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Minireview

Amyloid excess in Alzheimer's disease: What is cholesterol to be blamed for?

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Abstract A link between alterations in cholesterol homeostasis and Alzheimer's disease (AD) is nowadays widely accepted. However, the molecular mechanism/s underlying such link remain unclear. Numerous experimental evidences support the view that changes in neuronal membrane cholesterol levels and/or subcellular distribution determine the aberrant accumulation of the amyloid peptide in the disease. Still, this view comes from rather contradictory data supporting the existence of either high or low brain cholesterol content. This is of particular concern considering that therapeutical strategies aimed to reduce cholesterol levels are already being tested in humans. Here, we review the molecular mechanisms proposed and discuss the perspectives they open.

Keywords: Cholesterol; Alzheimer's disease; Membrane rafts; Amyloid peptide

1. Introduction

What causes Alzheimer's disease (AD), the most common form of dementia affecting up to 15 million individuals worldwide? Although there is not yet an answer to this question the search for it has provided a wealth of information that bring us closer to the understanding of this neurodegenerative disorder. Hence, its hystopathological hallmarks are well characterized: amyloid plaques and neurofibrillary tangles composed by the extracellular and intracellular accumulation in the brain of the amyloid peptide (Aβ) and the hyperphosphorylated protein Tau, respectively. Even though it is unclear whether these hallmarks are a consequence or a cause their analysis has been crucial towards the comprehension of the pathology. Thus, Aβ derives from the processing of the transmembrane amyloid precursor protein (APP) by the β- and γ-secretases [1]. Aβ production is a physiological event, and therefore it is necessary for relevant cellular functions including modulation of synaptic activity and facilitation of neuronal growth and survival [2]. On the other hand, abnormal increase in Aβ levels, even before its accumulation in plaques, appears to be toxic in vitro and in vivo [3]. Consistent with this peptide playing a key pathological role in AD, mutations in either APP or its secretases are a well established cause for the familial forms of the disease [4].

Being APP cleavage a membrane event the involvement of lipids in alterations of such cleavage is conceivable. The first link between a lipid defect and AD came with the observation that the inheritage of the e4 allele of the apolipoprotein E, the main cholesterol transport protein in the brain, constituted a risk factor for the disease [5]. Indeed, individuals who are homozygous for this allele and live longer than 80 years will almost invariably develop AD. Lately, genetic studies of the risk of AD have reported association with polymorphisms in three other cholesterol related genes: cholesterol 24-hydroxylase (CYP46A1), ATP-binding cassette transporter A1 (ABCA1) and lipoprotein receptor-related protein (LRP) [6]. In addition, other evidences support an involvement of cholesterol in the production and/or degradation of the amyloid peptide. However, no consensus arises on how defects in cholesterol homeostasis relate to AD. The purpose of this article is to review the data aimed at this understanding.

2. Why the brain regulates tightly the levels of cholesterol?

Cholesterol is an essential component of the cellular membranes determining the fluidity and biophysical properties by lowering the permeability and increasing the compacity. The distribution of this lipid in the membrane is not uniform but it is enriched in microdomains, the so-called rafts [7]. Numerous experimental evidences suggest that by means of raft formation cholesterol contributes to the dynamic compartmentalization of molecules allowing the fine-tuned modulation of events such as signaling and proteolysis [8]. In addition, cholesterol serves as a precursor or cofactor of several signaling molecules [9]. As the main component of rafts cholesterol also contributes to the sorting of certain membrane molecules to the right destination in polarized cells such as epithelia and neurons [7,10,11] and to raft molecule endocytosis [11,12]. More specifically, cholesterol is crucial for the most distinctive feature of neurons: their ability to communicate. First, it is required for the formation of synapses [13]. Second, it is a major component of the myelin sheath essential for an efficient electrical transmission [14]. Third, it serves as impermeant barrier against sodium leakage [15]. From the above it comes that the brain must posses a most robust mechanism to maintain the levels of cholesterol, in the neurons

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and their supporting cells, as independent as possible from the variations that occur in the circulation. In fact, to date there is no evidence that blood cholesterol crosses the brain–blood barrier [16].

3. How are cholesterol levels regulated in the brain?

The metabolism of cholesterol depends on three main aspects: synthesis, transport and catabolism. Cholesterol biosynthesis is a lengthy and energy consuming process that requires more than 20 reactions and intermediates. Production of mevalonate by the HMG-CoA reductase is the main regulatory step of the pathway. Developing neurons synthesize most of the cholesterol they need but the production is reduced once they reach maturation. At this point, neuronal cholesterol content becomes also dependent on the cholesterol synthesized and secreted by glial cells [16]. The transport of cholesterol among CNS cells appears to rely on the Apolipoprotein E that is synthesized by astrocytes [17]. ApoE-cholesterol complexes are then taken up by neurons via endocytosis through the LDL receptor related protein (LRP1) [18]. Assuming that the following step is conserved with that of other cells, endocytosed cholesterol-containing lipoproteins are hydrolyzed in the neuronal lysosomes allowing the intracellular release of free cholesterol. This can be utilized in two ways. On one hand, it is esterified in the endoplasmic reticulum by the acyl-coenzyme A cholesterol acyltransferase (ACAT), and stored in cytoplasmic droplets as a reserve pool [19]. On the other hand, free cholesterol provides feedback to the pathways regulating transcription factors that control the expression of cholesterol synthesizing enzymes and lipoprotein receptors. Examples of these are the sterol regulatory element-binding protein [20] or liver X receptors (LXRs). The later have been shown to enhance the release of cholesterol from cultured astrocytes and neurons by increasing the expression of the ATP-binding cassette transporter A1 (ABCA1) [21], a member of the family of ABC transporters, which mediates the transport of cholesterol from cells to Apolipoproteins, closing the cellular cycle of the lipid.

The fact that the neuronal membranes must be kept with a fairly constant amount of cholesterol, so to guarantee proper function, implies the existence of a mechanism for cholesterol removal. Because there is no degradation mechanism for this lipid any excess must exit the brain into the circulation. Hydroxylation of the side chain of cholesterol by the cholesterol 24-hydroxylase allows the sterol molecule to cross the blood–brain barrier freely [15]. This mechanism is responsible for the catabolism of most of the cholesterol that is turned over in the brain.

4. Is hypercholesterolemia a risk factor for AD because of higher cholesterol reaching the brain?

The striking reduction (almost 70%) in the prevalence of AD found in hypercholesterolemic patients treated with the cholesterol lowering drugs statins [22,23] led to establish the correlations ''high circulating cholesterol-predisposition to AD'' and its counterpart ''cholesterol lowering drugs-reduced AD risk''. This was further supported by studies in animals showing that high dietary cholesterol results in increased levels of Aβ and that treatment with cholesterol synthesis inhibitors leads to reduced amounts of Aβ40 and Aβ42 [24,25]. Mechanistically speaking the human and animal data can be explained in two different ways. The first is that high circulating cholesterol reaches the brain triggering neuronal dysfunction. The second is that neuronal dysfunction is the consequence of the numerous collateral defects that arise because of high blood cholesterol, most notably perturbed circulation and thus tissue oxygenation, without changes in the brain levels of this lipid. To distinguish between these possibilities it would be necessary to address certain issues. Most relevant is to know whether or not hypercholesterolemic individuals have more cholesterol in their brains than age-matched control individuals. Intuitively, this is not likely as brain cholesterol levels are controlled locally independently from the oscillations in peripheral cholesterol (see above). In agreement, high cholesterol diets or statin treatments in the experimental animals failed to change brain cholesterol levels significantly [26]. Although it could be argued that hypercholesterolemic individuals have, as consequence of this defect, a perturbation of the normal cholesterol-impermeant function of the blood–brain barrier, this possibility is also unlikely, as a permeability defect would lead to the leakage into the brain of blood components, not only cholesterol, producing rather acute syndromes but not AD.

From the above data and considerations one would have to conclude that there is no ground to think that hypercholesterolemia predisposes to AD because of the passage of circulating cholesterol to the brain making neurons ''fatter''. If this is accepted it then comes that hypercholesterolemia may lead to AD through a secondary event, most likely the poor-oxygenation of the brain due to cholesterol-clogged blood vessels. This would be consistent with the observation that people who had suffered brain trauma are also more prone to develop the disease [27]. Utilizing the same rationale, statins would not prevent or delay (if at all the case) the occurrence of the disease because of inhibiting cholesterol synthesis in brain cells or because of reducing the amount of peripheral cholesterol reaching the brain, but because of the overall improvement of circulation. Statins could also be beneficial because of their anti-inflammatory actions [28]. Considering all the above, and in following with the title of this review, high circulating cholesterol is not to be blamed for AD through a direct effect on the brain. Certainly, this does not rule out that changes in the content or distribution of cholesterol in neurons, by mechanism/s other than high circulating levels, occur in AD and are responsible for high amyloid production.

5. How does the e4 allele of the apolipoprotein E predispose to AD?

The only established molecular event directly linking cholesterol metabolism and AD comes from the discovery that the e4 allele of the ApoE predisposes to the disease [5]. The view that ApoE would contribute to AD by affecting Aβ levels came from the findings that mice lacking ApoE present reduced deposition of Aβ [29] whereas the expression of apoE3 and ApoE4 in a mouse model for AD resulted in higher accumulation of fibrilar Aβ substantially more abundant in the later [30]. While the early work did not make any assumption that

the inheritance of ApoE4 predisposed to AD because of higher brain cholesterol, this was later thought to be the case because apoE4-bearing individuals have high circulating cholesterol and hypercholesterolemia increases the risk for AD [31]. The same question asked in regard to the hyper-cholesterolemic human/animal models, applies therefore to the ApoE4 genetic correlation: do ApoE4 bearing people have increased brain cholesterol levels? The same arguments against this possibility utilized for the nonApoE4 hypercholesterolemic individuals apply here as well. Moreover, biochemical data from the hippocampal membranes of a few ApoE4-bearing AD patients indicate not only that there is no such increase in cholesterol but, on the contrary, that there is a moderate decrease [32]. While this is surprising if one takes the view that high circulating cholesterol will result in high neuronal cholesterol, it is predictable if one considers that ApoE4-derived glial cells have low ability to release cholesterol [33]. As described earlier, mature neurons are dependent on the cholesterol provided by glial cells.

The existing data make unlikely that ApoE4 predisposes to AD because of higher neuronal cholesterol content due to hypercholesterolemia. While it is beyond the scope of this review, we will briefly summarize below data on how the presence of ApoE4 can predispose to the disease in a high circulating cholesterol- independent manner. One possibility would be through the ability of ApoE to bind with high affinity to Aβ [34], thus acting as a scavenger for the extracellular peptide [35]. This mechanism could be less efficient for the E4 allele. This role would rely on the binding of Aβ-ApoE to the ApoE receptor LRP facilitating Aβ endocytosis and degradation [36]. Alternative mechanisms for the involvement of ApoE in AD derive from its roles in brain repair [37], regulation of antioxidative processes [38] or increased intracellular signaling of calcium in neurons [39].

Future work will determine whether a different modulation of neuronal cholesterol levels and transport by the diverse ApoE isoforms could participate in AD pathology. In line with this possibility a number of recent evidences suggest that changes in neuronal cholesterol, whether in its total content, free or sterified, or in its subcellular distribution can be blamed for high amyloid production. These results are discussed next.

6. Is high neuronal cholesterol responsible for high amyloid production?

In an early work Simons and colleagues showed that reduction of cholesterol from the plasma membrane of cultured APP overexpressing rat hippocampal neurons, through the addition of a cholesterol-synthesis inhibitor (statin) and a cholesterol-extracting drug (cyclodextrin), resulted in the drastic reduction of amyloid production [40]. This work was expanded by that of Ehehalt and coworkers who pointed to the critical involvement of cholesterol within lipid rafts. Thus, these authors showed, utilizing antibodies against APP and its β-secretase BACE in overexpressing neuroblastoma cells, that the two proteins co-cluster in such domains and that cholesterol reduction, upon statin and cyclodextrin treatments, diminished the amount of Aβ [41]. Because Ehehalt and colleagues also found that Aβ production was dependent on the expression of endocytic molecules, such as dynamin and Rab5, it was proposed a model for APP processing in which the small size and the dispersion of rafts at the cell surface maintain APP and BACE segregated in different rafts. Upon endocytosis raft co-cluster and APP and BACE1 will encounter thus favoring β-cleavage. In such scenario APP would be cleaved by raft BACE 1. Following with these authors' model, anomalous high levels of cholesterol in the brains would enhance raft co-clustering leading to higher Aβ production. In agreement, the expression of a GPI form of BACE capable of directing this protein to rafts, results in increased Aβ production in an APP overexpressing neuroblastoma cell line [42]. The significance of these results, which derive from over-expression of APP and BACE in undifferentiated cells, became challenged by work in cultured primary hippocampal neurons expressing constitutive levels of these proteins [43]. In this study it was shown that BACE is in rafts but not APP. In these cells a moderate reduction of cholesterol results in the enhancement of APP-β-cleavage and Aβ production not in its inhibition. From all these data two opposite models arise. In one, neurons of AD patients have some sort of defect, genetic or environmental, that makes them possess high plasma membrane cholesterol and clustered rafts. This results in increased raft BACE-mediated APP cleavage leaving this molecule ready for the action of the γ-secretase for Aβ generation. In the other, AD arises as a consequence of the opposite event, loss of neuronal cholesterol from rafts because of unknown intrinsic or extrinsic causes. This leads to the "escape" of BACE from the raft domains to non-raft territories where APP resides, therefore increasing cleavage. Although more work is needed to clarify which model is closer to reality some recent data have provided important cues. Thus, the analysis of brain membrane cholesterol content in a number of AD patients and control individuals revealed that the former present a moderate, still significant, reduction of cholesterol in areas particularly loaded with amyloid plaques [32]. This resulted in the inefficient activation of the amyloid degrading enzyme plasmin, which is a raft dependent event, in such brains. Moreover, when similar level of cholesterol reduction was achieved in cultured hippocampal neurons the plasmin deficit was also observed [32]. In the same line, Abad-Rodriguez and colleagues showed that the hippocampal membranes of AD patients with reduced cholesterol content present disorganized rafts with enhanced BACE and APP colocalization in non-raft membrane domains. These results, which would support that low membrane cholesterol induces increased Aβ production in AD, are in agreement with the observation that seladin 1, the enzyme responsible for the last step of cholesterol synthesis, is specifically downregulated in affected areas of AD brains [44]. Moreover, mice lacking one allele of seladin 1, which show similar reduction of brain membrane cholesterol than that reported in the AD patients, present raft disorganization, low plasmin activity and increased APP-β-cleavage and Aβ levels [45].

7. Is it changes in the intracellular distribution of cholesterol, rather than its absolute levels, that affect Aβ production?

As described earlier cellular cholesterol is present either as free cholesterol in the membrane or stored as cholesteryl-esters in the form of cytoplasmic droplets. It is thought that cholesterol-esters in cytoplasmic droplets serve storage and synthesis-

activating purposes [19]. Thus, in cases when free membrane cholesterol becomes reduced, replenishment is achieved through droplet mobilization and activation of the synthesis machinery. The importance of cholesterol ester content in the control of Aβ levels was suggested by the study of Puglielli and coworkers, who showed that genetic and pharmacological suppression of acyl-coenzyme A cholesterol acyltransferase (ACAT), the enzyme that catalyzes the formation of esters from free cholesterol, results in lower amount of esters and decreased Aβ production [46]. Consistently, the selective increase

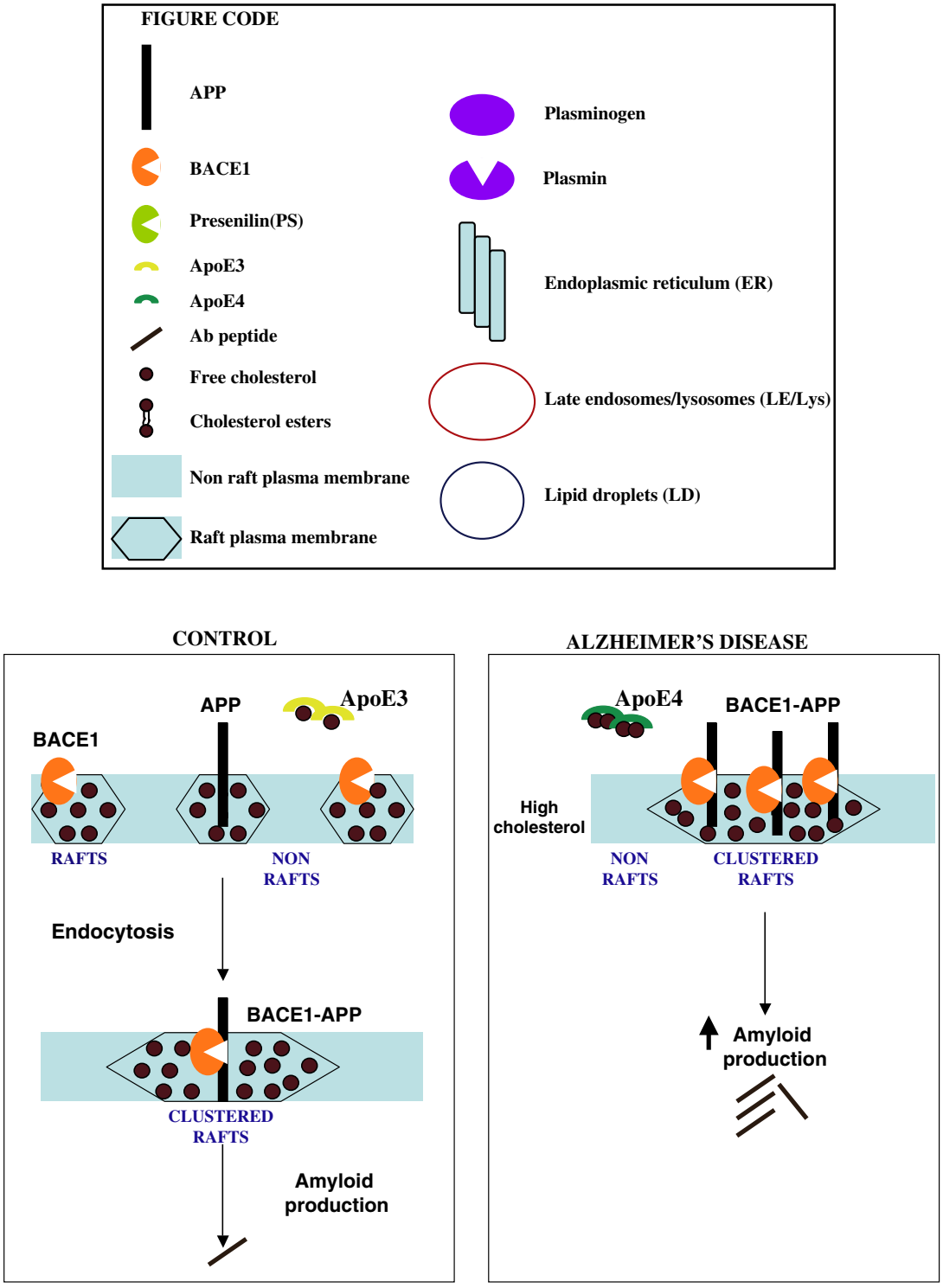

Fig. 1. High neuronal membrane cholesterol model for AD. In a control situation APP and its β-secretase BACE1 are located in different rafts at the plasma membrane of neurons. Endocytosis induces clustering of rafts favoring the interaction of these molecules, thus producing the physiological levels of Aβ. In contrast, the levels of cholesterol at the plasma membrane of neurons are aberrantly high in AD brains. This contributes to raft clustering and enhanced BACE1-APP interaction leading to increased Aβ production. ApoE4 could promote high levels of membrane cholesterol in neurons by increasing the circulating levels of this lipid compared to ApoE3.

in cholesteryl esters upregulated the generation of Aβ. Since the reduction in cholesterol esterification induced by ACAT inhibition leads to higher levels of free cholesterol in the membrane and since Aβ is generated in membranes that contain free cholesterol, it could be argued that cholesterol esters regulate Aβ production by modulating the amount of free membrane cholesterol. Work is needed to verify this prediction. Moreover, to give clinical relevance to these data it would be important to know whether or not neurons from AD patients have altered ACAT enzymatic activity, in gain or loss mode, or anomalous cholesterol ester amounts.

Another possible abnormality of brain cholesterol that could lead to AD is in situations of defective transport. In this regard, Runz and coworkers reported that drugs that arrest cholesterol trafficking in late endosomal/lysosomal compartments result in increased APP γ-cleavage activity and Aβ levels [47]. Since under such conditions presenilins accumulate in Rab7 positive vesicular compartments these authors proposed a model in which altered cholesterol distribution, increased in the late endocytic pathway, modifies secretase localization thus affecting Aβ production. Support for this model derives from the increased levels of Aβ and enhanced γ-secretase activity observed in mice lacking NPC1 [48], a transmembrane protein involved in the egress of unesterified cholesterol from late endosomes/lysosomes, which deficit cause the Niemann Pick disease type C (NPC). However, the fact that, different from AD, neurodegeneration in NPC occurs in early life makes unlikely that the same defect is to be blamed for both diseases. Alternatively, the extent of such defect would determine the onset and type of pathology.

Finally, changes in the asymmetric distribution of cholesterol in the exofacial and cytoplasmic leaflets of the membranes can be the cause of an abnormal "gain" in membrane cholesterol content, leading to increased Aβ production [26]. In this regard, ApoE4 knock-in mice show a twofold increase in exofacial leaflet cholesterol of synaptic plasma membranes compared with apoE3 knock-in mice and wild-type mice [49]. These results would be consistent with the view that high plasma membrane cholesterol in neurons could be a cause of AD but in contradiction with the work showing a reduced ability of ApoE4 glia to provide neurons with cholesterol.

8. Considerations about the conflictive results and many more open questions

The data on cholesterol and AD here summarized can be divided into two, well separated, aspects. The first aspect concerns how high blood cholesterol predisposes to AD. If it were not for the later appearance of data supporting the notion that high cholesterol in neurons increases amyloid production, the clinical correlation between hypercholesterolemic patients treated with statins and reduced risk to suffer the disease, would have been taken as an indication that high

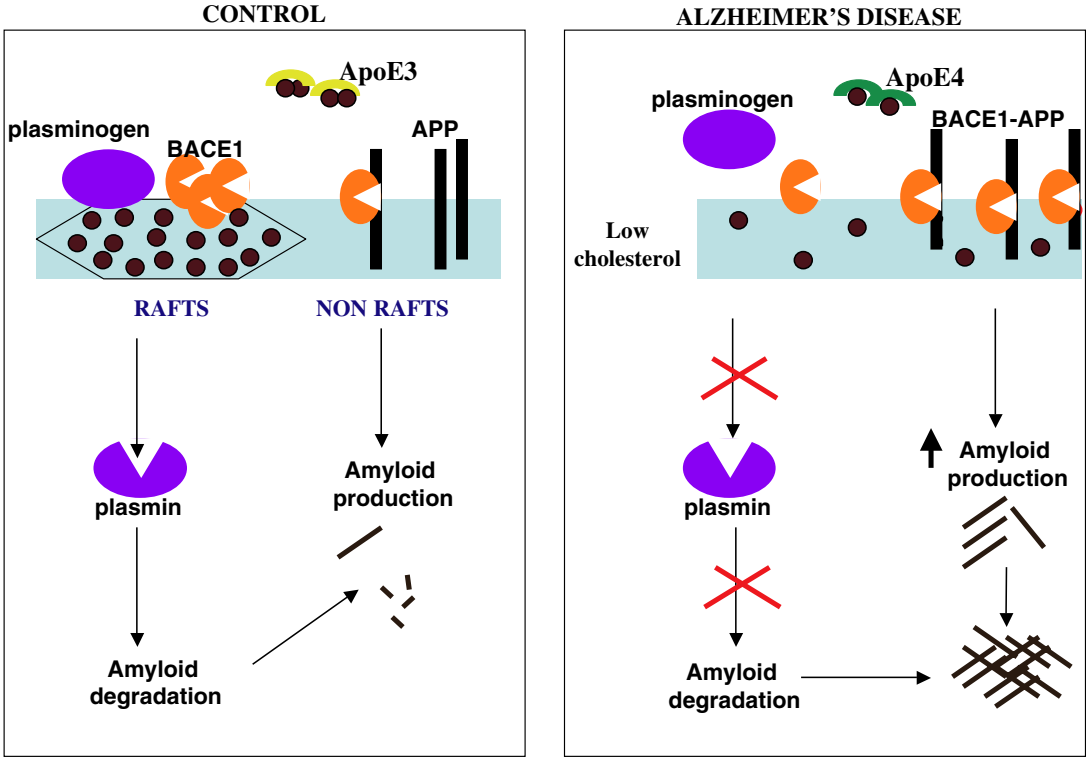

Fig. 2. Low neuronal membrane cholesterol model for AD. In a control situation the major pool of BACE1 is in rafts while its substrate APP is in non raft membranes of neurons. This segregation keeps the production of Aβ at physiological levels. In addition, plasminogen binding to the membrane and activation to the amyloid degrading enzyme plasmin occurs in rafts. This contributes to the degradation of the peptide and the maintenance of non deleterious steady state levels of Aβ. In contrast, raft domain disorganization occurs in AD brains due to low levels of membrane cholesterol. This favors the interaction of BACE1 and APP in non-raft membranes leading to increased amyloid production. Moreover, plasminogen binding to the membrane and activation to plasmin is impaired due to raft disorganization. This renders inefficient the clearance of Aβ contributing to its aberrant accumulation. The reduced ability of ApoE4 astrocytes to provide with cholesterol to neurons compared to ApoE3 astrocytes would contribute to the low amounts of membrane cholesterol in AD brains.

cholesterol in blood produces neurological defects secondarily from poor circulation. However, the in vitro data in pseudo-neuronal and neuronal cells led many researchers to think that high blood cholesterol individuals would have higher Aβ neuronal production because of increased brain levels of this lipid. This conclusion appears today weak. In fact, the literature is rich in demonstrating that peripheral cholesterol does not enter the CNS. Secondly, high brain cholesterol due to brain barrier leakage is as well unlikely as hypercholesterolemic patients do not present the symptoms of brain barrier permeability defects. Thus, a most reasonable explanation for why hypercholesterolemia predisposes to AD remains the indirect pathway, consequence of a deficient brain oxygenation secondary to vessel obstruction. This indirect mechanism would also explain why cholesterol synthesis inhibitors such as statins are a good strategy for the prevention of the brain side effects of high circulating cholesterol. Indeed, the potential beneficial effects of this type of drugs were seen for both brain–blood barrier permeable and non-permeable statins [26].

The second aspect concerns how cholesterol modulates Aβ production in neurons, irrespective of circulating cholesterol levels. This, for the time being, is an issue more interesting to the basic neurobiology community than to clinicians as most of the data derive from cell biological/biochemical approaches with very little correlation to the human scenario. These data consistently show however, that cholesterol does indeed play a role in Aβ production. This is of no surprise as Aβ generation is a membrane associated event and cholesterol a main membrane lipid. A prevalent view arising from these results, enthusiastically supported by the defenders of the hypercholesterolemia-AD predisposition, is that high neuronal cholesterol enhances Aβ production. A main drawback of this conclusion is that a large body, if not all, of the in vitro work supporting it (e.g. BACE-APP colocalization in rafts and diminished Aβ production upon cholesterol reduction) arises from cells overexpressing APP and/or its secretases. It is known that changes in protein distribution induced by over-expression occur in many cell types being especially evident in neurons, which have very tight axonal-dendritic sorting machinery. In fact, studies on APP membrane distribution in primary hippocampal neurons and human brains, under endogenous levels of expression, failed to find this protein within rafts. A second drawback of linking high cholesterol with high amyloid production is that the results supporting this view derive from cells with normal cholesterol levels induced to loose this lipid reaching concentrations far below physiological. The opposite type of approach aimed at increasing the levels of membrane cholesterol would have been more appropriate than its reduction. However, when such "gain of

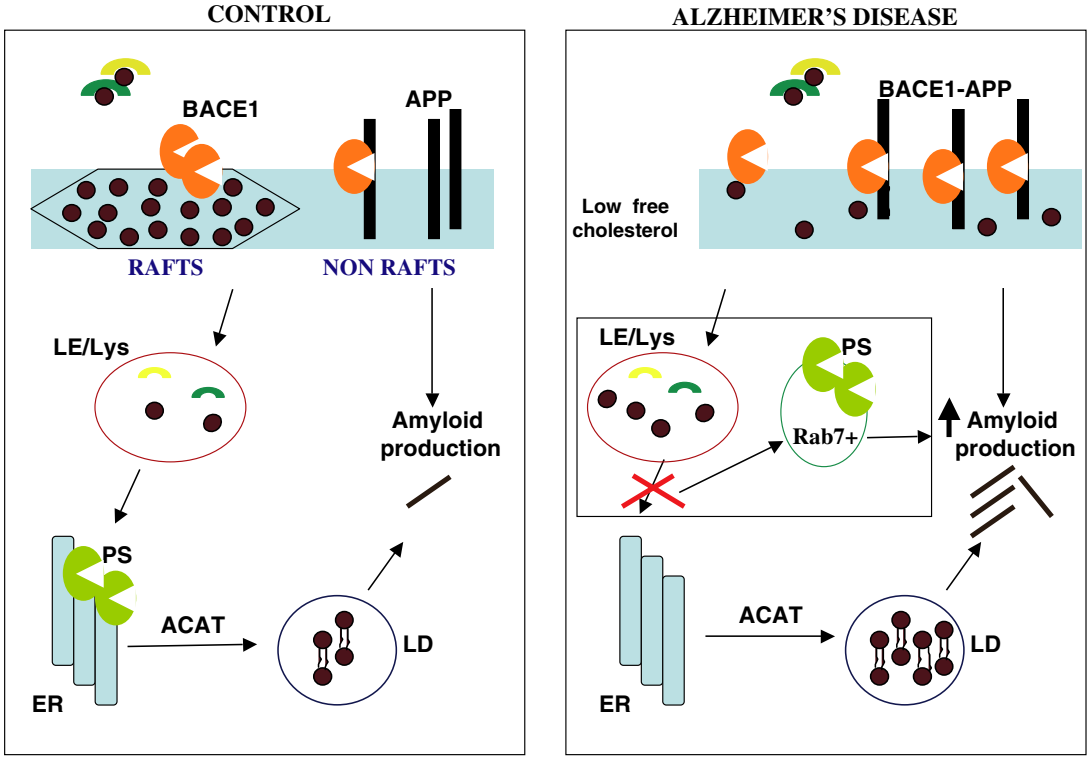

Fig. 3. Altered cholesterol transport and/or storage model for AD. In a control situation Apolipoprotein-cholesterol complexes are endocytosed and hydrolyzed in the late endosome/lysosome compartments of neurons. Free cholesterol traffics then to the endoplasmic reticulum where it can be recycled to form the cellular membranes or converted to cholesterol esters by ACAT to be stored in lipid droplets. Although the steady state distribution of APP secretases is not completely elucidated, it seems that the major pool of BACE1 resides at the plasma membrane while the major pool of presenilin is in the ER. This distribution of secretases and cholesterol results in the production of physiological levels of Aβ. In contrast, high levels of cholesterol esters correlate with increased Aβ production and low free cholesterol in the cellular membranes. A possible scenario in AD could be that low membrane cholesterol due to high cholesterol esterification impairs BACE1-APP segregation through raft disruption. This results in anomalous high Aβ levels. An alternative mechanism proposes the blockage of cholesterol transport from late endosome/lysosomal compartments (depicted in the black box). This blockage leads to the accumulation of cholesterol in these organelles and to the redistribution of the major pool of presenilin in Rab7 positive vesicles nearby late endosomes/lysosomes. The change in the intracellular localization of presenilin would lead to increased Aβ production.

function'' approach was taken the result was opposite to the expected one: decreased amyloid production [45]. In any event, even if controversial, the data linking cholesterol, Aβ levels and AD contributed to the idea that this disease may be, in its roots, the consequence of a basic membrane defect. In this view, an alteration in cholesterol levels, in more or less, would be sufficient to induce changes in membrane proteins' compartmentalization, affecting the normal segregation of APP and its secretases. Figs. 1 and 2 show how enhanced Aβ generation could be accomplished in situations of ''gain'' or ''loss'' of membrane cholesterol. On the other hand, the recent evidences that transport and/or storage defects of cholesterol can lead to changes in the subcellular compartments where APP and its secretases mainly reside, introduce new possible ways by which excessive amyloid production can occur (Fig. 3). In addition, these data open new perspectives of therapeutical targets for the cure or at least amelioration of the symptoms of the disease. In this regard, promising results have been recently obtained with an inhibitor of the cholesterol-ester forming enzyme ACAT, which markedly reduces amyloid pathology in a mouse model of AD [50].

Although most of the mechanisms proposed for the involvement of cholesterol point to a causative role in Aβ accumulation, recent work has demonstrated the ability of the peptide to regulate the synthesis of this lipid through the inhibition of HMGR [51]. Moreover, Aβ has the capacity to sequester cholesterol from the membrane [52]. These evidences raise the question on whether cholesterol alterations are a cause or a consequence in the disease. Indeed, the studies on cholesterol and AD bring about more questions than answers. For instance, if changes in the membrane fluidity are to be blamed for the link between cholesterol and alterations in Aβ generation/degradation, what role do other major lipids of the membrane play? In particular, and considering the postulated relevance of raft domains, could alterations in the raft-enriched sphingomyelin have the same effects? If rafts are altered, what are the consequences for other raft-mediated functions such as signaling or sorting of molecules? Defects in which of these produce the signs and symptoms of the disease? Given the relevance of cholesterol in synapse formation, could alterations in this lipid be responsible for the prominent synaptic loss in AD brains? And at the base of all these questions what is the cause for membrane cholesterol alterations in AD? Work on the above issues will certainly contribute to better understand the reasons behind this devastating disorder.

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