Emerging concepts of how β-amyloid proteins and pro-inflammatory cytokines might collaborate to produce an ‘Alzheimer brain’ (Review)

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Received January 11, 2008; Accepted January 25, 2008

Abstract. Three steps lead to the development of full-blown sporadic or late-onset Alzheimer’s disease or dementia (AD). In the young brain, amyloid β-(1-42) (Aß 42) is a normal aggregation-prone protein product of neuronal activity that is kept at a safe low level by proteolysis in neurons and glial cells, and by expulsion across the blood-brain barrier. But clearance declines with advancing age. Step 1: Because of the normal decline with age of the Aß 42-clearing mechanisms, toxic amyloid-derived diffusible ligands (ADDLs) made of dodecamers of the aggregation-prone Aß 42 start accumulating. These Aß 42 dodecamers selectively target the initially huge numbers of excitatory synapses of neurons and cause them to start slowly dropping, which increasingly impairs plasticity and sooner or later starts noticeably affecting memory formation. At a certain point, this increasing loss of synapses induces the neurons to redirect their still-expressed cell cycle proteins from post-mitotic jobs, such as maintaining synapses, to starting a cell cycle and partially or completely replicating DNA without entering mitosis. The resulting aneuploid or tetraploid neurons survive for as long as 6-12 months as quasi-functional ‘undead zombies’, with developing tangles of hyperphosphorylated τ protein disrupting the vital anterograde axonal transport of mitochondria and other synapse-vital components. Step 2: The hallmark AD plaques appear as Aß 42 clearance continues to decline and the formation of Aß 42 non-diffusible fibrils begins in the aging brain. Step 3: A terminal cytokine-driven maelstrom begins in the aging brain when microglia, the brain’s professional macrophages, are activated in and around the plaques. They produce pro-inflammatory cytokines, such as IFN-γ, IL-1β and TNF-α. One of these, IFN-γ, causes the astrocytes enwrapping the neuronal synapses to express their β-secretase (BACE1) genes and produce and release Aß 42, which can kill the closely apposed neurons by binding to their p75NTR receptors, which generate apoptogenic signals. Astrocytes are also stimulated by the same cytokines to turn on their nitric oxide synthase (NOS)-2 genes and start pouring large amounts of nitric oxide (NO) and its cytocidal derivative peroxynitrite (ONOO-) directly out onto the closely apposed neurons.

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Abbreviations: Aß 42, amyloid β-(1-42); ACIII, adenylyl cyclase III; AD, Alzheimer’s disease or dementia; ADDLs, amyloid-derived diffusible ligands; APOE, apolipoprotein E; APP, amyloid precursor protein; Arc, activity-regulated cytoskeleton protein; BACE1, β-secretase; Clq-R, receptor for complement Clq protein; CaMKII, Ca2+-calmodulin-dependent kinase II; CASR, Ca2+-sensing receptor; EOFAD, early-onset familial Alzheimer’s disease; IDE, insulin-degrading enzyme; IFN-γ, interferon γ; IL-1β, interleukin 1β; LOAD, late-onset (sporadic) Alzheimer’s disease; LRP1, low-density lipoprotein receptor-related protein 1; LTD, long-term depression; LTP, long-term potentiation; NEP, neprilysin; NMDA, N-methyl-D-aspartic acid; NO, nitric oxide; NOS-2, nitric oxide synthase-2; ONOO-, peroxynitrite; ORC, origin recognition complex; PS 1/2, presenilins-1/2; PSD, postsynaptic density-95 protein; ROS, reactive oxygen-species; TNF-α, tumor necrosis factor α

Key words: Alzheimer’s disease, amyloid-β peptides, human, neurons, astrocytes, microglia, primary cilium, pro-inflammatory cytokines

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1. Introduction
It was originally thought that the cause of AD was the over-production of Aß 42 and its aggregation into insoluble fibrillar plaques, which are neuronalcidal cytotoxic garbage dumps...
Figure 1. Aβ 42 released during normal synaptic activity is habitually cleared via destruction by neuronal and glial proteases and by being dumped into the circulation for disposal by the high capacity transendothelial carrier LRP1. With advancing age, Aβ clearance declines and the Aβ 42 level rises into the circulation for disposal by the high capacity transendothelial carrier LRP1. Aβ 42, amyloid β-(1-42); ADDLs, amyloid-derived diffusible ligands; ax, axon; dt, dendrite; FB-AD, full-blown Alzheimer’s disease; IDE, insulin-degrading enzyme; LRP1, low-density lipoprotein receptor-like protein; NEP, nephrilysin.

Aβ 42 is a normal metabolic product of neurons (8) (Fig. 1) that is secreted into the synaptic cleft along with the neurotransmitters (Fig. 1). Aβ 42-containing pre-synaptic vesicles were originally formed when membranes containing APP were first recycled along with the proteins of synaptic vesicle membranes, then sorted into separate vesicles with their APP being cleaved and reloaded with Aβ 42 (6). The aggregation of the co-released Aβ 42 fragments is likely to be driven by the large amounts of divalent cations, in particular Zn²⁺, released from synaptic vesicles together with glutamate transmitter (9,10) (Fig. 1). It is worth remembering that Zn²⁺ itself, released from neuronal pre-synaptic terminals during inflammatory brain damage, can also cause impaired astrocyte glutamate uptake and lead to increased neuronal vulnerability and/or to an exacerbation of neuronal injury (11). Indeed, preventing Zn²⁺ release together with neurotransmitters, by knocking out the transporter-3 that loads Zn²⁺ into synaptic vesicles, reduces plaque deposition. The Aβ fragments initially aggregate (oligomerize) into particularly toxic, but still diffusible, synapse-targeting dodecamers known as ADDLs (5,12) (Fig. 1). If allowed to accumulate to above critical levels, these ADDLs home selectively to receptors on excitatory post-synaptic sites of dendritic spines containing components such as glutamate receptors, postsynaptic density-95 (PSD) protein, and key parts of the hippocampal memory-forming machinery, such as Ca²⁺-calmodulin-dependent kinase II (CaMKII) (13,14). ADDLs stimulate the expression of activity-regulated cytoskeleton protein (Arc), the mRNA of which is normally selectively dispatched to synaptic spines, where it is promptly translated and briefly made as an immediate-early localized spine/synapse protein when synapses are activated (5,13,14). ADDLs impair memory formation by prolonged stimulation of Arc, which spreads throughout the dendrite instead of being restricted to the active synapses (5). Such overexpression and overextension of Arc impairs signaling by producing meaningless background static. This is accompanied by a drastic remodeling of the dendritic spines, with the loss of long-term potentiation (LTP)-mediating N-methyl-D aspartic acid (NMDA) and EphB2 receptors (5,13,14). Thus, the accumulating ADDLs start a decades-long elimination of hippocampal synapses and, concurrently, of the signal-processing machinery needed for plasticity and memory formation, as indicated by the blockage of LTP and the prolongation of long-term depression (LTD) (5). Unless kept below the critical threshold level, accumulating ADDLs will initiate in the target neurons the loss, at first imperceptible and probably reversible (5), of an increasing fraction of their normally huge numbers of synapses. This ADDL action does not kill the cells, but the loss of synapses and declining memory-forming plasticity eventually surface as a mild cognitive impairment, ultimately leading to irreversible full-blown AD (Fig. 2).

But what causes the Aβ 42 oligomers to start accumulating? The first obvious guess would be Aβ 42 overproduction (15). Indeed, the far less common early-onset familial Alzheimer’s disease (EOFAD; around 5% of AD cases) is certainly due to Aβ 42 overproduction, in which individuals most commonly carry a mutant gene encoding abnormally active γ-secretase that increases the release of Aβ 42 fragments from BACE1 pre-processed APP (16). However, it appears that the much slower accumulation of Aβ 42, responsible for the far more frequent LOAD, does not involve overproduction (17).

The Aßs and toxic ADDLs from busy neurons in a normal young brain are customarily kept below dangerous levels by the neurons themselves, by astrocyte networks contacting as many as 60% of the synapses in neuronal networks, by microglial macrophages, by proteases such as the membrane-
bound glycoprotein neprrisyn (NEP) and the cytosolic Zn-metallopestidase insulin-degrading enzyme (IDE), and by being carried out of the brain through the blood-brain barrier and dumped into the blood by the high-capacity low-density lipoprotein receptor-related protein 1 (LRP1) of the vascular endothelial cells (8,18-20) (Fig. 1). Unfortunately, the proteases and LRP1 decline with age and eventually the ADDLs reach a dangerous level and start driving a long, slow and - seemingly, at first - reversible synaptic loss (5). Along with it comes a cognitive decline ending in irreversible full-blown LOAD.

It follows that LOAD should be preventable if drugs could be found to prevent the decline of Aß 42 clearance before the irreparable loss of neurons has occurred. An example of these drugs may be the statins, prolonged treatment with which has been reported to prevent LOAD in individuals treated with the drugs for hypercholesterolemia (reviewed in refs. 21,22). At first, this was reasonably assumed to be due to the lowering of brain cholesterol. However, this is almost certainly not the case. Brain cholesterol is produced within the brain and controlled independently from circulating cholesterol; it is made by astrocytes, which package it in apolipoprotein E (APOE) and dumped into the blood by the high-capacity low-density lipoprotein receptor-related protein 1 (LRP1) of the vascular endothelial cells (8,18-20) (Fig. 1). Unfortunately, the proteases and LRP1 decline with age and eventually the ADDLs reach a dangerous level and start driving a long, slow and - seemingly, at first - reversible synaptic loss (5). Along with it comes a cognitive decline ending in irreversible full-blown LOAD.

Up to this point, increasing numbers of neurons have been disconnecting from other neurons as the mounting load of ADDLs destroys their synapses and reduces their plasticity. Nonetheless, although the individual will be beginning to show symptoms of cognitive impairment, these neurons are still alive. Mistakenly interpreting this gradual disconnection from the neuron network and the accompanying loss of trophic signaling as cell loss, the neurons - like fibroblasts in a scraped confluent monolayer or hepatocytes in the remnant of a partially-hepatectomized liver - initiate a cell cycle in an attempt to rectify the supposed neuronal loss (24,25). The demands for plasticity put pressure on post-mitotic neurons to constantly avoid starting a cell cycle when forming memories, because they use some of the same tools both for forming memories and for starting the buildup to chromosome replication. Thus, the synaptic plasticity mechanism uses cells from neuron cycle engine components, such as cyclins A, B, D and E, origin recognition complex (ORC) core components 2-6, but does so in the presence of the Cip/Kip cyclin dependent kinase inhibitors to prevent any attempt to start a cycle (24-26). For example, components of ORC - the chromosome replication origin starter - are accordingly needed for both dendrite arborization and spine formation, two major requirements for synapse formation and plasticity (26-28). It has been proposed by Arendt and Brückner (25) that ORCs normally signal the nucleus from the synapses in the dendritic spines to both suppress apoptogenesis and express products that are translocated to the spines to strengthen synapses. However, as the accumulating ADDLs destroy the synapses, the microtubule-associated, anterograde transport regulating τ protein is upregulated, and ORCs progressively signal the nucleus to start increasing the expression of other cell cycle engine kinases, such as Cdk s 2 and 4 and cyclins A, B, D, and E, as well as other replication drivers. Moreover, the ORC 2-5 core complexes are completed with ORC 1 and 6. As the activities of the Cdk s rise, the complexes are induced by Cdk phosphorylation to drive the buildup to the G1-S phase transition and DNA replication. Thus, restarting the build-up to DNA replication is an early ADDL-driven diversion of cell cycle components from their alternative synaptic functions. But DNA replication may or may not be completed; hence, the neuron becomes either aneuploid or fully tetraploid, but never enters mitosis nor, most important of all, does it undergo immediate apoptogenesis (26,29). However, the reactivation of the cell cycle may also include the hyperphosphorylation of the τ protein. This, when phosphorylated, separates from the microtubule trackways and collects into the neurofibrillary tangles, another cytopathological feature of AD and the cause of the failure of the anterograde transport down the axon of components needed for synaptic formation and maintenance, and of the mitochondria needed to fuel it (30).

Herrup and Yang (26) have calculated that neuronal death from cell cycle re-entry is a very slow process that may take as long as 6-12 months to occur. In the meantime, the increasing numbers of post-DNA-synthetic neurons in the high-plasticity parts of the ADDLed brain manage to stay alive and function quasi-normally. They have been likened to the injured ‘undead’ cells of (i) Drosophila imaginal discs prevented from apoptosing by the baculovirus pancaspase inhibitor p35; and (ii) of Caenorhabditis elegans expressing...
loss-of-function mutants of the apoptotic *ced-3* or *ced-4* genes, which cannot initiate developmentally-programmed apoptosis and can thus continue functioning (26,31,32). If the undead ADDLed neurons accumulating in the aging brain are like the undead cells of fly discs or worms, they may become contagious ‘zombies’ that release factors (e.g., *Drosophila* disc cell reaper- and hid-induced JNK that induces the proliferogenic wingless) that stimulate their healthy neighbors to start trying to cycle and, eventually, to join the undead cell pool (26,31,32). However, as the microtubules become unstable and the now hyperphosphorylated up-regulated t protein detaches from the microtubules and aggregates into neurofibrillary tangles, the synapse-provisioning anterograde transport of ORC-induced nuclear messages along the disintegrating microtubular trackways stops (30), and ORC messengers are sequestered in the tangles. Consequently, the suppression of apoptosis weakens and the undead cells become vulnerable to being killed by any further challenges (25,33).

3. The second hit: plaques appear and LOAD development shifts into overdrive

Eventually, the accumulating aggregation-prone Aß 42 fragments reach the point of fibrillization and start forming the insoluble fibrils that make up the neuritic plaques, the hallmarks of AD. In doing so, they deplete the pool of synapse-targeting/destroying ADDLs. As mentioned above, ADDL depletion should slow the progress of the disease as it does in transgenic mice expressing the human ‘Arctic’ mutant Aß (7). However, this is counterbalanced by the cytokines, and by other products emanating from the activated astrocytes and microglial cells surrounding or enmeshed in the plaques, which can give the second hit to the vulnerable undead ADDLed neurons. These pro-inflammatory and/or toxic agents are first released by the brain’s professional macrophages - the microglial cells - as they try to destroy the plaques (34,35). Because Aß 42 moieties activate microglial cell receptors like C1q-Rs and Ca^{2+}-sensing receptors (CASRs) (36,37), these cells typically start producing pro-inflammatory cytokines, such as IFN-γ, IL-1β, and TNF-α, as they surround, infiltrate, and try to destroy the plaques (35,36) (Fig. 2). The astrocytes, which normally have presenilins but do not make BACE1, are now able to process APP and make Aß 42 thanks to microglia-released IFN-γ, which activates their JAK2 and ERK1/2 pathways and thereby stimulates the BACE1 genes of the cells (38-40). However, this triggers a vicious cycle in which the rising exogenous Aßs stimulate the astrocytes (possibly via their CASRs and p75 NTR receptors) to express which the rising exogenous Aßs stimulate the astrocytes of the cells (38-40). However, this triggers a vicious cycle in such as IFN-γ, cells typically start producing pro-inflammatory cytokines, that is further reinforced by the astrocytes’ own IFN-γ-induced signaling from their CASRs - a response MAPKs-stimulating signals from their CASRs - a response that is further reinforced by the astrocytes’ own IFN-γ-induced production of Aßs (41). This cycle can have a major destructive impact on neuronal survival. Thus, in the rat hippocampal CA1 area for example, just a single astrocyte can contact up to 140,000 synapses (42) and is only part of an interconnected network of cells that contact neuronal synapses to form tripartite synapses. In this network, the astrocytes make the cholesterol needed to produce and maintain the synapses, sweep the synaptic clefts clear of neurotransmitters, make the thrombospondin needed for synapse formation, synthesize and recycle glutamate, control ionic homeostasis and neuronal excitability by buffering K^+, respond to released neurotransmitters with Ca^{2+} waves spreading through the ‘astro-network’, and release ‘gliotransmitters’ (ATP, glutamate, D-serine, taurine, TNF-α) that modify synaptic activity (43-51). It goes without saying that anything released by the astrocytes, such as NO and Aßs, can directly hit the contacted neurons.

The Aßs from the IFN-γ-activated BACE1-expressing APP-cleaving astrocytes, closely contacting undead neurons, can now bind to both monomeric and trimeric p75NTR receptors. Were Aßp75NTR complexes able to bind to TrkA co-receptors to form ternary complexes (where Aß would be passed over to and activate the TrkA receptors, as suggested by Barker (52)), a survival signal from the activated TrkA receptor would be generated, counterbalancing any apoptogenic signal emitted from the p75NTR receptor intracytoplasmic ‘death domain’. Regrettably, Aß does not bind to TrkA (53). Thus, Aß released from the IFN-γ-activated astrocytes closely contacting the neurons sends a caspase-mediated apoptogenic signal to the very same neurons via the death domains of the Aß-activated p75NTR neuron receptors (41,54,55).

Pro-inflammatory cytokines, such as TNF-α, IL-1β and IFN-γ, do a lot more. They synergize with endogenous Aß to induce the astrocytes in the neuron-astrocyte clusters to express their NOS-2 enzyme and massively produce NO, which generates a reactive oxygen-species (ROS), the neuron-killing ONOO^- (41,56-58). Indeed, since just a single astrocyte in a hippocampal astrocyte network is connected to a huge number of neurons, the production of large amounts of NO could, in and of itself, inflict extensive damage. Thus, when Aßs exceed a critical upper fibrillization level in an aging or mutant brain with accumulated vulnerable undead neurons, they trigger an accelerating vicious cytocidal cycle initiated by cytokine-activated Aß-producing astrocytes in functional neuron-astrocyte units consisting of pro-inflammatory cytokines, p75NTR receptor apoptogenic signals, and ONOO^- (Fig. 2). Hence, while the insoluble Aß 42 plaques are not correlated to cognitive decline in mice and men, the aggregates of accumulating Aß 42 fibrils give neurons the coup de grâce by activating pro-inflammatory cytokine-producing microglia, as well as astrocytes (Fig. 2).

4. Are the primary cilia of neurons and astrocytes involved in LOAD?

Pyramidal neurons in the hippocampal CA1 and CA3 regions and the granular neurons in the dentate gyrus are endowed with primary or solitary cilia, which can be visualized using rabbit polyclonal antibodies that bind to their particular marker, adenyl cyclase III (ACIII) (59,60). These non-motile cilia protrude like car aerials, not just from neurons but from almost every cell of the body (see ref. lists 60-62). They are busy organelles, with various cell-specific cargoes transported beneath their membranes along their 9 microtubule doublet trackways, toward the tips by a specific kinesin motor and downward to the body of the cell by a specific dynein motor. According to the type of cell from which they protrude, their surfaces are richly endowed with various receptors (e.g., serotonin receptor 6 and/or somatostatin receptor 3 for some...
neurons), signal complexes [patched (Pc)/smoothened (Smo)], and cyclic nucleotide-gated Ca\(^{2+}\) channels activated by the cyclic AMP made by receptor-activated adenylyl cyclase, such as neuronal ACIII (60-62). It appears that astrocytes also have primary cilia, some of which, like those of the neurons, have ACIII (61). Given our frustratingly limited knowledge, at present it is enticing to hypothesize that the primary cilia of neurons, like the primary cilial toggle switches of kidney tubule epithelial cells, cartilage chondrocytes and bone osteocytes (62,63), sense and respond to various agents released from the closely-contacting astrocytes and neighboring neurons (62). The astrocytes probably use their primary cilia to monitor and respond to various neuronal products in order to maintain neurons in functional plastic conditions. Indeed, neuronal plasticity and memory formation may actually be affected by signals from the primary cilia, triggered by factors coming from their associated astrocytes. Thus, finding out how primary cilium signaling affects neuronal function and what happens to the cilium in the ADDLed neuron could add a whole new dimension to our understanding of neuronal signaling and plasticity and of the damaging mechanisms that produce an Alzheimer brain.

5. Conclusion
The model that replaces the plaque-centered model, which has Aß fibrillar plaques as the initiators of LOAD, is one in which the neurons are, at first slowly and then increasingly, stripped of their synapses and disconnected from their intercommunicating network by mounting levels of diffusible dodecamers of their synapses and disconnected from their intercommunicating network by mounting levels of diffusible dodecamers of non-clearable Aß 42s. They are then converted into long-lived quasi-functional undead aneuploid or tetraploid cells, by fibrillization of non-clearable Aß 42. It follows that one key mechanisms before accumulating Aßs can inflict irreversible cognitive decline, to stop the waning of the Aß 42-clearing mechanisms of Alzheimer's disease may actually be affected by signals from the primary cilia, triggered by factors coming from their associated astrocytes. Thus, finding out how primary cilium signaling affects neuronal function and what happens to the cilium in the ADDLed neuron could add a whole new dimension to our understanding of neuronal signaling and plasticity and of the damaging mechanisms that produce an Alzheimer brain.

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