Apolipoprotein Aβ: Black Sheep in a Good Family

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Amyloid-β (Aβ) has for a long time been thought to play a central role in the pathogenesis of Alzheimer disease (AD). Analysis of available data indicates that Aβ possesses properties of a metal-binding apolipoprotein influencing lipid transport and metabolism. Protection of lipoproteins from oxidation by transition metals, synaptic activity and role in the acute phase response represent plausible physiological functions of Aβ. However, these important biochemical qualities which may critically influence the development of AD, have been largely ignored by mainstream AD researchers, making Aβ appear to be a "black sheep" in a "good apolipoprotein" family. New studies are needed to shed further light on the physiological role of Aβ in lipid metabolism in the brain.

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INTRODUCTION

Amyloid-β (Aβ) has for a long time been thought to play a central role in the pathogenesis of Alzheimer disease (AD). According to the amyloid cascade hypothesis which has dominated the field for longer than a decade, increased production of the Aβ peptide, especially of its longer and more amyloidogenic form $Aβ_{1-42}$, leads to the disease via formation of toxic amyloid plaques (66, 175, 176). The extracellular plaques, a pathological hallmark of AD, may subsequently cause formation of intracellular neurofibrillary tangles (NFT), another essential feature of the AD brain, and neuronal death. Experimental evidence in support of the primary role of Aβ in this temporal sequence has been primarily based upon links between familial forms of AD and mutations in the genes coding for amyloid-β precursor protein (APP), presenilin 1 and presenilin 2, all of which result in the elevation of either total Aβ or $Aβ_{1-42}$ in brain tissue (66, 175, 176).

However, an overwhelming majority of all AD cases (>90%) are not associated with such mutations and are classified as sporadic AD (184). The amyloid cascade hypothesis does not explain how amyloid plaques are formed in the absence of any genetically determined increase in Aβ production. In addition, toxicity of the plaques to neuronal cells has been frequently questioned (8, 114).

Most importantly, the hypothesis disregards the well-documented observation that sustained Aβ generation occurs in neurons, astrocytes, microglia, platelets and many other cells (64), suggesting that Aβ represents a hazardous by-product of APP metabolism (176). Such consideration of a ubiquitous peptide as an endogenous toxin led to the controversial idea that AD can be cured by using a vaccine raised against human Aβ (172). Despite numerous warnings (155), human trials were launched but interrupted shortly thereafter in Phase 2 after several patients developed severe brain inflammation (154); the interruption of the trials delivered a blow to the amyloid cascade hypothesis.

Potential limitations of the vaccine approach have been extensively discussed (162, 179, 182); it appears that a major conceptual drawback of these studies lies in their inability to recognize a physiologic role for Aβ. The peptide has been considered an enemy (51) that needs to be attacked (81). Many studies have addressed proteolytic mechanisms of Aβ cleavage from APP (45, 176); however, the physiological purpose of this highly sophisticated biochemical process (210) has often been overlooked. Meanwhile, physico-chemical properties of Aβ provide some intriguing insights into its preferential environment in living systems and its metabolic origin in senile plaques, whereas its biological activities suggest a plausible physiological role and alternative therapeutic implications.

PHYSICO-CHEMICAL PROPERTIES OF Aβ

Aβ, a peptide containing 39 to 43 amino acids (M_r about 4 kDa), is a major component of amyloid plaques (67). Aβ is produced in neuronal cells from APP under the action of β- and γ-secretases via intramembrane proteolysis (210). $Aβ_{1-40}$ is the predominant soluble species of Aβ in biological fluids, whereas $Aβ_{1-42}$ predominates in senile plaques and deposits associated with AD (108).

Lipid binding. Aβ possesses amphipathic properties which are related to its content of hydrophilic N-terminal and hydrophobic C-terminal fragments (Figure 1). The amphiphilic structure of Aβ results in low solubility in water and high propensity to aggregate in aqueous solutions (50). Aβ readily associates with lipids; as one of several known oblique-oriented peptides that have a hydrophobicity gradient, Aβ is partially inserted into lipids at its hydrophobic C-terminal tail (25). The N-terminal domain of $Aβ_{1-40}$ is exposed to the aqueous phase; it is unstructured between residues 1 and 14, forming a random coil with the Gly25 residue at the interface between the aqueous and lipid phases (37). At acidic pH, the rest of the peptide adopts an α-helical conformation between residues 15 and 36 with a kink at 25 to 27. At neutral pH, part of the helical region (residues 15-24) becomes less structured, unfolding to a random coil; deprotonation of 2 acidic residues Glu22 and Asp23 may account for this transition (37).

$Aβ_{1-42}$, a minor species of Aβ in biological fluids, is even more hydrophobic and less water-soluble as compared to $Aβ_{1-40}$, due to the presence of 2 additional hydrophobic residues of Ile41 and Ala42 at the C-terminus (50). $Aβ_{1-42}$ therefore aggregates and binds to lipids even more readily than $Aβ_{1-40}$; the large hydrophobic C-terminal domain of the peptide facilitates a strong binding. The structure of $Aβ_{1-42}$ at the water-lipid interface appears to be similar to that of $Aβ_{1-40}$, with 2 α-helical regions separated by a kink (40).

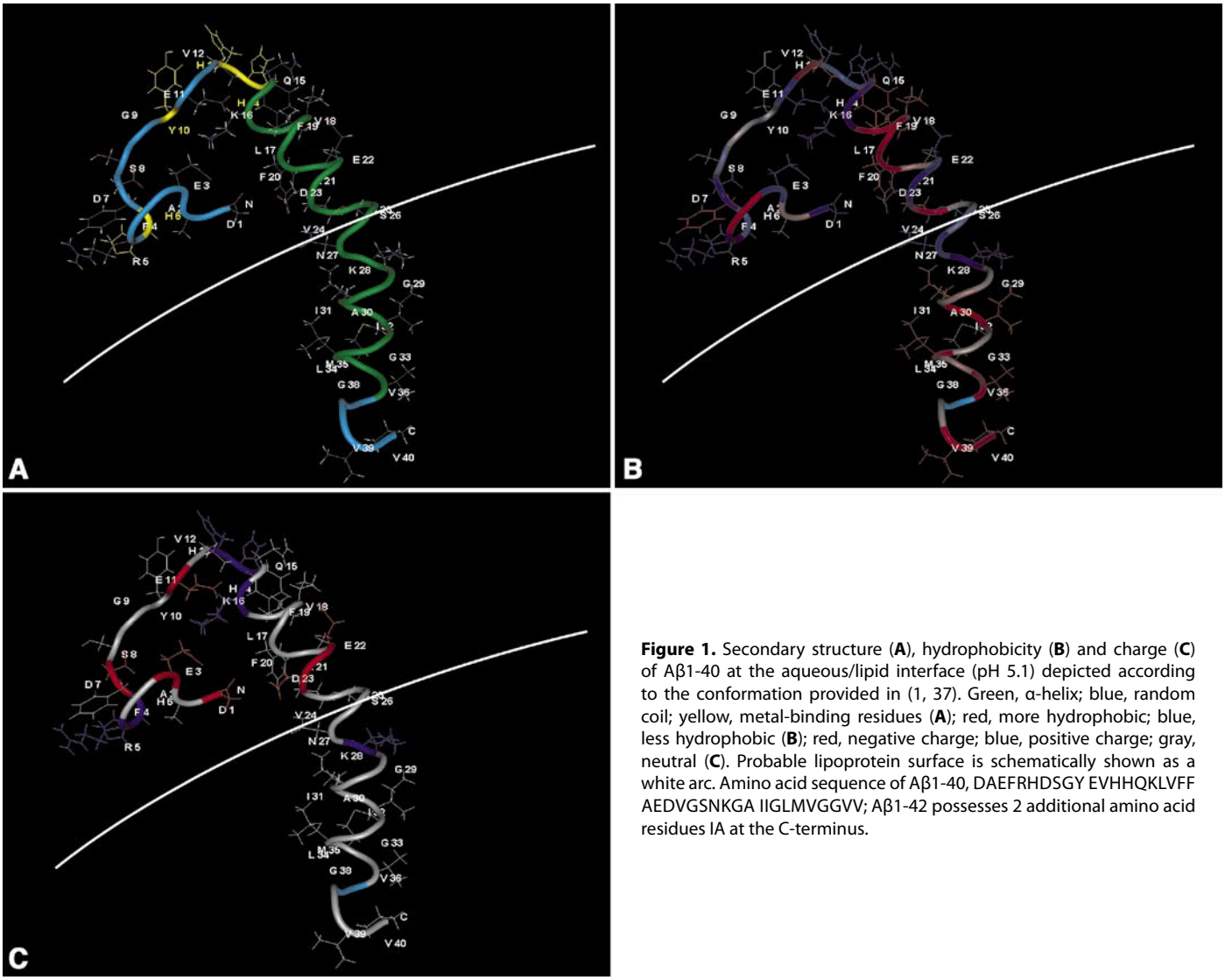

Figure 1. Secondary structure (**A**), hydrophobicity (**B**) and charge (**C**) of Aβ1-40 at the aqueous/lipid interface (pH 5.1) depicted according to the conformation provided in (1, 37). Green, α-helix; blue, random coil; yellow, metal-binding residues (**A**); red, more hydrophobic; blue, less hydrophobic (**B**); red, negative charge; blue, positive charge; gray, neutral (**C**). Probable lipoprotein surface is schematically shown as a white arc. Amino acid sequence of Aβ1-40, DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV; Aβ1-42 possesses 2 additional amino acid residues IA at the C-terminus.

Metal binding. In addition to its lipid-binding properties, Aβ possesses prominent metal-binding activity. Human Aβ is an exceptionally strong chelator for transition metal ions, particularly for copper (11). $Aβ_{1-42}$ has higher affinity to Cu(II) than $Aβ_{1-40}$ (apparent stability constants of Aβ-copper complexes, 2.0×10^{17} and 1.6×10^{10} M^{-1}, respectively [11]), which is comparable to the affinity of the best metal chelators known, such as ethyleneaminetetraacetic acids. Compared to copper, iron is a less suitable ligand for Aβ. The peptide appears to possess 2 binding sites for copper located between residues 6 to 14 in the hydrophilic N-terminal part, which differ in their affinity. The metal-binding sites consist of three histidine (His6, His13 and His14) and one tyrosine (Tyr10) residues (Figure 1); both histidine and tyrosine in free form are able to efficiently chelate transition metal ions (125). Copper presumably binds to nitrogen atoms of all three His residues of Aβ (42, 139) as well as to amide groups at the N-terminus (11).

Aggregability. Aβ is highly prone to aggregation; in the brains of AD patients, Aβ is found as extracellular deposits of β-sheet fibrils in the neuropil (senile plaques) and within cerebral blood vessels (amyloid congophilic angiopathy). Aβ may undergo a conformational transition from a soluble monomeric form to aggregated, fibrillary β-sheet structures. In vitro, Aβ exists as monomers, dimers, and higher oligomers; further aggregation yields protofibrils and then fully-fledged fibrils that resemble those found in amyloid plaques in AD brain tissue (204).

It has been shown that "spontaneous" Aβ aggregation to fibrils in vitro is caused by traces of transition metals present in laboratory buffers (141). Aβ is readily aggregated by transition metal ions, such as Cu(II), Fe(III), Zn(II) and Al(III) (7). In contrast, in the absence of metals, Aβ is monomeric, has α-helix conformation and does not form aggregates (119). Compareed to $Aβ_{1-40}$, $Aβ_{1-42}$ is more prone to aggregation, probably due to its higher metal affinity (7).

Molecular mechanisms implicated in Aβ aggregation by zinc or copper include formation of intermolecular crosslinks between β-sheets of Aβ by the atoms of metals. The crosslinks are formed between nitrogen atoms of His residues in Aβ (42, 139); His13 seems to be essential for Aβ aggregation (119). $Aβ_{1-40}$ aggregates possess about 3 to 4 metal atoms per molecule of Aβ (7). Incubation with Cu(II) causes extensive oxidation of Aβ (6); as a result,

Aβ molecules become cross-linked through tyrosine residues with formation of dityrosine (9).

Redox activity. Finally, Aβ displays significant redox activity associated with the Met35 residue in the lipophilic C-terminal domain (31). The sulfur atom of the methionine is redox-active, thereby accounting for the ability of Aβ to reduce transition metal ions (72). The redox activity of Met35 has been proposed to be related to its physical proximity to Ile31, whose oxygen atom may destabilize the electron system of the sulfur atom of Met35, facilitating electron transfer to transition metals (31). $A\beta_{42}$ is a more effective reductant than $A\beta_{40}$ (72), which can be related to its higher efficiency as a metal chelator (11). The efficiency of metal reduction by Aβ can therefore be influenced by the efficiency of metal binding to the peptide; consistent with this suggestion, Aβ reduces Cu(II) more efficiently than Fe(III). In comparison with chelation (which occurs instantly), reduction of transition metals by Aβ is slow (its rate constant can be estimated at about 10^1 $M^{-1}s^{-1}$ [72]) and is only efficient at high (micromolar) concentrations of Aβ.

Thus, the physico-chemical properties of Aβ suggest that the monomeric, non-aggregated peptide should be found in association with lipids in vivo, eg, with lipoproteins and/or cell membranes. Furthermore, Aβ can be expected to bind to lipids in such a way that the hydrophilic part of the lipid-anchored peptide is able to fulfill its metal-binding function in the aqueous phase, whereas Met35 could participate in redox reactions in the lipid environment. Indeed, monomeric, non-aggregated Aβ has been found in biological fluids in association with lipoprotein particles; in the brain, such particles can be termed "brain lipoproteins."

BRAIN LIPOPROTEINS

Brain is a site of high lipid turnover; neurons rely heavily on lipid supply which is essential for membrane synthesis and acetylcholine production. Even though the central nervous system (CNS) accounts for only 2.1% of body weight, it contains 23% of the cholesterol present in the whole body pool (49). Although neuronal cells are capable of de novo synthesis of lipid molecules, they can equally bind and internalize lipoproteins present in the extracellular fluid (15); lipid transport mediated by lipoproteins is thought to be of key importance for the proper functioning of the CNS. Equally, neurons need to dispose of excess lipids; lipoprotein-mediated lipid transport is therefore bidirectional and includes efflux from neuronal cells.

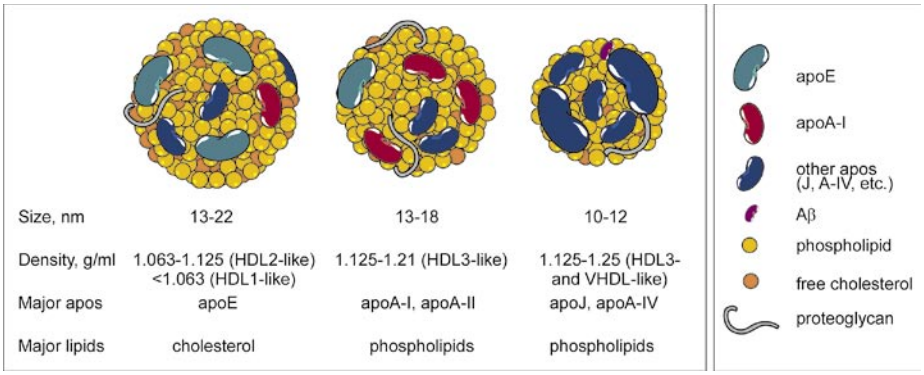

Figure 2. Major subclasses of CSF lipoproteins (from left to right): large, light particles enriched in apoE and cholesterol; smaller, denser particles enriched in apoA-I and phospholipids; small, dense, lipid-poor particles enriched in apoJ and apoA-IV.

CSF lipoproteins. Lipoproteins are macromolecular complexes consisting of lipids and amphiphatic proteins termed apolipoproteins. Plasma lipoproteins have been most extensively studied due to their central role in the development of atherosclerosis and cardiovascular disease (126); lipoproteins present in the CNS interstitium have not however been characterized so far. On the other hand, lipoprotein particles whose properties are similar to those of high and very high density lipoproteins (HDL and VHDL) in human plasma, are present in the cerebrospinal fluid (CSF) (24, 48, 88, 157). It appears reasonable to assume that the properties of lipoproteins from the extracellular fluid of the brain resemble those of lipoproteins from CSF, due to the existence of a passage between the 2 compartments.

Human CSF primarily contains spherical lipoproteins of approximately 10 to 22 nm in diameter with a density of 1.063 to 1.25 g/ml, which corresponds to the density of HDL and VHDL of human plasma (24, 48, 88, 157) (Figure 2); plasma HDL and VHDL (5-17 nm) are however smaller than CSF lipoproteins (5, 12). Both plasma HDL and CSF lipoproteins consist of a small non-polar core containing mainly cholesteryl esters which is surrounded by a monolayer of phospholipids and free cholesterol; apolipoproteins are anchored through insertion of their hydrophobic domains in the surface monolayer. Thus, CSF lipoproteins resemble plasma HDL in structure and density, yet are distinct from their counterparts in blood. Lipoprotein concentrations in CSF are much lower as compared to those in the plasma compartment; for example, levels of total cholesterol and phospholipids differ by 300- to 400-fold between CSF and plasma (74, 88). The low in vivo levels of CSF lipoproteins result in substantial difficulties in their isolation and separation into subclasses (48, 88). Apolipoproteins E and A-I (apoE and apoA-I) are the major apolipoproteins in CSF; apoE/apoA-I ratio in human CSF is about 1:2 (48, 88, 186). In addition, CSF contains apolipoproteins A-II, A-IV, J, D, C-II, C-III and H; major lipids are phospholipids, free cholesterol and cholesteryl esters (88, 157, 163).

Subclasses of CSF lipoproteins. In a similar manner to their plasma counterparts, CSF lipoproteins are highly heterogeneous, differing in size, density and chemical composition (88) (Figure 2). Different separation methods, including 2-dimensional gel electrophoresis, gel filtration chromatography, immunoaffinity chromatography and density gradient ultracentrifugation, allows isolation of up to 4 distinct lipoprotein classes (24, 48, 88, 104). Although the exact spectrum of lipoprotein subclasses strongly depends on the separation method, a certain level of consistency exists over different experimental approaches. The major lipoprotein subfraction in human CSF appears to consist of relatively large (13-22 nm) and light (HDL2-like, d 1.063-1.12 g/ml, and HDL1-like, d 1.006-1.063 g/ml) particles enriched in apoE; these lipoproteins carry

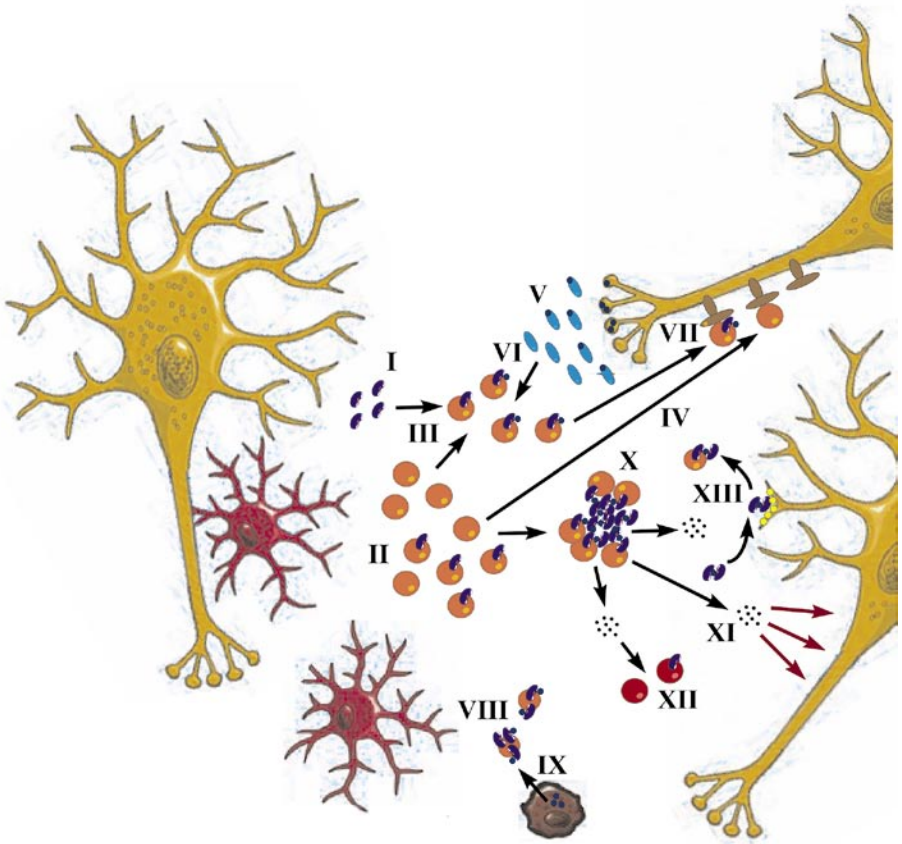

Figure 3. Physiological functions and dysfunction of apolipoprotein Aβ in the brain. Aβ (shown in violet) is secreted in the extracellular fluid either in a lipoprotein-free form by neurons (I) or in association with lipoproteins (orange) by astrocytes (II); lipoprotein-free Aβ subsequently bind to lipoproteins (III). Lipoproteins serve to deliver lipids to neurons via apoE receptors (brown) (IV). Synapses release in the synaptic gap transition metal ions (copper, zinc; dark blue) bound to metal chaperons (blue) (V); lipoprotein-associated Aβ chelates transition metals to protect lipoproteins from oxidation (VI) and to recycle metals back to axons (VII). Under stress conditions, neuronal cells increase secretion of Aβ, elevating its levels in lipoproteins (VIII); secreted Aβ might function as a chelator for metals released from dying cells (IX). Elevated Aβ levels in the extracellular fluid may result in the dissociation of the peptide from lipoproteins and in its excessive aggregation by transition metals, leading to the formation of amyloid aggregates (fibrils, plaques) (X). Some Aβ aggregates are able to generate reactive oxygen species (black), which are toxic to neurons (XI), and to oxidize biomolecules, including lipoproteins (XII), which become dysfunctional (red). Oligomers of Aβ can equally induce removal of cholesterol (yellow) from neurons, causing degeneration (XIII).

a majority of CSF cholesterol. By contrast, most of the phospholipids are present in smaller (13-18 nm) and denser (HDL3-like, d 1.12-1.21 g/ml) particles which are enriched in apoA-I and apoA-II. In addition, CSF contains low levels of small (10-12 nm), dense (HDL3- and VHDL-like, d 1.125-1.25 g/ml), lipid-poor lipoproteins enriched in apoJ and apoA-IV (24, 47, 53, 88, 98, 104, 194). Thus, there is a clear trend for apoE to associate with large, cholesterol-rich particles, whereas apoA-I tends to be present in smaller, phospholipid-rich lipoproteins.

Metabolism and functions. Metabolism of CSF lipoproteins remains poorly characterized; available data indicate that it is distinct from that of plasma lipoproteins. Large, light, apoE-rich lipoproteins are synthesized locally in CNS and secreted by astrocytes as discoidal complexes enriched in free cholesterol (53, 76, 109); other glial cells, including microglia, are equally able to produce apoE (15). Neurons require a continuous supply of cholesterol for normal functioning; this task appears to be accomplished by astrocytes through secretion of apoE- and cholesterol-rich particles (156). High apoE content probably targets these lipoproteins towards specific apoE receptors, particularly the low density lipoprotein (LDL) receptor-related protein (LRP), which are abundantly expressed on the surface of neurons (15). Indeed, CSF-derived, apoE-containing lipoproteins are able to deliver cholesterol to neurons through direct interaction with lipoprotein receptors on the cellular surface in vitro (52, 161). ApoE-containing lipoproteins isolated from CSF mainly contain esterified cholesterol (109); free cholesterol may therefore be esterified in nascent brain lipoproteins by lecithin-cholesterol acyltransferase (LCAT) (80) whose presence in CSF has been recently demonstrated in association with small particles (48); apoA-IV located to the same lipoproteins may act as an activator for this reaction. Alternatively, nascent lipoproteins may acquire cholesteryl esters upon interaction with neuronal cells before reaching the CSF (109). Additional remodelling of CSF lipoproteins may be mediated by phospholipid transfer protein (PLTP) (48, 202) which is involved in the interconversion of HDL subclasses in plasma (198). By contrast, cholesteryl ester transfer protein (CETP), another important determinant of the composition and levels of plasma HDL (111), is absent from CSF (48), consistent with the virtual absence of triglycerides from this body compartment (88).

Small, apoA-I-rich CSF lipoproteins are most probably derived from a subclass of small, dense plasma HDL that enters CNS by crossing the blood-brain barrier (88). Indeed, apoA-I is a major protein component of plasma HDL (56) which is not synthesized in the CNS (15); in subjects with an intact blood-brain barrier, the percentage of apoA-I levels in CSF relative to plasma (0.26%) is similar to the percentage of albumin (0.50%) but much lower than the percentage of apoE (4.4%), thus underscoring the local synthesis of the latter (88). Reverse cholesterol transport from peripheral cells to the liver is a major function of plasma HDL; consistent with this pathway, HDL-like CSF lipoproteins are able to induce efflux of cholesterol from neuronal cells (48), an effect which may be specifically mediated by apoA-I-containing particles (76). Scavenger receptor type B-I (SR-BI) (190) and ATP-binding cassette transporter subfamily A1 (ABCA1) (203) may represent major players participating in this pathway; these well-characterized HDL-binding proteins are expressed in brain tissues.

Thus, removal of cholesterol and other molecules (eg, Aβ) from neuronal cells and transport to the liver across the blood-brain barrier might constitute a major function of apoA-I-rich CSF lipoproteins. Cholesterol may be removed from neurons by apoA-I-containing particles both in unmodified and hydroxylated form (as 24S-hydroxycholesterol) (21); direct evidence supporting the existence of this pathway is however lacking. By contrast, apoE-containing particles may be involved in the reverse transport of cholesterol and Aβ from the brain to the liver which is mediated by LRP (199). In plasma, both excessive cholesterol and excessive Aβ from the brain could be transported to the liver on HDL; consistent with this mechanism of reverse Aβ-cholesterol transport, increased levels of plasma HDL seem to protect against the development of AD (136).

The function of small, dense, lipid-poor, apoJ-rich lipoproteins is unknown; there is some evidence that these particles may play a role in the acute phase response (see below). Intriguingly, small, dense, HDL-like lipoproteins appear to carry monomeric Aβ in brain extracellular fluid, suggesting that Aβ is truly an apolipoprotein.

Aβ AS AN APOLIPOPROTEIN

Normally, monomeric Aβ is present in biological fluids and tissues and is constitutively produced by a variety of cell types (64). Neuronal cells are the major source of Aβ in the brain (27, 64). Neurons appear to secrete more Aβ as compared to astrocytes and glial cells (59, 113); other cell types, including platelets and hepatocytes, are equally known to constitutively produce Aβ (35, 64, 100).

In biological fluids, monomeric Aβ is carried by lipoproteins. Although the size and density of Aβ-carrying lipoproteins may vary, they are typically within the range of size (5-17 nm) and density (1.063–1.21 g/ml) (5, 12) of plasma HDL. In human CSF, Aβ is associated with spherical, HDL-like lipoproteins of approximately 17-nm diameter and 200-kDa molecular mass (104). The main Aβ species associated with CSF-HDL is $A\beta_{1\text{-}40}$, consistent with the observation that this species is the main form of Aβ in human CSF (131). In AD brains, soluble Aβ is monomeric and co-elutes upon separation by FPLC with lipoprotein particles of >200 kDa molecular mass (132). Similarly, monomeric Aβ co-isolates with lipoprotein particles from human plasma, in particular with the HDL and VHDL fractions (97). Finally, under in vitro conditions of cell culture, Aβ is detected in the culture medium as a part of 200-300 kDa lipoprotein complexes (100). Human hepatoma HepG2 cells secrete Aβ in the culture media in association with apoA-I, apoJ, phospholipids, triglycerides and free and esterified cholesterol.

It is unknown whether Aβ is produced as a part of lipoprotein complexes, or whether it associates with lipoproteins in the extracellular space after secretion. Astrocytes are able to secrete both apoE-containing lipoproteins and Aβ (53, 59, 76, 109, 113); Aβ synthesized by astrocytes is probably secreted within a lipoprotein complex (Figure 3). Aβ production in neuronal cells is known to parallel synthesis of cholesterol. Lipid-lowering drugs (statins) decrease both neuronal cholesterol content and Aβ production (211), whereas diet-induced hypercholesterolemia acts in the opposite fashion (178, 187). These observations support a mechanism of coordinated production of Aβ and cholesterol for subsequent secretion as a lipoprotein complex. By contrast, distal axons of sympathetic neurons are incapable of cholesterol production and lipoprotein secretion (156); neuron-produced Aβ may then associate with lipoproteins secreted by astrocytes in the extracellular space (Figure 3).

Aβ is a rather minor component of brain lipoproteins; on average, only one of 100 lipoprotein particles carries a molecule of the peptide (90). However, the importance of Aβ as a component of brain lipoproteins increases if one takes into account that the peptide is specifically associated with a subclass, rather than with all CSF lipoprotein particles. In CSF, most Aβ is found in small HDL-like particles, whose density corresponds to that of plasma HDL3 and VHDL (98, 104); these particles are enriched in apoJ and contain apoA-I (98, 104, 194). In plasma HDL, Aβ is equally complexed to apoJ and, to a lesser extent, to apoA-I (97). Furthermore, Aβ co-isolates with apoJ also from human CSF (62), implying that the peptide is preferentially associated with small, dense, apoJ-carrying HDL-like particles (Figure 2) in CSF and plasma.

Thus, small HDL-like particles represent a major carrier of monomeric Aβ in vivo. This important conclusion emphasizes the importance of methodological approaches dealing with lipidated or lipoprotein-associated Aβ (92, 110, 133); in addition, it casts doubts on the physiological relevance of frequent attempts to study in vitro biochemical actions of Aβ directly added to the reaction mixture in the absence of a lipoprotein carrier (192). Indeed, differential biological activities of free- and lipid-associated species have been widely documented in plasma apolipoproteins (56). The obligatory presence of lipids in experiments involving apoE has been proposed (175); this requirement needs to be widened to include Aβ.

It is noteworthy that no Aβ reactivity is detected in lipoprotein-free fractions upon ultracentrifugal fractionation of CSF or plasma obtained from control subjects, and of cell culture medium (97, 100, 104). Since ultracentrifugation is well-known to cause a dissociation of weakly bound apolipoproteins from the lipoprotein surface (106), Aβ appears to be tightly bound to lipoproteins in biological fluids.

The amphiphilic structure of Aβ may form the basis for its association with lipoproteins; Aβ is probably anchored to the lipoprotein surface via its hydrophobic C-terminal domain (Figure 1). However, the association of Aβ with lipoproteins is stronger than can be expected for purely hydrophobic interactions with lipids, suggesting that the peptide may equally bind to apolipoproteins—to apoE (110, 127) and/or apoJ (62, 133)—strengthening the association with lipoprotein particles. Importantly, Aβ binding to lipoproteins may play a key role in maintaining the peptide in solution. In vitro, HDL phospholipids (103), native HDL (151), reconstituted protein-free HDL particles (99) and apoE-containing liposomes (16) all efficiently bind Aβ and inhibit its aggregation. Interestingly, plasma lipoproteins have been reported to carry high amounts of aggregated Aβ covalently bound to lipoproteins (107); the biological relevance of this finding remains to be determined.

Together, these data leave little doubt that Aβ is a normal and physiological protein component of lipoproteins. Webster's Dictionary defines apolipoproteins as "protein components of lipoproteins which re-

main after the lipids to which the proteins are bound have been removed... they play an important role in lipid transport and metabolism" (2). Aβ clearly fulfils the first part of this definition and can therefore be regarded as a candidate to enter the apolipoprotein family (100). Aβ can be considered as a small metal-binding apolipoprotein; the molecular mass of Aβ is close to that of apoC-I (about 6 kDa), the smallest known apolipoprotein. As to the second part of the apolipoprotein definition, available data show that Aβ performs important functions in lipid transport and metabolism.

FUNCTIONS OF APOLIPOPROTEIN Aβ

Plausible physiological functions of Aβ are closely related to its role as an apolipoprotein, as well as to its physico-chemical properties.

Protection from oxidation by transition metals. The potent metal-binding capacity of Aβ together with its location in lipoproteins implies a strong influence on lipoprotein oxidation. Lipids carried by CSF lipoproteins (polyunsaturated fatty acid moieties of phospholipids; free cholesterol) are easily oxidisable; similar to plasma lipoproteins (191), CSF lipoproteins can therefore be oxidatively modified in vitro (4). Oxidation of CSF lipoproteins can have similar pathophysiological consequences as oxidation of their plasma counterparts, which substantially perturbs normal lipoprotein metabolism (36). Most importantly, lipid delivery to neurons can be impaired as a consequence of altered recognition of oxidized lipoproteins by lipoprotein receptors at the neuronal surface, resulting in insufficient lipid supply which could have potentially deleterious effects on cell viability. In addition, plasma lipoproteins accumulate high amounts of pro-inflammatory, pro-apoptotic lipids upon oxidation (167). Oxidized lipids exert a plethora of toxic effects towards neuronal cells (86); it has been accordingly demonstrated that oxidized CSF lipoproteins are neurotoxic by disrupting neuronal microtubule organization in neuronal cell culture (13). Interestingly, CSF lipoproteins of AD patients are more sensitive to in vitro oxidation than those of controls (13, 173, 174), indicating insufficient antioxidant protection which can however be normalized by antioxidant supplementation in vivo (95).

The brain is a specialized organ that concentrates transition metals (28). Transition metal ions (Cu(II), Fe(III)) in a redox-active free form are potent catalysts of adverse oxidation of biomolecules; therefore, efficient mechanisms must exist in the brain to prevent abnormal distribution of metals. Normally, transition metal ions are tightly bound in a redox-inactive state to their transport or storage proteins. Under some conditions, transition metals may be pathologically released and/or reduced to their highly active low-valency form, which makes them potent oxidants. For example, human ceruloplasmin, which contains 6 to 7 copper atoms per molecule of protein, can efficiently promote oxidation in the presence of superoxide-generating systems that reduce ceruloplasmin copper (55). Brain homeostasis of transition metals is heavily impaired in AD (28), suggesting that metal-catalyzed oxidation is particularly important for the development of this disorder.

Transition metals accumulate in both types of AD lesions, amyloid plaques and NFT (123). Significant increases in iron and zinc content are observed in multiple regions of AD brain as compared to controls (38), and correlate with the severity of histopathologic alterations (46). Accordingly, copper, iron and zinc are present at increased levels in CSF of AD patients (14, 69). Increased secretion of ceruloplasmin under inflammatory conditions (20), transition metals released into extracellular space from synapses upon depolarization (83) as well as iron produced from mitochondrial heme upon its cellular degradation (183) may serve as potential sources for excessive metals in AD brains.

Brain homeostasis of iron is disrupted in AD. Both amyloid plaques and NFT contain redox-active iron, which is not bound to normal iron-binding proteins and can catalyze oxidation in situ (181). Brain homeostasis of copper is also severely affected. Ceruloplasmin is increased by more than 60% in all regions in AD brain compared to elderly controls (121); in addition, ceruloplasmin is increased in CSF (120). Moreover, metallothionein III, a strong chelator for copper and zinc, is reduced in AD cortex (214).

In parallel, oxidative stress is highly elevated in AD brains. Brain tissues of AD patients reveal elevated levels of products of lipid peroxidation including thiobarbituric acid-reactive substances (122), 4-hydroxy-2-nonenal (130), acrolein (124) and F2-isoprostanes (159). Interestingly, increase in F2-isoprostanes is highest in the temporal and frontal cortex, brain regions which are particularly affected in AD. Furthermore, increased accumulation of oxidation products is found in brain proteins and DNA (30, 185).

Consistent with these findings, antioxidative vitamins C and E as well as easily oxidizable polyunsaturated fatty acids are decreased in CSF of AD patients (173, 174), whereas CSF F2-isoprostanes are increased (142, 159). Elevated levels of oxidative damage in AD are not restricted to the brain and CSF compartments but to some extent are also observed as systemic oxidative stress in plasma (173, 174) and urine (197), additionally documenting elevated oxidative stress.

Together, these data strongly argue for a key role of transition metals in elevated oxidative stress in AD; protection against such oxidative stress appears to be of key importance. In lipoproteins, the metal-binding region of Aβ extends into the aqueous phase, where it can bind transition metals; chelation of transition metals in a redox-inactive form at the metal-binding site of Aβ may therefore serve to inhibit metal-catalyzed oxidation and to decrease oxidative damage.

Consistent with this hypothesis, exogenously added Aβ efficiently inhibits metal-catalyzed oxidation of lipoproteins from human CSF and plasma (90, 92). Importantly, the antioxidant effect is observed at the peptide concentration measured in these biological fluids (0.1-1.0 nM), when Aβ is known to be monomeric; at higher concentrations, the antioxidant action is abolished. In addition, aging of Aβ solutions (which results in Aβ aggregation), abrogates its antioxidative activity, suggesting that the peptide is only active in the monomeric form, which possesses vacant binding sites for transition metals.

Both $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ are efficient antioxidants, whereas the $A\beta_{25\text{-}35}$ fragment is much less effective and $A\beta_{40\text{-}1}$ does not possess antioxidative activity (90, 92). Similarly, $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}28}$—but not

$A\beta_{40-1}$—protect cortical membranes from ascorbate-stimulated, metal-dependent oxidation in vitro (3). In contrast, all Aβ peptides are unable to considerably influence metal-independent lipoprotein oxidation (90, 92), suggesting that the antioxidative activity of Aβ is mainly mediated by the chelation of transition metal ions by its hydrophilic moiety. In addition, Aβ complex with Cu(II) displays a superoxide dismutase-like activity in vitro, metabolizing superoxide radical (42); this mechanism might additionally contribute to antioxidative activity of Aβ.

Endogenous Aβ present in CSF can also act as an antioxidant in vitro, as suggested by the positive correlation between CSF resistance to oxidation and its levels of Aβ (94). The level of $A\beta_{1-42}$ better correlates with CSF oxidative resistance than that of $A\beta_{1-40}$ (94), which is in accordance with stronger metal binding to $A\beta_{1-42}$ as compared to $A\beta_{1-40}$ (11). CSF from AD patients has lower oxidative resistance than CSF from control subjects (173), in accordance with increased oxidative stress known to occur in AD (185). Since Aβ at its CSF concentrations has antioxidative properties, this is in agreement with the lower Aβ levels in CSF typically measured in AD patients as compared to control subjects (23, 94) .

An antioxidant role for Aβ in vivo is consistent with data on the distribution of oxidative damage to AD neurons. 8-Hydroxyguanosine (8OHG), a major product of nucleic acid oxidation, markedly accumulates in the cytoplasm of cerebral neurons in AD. Unexpectedly, an increase in Aβ deposition in AD cortex is associated with a decrease in neuronal levels of 8OHG, ie, with decreased oxidative damage (148). A similar negative correlation between Aβ deposition and oxidative damage is found in patients with Down syndrome (DS) (149). These findings indicate that in brains of patients with AD and DS, Aβ deposition is related to decreased oxidative damage. Thus, formation of amyloid plaques may be considered as a compensatory response that reduces oxidative stress (114, 185).

Finally, the antioxidative function of apolipoprotein Aβ is consistent with the observation that neuronal cell cultures secrete a high molecular weight product, presumably a lipoprotein complex, that possesses antioxidative activity (18). Thus, Aβ may function as an antioxidant secreted as part of a lipoprotein complex, which prevents oxidation by binding transition metal ions in inactive form (Figure 3). Interestingly, a small, dense HDL subfraction from human plasma has recently been reported to possess potent antioxidative and anti-inflammatory activity (93). Consistent with this finding, Aβ is preferentially associated with small, dense, apoJ-carrying HDL-like particles in CSF and plasma.

Antioxidative protection provided by monomeric Aβ is not confined to lipoproteins; the peptide is equally able to inhibit metal-induced oxidation in neuronal cells (217). The antioxidative activity of monomeric Aβ underlies protection of cells from the toxic action of transition metals. Moreover, Aβ at low concentrations possesses anti-apoptotic activity (34). These data indicate that Aβ may serve as a ubiquitous preventive antioxidant protecting various biological systems.

Synaptic activity. Cholinergic synaptic dysfunction is a prominent feature of AD (101); loss of synapses in AD is greater than could be explained by the loss of neurons (44). Cholinergic synapses must therefore be efficiently preserved in AD. The metal-chelating properties of Aβ may be related to its potential protective function in synapses.

Neurons release high (micromolar) amounts of copper and zinc during depolarization of synaptic membranes (68, 70, 83, 115) (Figure 3). Synaptic copper may be important for neurotransmission (169, 201); copper ions may regulate fusion of synaptic vesicles with plasma membranes (71). It is unlikely that the metals are released in a free form, rather they are bound to specialized metal chaperones; however, presence of abnormally bound metals cannot be excluded.

On the other hand, cholinergic synaptic activity relies heavily on lipid supply which is essential to synthesize acetylcholine. ApoE- and cholesterol-containing lipoproteins produced by astrocytes represent a synapse-promoting factor (134); moreover, most synapses develop after the differentiation of astrocytes (156). These observations presume the presence of lipoproteins in the synaptic cleft.

It is plausible that Aβ present in the synaptic cleft as an apolipoprotein within lipoprotein complexes could participate in the binding of transition metal ions released from synapses, thereby regulating their redistribution and uptake by neurons (Figure 3). The high affinity of Aβ for metals may be important for these processes, allowing Aβ to participate in metal clearance from the brain as suggested by studies in hyperlipidemic rabbits (188) and AD transgenic mice (135). Association of Aβ with lipoproteins suggests that metals are transported bound to lipoproteins; Aβ-mediated binding of redox-active copper could protect lipoproteins and neurons from adverse oxidation. The recently reported down-regulation of synaptic excitatory transmission by Aβ secreted by neuronal cells in response to activity (82) might be related to chelation of metals essential for synaptic activity.

Acute phase activity. Various stress conditions are known to increase Aβ production. For example, Aβ production increases under oxidative stress induced in different mechanisms. Both H_2O_2 and UV irradiation elevate production of Aβ peptides in monkey eye lenses (58) and neuroblastoma cells (152, 216). H_2O_2 upregulates both secretion of Aβ in the cell medium (152) and levels of Aβ in the cell; antioxidants Trolox and dimethyl sulfoxide are able to block the effect (138). Increased production of Aβ in the presence of H_2O_2 is not related to increased synthesis of APP but rather to increased generation of Aβ from APP (138) mediated by elevated expression of β-secretase in Golgi apparatus (196). Similarly, increased levels and activity of β-secretase BACE1 occur following transient cerebral ischemia in rats (207).

Other sources of oxidative stress, less common than H_2O_2, similarly lead to increased Aβ production in cell culture. Inorganic mercury decreases cellular glutathione and increases release of Aβ from neuroblastoma cells (153). Paired helical filaments from AD patients generate superoxide radicals and increase release of Aβ from neurons (212). Interestingly, secretion of Aβ is also increased when oxidative stress is induced by micromolar concentrations of Aβ itself (152), thereby providing a feedback loop mechanism which allows aggregated Aβ to increase its own production—a vicious circle (216).

Aβ generation can be equally increased when cells are subjected to a more general metabolic stress. For example, serum deprivation increases Aβ production by human neurons (112), and inhibition of energy metabolism results in increased amyloidogenic APP processing by β-secretase (60).

Finally, Aβ production increases in vivo after brain injury. In patients with head injury, both $Aβ_{1-40}$ and especially $Aβ_{1-42}$ increase in CSF during the first week following the trauma (160). Fatal head injury results in the formation of diffuse parenchymal deposits of Aβ in the brain, all of which contain $Aβ_{42}$ as a major component (61). Notably, the post-traumatic deposits of Aβ do not arise as a result of passive leakage from damaged cerebral blood vessels but are similar to the early $Aβ_{42}$ deposits observed in AD and DS. In addition, Aβ accumulates in the brain as a response to ischemic/hypoxic injury localized to cerebral cortex (79).

Taken together, these data strongly suggest that Aβ behaves as a positive acute-phase protein whose synthesis is increased under stress conditions (Figure 3). Again, antioxidant metal-chelating properties of Aβ may form a rationale for this phenomenon. Indeed, an increase in Aβ production may be aimed at chelating potentially harmful transition metal ions which can be released, eg, from metal-binding proteins, during abnormal cellular metabolism and otherwise catalyze adverse oxidation of biomolecules (Figure 3). This mechanism has been recently proposed (19), and is supported by the fact that increased levels of oxidative damage (measured as neuronal 8OHG immunoreactivity) occur prior to the onset of Aβ deposition in brains of patients with Down syndrome (148). Moreover, elevated oxidative stress precedes amyloid deposition in brains of transgenic mice carrying mutant APP (158); antioxidant supplementation decreases amyloidosis in young but not aged animals (193). Increased oxidative stress in the brain can be induced by dysfunctional mitochondria; high levels of mutations have been recently reported in mitochondrial DNA from AD brain (39). This pathway can lead to accelerated synthesis of Aβ and account for the development of sporadic AD as has been recently proposed (90, 183, 195).

Increase in Aβ production may be a regulatory response which helps cells to cope with abnormal metabolism of transition metals (183). Processing of APP to Aβ has been suggested more than a decade ago to focus on a release of an active peptide ligand, constituting a part of reactive plasticity response to neuronal loss (209). Now, when Aβ production is known to occur by a fundamental mechanism of regulated intramembrane proteolysis (210), its obligatory physiologic significance is even more apparent.

None of the studies on Aβ synthesis under stress conditions cited above has investigated whether the peptide is secreted as a part of a lipoprotein complex; the consistency of data on the association between Aβ and HDL-like lipoproteins in biological fluids suggests that this is probably the case. Moreover, secreted Aβ might be associated with small, dense, HDL-like lipoproteins; selective increase in small lipoprotein particles rich in apoA-I, phospholipids and free cholesterol is observed in patients with subarachnoid hemorrhage (85). Similarly, the relative concentration of small apoA-I-containing lipoproteins is elevated following traumatic brain injury (84).

Plasma HDL is known to undergo a major rearrangement in the acute phase which is accompanied by dramatic alteration in apolipoprotein composition, including replacement of apoA-I by serum amyloid A (SAA) (145). Such HDL rearrangement has been proposed to play an important role in innate immunity (146). In CSF, SAA is found in the lipoprotein fraction; SAA levels in CSF are significantly elevated in AD (87). One can hypothesize that innate immune response may involve increase in Aβ levels as recently proposed (32). Consistent with this hypothesis, increase in Aβ production is mediated by the pro-inflammatory cytokines TNFα and IFNγ, and can be reversed by anti-inflammatory drugs (22). Infection with *C. pneumoniae* induces AD-like amyloid plaques in brains of non-transgenic mice (77, 118); herpes simplex virus type 1 and cytomegalovirus are present in brains of patients with AD and vascular dementia (116, 117). Elevated secretion of Aβ might therefore represent an important component of innate immunity.

Regulation of lipid transport and metabolism. Aβ production is often regulated in parallel with cholesterol synthesis (211); in turn, Aβ has been shown to influence synthesis of cholesterol. Elevation in Aβ concentrations has been reported to accelerate synthesis of cholesterol in neuronal cell culture and fetal brain (102). Several studies have found that at low concentrations, Aβ is non-toxic and has beneficial effects on neuron survival, axonal length and neurite outgrowth (96, 208, 213). These trophic activities may be related to increased cholesterol supply to neurons induced by monomeric Aβ.

This brief review reveals several potential functions for apolipoprotein Aβ in vivo; there is evidence that these important activities may be impaired in AD.

DYSFUNCTION OF APOLIPOPROTEIN Aβ IN AD

Dissociation from lipoproteins. In non-demented control subjects, levels of Aβ in biological fluids are low and all Aβ is detected in the lipoprotein fraction. In AD, Aβ production in the brain can be considerably increased, due to the presence of mutations in APP and/or γ-secretase associated with familial AD, or under stress conditions as a compensatory response (90, 92). It is tempting to speculate that the association of excessive Aβ with lipoproteins can be abnormal, causing its dissociation from the particles.

Indeed, appreciable levels of $Aβ_{1-42}$ are found in ultracentrifugally isolated, lipoprotein-depleted plasma of patients with AD (131) and of aged subjects (132), pointing out that $Aβ_{1-42}$ association with lipoproteins can be weakened in AD and aging. Consistent with these results, distribution of $Aβ_{1-40}$ between lipoprotein subpopulations is equally altered in CSF from AD subjects, resulting in peptide redistribution from small to large particles; other apolipoproteins reveal a similar trend (98).

Aggregation and pro-oxidative activity. The pathological aggregation of the peptide has been proposed to follow its dissociation from lipoproteins in AD (132) (Figure 3). Aggregation of Aβ leads to the loss of its biological functions. First, antioxidative activity may evolve into pro-oxidative (10, 29, 89-91, 217). Pro-oxidative properties of Aβ have been known for about a decade (129, 200). Importantly, to induce oxida-

tion, Aβ must be present at concentrations greatly exceeding those normally measured in biological fluids (ie, micromolar vs. nanomolar; see (90, 92, 129)). In addition, Aβ preparations must be "aged" (incubated for a relatively long time at room temperature) to become aggregated and fibrillated (129, 200). Production of H_2O_2 is thought to be central for pro-oxidative activity and toxicity of Aβ (17).

The presence of transition metals is required for both Aβ aggregation and pro-oxidative activity. Iron is required for the toxicity and pro-oxidative activity of aged preparations of $Aβ_{1-42}$ to neuronal cells, whereas iron chelators protect cells from Aβ (166). Incubation of $Aβ_{1-40}$ and $Aβ_{1-42}$ with transition metals results in the generation of H_2O_2 (72). Therefore, Aβ toxicity is likely to be mediated by a direct interaction between Aβ and transition metals with subsequent generation of reactive oxygen species (73, 166) (Figure 3). Another factor essential for the pro-oxidative activity of Aβ seems to be the presence of Met35 (206).

The triple requirement of fibrillation, transition metals and presence of Met35 for the pro-oxidative activity of Aβ can be understood when its redox properties are taken into account. In order to function as a pro-oxidant, Aβ must first bind metals to its metal-binding site(s) and then reduce them at its metal-reducing site in order to produce reactive oxygen species (eg, hydroxyl radicals from H_2O_2). However, metals are bound to the N-terminal hydrophilic domain of Aβ, whereas metal reduction occurs at the C-terminal hydrophobic domain. Since metals must be placed in the vicinity of the reductant to be reduced, dissociation of Aβ from lipoproteins followed by aggregation are likely to fulfill this task by forming complexes in which metal atoms bound to the N-terminal part of one molecule of Aβ can be simultaneously available for the reductive Met35 residues belonging to other Aβ molecules (89, 90). Reduced transition metal ions formed can participate in further redox reactions, generating various free radical species. Due to the relatively slow reduction of metals by Aβ (72), this mechanism can only be operative at high (micromolar) concentrations of the peptide.

Evolution of the antioxidative into pro-oxidative activity of Aβ represents a typical gain-of-function transformation; this can further stimulate Aβ production, providing a feedback loop mechanism to accelerate plaque growth by a "seeding" mechanism (75). As a result, levels of protective monomeric Aβ decrease, whereas levels of deleterious oligomeric Aβ, which can no longer chelate copper and protect against oxidation, increase. Massive accumulation of Aβ in brains of AD patients may be accordingly considered as a hyper-response to increased oxidative stress in aging.

Deleterious Aβ-metal complexes must be efficiently removed, a process which may occur through lipoprotein receptors known to be abundant in CNS (43). A fine balance exists between synthesis and degradation, since Aβ accumulation is caused by only about 50% increase in Aβ anabolism in most early onset familial AD cases (78). At some stage, efficient removal of Aβ-metal complexes can be overtaken by their disproportionably high generation in turn resulting in their accumulation in the form of Aβ oligomers and, subsequently, early (diffuse) amyloid plaques.

Taking into account the extracellular location of amyloid plaques, one can assume that plaque Aβ originates from brain lipoproteins (Figure 3). The lipoprotein origin of Aβ in senile plaques is supported by a close correspondence between the deposition of apoE, cholesterol and Aβ in amyloid plaques (26, 65, 143). Since transition metal ions are highly enriched in plaques (123), aggregation of lipoprotein-derived Aβ by transition metals seems to represent a plausible mechanism of plaque formation. Zinc ions may play a critical role in plaque formation, causing entombment of otherwise toxic Aβ oligomers (41, 57). Oligomeric, transition metal-carrying Aβ, in the form of early protofibrils (205) and/or late (compact) amyloid plaques (170), appears to be the toxin responsible for neurodegeneration in AD brains.

Dysregulation of lipid transport and metabolism. In addition to pro-oxidative activity, oligomerized Aβ can inhibit cholesterol synthesis and promote lipid release from brain neurons in complex with GM1 ganglioside (137), resulting in the formation of NFT in the cells (63, 128). Aβ oligomers can be produced intracellularly and then secreted (140); alternatively, they can accumulate extracellularly as a result of Aβ aggregation by transition metals, eg, by copper (188). Whatever the case, both pathways may lead to the disruption of neuronal lipid homeostasis, intracellular pathology, dysregulation of synaptic homeostasis and loss of neuronal function.

Thus, oligomeric Aβ can cause multiple metabolic dysfunctions in the brain; knowledge of their mechanisms may have critical therapeutic implications.

THERAPEUTIC IMPLICATIONS FOR AD

Although the principal physiological function of Aβ still remains to be determined, the view of this peptide as an apolipoprotein offers new intriguing perspectives in the treatment of AD which primarily include targeting lipid metabolism in the brain.

There is no doubt that to develop an effective therapy, brain lipid metabolism must be understood in much more detail, particularly the role played by Aβ. Individual lipoprotein subclasses, such as potentially deleterious (delivering Aβ to oligomers) and protective (removing Aβ from oligomers) particles, need to be identified; modulation of their levels in CSF may prove beneficial. Applicability of approaches developed in the field of the lipidology of cardiovascular diseases (HDL raising by fibrates (189), niacin (164), and apoA-I mimetics (144)) should be critically evaluated; effects of lipid-modulating drugs, particularly statins, need to be extensively studied at the lipoprotein level.

Targeting oxidative stress using classical antioxidants, such as vitamin E or vitamin C (95, 168), appears to be unselective and may probably be used only as a supplementary approach, despite apparent benefits (215). By contrast, attempts to block formation of Aβ oligomers, eg, using metal chelators (54) or β-sheet breaker molecules (33), are promising. Phospholipid preparations which display remarkable anti-inflammatory activity and activate reverse cholesterol transport (147), equally represent an intriguing possibility (103).

Attempts to remove Aβ from the brain (eg, using a vaccine developed against the peptide [171]) and to decrease Aβ production (eg, using inhibitors of γ- and/or β-secretase [177]) should be undertaken under the assumption that brain levels of monomeric Aβ will not be excessively decreased (180), and that only oligomeric

Aβ will be targeted (105). Indeed, BACE1 deficiency results in decreased Aβ levels in the brain and impaired performance in the Y maze test in a transgenic mice model of AD (150). Potential physiological roles of Aβ in protection against oxidative stress, synaptic function and innate immunity point out that great caution must be exercised regarding therapeutic approaches targeting monomeric Aβ.

CONCLUSION

Analysis of available data indicates that Aβ possesses the properties of an authentic apolipoprotein associated with lipoproteins and influencing lipid transport and metabolism. However, these important biochemical qualities, which appear to critically contribute to the development of AD, have been largely ignored by the mainstream of AD researchers for more than a decade, making Aβ appear to be a "black sheep" in a physiologically important "good apolipoprotein" family. The treatment of Aβ as an enemy (51) should be reconsidered; the peptide should be "forgiven" and its negative image replaced by a more balanced view (165) which would take into account both beneficial and deleterious activities. New unprejudiced studies are desperately needed to shed further light on the physiological role of Aβ and to secure its well-deserved place among other more classical apolipoproteins—despite a suspicion that it will still remain at the core of our understanding of AD.

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