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Three dimensions of the amyloid hypothesis: time, space and 'wingmen'

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The amyloid hypothesis, which has been the predominant framework for research in Alzheimer's disease (AD), has been the source of considerable controversy. The amyloid hypothesis postulates that amyloid-β peptide (Aβ) is the causative agent in AD. It is strongly supported by data from rare autosomal dominant forms of AD. However, the evidence that Aβ causes or contributes to age-associated sporadic AD is more complex and less clear, prompting criticism of the hypothesis. We provide an overview of the major arguments for and against the amyloid hypothesis. We conclude that Aβ likely is the key initiator of a complex pathogenic cascade that causes AD. However, we argue that Aβ acts primarily as a trigger of other downstream processes, particularly tau aggregation, which mediate neurodegeneration. Aβ appears to be necessary, but not sufficient, to cause AD. Its major pathogenic effects may occur very early in the disease process.

In their original edition of the amyloid cascade hypothesis in 1992, Hardy and Higgins postulated that "amyloid-β protein (Aβ)…is the causative agent in Alzheimer's disease (AD) pathology and that neurofibrillary tangles, cell loss, vascular damage and dementia follow as a direct result of this deposition"[1]. Although two decades of research have revealed many layers of complexity, we will argue that the bulk of data still supports a role for Aβ as the primary initiator of AD pathogenesis. We will discuss data that support the amyloid hypothesis, address some key arguments against the amyloid hypothesis and present an updated framework for AD pathogenesis that attempts to reconcile the existing data. It has become clear that Aβ does not exert its effects in a vacuum and that a complex network of pathologic processes converge to produce the neuropathologic and clinical syndrome that is AD. However, multiple lines of evidence still support the concept that Aβ aggregation has a unique and critical role as the key initiator of AD pathology. By our interpretation of the existing data, Aβ appears to be necessary, but not sufficient, for AD, although it may be necessary and sufficient for cerebral amyloid angiopathy (CAA). This argument over the role of Aβ has important implications regarding the development of therapies, the allocation of research funding, and the focus of public and private research efforts.

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Genetic factors that increase Aβ aggregation cause AD

The strongest data supporting the role of Aβ as disease initiator come from human genetics. Autosomal dominant familial AD (fAD) is caused by mutations in three genes, *APP*, *PSEN1* and *PSEN2*, which are all integrally involved in Aβ production[2]. *APP* encodes the amyloid precursor protein, which is the precursor to Aβ, whereas *PSEN1* and *PSEN2* encode Presenilin 1 and 2, catalytic subunits of the γ-secretase complex that cleaves APP to generate Aβ. fAD mutations lead to accelerated accumulation of Aβ plaques and cause early-onset dementia, CAA or both[2–6]. Duplication of the *APP* locus on chromosome 21 increases Aβ production through increased gene dosage and causes age-related dementia with brain parenchymal Aβ deposits and CAA[7–9]. Down syndrome patients, who have trisomy 21 and thus have an extra copy of *APP*, develop age-related amyloid plaque deposition and dementia in an APP-dependent manner[10]. In general, pathogenic mutations in all three genes are specifically clustered in regions that serve to increase the level of amyloidogenic APP cleavage by β- or γ-secretase or increase the relative production of amyloidogenic $A\beta_{1-42}$ versus shorter Aβ species[2,11–13]. Notably, several APP mutations that reside in the middle of the Aβ coding region have been identified that cause fAD or CAA by increasing the aggregation propensity or inhibiting degradation of the Aβ peptide and do not affect Aβ production[5,6]. This suggests that it is Aβ aggregation, rather than some other alteration in APP processing or presenilin function, which instigates fAD. Thus, fAD provides an opportunity to examine a pure Aβ-driven disease in relatively young, often healthy patients. Consistent with the amyloid hypothesis, fAD patients first develop fibrillar amyloid plaques, then subsequently accumulate neurofibrillary tau pathology, neuronal loss and dementia[14,15]. Thus, it is relatively easy to argue that the amyloid hypothesis, or a variant of it, is generally true for fAD. Notably, fAD-linked mutations clearly affect age of onset, but do not strongly affect rate of progression of symptomatic disease. Although some of these mutations cause large increases in the production of Aβ, they do not substantially accelerate progression, suggesting that Aβ serves as an initiator of pathology, but not a major mediator of downstream neurodegeneration[16].

Fortunately, fAD is extremely rare, and the vast majority of AD cases are considered to be sporadic (sAD). The genetics of sAD are more complex, and the strongest genetic risk factor is *APOE*. The *APOE* ε4 allele increases risk for AD and CAA, and the ε2 allele decreases risk for AD relative to the ε3 allele[17,18]. Approximately 20–25% of the population carries at least one copy of ApoE4, which increases the risk of AD by ~4-fold (as compared to those with the more common ApoE3/E3 genotype), whereas 2% of the population carries two E4 alleles, imparting a ~12-fold increased risk[19]. Although ApoE4 appears to exert a variety of effects in the brain, it is a strong

modulator of Aβ pathology, and knock-in mice expressing human ApoE4 along with FAD-linked APP transgenes have greatly increased Aβ plaque pathology and reduced Aβ clearance[19,20], whereas human ApoE2–expressing mice have decreased Aβ plaque pathology[21]. Numerous studies have demonstrated accelerated amyloid plaque accumulation in human ApoE4 carriers, and preclinical studies have shown that ApoE influences Aβ metabolism, with ApoE4 promoting amyloid aggregation and deposition[20–25]. Reducing ApoE levels, either genetically or with antibody therapy, also reduces Aβ plaque burden in mice[26–29]. Furthermore, human ApoE4 is associated with pathogenic changes in cerebrospinal fluid (CSF) $A\beta_{42}$ in cognitively normal subjects, but not changes in tau, again suggesting that ApoE4 exerts its main effects in AD by modulating Aβ upstream of tau[23,30]. Thus, the predominant genetic risk factor for sAD clearly exacerbates Aβ aggregation. Recently, a rare protective mutation in the APP gene, which diminishes amyloidogenic Aβ production, has been identified in humans and linked to a decreased risk of developing AD[31], providing another strong genetic link between Aβ and sAD.

Tau pathology and neurodegeneration: anatomical discord

One key argument against the amyloid hypothesis is based on the poor correlation, both temporally and anatomically, between Aβ plaque deposition, neuronal death and clinical symptoms in sAD. Neuroanatomically, fibrillar Aβ deposition occurs first and most severely in regions such as the precuneus and frontal lobes, whereas neuronal death begins and occurs most readily in the entorhinal cortex and hippocampus, regions with relatively few Aβ plaques[32,33]. Tau pathology correlates much more closely with neuronal loss, both spatially and temporally, than amyloid plaques[33–36]. According to the work of Braak and others, Aβ pathology appears to begin in the cortex and spread inward, whereas tau pathology exhibits an opposite progression[32,37]. This has prompted critics to suggest that Aβ must not mediate neurodegeneration in AD and is merely a bystander. If this were true, one would expect that, in fAD, in which disease pathogenesis is more clearly driven by Aβ, that there would be a stronger anatomical correlation between plaque deposition and neuronal loss. This is not the case, as fAD pathology closely resembles that of sAD, with Aβ plaques anatomically disconnected from areas of severe neuronal loss[14,15]. Thus, genetic data again demonstrate that it is possible for Aβ to drive tau pathology and neuronal loss without inherently obvious anatomic colocalization between plaques and areas of neurodegeneration. This anatomic disconnect between fibrillar Aβ plaques, neurofibrillary tangles and neuronal loss is still not fully explained, but appears to be consistent between fAD and sAD.

In sAD, the relationship between Aβ and tau pathology is complex, but ultimately supportive of the amyloid hypothesis. Aggregated, phosphorylated tau pathology is present in the brainstem and entorhinal cortex of young, normal, asymptomatic people and precedes the onset of amyloid accumulation[38]. With age, hippocampal neurofibrillary tau pathology is nearly ubiquitous, but remains confined to limbic regions in cognitively normal, amyloid-free individuals[39,40]. However, tau appears to spread into neocortical regions almost solely in people with coexistent Aβ pathology[39,41]. These individuals generally go on to develop AD dementia. In several studies examining the cognitively normal elderly or patients with mild cognitive impairment or AD, widespread cortical tau pathology (Braak stage ≥3) was commonly observed in patients with concomitant Aβ plaques; it was never observed in those without plaques, suggesting that the presence of Aβ aggregation is required for the appearance of high-grade cortical tau pathology[39,41,42]. Furthermore, although hippocampal tau phosphorylation is commonly observed in cognitively normal adults, studies have found no evidence of neuronal loss in these regions with normal aging[43,44]. However, neurodegeneration does occur prominently in these regions in AD patients with amyloid plaques[43,44], suggesting that tau-mediated toxicity again requires some trigger from Aβ. Accordingly, 'neuritic plaques', which are amyloid plaques with associated neurofibrillary tau pathology, correlate more closely with neuronal loss and dementia in AD than either plaques or tangles alone[45]. These neuropathologic observations support the idea that while fibrillar Aβ and neurofibrillary tau pathologies are anatomically separated, Aβ aggregation appears to somehow result in accelerated neurofibrillary tau pathology and neuronal toxicity. As noted above, fAD mutations, which directly influence Aβ aggregation, cause tau pathology that is similar to that seen in sAD and correlates closely with neuronal death. However, although mutations in the gene encoding tau (*Mapt*) cause neurodegenerative disease, they do not induce Aβ pathology[46,47]. Accordingly, polymorphisms in *Mapt* are associated with increased CSF phospho-tau levels, but only in patients with CSF evidence of Aβ pathology[48]. Thus, human neuropathological data supports a key tenet of the amyloid hypothesis: that Aβ triggers or exacerbates downstream tau pathology, although it appears to do so without anatomic colocalization.

Preclinical data from cell culture and animal experiments also support Aβ as the initiator of tau pathology and downstream neuronal injury. Exposure of primary mouse neuronal cultures to aggregated Aβ species, derived either from synthetic Aβ or isolated from human brain, induces tau phosphorylation, neuritic damage and dendritic spine loss[49,50]. Notably, tau knockout neurons were resistant to neuritic degeneration induced by synthetic or human-derived Aβ species, whereas tau overexpression exacerbated Aβ-induced damage[51,52]. It is notable that a developmental effect of tau deletion cannot be excluded in these and other experiments, as experiments using postnatal tau deletion have not been reported. In human neurons grown in a matrigel culture system, expression of fAD mutation–bearing APP elicited extracellular Aβ aggregation and caused subsequent tau pathology[53]. This tau pathology could be blocked with β- or γ-secretase inhibitors, illustrating the dependence on APP cleavage. In mice, coexpression of mutant human *APP* and *MAPT* caused increased severity and spread of neurofibrillary tau pathology, but did not alter Aβ pathology, again demonstrating that Aβ aggregation appears upstream of tau[54,55]. Accordingly, injection of synthetic Aβ fibrils into the brain of mutant human tau–expressing mice led to markedly increased tau pathology[56]. Aβ also impairs axonal transport in cultured neurons in a tau-dependent manner, whereas behavioral deficits observed in human APP–expressing mice were alleviated by tau deletion. Again, in these experiments, tau had no effect on Aβ pathology[57,58]. In total, substantial preclinical data demonstrates that Aβ species can directly trigger tau pathology in cells and mice, whereas human neuropathologic data supports a model in which tau pathology does not spread into the neocortex until after substantial Aβ pathology has developed. Aβ aggregation appears to somehow be triggering an exacerbation of tauopathy, which may in turn cause neuronal dysfunction and death. Although Aβ and tau pathology are anatomically separate in the AD brain, considerable evidence demonstrates that Aβ can still trigger tau pathology and neurodegeneration in regions with minimal fibrillar Aβ pathology.

Induction of toxic protein misfolding by Aβ is not isolated to tau. Many individuals with either fAD or sAD also develop α-synuclein aggregation in limbic and cortical regions, suggesting that Aβ may also accelerate synuclein pathology[59,60]. Accordingly, preclinical models demonstrate that Aβ can induce synuclein pathology[61,62]. TDP-43

pathology is also commonly seen in AD patients and may contribute to neurodegeneration[63]. Thus, the ability of Aβ to induce misfolding of other toxic proteins, such as tau, synuclein and TDP-43, may be critical for AD pathogenesis, although the exact contribution of some of these protein aggregates is not fully understood.

Tau pathology and neurodegeneration: temporal discord

Temporal discrepancy between the appearance of amyloid plaques, tau tangles, neuronal loss and clinical dementia is another point of frequent contention with the amyloid hypothesis. Many clinically asymptomatic older individuals are known to have substantial amyloid plaque pathology, either at autopsy or by PET imaging. Critics claim that this shows that Aβ does not cause dementia. Again, it should be noted that fAD patients also develop fibrillar amyloid plaque pathology years before symptom onset, but clearly have Aβ-driven disease[15]. Large cross-sectional biomarker studies suggest that amyloid plaque pathology is not asymptomatic, but instead represents preclinical AD, although the pathologic 'incubation time' between the appearance of plaques and onset of tau pathology can be several years, and the time to onset of clinical dementia can be over a decade[64–68]. Emerging longitudinal evidence from several groups supports this concept, as asymptomatic individuals with biomarker evidence solely of amyloid plaques (either by CSF or PET amyloid imaging markers, with no tau or other cell injury markers) are ~4-fold more likely than those without plaques to develop clinical dementia within the ensuing 2–7 years, although the length of follow up is likely still too short to fully appreciate this risk[66–71]. If amyloid plaques are present and there is biomarker evidence of brain injury (such as decrease glucose uptake, hippocampal atrophy or elevated CSF tau), risk of conversion to clinical dementia is even higher, with relative risks exceeding 10 (refs. 66,67,71,72). People who are ostensibly cognitively normal but have amyloid plaques also exhibit very subtle cognitive deficits on detailed neuropsychometric testing and accelerated hippocampal atrophy when compared with plaque-free controls, suggesting that Aβ deposition may cause a mild, but progressive, pathological state before the onset of more rampant neurodegeneration with tauopathy and synucleinopathy[73,74]. Although not conclusive evidence of the central role of Aβ in AD, the bulk of human biomarker data supports the amyloid hypothesis, as Aβ-related changes precede tau biomarker changes and cognitive markers by years, are associated with mild, but progressive, neuropathology, and are predictive of subsequent dementia.

How does Aβ initiate AD pathology?

So how do we reconcile this anatomic and temporal discordance between fibrillar Aβ pathology, tau aggregation and neurodegeneration in both fAD and sAD? One explanation is that in addition to fibrillar plaques, oligomeric forms of Aβ may mediate further pathology in AD. Indeed, a variety of cytotoxic oligomeric Aβ species have been identified in AD brain lysates which can exert a wide variety of pathogenic effects *in vitro* and in the mouse brain[75–77]. These oligomeric Aβ species do not necessarily correlate temporally or spatially with plaques, and can be purified from brain regions which are subject to intense neuronal loss, such as the hippocampus[78–80]. Indeed, there is some evidence that the presence of Aβ oligomers predicts the presence of dementia more accurately than plaque burden[78,80]. In humans, Aβ oligomers appear to accumulate with age, and are correlated with the appearance of tau pathology[81,82]. Mice which accumulate Aβ oligomers but not fibrillar plaques also develop synaptic damage, inflammation and cognitive impairment[83,84] However, it is difficult to rectify the oligomer hypothesis with the long prodromal phase of human AD during which plaques are evident but significant neurodegeneration is not, or to explain why oligomers would not lead to more significant neuronal loss in animal models of Aβ deposition. Furthermore, the specific species of Aβ which mediate downstream pathology remain an open question[75,85]. While the direct neurotoxicity of Aβ oligomers *in vivo* is unclear, these species have been shown to directly initiate tau phosphorylation both *in vitro* and *in vivo*[52,83,86]. At a molecular level, oligomeric Aβ may initiate tauopathy through specific signaling events in neurons, such as activation of the Src kinase Fyn[58,87,88], inducing proteases which modify tau[89], or activating specific tau-targeting kinases[90], though none of these have been adequately demonstrated in humans.

Given increasing evidence that Aβ, tau and α-synuclein aggregates all have prion-like properties and can potentially seed normal forms of these proteins and spread through the brain, it may also be important to investigate the ability of Aβ species to exacerbate the prion-like spread of tau and synuclein[91–96]. Finally, Aβ could cause large-scale alterations in the brain's ability to maintain proper protein quality control (proteostasis), eventually prompting aggregation of tau, synuclein and other intracellular proteins and causing toxicity[97–99]. Proteostatic failure could explain α-synuclein in the brains of fAD patients, many of whom are too young to have accumulated such deposits as a function of age, or coexistent α-synuclein and TDP-43 aggregates in sAD patients[63]. Aβ-induced aggregation and subsequent propagation and cross-templating of other toxic proteins could overwhelm proteostatic mechanisms. As many cytoprotective and proteostatic systems in neurons and glial decline with age, aging might thus set the stage for disseminated Aβ toxicity[100,101]. Other age-related effects or comorbid pathologies (such as diabetes, head trauma, oxidative stress, inflammation, vascular factors, or even sleep deprivation) might influence the rate of Aβ aggregation and accumulation, accelerate the rate at which Aβ triggers downstream pathology, or directly exacerbate downstream pathology, thereby modulating the onset of dementia.

Aβ does not operate in a vacuum

A shortcoming of the initial amyloid hypothesis is the suggestion that Aβ itself could directly cause the panoply of pathologic changes observed in AD. Aβ critics have frequently and correctly pointed out the many other destructive phenomena, from oxidative stress to impaired autophagy, that occur in AD brain. Certainly when one uses the clinical endpoint of cognitive impairment, many factors appear to accelerate AD, cerebrovascular disease being foremost among them. The relationship between these factors, Aβ and neurodegeneration is unclear. Mice harboring fAD mutations develop neuroinflammation, oxidative stress, mitochondrial dysfunction and other pathologic alterations that appear to be downstream changes that indicate Aβ-induced neuronal or glial injury, although some of these markers appear before substantial plaque deposition[102–104]. However, preclinical data suggest that neuronal stressors, such as oxidative and nitrative stress[105,106], mitochondrial dysfunction[107] and inflammation[108], can also directly influence APP metabolism and Aβ accumulation, indicating the existence of a possible feedforward mechanism by which Aβ-induced injury can facilitate Aβ production, or by which other stressors, such as ischemia or trauma, might exacerbate Aβ production and toxicity.

Although it may be tempting to implicate these other processes as the causative factors in AD, it is important to consider that Aβ plaque pathology is uniquely indicative of the clinical syndrome of AD, whereas oxidative damage, inflammation, mitochondrial dysfunction and other pathogenic markers are observed in many neurologic diseases. Furthermore, there is no strong evidence from humans that genetic mutations that increase oxidative stress, inflammation, mitochondrial

dysfunction or inhibit autophagy lead to Aβ pathology in the brain. Risk factors such as smoking can have Aβ-dependent and Aβ-independent effects on AD pathogenesis, as smoking causes systemic oxidative stress, vascular injury and may increase Aβ accumulation[109,110]. Similarly, polymorphisms in *TREM2* affect microglial activation and increase the risk of AD, yet *TREM2* hemizygous mice have no increase in Aβ plaques, suggesting that microglial dysfunction could influence AD pathogenesis independently of the amount of Aβ accumulation[111]. In both cases, it is likely that these risk factors influence pathogenic mechanism downstream of Aβ and are not unique to AD. Accordingly, smoking increases the risk of many diseases of the brain, including vascular dementia, whereas *TREM2* likely does the same (it has recently been linked to increased risk of ALS, frontotemporal dementia and Parkinson disease)[112,113]. It would be foolish to argue that these many processes are not important in AD, as they clearly contribute to disease pathogenesis and might provide excellent therapeutic targets. But the existence of these other mechanisms of neural injury in AD does not mean that they are causative, nor does it negate the central importance of Aβ.

Another alternative concept of AD pathogenesis suggests that APP cleavage is indeed critical to AD, but that APP fragments other than Aβ are the pathogenic species. Mice with inducible expression of APP develop Aβ plaques and cognitive impairment. The cognitive impairment resolved in part when the APP transgene expression was turned off, despite persisting Aβ plaques[114]. Conversely, mice that express Aβ from a truncated transgene (and not full-length APP) accumulate multiple Aβ species as well as plaques, but do not develop cognitive impairment[115]. Several studies have implicated the APP β-C-terminal fragment (β-CTF) as a possible mediator of cognitive impairment in APP-overexpressing mice[116,117]. However, it should be noted that antibodies targeting Aβ reduce plaque burden and rescue cognition in APP transgenic mice without eliminating plaques[118]. Moreover, as mentioned earlier, APP mutations in the Aβ coding region that enhance Aβ aggregation without influencing APP cleavage still cause human fAD[5,6]. Thus, the data implicating alternative APP fragments in AD is intriguing, but it is still largely restricted to mouse models and does not discount the contribution of Aβ.

APP is a complex molecule with a variety of neurobiological functions[119]. Thus, it is important to note that cognitive and behavioral changes in APP-overexpressing mice are not fully correlated with Aβ pathology and are likely to be greatly influenced by both the APP transgene and marked overexpression from the promoter used. Cognitive impairment in APP mice can appear before, after or without substantial Aβ deposition, and is generally reversible[85,118]. In human AD, however, cognitive decline is notable years or even decades after extensive plaque pathology accumulates, is almost always associated with the onset of tau pathology and neurodegeneration, and has not been shown to be reversible. Thus, the nature of cognitive impairment in mouse APP models and humans may be fundamentally different, and mouse results should be interpreted carefully.

What initiates Aβ aggregation?

If Aβ is the initiator of AD pathology, what initially triggers the aggregation and accumulation of Aβ in sAD? How is this related to age? Currently, we have no iron-clad explanation for why Aβ initially accumulates and forms plaques in sAD or why this pathology occurs late in life. Aβ might accumulate in a concentration-dependent manner throughout life, with increased neuronal activity in certain brain regions leading to excessive Aβ production, ultimately causing aggregation and seed formation that then propagates[91,120]. Factors that enhance Aβ production or aggregation or that suppress Aβ clearance could contribute to this over a lifetime. Impaired sleep-wake cycle, for example, could contribute to gradual Aβ accumulation by promoting a higher level of neuronal Aβ release and by disrupting normal diurnal oscillations in extracellular Aβ levels[121]. Sleep deprivation could also impair the clearance of Aβ from the brain by bulk fluid movement, or 'glymphatic' flow[122]. As another example, age-related oxidative and nitrative stress could also exert a gradual influence on Aβ production by altering secretase function[105,106] and by promoting Aβ aggregation through oxidative modification of the peptide[123,124]. Another possibility is that age-related decline in the clearance of Aβ causes initial Aβ accumulation. Glial uptake of Aβ, as well as the aforementioned glymphatic bulk-flow removal of proteins from the brain, both decline with age[125,126]. Studies in humans have shown diminished Aβ clearance in AD patients[127], although this has not yet been demonstrated to precede plaque deposition. Age-related disturbances in proteostasis and neuronal stress response signaling might also shift the balance of Aβ metabolism toward aggregation[100,101,128].

We propose a model in which Aβ serves as the primary initiator of AD pathogenesis (**Fig. 1**). In our model, Aβ levels exist in a carefully orchestrated homeostasis throughout life, and factors during middle age that disturb that balance facilitate Aβ aggregation. These factors include increased synaptic activity and sleep disruption, which lead to increased Aβ release, psychological stress (which can increase Aβ production via different mechanisms), the function or genotype of proteins (for example, ApoE, Lrp1 and SorL1) that regulate Aβ trafficking and clearance, age-related declines in Aβ clearance mechanisms, and cellular stressors such as reactive oxygen species and ischemia that might facilitate Aβ production or aggregation. As Aβ homeostasis is lost, oligomeric and fibrillar Aβ species begin to accumulate, and the first plaques appear, providing the first biomarker evidence of AD in humans, although the patient remains clinically asymptomatic. After several years of Aβ aggregation, Aβ somehow triggers the acceleration of AD-type tau pathology, as neurofibrillary tangles begin to spread outside the limbic system and into the neocortex, prompting increases in neurodegeneration that are reflected as an increase in CSF tau and p-tau levels as well as other neuronal markers (for example, VILIP-1)[72]. This 'tau trigger' event is still poorly understood and may be mediated by the appearance of particular toxic Aβ species, the activation of an intermediary signaling process or kinase cascade, or changes in the innate immune system. These events may lead to a breaking point at which the proteostatic capacity of the brain has been overwhelmed, facilitating tau aggregation and spreading. Aggregation of other toxic proteins such as synuclein and TDP-43 may also begin at this stage. At this point, the cascade is set in full motion, as neuronal loss, oxidative damage, inflammation and clinical symptoms become evident, and neutralizing or removing Aβ is less likely to have a major effect. Age-related factors and comorbid pathologies likely contribute to the occurrence of AD by injuring neurons in parallel with Aβ and tau, thereby accelerating the appearance of symptoms, although some such factors may directly regulate Aβ levels or affect the Aβ-tau interaction. Thus, preventative strategies for AD would focus on treatment of conditions that promote Aβ accumulation in middle age (for example, sleep disorders). Anti-Aβ therapies would need to be delivered as early in the process as possible, whereas the appearance of positive Aβ biomarkers would be most helpful for timing the initiation of therapies targeting downstream pathologies, such as anti-tau agents.

Has the amyloid hypothesis been tested pharmacologically?

Mention of AD therapeutics elicits one of the more recent arguments against the amyloid hypothesis: the failure of clinical trials targeting Aβ. The possibility that Aβ initiates pathology very early

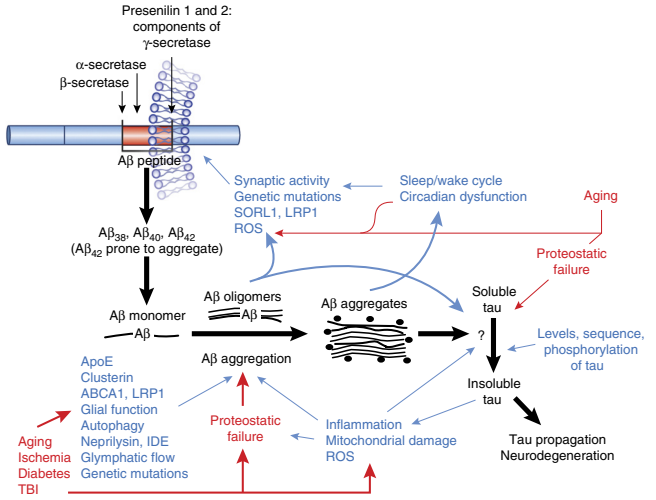

Figure 1 An updated framework of the amyloid hypothesis. The black arrows illustrate the processing of APP by β- and γ-secretases to yield Aβ species, which subsequently aggregate, ultimately triggering tau aggregation and downstream toxicity. Blue text and arrows illustrate proposed modifiers of the Aβ cascade, and red text and arrows show the influence of aging and comorbid pathologies. Note that several feedforward cycles are hypothesized, including one involving disturbed sleep promoting Aβ production (and perhaps Aβ clearance, although not depicted), whereas Aβ aggregation in turn disrupts sleep cycles. Multiple factors, from aging to oxidative stress, contribute to proteostatic failure, which in turn promotes aggregation of Aβ, tau and likely other toxic proteins. Many of the Aβ-modifying factors interact with each other (such as ApoE modulating inflammation), although this is not depicted. IDE, insulin-degrading enzyme; ROS, reactive oxygen species; TBI, traumatic brain injury.

in the disease suggests that only early anti-Aβ therapy is likely to be effective, and, to date, no completed trials have attempted therapy early in the Aβ cascade. Furthermore, Aβ target engagement has not yet been adequate to truly test the amyloid hypothesis. The Elan AN1792 active Aβ immunization study (which was halted as a result of several cases of meningoencephalitis) is often cited as an indictment of the amyloid hypothesis, as several remaining patients were followed after immunization and failed to show any clinical benefit despite evidence of reduced plaque burden at autopsy[129]. However, these patients were immunized late in the course of disease, well after dementia was clinically apparent, and the small sample size in a phase I trial primarily assessing safety that was aborted after only a few vaccinations at most makes it difficult to draw any firm conclusions from this work. If one insists on considering this data, it should be noted that analysis of this same cohort did show a modest decrease in tau pathology, while surviving immunized participants had a slight improvement in functional outcomes, despite late treatment initiation[130–132]. Ongoing studies, however, may put the amyloid hypothesis to a much more rigorous test. The Dominantly Inherited Alzheimer Network (DIAN) treatment trial and Alzheimer's Prevention Initiative (API) should address the role of Aβ in fAD most directly, as both are designed to test if anti-amyloid therapy in primarily presymptomatic fAD patients can prevent pathologic changes and dementia[133,134]. This represents the earliest therapy that is currently possible and employs a patient population with more 'pure' Aβ-driven disease. However, one concerning possibility that arises from the Aβ as initiator hypothesis is that the appearance of fibrillar Aβ pathology could represent a point in the pathogenic cascade that is already too late for effective anti-Aβ therapy. Ultimately, prior prevention of Aβ deposition could prove to be the most effective treatment to target Aβ, but whether such an approach will be tested is unclear. These studies, along with similar trials in sAD, such as the A4 trial, may dictate, in part, the future of Aβ-targeted therapies.

There are also several ongoing trials in very mild and mild dementia resulting from AD using either BACE inhibition or anti-Aβ antibodies that will also be very important to evaluate. Considering the therapeutic regimens employed in diseases such as diabetes, coronary atherosclerosis and cancer, it seems reasonable that a multi-target therapeutic approach will be needed for AD, with the selection of targets dictated by the stage of the pathology. Thus, regardless of the accuracy of the amyloid hypothesis, target elucidation and therapy development for a variety of pathogenic processes in AD, from tau aggregation to microglial activation to mitochondrial dysfunction, is critical.

Conclusions

In the past 22 years, it has become clear that the idea that Aβ causes AD via a simple, linear model of toxicity is very likely incorrect. Our interpretation of the data places Aβ not as the primary direct neurotoxin that itself alone causes AD, but rather as the initiator of a complex network of pathologic changes in the brain, many of them tau-dependent, that culminates years later in neurodegeneration. To successfully forestall the progression of this cascade, multiple therapies targeting several nodes of the neurodegenerative network may be needed, and the timing of these therapies in the disease progression will likely be critical. Many important questions remain to be answered.

What instigates Aβ aggregation in sAD? Are there earlier precipitating events that precede Aβ aggregation and are related to age or other disease processes? How can we prevent this?

How does Aβ aggregation trigger spread of tau and synuclein pathology in the human brain? Are there specific therapeutic targets to prevent this process?

At what point in the cascade is it too late to intervene by targeting Aβ? Once Aβ plaques are apparent by imaging or CSF studies, has Aβ already triggered the critical downstream cascades?

Which downstream pathologies are legitimate therapeutic targets and which are epiphenomena? Post-mortem AD brain tissue reveals a dizzying array of cellular and biochemical alterations, but which can be successfully targeted to mitigate disease?

What is the pathogenesis of AD-like dementias without amyloid plaques (such as suspected non-amyloid pathology, SNAP), or 'tangle-predominant AD'? Are these new subtypes of dementia that appear clinically similar to AD or biologically separate entities that will require unique therapeutic approaches? If there are no plaques, should we still call it AD?

The answers to these questions, as well the outcomes of the many anti-Aβ therapeutic trials currently in progress, should bring us closer to a comprehensive understanding of the role and therapeutic potential of Aβ in AD, and guide the development of the next generation of AD therapeutic regimens.

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The authors declare competing financial interests: details are available in the online version of the paper.

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