

Lipid peroxidation and Alzheimer's disease: Key role of Amyloid- β

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Abstract: Increased lipid peroxidation and elevated oxidative stress represent well-established characteristics of Alzheimer's disease (AD). Amyloid- β (A β) peptide, a major component of amyloid plaques, can strongly influence oxidative processes. In aggregated form, A β has prooxidative properties, whereas in monomeric form it functions as an antioxidant. The antioxidative properties of monomeric A β are related to its ability to chelate transition metal ions, which are potent catalysts of oxidation. A β possesses an amphiphilic structure, associates with lipoproteins in vivo and may therefore function as a preventive antioxidant which protects lipoproteins from oxidation by transition metal ions. Increased production of A β in response to elevated oxidative stress has been documented in a number of in vitro studies, implying that production of monomeric A β as a lipoprotein antioxidant can be abnormally increased in response to elevated oxidative stress in aging. Subsequent accumulation of A β -metal aggregates, production of reactive oxygen species and toxic action to neuronal cells may represent a gain-of-function transformation and form temporal sequence of events in the development of AD.

Key words: Alzheimer's disease, amyloid- β , oxidative stress, antioxidant, prooxidant, lipoproteins, lipids, transition metals, oxidation, antioxidants

Introduction

Oxidative stress is thought to be an important mechanism in many degenerative diseases including atherosclerosis [1], diabetes [2] and neurodegenerative disorders [3]. It can affect biomolecules including proteins, DNA and lipids, and may lead to their dysfunction. Human brain is especially vulnerable to oxidative stress due to its high oxygen consumption and elevated concentrations of easily oxidizable lipids. Markers of lipid peroxidation are elevated in brain tissue and body fluids in several neurodegenerative diseases and the role of lipid peroxidation has been extensively discussed in the pathogenesis of Alzheimer's disease (AD), Parkinson's disease, amyotrophic lateral sclerosis and prion diseases [3]. It is controversial whether elevated markers of lipid peroxidation in neurodegenerative disorders reflect distinct mechanisms of these diseases or are merely an epiphenomenon. While our knowledge about the exact role of lipid peroxidation in the mechanisms of most neurodegenerative diseases remains fragmentary, in AD there seems to be a direct link between lipid peroxidation and the putative disease-causing agent, amyloid- β (A β) peptide.

A β possesses metal- and lipid-binding properties

A β , a 39-43 amino acid peptide, is a major component of amyloid plaques which are a hallmark of AD. Distinctive structure of A β

results in a unique combination of metal-binding and lipid-binding properties (figure 1). Human A β contains three histidine and one tyrosine residues which in free form are able to efficiently chelate transition metal ions [4]. All the residues (His6, His13, His14, Tyr10) are located in the hydrophilic N-terminal part of A β (figure 1); as a result, the N-terminal part contains two binding sites for copper located between amino acid residues 6 and 14 [5, 6]. Nitrogen atoms of the His residues [5, 6] as well as amide groups at the N-terminus of A β [7] ensure strong binding of transition metal ions. Human A β therefore possesses high affinity for transition metals, particularly for copper [7]; apparent stability constant of A β -copper complexes reaches $2.0 \cdot 10^{17} \text{ M}^{-1}$, a value reported for human A β 1-42 [7].

Amino acid sequence of A β is equally characterized by a distinct hydrophobicity gradient which accounts for its amphiphatic properties (figure 1). The presence of a hydrophobic C-terminal fragment leads to prominent lipid-binding properties of A β ; as a result, A β readily associates with lipids. At a lipid-water interface, A β is partially inserted into lipids via the C-terminal tail in such a way that the hydrophilic N-terminal domain of A β 1-40 which contains the metal-binding sites is exposed to the aqueous phase [8].

A β is associated with lipoproteins

Lipid-binding properties of A β form the basis for its association with lipoproteins. In biological

fluids, monomeric A β is not present in a free form but carried by lipoproteins. A β -carrying lipoproteins are spherical and typically display size (5-17 nm) and density (1.063-1.21 g/mL) in the range of plasma high density lipoprotein (HDL) [9, 10]. In human cerebrospinal fluid (CSF), A β is found in HDL-like lipoproteins of approximately 17 nm diameter and 200 kDa molecular mass [11]. Similarly, monomeric A β co-isolates with lipoprotein particles from human plasma, in particular with the HDL and very high density lipoprotein (VHDL) fractions [12], and from AD brains [13]. Finally, human hepatoma HepG2 cells secrete A β in the culture medium as a part of 200-300 kDa lipoprotein complexes in association with apolipoprotein (apo) A-I, apoJ, phospholipids, triglycerides and free and esterified cholesterol [14].

In addition to hepatocytes, A β is constitutively synthesized and secreted by a variety of other cells types [15]. In the brain, neuronal cells, particularly neurons but also astrocytes and glial cells [16, 17], are the major source of A β [15, 18] (figure 2); however, it is unknown whether A β is secreted by neuronal cells in a free form or as a part of lipoprotein complex. Since astrocytes are able to secrete both apoE-containing lipoproteins and A β [16, 17, 19-21], A β synthesized by astrocytes might be secreted as a part of lipoprotein particles. By contrast, distal axons of sympathetic neurons are incapable of cholesterol production and lipoprotein secretion [22]; neuron-produced A β might then associate with lipoproteins secreted by astrocytes in the extracellular space.

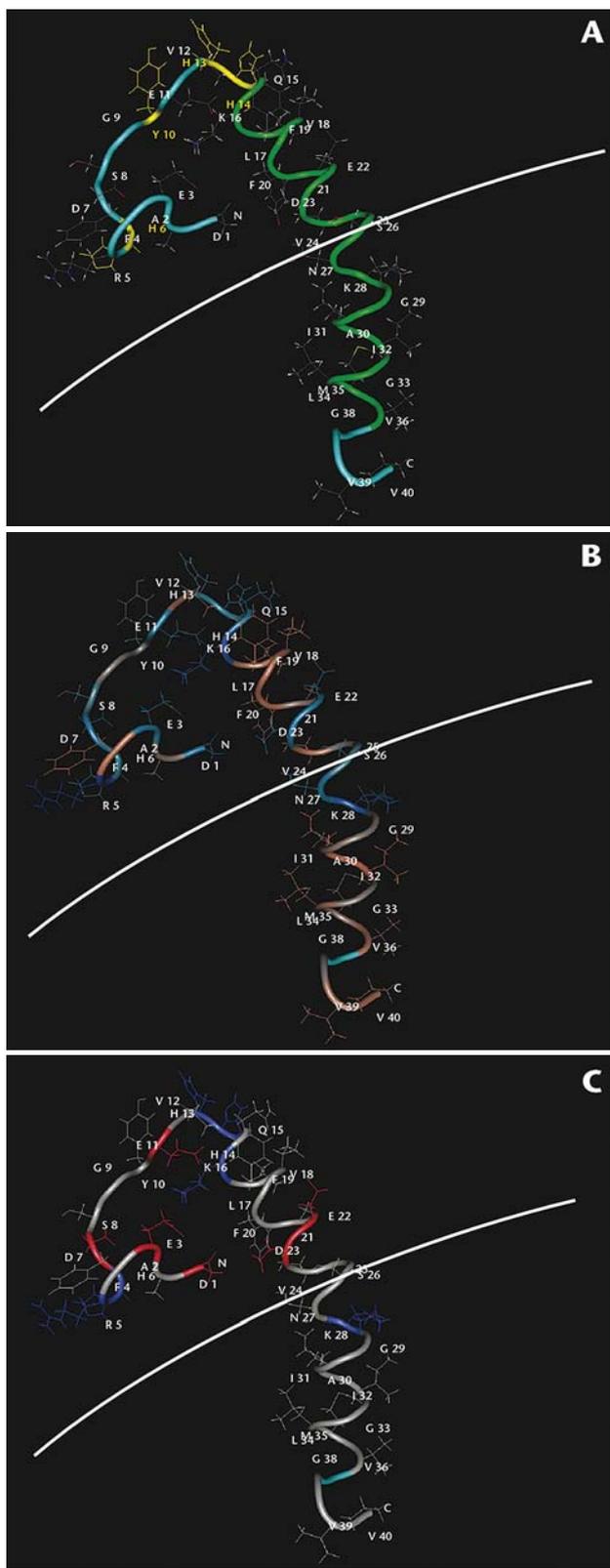


Figure 1. Secondary structure (A), hydrophobicity (B) and charge (C) of Ab1-40 at the aqueous/lipid interface (pH 5.1). Green, α -helix; blue, random coil; yellow, metal-binding residues (A); red, more hydrophobic; blue, less hydrophobic (B); red, negative charge; blue, positive charge; gray, neutral (C). Probable lipoprotein surface is schematically shown as a white arc. Amino acid sequence of A β 1-40, DAEFRHDSGY EVHHQQLVFF AEDVGSNKGAIIGLMVGGVV; A β 1-42 possesses two additional amino acid residues IA at the C-terminus.

Brain lipoproteins are highly heterogeneous [23]; A β appears to be specifically associated with a subclass, rather than with all lipoprotein particles. In CSF, most A β is found in small, dense HDL-like particles, whose density corresponds to that of plasma HDL3 and VLDL [11, 24]; these particles are enriched in apoJ and contain apoA-I [11, 24, 25]. In plasma HDL, A β is equally complexed to apoJ and, to a lesser extent, to apoA-I [12]. Furthermore, A β co-isolates with apoJ also from human CSF [26], implying that the peptide is preferentially associated with small, dense, apoJ-carrying HDL-like particles in CSF and plasma.

A β appears to be tightly bound to lipoproteins in biological fluids, since no A β reactivity is detected in lipoprotein-free fractions upon ultracentrifugation of CSF or plasma obtained from control subjects [11, 12, 14], which is well-known to cause a dissociation of weakly bound apolipoproteins from the lipoprotein surface [27]. Such tight association of A β with lipoproteins is stronger than can be expected for purely hydrophobic interactions with lipids, suggesting that the peptide may equally bind to apolipoproteins (*e.g.*, to apoE [28, 29] and/or apoJ [26, 30]), strengthening the association with lipoprotein particles.

Together, these data demonstrate that A β is a physiological protein component of lipoproteins and can be therefore regarded as an apolipoprotein. Since the molecular mass of A β (about 4 kDa) is close to that of apoC-I (about 6 kDa), the smallest known apolipoprotein, A β can be considered as a small metal-binding apolipoprotein.

A β production increases under oxidative stress

A β synthesis and secretion increase under conditions of oxidative stress (*figure 2*). Normally, A β is produced from amyloid precursor protein (APP) under the action of β - and γ -secretases via the mechanism of intramembrane proteolysis [31]. A β production in the brain can be considerably increased due to the presence of mutations in APP and/or γ -secretase associated with familial AD [32].

Oxidative stress induced by different agents equally increases A β production in cell culture. Treatment with H₂O₂ and irradiation with UV rise production of A β peptides in monkey eye lenses [33] and neuroblastoma cells [34, 35]; H₂O₂ upregulates both secretion of A β in the cell medium [35] and levels of A β in the cell. Increased production of A β in the presence of H₂O₂ is related to increased generation of A β from APP rather than to increased synthesis of APP [36] mediated by elevated expression of

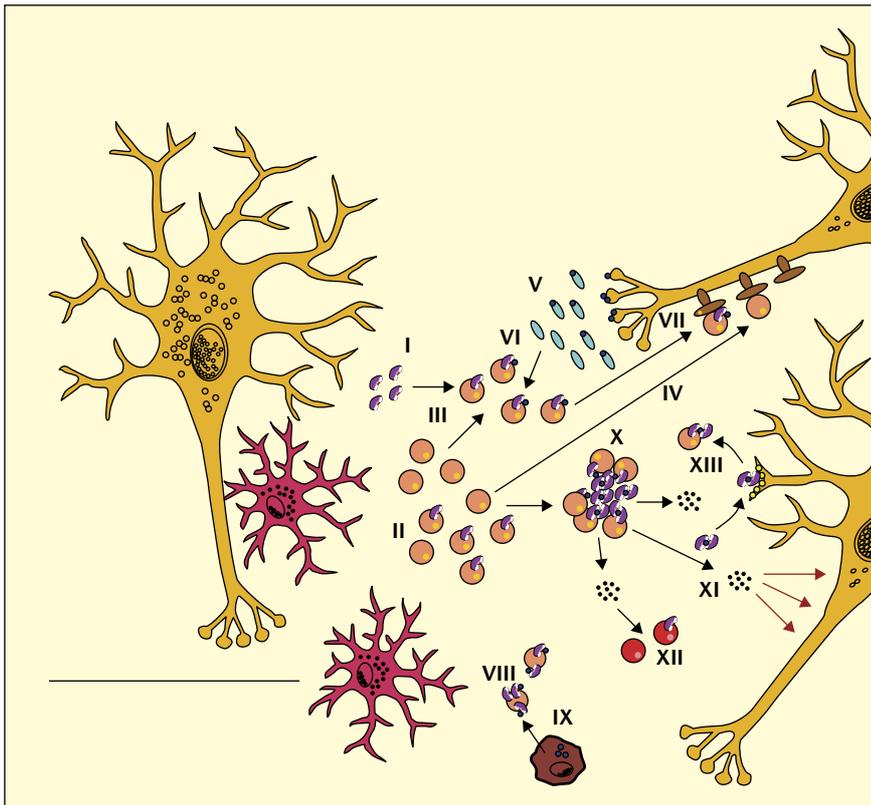


Figure 2. Physiological functions and dysfunction of A β in the brain. A β (shown in violet) is secreted in the extracellular fluid either in a lipoprotein-free form by neurons (I) or in association with lipoproteins (orange) by astrocytes (II); lipoprotein-free A β subsequently bind to lipoproteins (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 A β 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 A β , elevating its levels in lipoproteins (VIII); secreted A β might function as a chelator for metals released from dying cells (IX). Elevated A β 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 A β 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 A β can equally induce removal of cholesterol (yellow) from neurons and are toxic causing degeneration (XIII).

β -secretase in Golgi apparatus [37]. Consistent with this mechanism, increased levels and activity of β -secretase BACE1 occur following transient cerebral ischemia in rats [38].

Other sources of oxidative stress similarly lead to increased A β production in cell culture. Inorganic mercury decreases cellular glutathione and increases release of A β from neuroblastoma cells [39]. Paired helical filaments from AD patients generate superoxide radicals and increase release of A β from neurons [40]. Interestingly, secretion of A β is also increased when oxidative stress is induced by micromolar concentrations of A β itself [35], thereby providing a feedback loop mechanism which allows A β to increase its own production – a vicious circle [34].

A β generation can be equally increased when cells are subjected to a more general metabolic stress. For example, serum deprivation increases A β production by human neurons

[41], and inhibition of energy metabolism results in increased amyloidogenic APP processing by β -secretase [42]. Finally, A β production increases in vivo after brain injury. In patients with head injury, both A β 1-40 and especially A β 1-42 increase in CSF during the first week following the trauma [43]. Fatal head injury results in the formation of diffuse parenchymal deposits of A β in the brain, all of which contain A β 42 as a major component [44]. Notably, the post-traumatic deposits of A β do not arise as a result of passive leakage from damaged cerebral blood vessels but are similar to the early A β 42 deposits observed in AD and Down's syndrome. In addition, A β accumulates in the brain as a response to ischemic/hypoxic injury localized to cerebral cortex [45]. Taken together, these data strongly suggest that A β behaves as a positive acute-phase reactant whose synthesis is increased under stress conditions.

Monomeric A β protects against oxidative stress

Metal-chelating properties of A β may form a rationale for increased A β production in the presence of oxidative stress. In lipoproteins, the metal-binding region of A β 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 monomeric A β may therefore serve to inhibit metal-catalyzed oxidation and to decrease oxidative damage. An increase in A β production may be therefore aimed at chelating potentially harmful transition metal ions which can be released, *e.g.* from metal-binding proteins, during abnormal cellular metabolism and otherwise catalyze adverse oxidation of biomolecules [46, 47] (figure 2). Indeed, brain homeostasis of transition metals is heavily impaired in AD [48], suggesting that metal-catalyzed oxidation is particularly important for the development of this disorder. Available data strongly argue for a key role of transition metals in elevated oxidative stress in AD [49]. Increase in A β production may then constitute a regulatory response which helps cells to cope with abnormal metabolism of transition metals [50, 51].

Consistent with this hypothesis, *in vitro* actions of A β at physiological concentrations (*i.e.* in a monomeric form) include inhibition of metal-induced oxidation of lipoproteins from human CSF and plasma [46, 47], protection of neuronal cells against toxic action of transition metals [52] and attenuation of apoptosis in neuronal cells [53]. The metal-chelating properties of A β may be especially relevant in synaptic endings which may release high amounts of copper and zinc during depolarization [54-57]. The recently reported down-regulation of synaptic excitatory transmission by A β secreted by neuronal cells in response to activity [58] might be related to chelation of transition metals essential for synaptic activity. It remains indeterminate whether A β is present in biological fluids as a part of a lipoprotein complex under stress conditions. Existence of such acute-phase A β -lipoprotein complexes can be postulated taking into account consistent data on the association between A β and HDL-like lipoproteins under normal conditions [11-14]. As described above, secreted A β might be associated with a subset of small, dense, HDL-like lipoproteins. Plasma HDL undergoes 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) [59]. Reverse cholesterol transport (RCT) from peripheral tissues to the liver primarily provided by apoA-I represents one of the major

functions of plasma HDL [60]. The replacement of apoA-I by SAA redirects cholesterol from the liver to macrophages and other cells of the immune system and thereby reverts the RCT, providing optimal host defense [61]. Such HDL rearrangement has been proposed to play an important role in innate immunity [62]. In CSF, SAA is primarily present in the lipoprotein fraction; SAA levels in CSF are significantly elevated in AD [63]. It has been proposed that an innate immune response may involve increase in A β levels [64]. This hypothesis can now be refined taking into account the preferential association of both SAA [65-67] and A β [11, 24] with HDL, particularly with small, dense HDL particles. Compositional alterations in HDL particles which include their enrichment in SAA and A β may constitute an important element of innate immune response aimed at providing optimal cellular function, sufficient supply with nutrients and necessary protection from harmful agents, such as free transition metal ions. Interestingly, the proposed role of A β as an important component of immune system is consistent with the development of brain inflammation in AD patients vaccinated against human A β [68].

A β overproduction may result in the formation of amyloid aggregates

Mechanistically, stress-related increase in A β production is mediated by pro-inflammatory cytokines. Systemic inflammatory markers are elevated in AD patients; AD can be thus regarded as a chronic inflammatory disease [69]. Interleukin-6 [70], transforming growth factor β 1 [71], combination of tumor necrosis factor α and interferon γ [72], and combination of interleukin-1 β and interferon γ [73] can all induce A β production in neuronal and extra-neuronal cells which can be partially reversed by anti-inflammatory drugs [69]. Such increased production of A β aimed to provide protection against oxidative stress via chelation of transition metal ions may underlie accumulation of amyloid plaques and pathogenesis of sporadic AD.

Casual relationship between oxidative stress and amyloid deposition is supported by the fact that increased levels of oxidative damage (measured as neuronal 8OHG immunoreactivity) occur prior to the onset of A β deposition in brains of patients with Down's syndrome [74]. Moreover, elevated oxidative stress precedes amyloid deposition in brains of transgenic mice carrying mutant APP [75]; antioxidant supplementation decreases amyloidosis only in young but not in aged animals [76]. Increased oxidative stress in the brain can be induced by dysfunctional mitochondria; high levels of muta-

tions have been recently reported in mitochondrial DNA from AD brain [77]. This pathway can lead to accelerated synthesis of A β and account for the development of sporadic AD as has been hypothesized [46, 50, 78].

In AD, excessively produced A β may be less tightly associated with brain lipoproteins which can cause its dissociation from lipoprotein particles. Indeed, appreciable levels of A β 1-42 are found in ultracentrifugally isolated, lipoprotein-depleted plasma of patients with AD [79] and of aged subjects [13], pointing out that A β association with lipoproteins can be weakened in AD and aging. A β binding to lipoproteins may play a key role in maintaining the peptide in solution. *In vitro*, HDL phospholipids [80], native HDL [81], reconstituted protein-free HDL particles [82] and apoE-containing liposomes [83] all efficiently bind A β and inhibit its aggregation.

In the presence of free transition metal ions, A β dissociation from lipoproteins in AD can be followed by pathological oligomerization and aggregation of the peptide [13] (*figure 2*). A β aggregation can be initiated by the interaction with transition metal ions. A β is readily aggregated *in vitro* by Cu(II), Fe(III), Zn(II) and Al(III) [84]. As a result, the peptide may easily undergo a conformational transition from a soluble monomeric form to aggregated, fibrillary β -sheet structures. *In vitro*, A β exists as a mixture of monomers, dimers, and higher oligomers; further aggregation yields protofibrils and then fully-fledged fibrils that resemble the bulk of the amyloid plaques in AD brain tissue [85]. In contrast, in the absence of metals, A β is monomeric, has α -helix conformation and does not form aggregates [86].

The molecular mechanism of *in vitro* A β aggregation by zinc or copper includes formation of intermolecular crosslinks between β -sheets of A β by the atoms of metal. The crosslinks are formed between nitrogen atoms of all three histidine residues in A β [5, 6]. Such A β 1-40 aggregates typically possess about 3-4 metal atoms per molecule of A β [84].

Aggregated A β promotes oxidative stress

Oligomeric (dimeric, trimeric, etc.) A β that carries transition metal ions may lose its normal biological functions to acquire deleterious activities (*figure 2*). First, results of *in vitro* experiments suggest that antioxidative activity of monomeric A β evolves into prooxidative activity of aggregated A β [46, 52, 87-90]. In order to function as a prooxidant, A β binds metals to its metal-binding site(s) and then reduces them at its metal-reducing site (methionine residue at a position 35) in order to produce reactive oxygen species (*e.g.*,

hydroxyl radicals from H₂O₂). Metals are bound to the N-terminal hydrophilic domain of A β , 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 A β 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 A β can be simultaneously available for the reductive Met35 residues belonging to other A β molecules [46, 87].

In addition to prooxidative activity, oligomerized A β can inhibit cholesterol synthesis and promote lipid release from brain neurons in complex with GM1 ganglioside [91], resulting in the formation of neurofibrillary tangles in the cells [92, 93]. A β oligomers can be produced intracellularly and then secreted [94]; alternatively, they can accumulate extracellularly as a result of A β aggregation by transition metals, *e.g.* by copper [95]. 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. Oligomeric A β has been accordingly proposed to be the toxin responsible for neurodegeneration in AD brains [96].

Deleterious A β -metal complexes must be efficiently removed, a process which may occur through lipoprotein receptors known to be abundant in CNS [97]. A fine balance exists between synthesis and degradation, since A β accumulation is caused by only about 50% increase in A β anabolism in most early onset familial AD cases [98]. At some stage, efficient removal of A β -metal complexes can be overtaken by their disproportionately high generation in turn resulting in their accumulation. Soluble A β oligomers can be detoxified by zinc ions, forming non-toxic precipitate which can subsequently evolve into amyloid plaques [99, 100]. Taking into account the extracellular location of amyloid plaques, one can assume that plaque A β originates from brain lipoproteins. The lipoprotein origin of A β in senile plaques is supported by a close correspondence between the deposition of apoE, cholesterol and A β in the plaques [101-103]. Since transition metal ions are highly enriched in plaques [104], aggregation of lipoprotein-derived A β by transition metals seems to represent a plausible mechanism of plaque formation. The entombment of otherwise toxic A β oligomers promoted by zinc may represent the final non-pathological phase of this stress-related pathway [99, 100]. Consistent with this mechanism, infection with *C. pneumoniae* induces AD-like amyloid plaques in brains of non-transgenic mice [105, 106]; moreover, herpes simplex virus type 1 and cytomegalovi-

rus are present in brains of patients with AD and vascular dementia [107, 108].

Therapeutic insights in AD

The view of A β as a metal-binding apolipoprotein capable of developing both anti- and prooxidative activities can provide an important re-evaluation of current perspectives in the treatment of AD. First, attempts to block formation of A β oligomers (e.g. using metal chelators [109] or β -sheet breaker molecules [110]) are of a special interest, because of their attempt to selectively target the toxic form of the peptide. Second, anti-inflammatory strategies may prove useful to decrease A β production and delay AD [111]. Third, the applicability of lipid-modulating drugs developed in the field of the lipidology of cardiovascular diseases (statins [112], ACAT inhibitors [113, 114], fibrates [115], niacin [116], CETP inhibitors [117] and apoA-I mimetics [118]) should be evaluated at the level of the metabolism of brain lipoproteins. Fourth, decreasing oxidative stress using classical antioxidants, such as vitamin E or vitamin C [119, 120], may provide benefits as a supplementary approach [121]. By contrast, unselective attempts to decrease brain levels of both monomeric and oligomeric A β (e.g. using a vaccine developed against A β [122] or inhibitors of γ - and/or β -secretase [123]) appear to be dangerous, since they can target a physiologically important molecule of monomeric A β . Indeed, vaccination of AD patients against human A β may cause brain inflammation [68], whereas BACE1 deficiency results in impaired performance in the Y maze test in a transgenic mice model of AD [124]. New studies on the possible physiological functions of A β , particularly on its role in brain lipoprotein metabolism and acute phase response, are urgently needed and may lead to novel promising anti-AD therapies.

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