Emerging concepts of how ß-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 ß-(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 *a*myloid-*d*erived *d*iffusible *l*igands (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 maëlstrom 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-1ß and TNF-α. One of these, IFN-γ, causes the astrocytes enwrapping the neuronal synapses to express their ß-secretase (BACE1) genes and produce and release Aß 42, which can kill the closely apposed neurons by binding to their $p75^{NTR}$ 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 ß-(1-42); ACIII, adenylyl cyclase III; AD, Alzheimer's disease or dementia; ADDLs, *a*myloid-*d*erived *d*iffusible *l*igands; APOE, apolipoprotein E; APP, amyloid precursor protein; Arc, activity-regulated cytoskeleton protein; BACE1, ß-secretase; C1q-R, receptor for complement C1q protein; CaMKII, Ca^{2+}-calmodulin-dependent kinase II; CASR, Ca^{2+}-sensing receptor; EOFAD, early-onset familial Alzheimer's disease; IDE, insulin-degrading enzyme; IFN-γ, interferon γ; IL-1ß, interleukin 1ß; 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-ß peptides, human, neurons, astrocytes, microglia, primary cilium, pro-inflammatory cytokines

Contents

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 neuronocidal cytotoxic garbage dumps

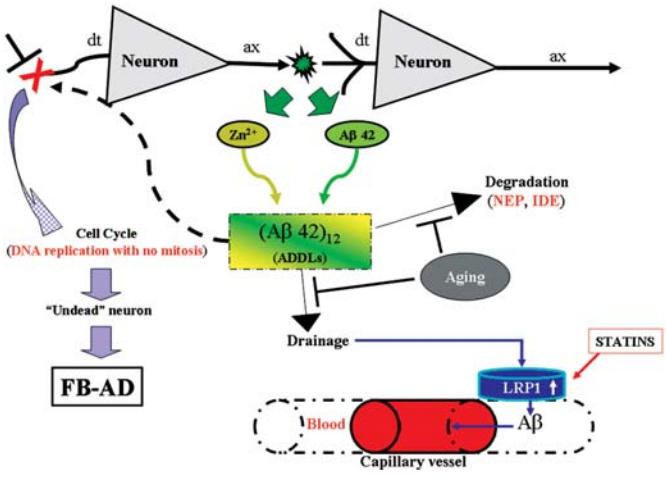

Figure 1. Aß 42 released during normal synaptic activity is habitually cleared via destruction by neuronal and glial proteases and by being dumped into the circulation for disposal by the high capacity transendothelial carrier LRP1. With advancing age, Aß clearance declines and the Aß 42 level rises and is induced by coreleased Zn^{2+} to aggregate into toxic dodecamers called ADDLs, which selectively target and destroy synapses. The resulting disconnection of the neurons from their networks induces them to try to proliferate to reconnect to the network. Neurons can partially or completely replicate their DNA, but cannot enter mitosis or immediately die. Instead, they lapse into a quasi-normal, long-lived (6 months to 1 year) limbo as 'undead' cells. In this state, they are vulnerable to being killed by the flurry of second hits shown in Fig. 2. This initial progression toward late-onset AD should be prevented by stopping the age-dependent decline of the clearance mechanisms by, for example, administering statins that stimulate vascular endothelial cells to make LRP1. Aß 42, amyloid ß-(1-42); ADDLs, *a*myloid-*d*erived *d*iffusible *l*igands; ax, axon; dt, dendrite; FB-AD, full-blown Alzheimer's disease; IDE, insulin-degrading enzyme; LRP1, low-density lipoprotein receptor-like protein; NEP, neprilysin.

(1,2). Aß 42 is chopped out of the membrane-embedded amyloid precursor protein (APP) by serially-acting proteases, first BACE1 and then γ-secretase (PS-1/2; presenilins-1/2) (2). It has long been believed that AD is caused by fibrillar Aß 42-attracting and activating astrocytes and microglial cells, the toxic products of which kill neurons trapped in the fibrillar net. But this 'plaques-centered' model has recently been dethroned because of the weak connection between plaques and cognitive impairment in humans, as well as the unexpected lack of a requirement for Aß 42 plaques to impair memory in transgenic mouse models of AD (1-5). Now, it appears that the culprit is a diffusible pre-plaque dodecamer of Aß 42 fragments released from normally-functioning neurons (6) (Fig. 1). In fact, accelerated Aß 42 fibrillization and plaque formation in transgenic mice expressing the human 'Arctic' Aß 42 mutant locks up, and thus depletes, the pool of soluble oligomers, actually reducing functional deficits (7).

2. The first drivers of sporadic or late-onset Alzheimer's disease (LOAD)

Aß 42 is a normal metabolic product of neurons (8) (Fig. 1) that is secreted into the synaptic cleft along with the neurotransmitters (Fig. 1). Aß 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 Aß 42 (6). The aggregation of the co-released Aß 42 fragments is likely to be driven by the large amounts of divalent cations, in particular Zn^{2+}, released from synaptic vesicles together with glutamate transmitter (9,10) (Fig. 1). It is worth remembering that Zn^{2+} in 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 Zn^{2+} release together with neurotransmitters, by knocking out the transporter-3 that loads Zn^{2+} into synaptic vesicles, reduces plaque deposition. The Aß 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 postsynaptic 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 Ca^{2+}-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 prolongedly stimulating 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 Aß 42 oligomers to start accumulating? The first obvious guess would be Aß 42 overproduction (15). Indeed, the far less common early-onset familial Alzheimer's disease (EOFAD; around 5% of AD cases) is certainly due to Aß 42 overproduction, in which individuals most commonly carry a mutant gene encoding abnormally active γ-secretase that increases the release of Aß 42 fragments from BACE1-pre-processed APP (16). However, it appears that the much slower accumulation of Aß 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-

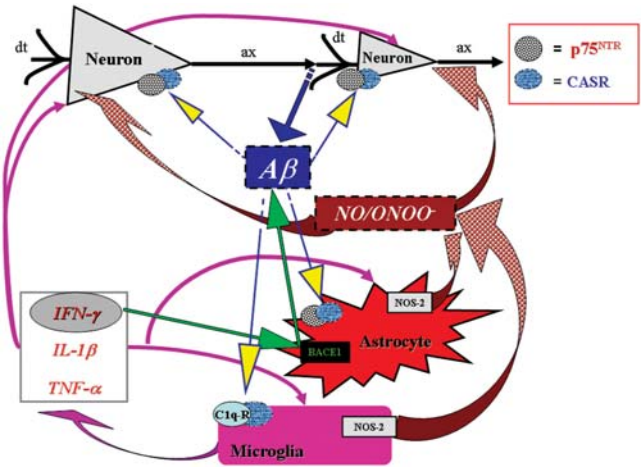

Figure 2. With time, aggregation-prone Aß 42 forms insoluble fibrillar plaques that are the hallmarks of the AD brain. The response of the glial cells to the plaques triggers a maëlstrom of events, including the release of pro-inflammatory cytokines, a vicious cycle of Aß 42 generation by astrocytes contacting the neurons, and the massive production of NO/ONOO$^-$, which combine to give the *coup de grâce* to the vulnerable ADDLed cells. Aß 42, amyloid ß-(1-42); ax, axon; BACE1, ß-secretase; C1q-R, receptor for complement C1q protein; CASR, Ca^{2+}-sensing receptor; dt, dendrite; IFN-γ, interferon γ; IL-1ß, interleukin 1ß; NO, nitric oxide; NOS-2, nitric oxide synthase-2; ONOO$^-$, peroxynitrite; TNF-α, tumor necrosis factor α.

bound glycoprotein neprilysin (NEP) and the cytosolic Zn-metallopeptidase 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 deliver it directly to the closely apposed neurons. Moreover, astrocyte-made cholesterol has an extremely long 5-year half life; a statin treatment that rapidly reduces the level of short-lived circulating cholesterol would not affect brain cholesterol levels so swiftly (21,22). Instead, Dean and Zlokovic (23) have found that 5 μM lovastatin and simvastatin can increase the expression of LRP1 by 3.5 to 4.5-fold in fetal bovine vascular endothelial cells. This suggests that taking one of these drugs each day to prevent or manage hypercholesterolemia could confer the added benefit of thwarting the age-dependent buildup of ADDLs by preventing, in this high-capacity transporter, the age-dependent decline of Aß 42 across the blood-brain barrier (Fig. 1).

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 Cdks 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 Cdks 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 apoptogenic *ced-3* or *ced-4* genes, which cannot initiate developmentally-programmed apoptogenesis 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 τ 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 apoptogenesis 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-1ß, 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 NOS-2, and then activate the nascent NOS-2 protein with 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 $p75^{NTR}$ receptors. Were Aß•$p75^{NTR}$ 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 $p75^{NTR}$ 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 $p75^{NTR}$ neuron receptors (41,54,55).

Pro-inflammatory cytokines, such as TNF-α, IL-1ß 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, $p75^{NTR}$ 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, adenylyl 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 (Ptc)/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 non-clearable Aß 42s. They are then converted into long-lived quasi-functional undead aneuploid or tetraploid cells, by re-starting a cell cycle in a futile effort to restore the seemingly depleted neuronal network. These neurons are ultimately killed by apoptogenic signals from their $p75^{NTR}$ receptors activated by Aßs, and by a massive flood of NO from both the astrocytes, which have been activated by cytokines such as IFN-γ, IL-1ß and TNF-α, and from microglial cell attacking plaques produced by the mounting accumulation and further fibrillization of non-clearable Aß 42. It follows that one key to preventing LOAD is to find drugs that can be administered over many years, from the earliest indication of impending cognitive decline, to stop the waning of the Aß 42-clearing mechanisms before accumulating Aßs can inflict irreversible damage.

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