



The Amyloid Hypothesis of Alzheimer's Disease: Progress and Problems on the Road to Therapeutics

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It has been more than 10 years since it was first proposed that the neurodegeneration in Alzheimer's disease (AD) may be caused by deposition of amyloid β -peptide ($A\beta$) in plaques in brain tissue. According to the amyloid hypothesis, accumulation of $A\beta$ in the brain is the primary influence driving AD pathogenesis. The rest of the disease process, including formation of neurofibrillary tangles containing tau protein, is proposed to result from an imbalance between $A\beta$ production and $A\beta$ clearance.

Amyloid β -peptide ($A\beta$), the sticky peptide prominent in the brain plaques characteristic of Alzheimer's disease (AD), was first sequenced from the meningeal blood vessels of AD patients and individuals with Down's syndrome nearly 20 years ago (1, 2). A year later, the same peptide was recognized as the primary component of the senile (neuritic) plaques of AD patient brain tissue (3). These discoveries mark the beginning of the modern era of research on this common, devastating neurodegenerative disease. The subsequent cloning of the gene encoding the β -amyloid precursor protein (APP) and its localization to chromosome 21 (4–7), coupled with the earlier recognition that trisomy 21 (Down's syndrome) leads invariably to the neuropathology of AD (8), set the stage for the proposal that $A\beta$ accumulation is the primary event in AD pathogenesis. In addition, the identification of mutations in the APP gene that cause hereditary cerebral hemorrhage with amyloidosis (Dutch type) showed that APP mutations could cause $A\beta$ deposition, albeit largely outside the brain parenchyma (9, 10).

Soon, the first genetic mutations causing AD were discovered in the APP gene (11–14). The contemporaneous discovery that $A\beta$ was a normal product of APP metabolism throughout life and could be measured in culture medium, cerebrospinal fluid, and plasma (15–17) allowed scientists to quickly establish the biochemical abnormalities caused by APP mutations. Most of the mutations cluster at or very near the sites within APP that are normally cleaved by proteases called the α -, β -, and γ -secretases. In accordance with this, these mutations promote

generation of $A\beta$ by favoring proteolytic processing of APP by β - or γ -secretase (18–20). Furthermore, APP mutations internal to the $A\beta$ sequence heighten the self-aggregation of $A\beta$ into amyloid fibrils (21). These exciting developments provided a genetic framework for the emerging amyloid hypothesis (Fig. 1) (22, 23).

In the past 10 years, bolstered particularly by the cloning of the presenilin (PS) proteins (24, 25) and the demonstration that AD-causing mutations in PS1 and PS2 also enhance the processing of APP to form amyloidogenic $A\beta$ (26), the amyloid hypothesis has become the focus of much AD research. Now that therapies based on this idea are beginning to enter human trials, it is important that we critically reexamine the amyloid hypothesis and address its strengths and weaknesses, both real and perceived.

Recent Progress Supports the Amyloid Hypothesis

In addition to the cloning of PS1 and PS2 and the discovery that they alter APP metabolism (27–29) through a direct effect on the γ -secretase protease (30, 31) (Fig. 2), there have been four conceptually important observations that strongly support the amyloid hypothesis. First, mutations in the gene encoding the tau protein cause frontotemporal dementia with parkinsonism (32–34). This neurodegenerative disorder is characterized by severe deposition of tau in neurofibrillary tangles in the brain, but no deposition of amyloid. The clear implication is that even the most severe consequences of tau alteration—profound neurofibrillary tangle formation leading to fatal neurodegeneration—are not sufficient to induce the amyloid plaques characteristic of AD. Thus, the neurofibrillary tangles of wild-type tau seen in AD brains are likely to have been deposited after changes in $A\beta$ metabolism and initial plaque formation, rather than before (35). Second, transgenic mice overexpressing both

mutant human APP and mutant human tau undergo increased formation of tau-positive tangles (as compared with mice overexpressing tau alone), whereas the structure and number of their amyloid plaques are essentially unaltered (36). This finding suggests that altered APP processing occurs before tau alterations in the pathogenic cascade of AD, a notion bolstered by the recent observation that in mouse hippocampal primary neuronal cultures, $A\beta$ toxicity is tau dependent (37). Third, crossing APP transgenic mice with apolipoprotein E (apoE)-deficient mice markedly reduces cerebral $A\beta$ deposition in the offspring (38), providing strong evidence that the pathogenic role of genetic variability at the human apoE locus (39) is very likely to involve $A\beta$ metabolism. And fourth, growing evidence indicates that genetic variability in $A\beta$ catabolism and clearance may contribute to the risk of late-onset AD (40–44). Taken together, these four findings are consistent with the notion that cerebral $A\beta$ accumulation is the primary influence in AD and that the rest of the disease process, including tau tangle formation, results from an imbalance between $A\beta$ production and $A\beta$ clearance.

Concerns with the Amyloid Hypothesis

Although the amyloid hypothesis offers a broad framework to explain AD pathogenesis, it is currently lacking in detail, and certain observations do not fit easily with the simplest version of the hypothesis. The most frequently voiced objection is that the number of amyloid deposits in the brain does not correlate well with the degree of cognitive impairment that the patient experienced in life. Indeed, some humans without symptoms of AD have many cortical $A\beta$ deposits. However, the latter are almost exclusively diffuse forms of amyloid plaques that are not associated with surrounding neuritic and glial pathology. Such diffuse $A\beta$ deposits may be analogous to early fatty streaks of cholesterol that are the harbingers of mature, symptom-producing atherosclerotic plaques. Moreover, the degree of dementia in AD correlates much better with $A\beta$ assayed biochemically than with histologically determined plaque counts, and the concentration of soluble $A\beta$ species (which are invisible to immunohistochemistry) appears to correlate with cognitive impairment (45–48).

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Another concern arises from the fact that all AD-causing mutations in APP, PS1, or PS2 increase A β deposition, yet the degree to which a particular mutation affects A β production in cell culture shows no simple correlation with the age at which it first produces symptoms (29, 49). Indeed, some PS mutations that strongly increase A β metabolism seem to be associated with special symptoms such as spastic paraparesis (weakness affecting the lower extremities) (50–54) and the occurrence of large “cotton wool” plaques, rather than with a particularly early AD onset. Furthermore, in cell culture models of AD, the A β -elevating effects of PS mutations seem to be similar to those of the COOH-terminal APP mutations (26); however, the age at which symptoms appear in the latter cases, but not the former, is accelerated by inheritance of apoE4 alleles (55). The reasons for these phenotypic discrepancies are not clear, but they may relate to the fact that cell culture systems do not adequately reflect the complexity of A β economy in the human brain.

In considerable part, the amyloid hypothesis remains controversial because a specific neurotoxic species of A β and the nature of its effects on neuronal function have not been defined *in vivo*. However, several lines of evidence have converged recently to demonstrate that soluble oligomers of A β , but not monomers or insoluble amyloid fibrils, may be responsible for synaptic dysfunction in the brains of AD patients and in AD animal models. Metastable intermediates in the formation of fibrils by synthetic A β —referred to as AD diffusible ligands (ADDLs) (56) or protofibrils (57)—cause subtle injury to cultured neurons. That such prefibrillar assemblies might also be neurotoxic *in vivo* seems plausible in view of the synaptic, electrophysiological, and behavioral changes documented in young APP transgenic mice before plaque formation (58, 59). However, the presence of mixtures of A β assemblies, ranging from monomers to mature fibrils, in the brains of both transgenic mice and humans with AD has made it difficult to ascribe synaptotoxicity principally to one or another A β species. Recently, microinjection into living rats of culture medium containing naturally secreted human A β revealed that A β oligomers (in the absence of monomers and amyloid fibrils) can inhibit long-term potentiation in the hippocampus, which is required for memory formation (60). The combined use of biochemical, electrophysiological, and pharmacological methods showed that this interference with synaptic plasticity was attributable specifically to soluble oligomers of A β , and not to monomers or fibrils.

Such findings are in accord with the concept that neurodegeneration in diseases such as Alzheimer's, Parkinson's, Huntington's,

and the spinocerebellar ataxias arises from injury caused by small, diffusible oligomeric assemblies of the respective misfolded proteins. According to this hypothesis, large polymeric aggregates (such as the amyloid plaques in AD and Lewy bodies in Parkinson's disease) represent inactive reservoirs of species that are in equilibrium with the smaller, putatively neurotoxic assemblies. Nevertheless, we cannot view large, mature lesions such as plaques and Lewy bodies as necessarily protective, because their presence signifies that the host has accumulated a reser-

vement of human-type inflammatory mediators (for example, certain cytokines), and the relatively short duration of exposure to A β . Indeed, the finding that an APP transgene clearly accelerates neurofibrillary tangle formation in APP/tau double transgenic mice (36) suggests a causal connection between A β accumulation and neurofibrillary degeneration, even in mouse models.

A final criticism of the amyloid hypothesis has arisen from the interpretation of the work of Braak and Braak (65), who showed that neurofibrillary degeneration of cell bodies and their neurites increases gradually with the age in humans and that these changes predate morphologically detectable amyloid plaques. However, the earliest cases examined in these postmortem studies (cases on which the definition of Braak Stage I neuropathology was based) were actually nondemented older individuals, and it is impossible to know whether their neurofibrillary changes represented the prodrome of AD. Indeed, recent analyses suggest that the formation of neurofibrillary lesions during late middle age can often represent a process separate from AD (66). Moreover, studies of patients dying with Downs syndrome at varying ages have consistently shown that A β deposition predates neurofibrillary tangle formation in this disorder (67, 68). Finally, in an Australian family with a spastic paraparesis variant of familial AD, an individual died of unrelated causes after the onset of the paraparesis but before the onset of dementia, and she was found on neuropathological examination to have fulminant amyloid deposition but no neurofibrillary tangles (54). Thus, in this patient, altered APP processing and A β accumulation clearly predated tau changes and frank neuronal injury.

In summary, none of the currently perceived weaknesses of the amyloid hypothesis provides a compelling reason to abandon this idea, although together they certainly point to important gaps in our understanding of AD.

Treatment Strategies Based on A β Biology

If AD represents the effects of a chronic imbalance between A β production and A β clearance and this imbalance can be caused by numerous distinct initiating factors, how should we treat and prevent the disorder? Six broad therapeutic strategies have been proposed. First, one could attempt to partially inhibit either of the two proteases, β - and γ -secretase, that generate A β from APP. In the case of β -secretase, compound screening and medicinal chemistry are being pursued vigorously to identify potent small-molecule inhibitors that can fit the large active site of this aspartyl protease and still penetrate the blood-brain barrier. In the case of γ -secretase, potent membrane-permeable inhibitors

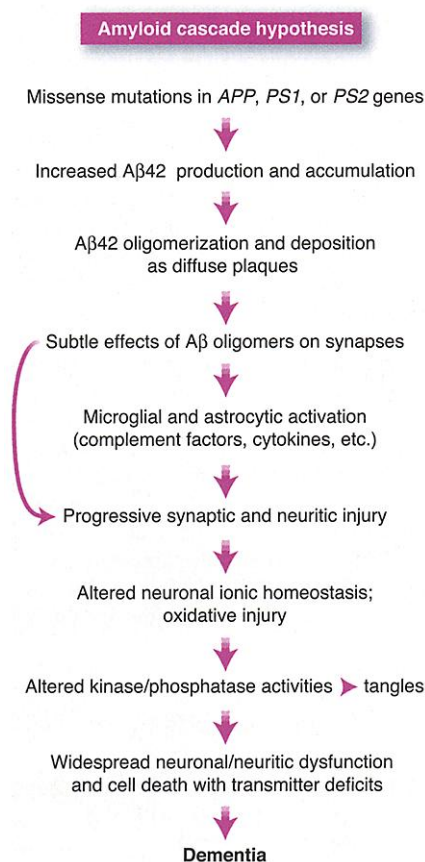
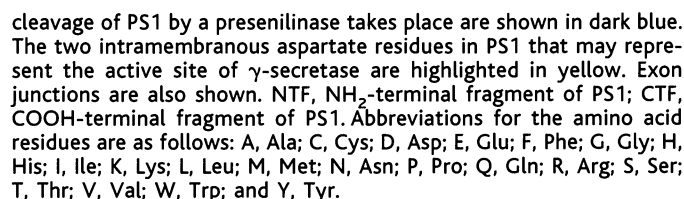


Fig. 1. The sequence of pathogenic events leading to AD proposed by the amyloid cascade hypothesis. The curved violet arrow indicates that A β oligomers may directly injure the synapses and neurites of brain neurons, in addition to activating microglia and astrocytes.

voir of the potentially injurious protein in the brain.

Yet another concern is that transgenic mice undergoing progressive A β deposition (61, 62) often do not show clear-cut neuronal loss (63, 64). The reasons for the failure of mice expressing only an APP transgene to show neurofibrillary degeneration and substantial neuronal loss are not yet clear, but there are several plausible explanations. These include species differences in neuronal vulnerability, the absence of human tau molecules in these mice, the lack of a full com-

The fifth strategy is based on the observation that A β aggregation is, in part, dependent on the metal ions Cu $^{2+}$ and Zn $^{2+}$ (84). This strategy reasons that chelation of these ions *in vivo* may prevent A β deposition. A β deposition was impeded in APP transgenic mice treated with the antibiotic clioquinol, a known Cu $^{2+}$ /Zn $^{2+}$ chelator (85). This strategy has now reached the clinical trial stage. The sixth broad amyloid-based strategy is to prevent the synaptotoxic and neurodegenerative effects putatively triggered by A β accu-



mulation. Numerous approaches have been contemplated, including the use of compounds with antioxidant, neuroprotective, and/or neurotrophic properties, but again, no slowing of cognitive decline has been documented in humans to date. Thus, for now, clinically validated treatments for AD remain confined to symptomatic interventions such as treatment with acetylcholinesterase inhibitors and drugs that ameliorate behavioral disturbances.

Conclusion

In the more than 10 years that the amyloid hypothesis has been formally articulated, a wealth of studies from many laboratories worldwide has supported its broad outlines. The development of anti-A β therapeutics remains a rational approach to treating AD, based on our current understanding of the earliest features of this disease. Several perspectives on the deficiencies of the hypothesis have been put forward [see, for example, (86–88)], but an alternative hypothesis explaining the cause and early pathogenesis of AD that has as much experimental support as the A β hypothesis has not emerged. Each new gene implicated in the etiology of AD will need to go through the same genotype-to-phenotype analysis that has already occurred for APP, PS1, PS2, and apoE4. Through this iterative process, and through further trials of anti-amyloid therapies, we will learn whether the amyloid hypothesis indeed explains the fundamental basis of AD, the most common and devastating disorder of human intellect.

References and Notes

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