. 2 Schematic diagram of cholesterol metabolism in a model cell. Cholesterol bound to ApoE in LDLs is taken up into the cell via a LDLR-dependent mechanism, transported through the endosomal system and delivered to the ER via NPC-1. Endogenous synthesis of cholesterol also occurs in the ER. Excess cholesterol inhibits further synthesis by inhibiting HMGR while low levels stimulate cleavage of SRBP in the ER, to generate transcription factors which induce SRE-1-dependent transcription of HMGR, LRLR and other genes involved in cholesterol metabolism. Excess cholesterol can also be esterified by ACAT, oxidised to 24-OHC by CYP-46 (in neurons) or released together with ApoE (in astrocytes). ApoE transcription is enhanced by the nuclear LXR receptors, which are activated by 24-OHC

cross the blood-brain barrier into the plasma, and is subsequently excreted by the liver. Oxysterols also have important regulatory functions on cholesterol metabolism within the brain. Circulating 24-OHC derives almost exclusively from the brain and is used as a marker of brain cholesterol metabolism [15]. Cholesterol metabolism in the brain has been recently reviewed by Dietschy [17].

Brain cholesterol is mainly unesterified; most of it is associated with myelin, but it is present also in the membranes of astrocytes and neurons.

Cholesterol synthesis occurs mainly in glial cells [18], together with synthesis of ApoE, the main lipoprotein in the CNS (which also has ApoA-I, ApoJ and ApoD [19]). The ApoE-cholesterol complex is secreted, in a process involving the ABCA-1 [20]. After the first stages of brain development, most mature neurons synthesise only small amounts of cholesterol, they become heavily dependent on uptake of cholesterol synthesised in astrocytes, which they internalize via a member of the LDL receptor (LDLR) family - mainly the LDLR itself and the LDL receptor-related protein, LRP, but also VLDL receptor, ApoE receptor 2, megalin and others [19] (Fig. 2). It has been shown that neurons require ApoE-cholesterol to develop numerous and efficient synapses in vitro [21].

Cholesterol homeostasis is regulated by the dynamic equilibrium of uptake, de novo synthesis, esterification, catabolism (oxidation) and release. The genes for both ApoE and ABCA-1 are among those controlled by the Liver X receptors (LXR) [22], nuclear receptors (originally identified in the liver) widely expressed throughout the body, which are activated by oxysterols [23]—these are therefore major regulators of cholesterol homeostasis, since they switch on the mechanism of efflux and excretion via ABCA-1 and ApoE [24], and also inhibit HMGR [25], therefore cholesterol synthesis (Fig. 2).

Cholesterol and AD

A number of studies point to a deleterious effect of cholesterol in the development of AD. As mentioned above, the risk of developing AD is increased in individuals expressing the ε4 form of the cholesterol-carrying ApoE, and is decreased in patients treated with cholesterol-lowering statins. Cholesterol accumulation has been observed in association with neuritic plaques in the brain in AD and in a transgenic AD mouse model [26]. In addition to the decreased AD incidence with statin treatment mentioned above, elevated total serum cholesterol has been associated

with risk of AD in later life [27–29]. Increased cholesterol has been found in brain membranes in aging mice [30] and increased flux of cholesterol across the CNS in aging [16] and in early AD. 24-OHC levels increase in mild cases, while it decreases in more severe cases probably due to the loss of neurons expressing CYP46 [31]. Breaking down of neuronal membranes along with a reduction in the expression of CYP-46 would lead to accumulation of cholesterol in the brain [31]. A preliminary clinical study lowering plasma cholesterol with atorvastatin in mild-moderate AD indicated a positive effect on cognition and behaviour [32]. Staining of sections from AD cortex with a cholesterol-specific fluorescent probe revealed that tangle-bearing neurons contain more free cholesterol than adjacent tangle-free neurons [33].

However, the picture is more complex. On the other hand, cholesterol affects membrane fluidity and is essential for membrane function; adequate levels of cholesterol imported from astrocytes are necessary for synaptogenesis and neuronal functionality [21]. Other groups found that cholesterol synthesis decreases with age in human hippocampus, with no change in brain levels [34], or no change in hippocampal levels in AD [35]. In some cases increased levels of total plasma cholesterol in late life was associated with decreased risk of dementia [36], while in others no association was found [37, 38]. For a review, [39].

Recently it has been shown that polymorphisms in a cluster of genes related to cholesterol metabolism confer susceptibility to AD [40], including CYP46 [41] and ABCA1 [42], although other groups have found no link [43, 44]. Others have found that CYP46 expression shifts from neurons to astrocytes in AD [45].

Cholesterol and Aβ generation

The deleterious effects of cholesterol in AD have been attributed to its ability to increase Aβ generation. Aβ is produced by sequential cleavage of the precursor protein APP by the amyloidogenic β-secretase (as alternative to the non-amyloidogenic α-secretase) followed by cleavage by γ-secretases (presenilins). These processes take place mainly in the ER and plasma membranes.

High cholesterol diets caused higher brain accumulation of Aβ in rabbits [46, 47] and in a transgenic mouse model [48], while lowering cholesterol levels leads to an inhibition of Aβ generation both in cultured neurons and in vivo in guinea pigs and mice [49, 50]. In humans a reduction of Aβ in the CSF has been found with simvastatin in mild AD but not in more severe cases [51–53]. Lowered membrane cholesterol inhibits β-secretase activity in hippocampal neurons [54] and increases Aβ binding to membranes, and Aβ toxicity [55, 56].

At least a fraction of the cell APP, as well as the β-secretase and γ-secretases, are localised in cholesterol-rich lipid rafts [57–59], while the non-amyloidogenic α-secretase is associated with the membrane surface, outside raft domains [60]. A change in cholesterol levels or distribution within different membrane pools (i.e. raft vs. non-raft) alters the localization of APP molecules and their availability to the action of the various secretases [61, 62]. Lipophilic statins have been shown to decrease cholesterol levels in raft environment, and to decrease the expression of the raft marker protein flotillin [63]. However, drastic reductions (over 35%) in membrane cholesterol decreases Aβ generation [61], which may explain why some groups have found increased Aβ in the brain of transgenic mice treated with lovastatin [64]. APP processing can be altered by changes in the dietary cholesterol content in APP gene-targeted mice, by a mechanism dependent on ApoE [65]. This group found that high cholesterol diets in AD transgenic mouse models decreased brain Aβ [65].

Kalvodova and colleagues have found that the proteolitic activity of β-secretase is also directly increased by cholesterol, as well as by neutral glycosphingolipids and anionic glycerophospholipids [66].

Another mechanism linking cholesterol and Aβ secretion may be ABCA1, the transporter involved in cholesterol release, which is present in neurons. Oxysterols, as well as other agonists of the LXR receptors, which increase ABCA-1 expression, also increase Aβ secretion, especially the more hydrophobic $A\beta_{1\text{-}42}$, suggesting that the peptide may be released bound to cholesterol [67]. Others however have found that increasing ABCA1 expression with oxysterols inhibits Aβ generation [68].

The levels of cholesteryl esters (CE) have also been linked to Aβ generation rather than free cholesterol (FC). In cells lacking ACAT, which cannot generate CE and accumulate FC, Aβ generation is inhibited [69]. Treatment with an ACAT inhibitor also leads to decreased brain Aβ load, as well as cognitive function, in a transgenic mouse model of AD [70].

Statin as a therapy for AD

As mentioned above, retrospective studies of cardiovascular patients taking statins have shown a decreased incidence of AD in this population [6, 7]; since then, a large part of the cholesterol inhibition research has been done using these drugs. Statins are inhibitors of

HMG-CoA reductase, the regulatory step in the biosynthetic pathway for cholesterol; they have been used for a long time to treat hypercholesterolaemia, and are well tolerated. However, results in connection with AD have been inconsistent. Some recent prospective studies have found no association between statin treatment and AD risk [37, 71]. Statins also seem to have no effect on the Aβ burden in human AD brains [72].

Statins in mice decrease brain cholesterol by a mechanism that seems to involve ApoE, since no effect is observed in ApoE deficient animals [30]. Statins also decrease 24-OHC, indicating an effect on brain cholesterol [73, 74].

One problem in interpreting the statin treatment results is that where an effect is seen, all types of statins tested seem to have a similar impact on risk of AD and brain cholesterol [7, 74], irrespective of the fact that the more lipophilic drugs (lovastatin, simvastatin) cross the blood brain barrier much more easily than the more hydrophilic ones (pravastatin, atorvastatin) [75]—raising the question that the effect may be indirect.

It is possible that statin-induced reductions in peripheral cholesterol affect brain cholesterol via the degradation product 27-hydroxycholesterol (27-OHC), which can cross the blood brain barrier and is actively taken up by the brain [76].

Michikawa [77] ascribes the conflicting data to the existence of different cholesterol pools, pointing out that total serum cholesterol does not affect CSF or brain levels, and that statins affected brain cholesterol only at high doses. Cholesterol is transported in the CSF mainly in HDL, the form released from cells: this pool is low in AD patients and is *increased* by statins, both in serum and CSF. It is also the high HDL cholesterol levels that correlate with increased AD pathology, rather than the total or LDL pool [78]. The two pools are regulated in opposite ways also by the ApoE phenotype, i.e. higher total and LDL cholesterol and lower HDL cholesterol are found in the subjects with the ApoE4 phenotype compared to those with the ApoE3.

Another interesting set of data showed that cholesterol distribution in the plasma membrane was uneven and that statins caused cholesterol to translocate from the cytofacial leaflet to the exofacial leaflet. This change is accompanied by decreased Aβ levels in vivo, suggesting that cholesterol distribution and not total cholesterol levels may be important to Abeta production in the CNS [61, 79].

Finally, a side effect of statin treatment in a small proportion of cases is a myopathy, which seems to be related to the inhibition of the synthesis of ubiquinone, which shares the HMGR step with the biosynthetic pathway of cholesterol [80]. It is possible that an adverse effect on this important mitochondrial electron carrier may contribute to the variability of results obtained with statin therapies, especially in a situation such as AD where the antioxidant balance and mitochondrial function may already be compromised.

Statins have pleiotropic effects on top of lowering cholesterol. One of these is the prevention of the isoprenylation of small G-proteins such as RhoA and Rac1, which results in the inhibition of their translocation to the membrane [81]. This in turn regulates, among other things, the activities of NOS and NADPH oxidase, to generate NO and superoxide respectively [82, 83], resulting in alterations in the vascular tone and in the anti-inflammatory action. Statins have also direct immunological effects on microglia [84], and anti-apoptotic effects [85]. Some effects on APP processing have been attributed to inhibition of isoprenoid products [86]. Wolozin [87] has in fact proposed that the primary action of statins could be actually to reduce inflammation by inhibiting microglial activation, rather than to decrease the Aβ load. The implication would be that the treatment would prevent the progression of AD towards the more severe stages, rather than reducing the incidence, as the role of inflammation seems to be less important in the early stages.

However, at least part of the effects observed are attributable to statins' cholesterol lowering action. In cultured neurons, lowering cholesterol with a completely different mechanism, i.e. binding the lipid with methyl-β-cyclodextrin, also lowered Aβ levels, indicating that at least in this model the effect is related to cholesterol levels [50]. In addition, a non-statin cholesterol lowering drug, BM15.766, also decreased brain Aβ in transgenic mice [88].

Micro-array experiments have revealed alterations in gene expression patterns in the brain of mice after statin treatment, mostly genes involved in cell growth and signaling and trafficking that were similarly changed by three drugs of this class [89].

Further clinical and laboratory trials are currently under way which will hopefully help clarify the mechanism of action of these drugs.

Niemann-Pick Disease type C

It is also worth pointing out the pathological similarities between AD and Niemann-Pick Disease type C (NPC), an autosomal recessive disorder caused by a failure in cholesterol trafficking, due to a mutation in the NPC-1 protein (Fig. 2). Both present neurofibrillary tangles and tauopathy, endosomal abnormalities, and

increased Aβ generation [90]. In NPC cholesterol accumulates in late endosomes without being able to proceed towards the plasma membrane and ER, which as a result are cholesterol-poor. Mice expressing a pathogenic mutant NPC-1 display increased γ-secretase activity and accumulate $A\beta_{1-40}$ and $A\beta_{1-42}$ [91]. Cells treated with a drug which inhibits NPC-1, U18666A, also accumulate Aβ, and the effect disappears on removal of the drug [92].

Cholesterol and Aβ toxicity

Since Aβ toxicity is correlated to its state of aggregation, many studies have investigated the relationship between cholesterol and the toxic effects of Aβ. Aβ is known to interact with and disrupt the order and fluidity of lipid bilayers [93], according to their lipid composition, and this interaction also affects the rate of peptide aggregation. Cholesterol has been reported to favour the formation of amyloid polymerisation ''seeds'' which initiate peptide aggregation [94]. Arispe and Doh [95] found that lowering membrane cholesterol increased the membrane incorporation of Aβ and cytotoxicity. This is in agreement with the findings of many groups, who also showed that higher cholesterol reduced the effects of Aβ on calcium signalling and neurotoxicity [55, 56, 96–98].

On the other hand, cholesterol was necessary for Aβ binding and toxicity in other models [99, 100]. Oxidised cholesterol metabolites promote Aβ aggregation in vitro [101]. Recently, Schneider and colleagues [102] showed that low cholesterol reduces the ability of Aβ to form oligomeric aggregates, which are now considered the most toxic species of the peptide, without affecting the generation of Aβ monomers.

The interactions between membrane cholesterol and Aβ are undoubtedly very complex and subtle changes in the concentration and/or distribution of this lipid in the various cell compartments have often contrasting effects on Aβ binding and toxicity, depending also on the aggregation state of Aβ [103]. This has been brilliantly reviewed by Eckert and colleagues [104, 105].

Aβ on lipid metabolism

Interesting recent findings show that Aβ in turn also affects lipid metabolism. $A\beta_{1-42}$ induces accumulation of cholesterol and ceramides in hippocampal neurons [106]. Ceramides are the products of sphingomyelin metabolism by sphingomyelinases (SM), and are involved in various signaling pathways including those leading to apoptosis. Aβ oligomers have been shown to induce neuronal apoptosis mediated by a SM-ceramide pathway [107]. Other groups have also shown that while $A\beta_{1-42}$ stimulates sphingomyelinase, $A\beta_{1\text{-}40}$ inhibits HMG reductase (the target of statins), and therefore inhibits cholesterol synthesis [108]. Changes in the 1–40/1–42 ratio in AD therefore may in turn alter membrane lipid composition, alter the activity of proteins associated with membranes, and affect cell viability, and initiate a vicious Aβ-cholesterol loop.

Grimm and collaborators have shown that detergent resistant membranes from presenilin KO mice differ from the wild type in the cholesterol content and also show altered membrane fluidity, demonstrating that Aβ and presenilin not only regulate lipid metabolism but also are involved in the regulation of membrane structure and function [109].

ApoE and neurodegeneration

The ApoE4 phenotype is associated with higher risk of AD [4], earlier age of onset of both AD [110] and Down's syndrome (where there is an additional copy of the chromosome carrying the APP gene) [111], and also with a worse outcome after head trauma [112] and stroke, both in humans [113] and in transgenic mice expressing the human ApoE4 [114]. Individuals carrying ApoE4 have higher total and LDL cholesterol [115], greater amyloid and tangle pathology [116, 117] and show worse mitochondrial damage [118] compared to those carrying other forms.

In cultured neurons, cholesterol uptake is lower when the lipid is bound to ApoE4 compared to ApoE2 and ApoE3 [119]. ApoE4 is less efficient than the other forms in promoting cholesterol efflux from both neurons and astrocytes [120]. Lipidated ApoE binds aggregated Aβ in a isoform-specific manner, ApoE4 being much more effective than the other forms, and may cause enhanced deposition of the peptide [121].

ApoE knockout mice (ApoE–/–) have been used extensively for cardiovascular research. Brain cells from these animals are more sensitive to excitotoxic and age-related synaptic loss [122], while Aβ induced synaptosomal dysfunction was also exaggerated compared to material from control animals [123]. Human ApoE isoforms have been expressed in ApoE–/– mice. Expression of ApoE3, but not ApoE4, is protective against excitotoxicity and age related neurodegeneration [122] and Aβ toxicity [124]. Astrocytes from ApoE–/– mice expressing human ApoE3 release more cholesterol into the medium than those expressing ApoE4, and therefore may modulate the amount of the lipid available for neurons. In other cases ApoE3 was

found to bind Aβ more avidly than ApoE4 when associated with lipids [125], and may therefore affect Aβ removal from the extracellular space.

The wide-ranging studies on the role of ApoE in AD have been reviewed extensively [19, 126, 127], including some suggestions that ApoE may be a direct pathogenic factor in AD independently from its effect on amyloid-related mechanisms [128], including neurotoxic effects of ApoE4 involving mitochondrial damage [129, 130].

Neuronal-glial interaction

Most of the research involving Aβ is focused on its interaction with neurons, since these are the cells that die in AD and after treatment with the peptide. However, the living brain is a complex structure where neurons depend heavily on surrounding glial cells for their survival. It is becoming increasingly clear that glial cells, notably astrocytes, far from having just a structural function, play pivotal roles in the metabolic support of neurons and in cell signaling. Walsh and collaborators [131] have found in retina that Aβ-induced dysfunction of glial cells caused secondary neuronal death in the ganglion cell layer.

We have found that in brain primary mixed cultures, Aβ selectively induced calcium oscillations surprisingly in astrocytes and not in neurons, following a delay of 10–15 min [132]. It has been shown previously that Aβ will insert into lipid bilayers and form calcium permeant channels [95, 133] and our data strongly suggested that the astrocytic calcium fluctuations were dependent on calcium influx from the extracellular space, probably through membrane channels formed by the peptide itself. In lipid bilayers, peptide aggregation and insertion depends strongly on the lipid composition of the membrane, in particular on the cholesterol content [95], raising the possibility that the lipid composition of neuronal and astrocytic membranes may differ and so determine the selective action of the peptide. At 24 h we observed cell death in neurons but not in astrocytes, although a decrease in glutathione content was observed in both cell types [132]. The functional importance of this response was underlined by the rescue of cells by glutathione precursors. Subsequent work showed that Aβ treatment also caused mitochondrial depolarisation but again only in astrocytes. This response was dependent on both the calcium response and on oxidative stress. We traced the source of oxidant species to the activation by Aβ of an NADPH oxidase expressed by astrocytes [134], which we described for the first time [135]. This would cause an oxidative stress in the astrocytes, followed by a secondary impact on neuronal viability, as neuronal antioxidant defences are maintained mainly by astrocytes [136]. Activation of this enzyme by Aβ has been observed in microglia [137], but has not been described before in astrocytes.

Aβ and NADPH oxidase

NADPH oxidase is primarily expressed in immune competent cells—neutrophils, monocytes and microglia—where it is responsible for the superoxide burst and bacterial killing. A range of isoforms have more recently been described in a wide range of cells and tissues. The microglial enzyme is activated by Aβ, as mentioned above [137], and expression is elevated in AD brains [138]. The enzyme consists of a membrane-bound cytochrome b_{558} (subunits p22phox and gp91phox), and cytosolic subunits (p40phox, p47phox, p67phox, Rac and Rap1A); the latter translocate to bind the membrane-bound subunits during activation.

We have characterized the properties of the astrocytic NADPH oxidase by Western blot analysis and by immunofluorescence which showed coexpression with the astrocyte-specific marker glial fibrillary acidic protein (GFAP), using antibodies against gp91 and p67, both in cultures and in vivo [135]. Recent studies [139, 140] have shown that the cytochrome b_{558} is localised in the cholesterol-rich lipid rafts, which are required for activation: cholesterol depletion reduces translocation of cytosolic subunits and superoxide generation. Statins also inhibit Rac1 independently from their effect on cholesterol [141]. Conversely, in mice lacking ApoE, which have higher levels of oxysterols, macrophages show increased p47 translocation and generation of reactive oxygen species [142].

It is also interesting to note that activation of sphingomyelinases and apoptosis by Aβ in neurons has been proposed to be mediated by activation of NADPH oxidase [143].

We believe that activation of NADPH oxidase may play a major role in the Aβ-induced neurodegeneration in Alzheimer's Disease, raising the possibility that modulating the activity of this enzyme may prove beneficial.

Conclusion

There are multiple strands of research pointing at an important role for cholesterol in the pathogenesis of Alzheimer's Disease. The picture is however far from clear, ad there is still disagreement in several areas, the

difficulties rising among other things, from the fact that AD is a complex multifactorial disease, and that cholesterol metabolism is subject to a complex regulation at many different levels and in different compartments, so that increases or decreases in cholesterol levels bring about re-adjustments of the system in way that may not be immediately evident. Certainly more research is needed in this rapidly evolving and promising field.

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