

# Alzheimer's disease results from the cerebral accumulation and cytotoxicity of amyloid $\beta$ -protein

*A reanalysis of a therapeutic hypothesis*

*Dedicated to the memory of Henryk M. Wisniewski, MD, PhD, an international leader in the scientific assault on Alzheimer's disease*

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## 1. Introduction

The progressive cognitive and behavioral symptoms which characterize Alzheimer's disease (AD) derive from profound functional and structural changes observed in neurons, their processes and synapses, and the microgliosis and astrocytosis which accompany these changes. This multicellular dysfunction appears to represent a common cytopathological response to several distinct genetic defects and perhaps also to certain environmental precipitants that remain poorly understood. In short, AD is actually a syndrome in which multiple molecular etiologies can trigger a somewhat varied but largely stereotyped pathogenetic cascade. From this perspective, AD resembles other common, multigenic degenerative pathologies of late life, such as atherosclerosis. Because the AD syndrome has multiple molecular causes and a gradual, chronic evolution, one may anticipate several distinct classes of therapeutic molecules that could interfere with one or another step in the disease cascade.

A central goal for AD research during the last quarter century has been to learn enough about the etiologies and the shared biochemical mechanism of this syndrome to be able to identify or design small, brain-permeable molecules which inhibit relatively early and obligatory molecular events that occur in most, if not all, AD patients. Because clearcut environmental causes of AD have been so difficult to identify, we are forced to focus on elucidating the mechanisms of those genetic defects which are already known to cause AD or which will be discovered in the future. Although such specific genetic forms of AD have been said to be relatively uncommon, their usefulness as a starting point for achieving therapeutic insights into all forms of AD is supported by at least three considerations. First, the neuropathological and clinical phenotypes of genetically distinct forms of AD appear to be highly similar to and often indistinguishable from the widespread "sporadic" form, save for age of onset. This commonality suggests that therapeutics found to slow the mechanism of the disease in one genetic form may be applicable to numerous other forms. Second, both epidemiologic research and anecdotal clinical experience suggest that a much larger portion of AD cases may be genetically based than has generally been thought. Again, this suggests that insights gained from research on the biochemistry and pharmacotherapeutics of familial forms of AD could be extended to many, if not most, AD patients. Third, the scientific tools needed to decipher the specific molecular events that underlie the known genetic forms of AD are at hand, so that the mechanism(s) of these relatively infrequent forms should be elucidated as fully as possible, even if we do not yet understand the causes of many other forms of the syndrome.

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## 2. The amyloid $\beta$ -protein hypothesis of Alzheimer's disease

A unifying hypothesis intended to explain all forms of AD proposes that progressive cerebral accumulation of amyloid  $\beta$ -protein ( $A\beta$ ) initiates a complex multi-cellular cascade that includes neuritic dystrophy, microgliosis, astrocytosis, neuronal dysfunction and loss, and the synaptic alterations that result in neurotransmitter deficits and impaired mnemonic and cognitive functions (see Fig. 1). Support for this hypothesis has come from virtually every line of investigation on the pathobiology of Alzheimer's disease during the last one and a half decades. The  $A\beta$ -initiated pathogenic cascade postulated by this hypothesis provides a comprehensive theory for and can explain almost all known features of the disease. Moreover, the multi-step cascade which the hypothesis posits also provides specific molecular targets that can be screened against in order to develop treatments and, ultimately, preventions for AD and age-related cerebral  $\beta$ -amyloidosis.

The mainstay of the amyloid  $\beta$ -protein hypothesis of Alzheimer's disease is that a gradual and chronic imbalance in the production versus the clearance of  $A\beta$  leads to a slow rise in its steady state levels in brain tissue that leads to  $A\beta$  accumulation and subsequently, to the complex molecular and cellular changes of the disease. While the hypothesis has been phrased in the past as dependent on the cerebral deposition of  $A\beta$  as amyloid, recently evolving knowledge suggests that it is the accumulation of the peptide in various forms that may relate most closely to glial and neuronal dysfunction, rather than just its deposition in myriad plaque-like lesions. Nevertheless, it is only when such plaque-like lesions are present (presumably signifying accumulation of sufficient critical mass of  $A\beta$  peptides) that there are enough of the cytotoxic  $A\beta$  species (which are not yet clearly defined) to initiate progressive cellular changes.

The hypothesis proposes that  $A\beta$  accumulation, i.e. a rise in its steady state cerebral levels, is the central early event in the pathogenesis of AD. It is important to emphasize that the words "cause of AD" cannot necessarily be applied directly to the  $A\beta$  accumulation, because we already know that a number of discrete genetic mutations or polymorphisms (e.g. in presenilins or apolipoprotein E) can cause the rise in  $A\beta$  and thus precede it. Nevertheless, it has become increasingly clear that all of the genetic events currently known to lead to the development of AD act to alter the economy of  $A\beta$  in brain tissue [8,17]. Thus, it becomes largely

a matter of semantics as to whether  $A\beta$  accumulation should be considered "causative" or "a very early response event" that is necessary to drive the disease forward. The resolution of this semantic argument is not as important as incorporating all known pathogenetic events into a comprehensive mechanism of disease.

In considering this hypothesis, one must bear in mind the distinction between  $A\beta$  and amyloid.  $A\beta$  is a peptide fragment, principally 40 or 42 amino acids long, that is proteolytically cleaved from a large precursor polypeptide,  $\beta$ -amyloid precursor protein (APP). Once generated,  $A\beta$  can apparently exist in a number of forms, including as monomers, dimers, higher oligomers and polymers, the latter including those which constitute the  $\sim 8$  nm amyloid fibrils that accumulate in the disease. The term amyloid refers solely to the latter (fibrillar) form and, in particular, to large masses of fibrils that accumulate as deposits in the extracellular space of the brain and its microvasculature. While it has been convenient to use the term "amyloid hypothesis of Alzheimer's disease", it is more correct to speak of "the  $A\beta$  hypothesis of Alzheimer's disease", since this term incorporates all forms in which the peptide might accumulate.

The hypothesis I discuss here requires some change to occur in the steady state levels of  $A\beta$  in the brain, initially as a soluble monomer in brain interstitial fluid and perhaps also intracellularly. When levels of  $A\beta$  monomers rise appreciably above normal concentrations intra- and/or extracellularly, oligomer formation is favored, and then dimers and higher oligomers become the principal form in which  $A\beta$  accumulates stably and progressively. It appears increasingly likely that at least some forms of  $A\beta$  oligomers (as opposed to mature fibrillar polymers) can exert stimulating effects on microglia and/or subtle toxic effects on neuronal processes (dendrites and axons). The earliest neuropathological change, if the word pathology is classically defined as a morphological abnormality in the tissue that is apparent by light microscopy, is presumably the  $A\beta_{42}$ -containing diffuse plaque. This may begin to appear in very faint, wispy forms during the course of cortical accumulation of  $A\beta$  oligomers. Indeed, we know that diffuse  $A\beta_{42}$  deposits can occur in neurologically normal elderly individuals, sometimes in high abundance. Therefore, such deposits likely represent an immature lesion that does not by itself induce substantial local cytotoxicity. A rough analogy can be made to very early fatty streaks of lipids that may initiate the process leading to mature atherosclerotic plaque formation in systemic arteries. It is assumed that some

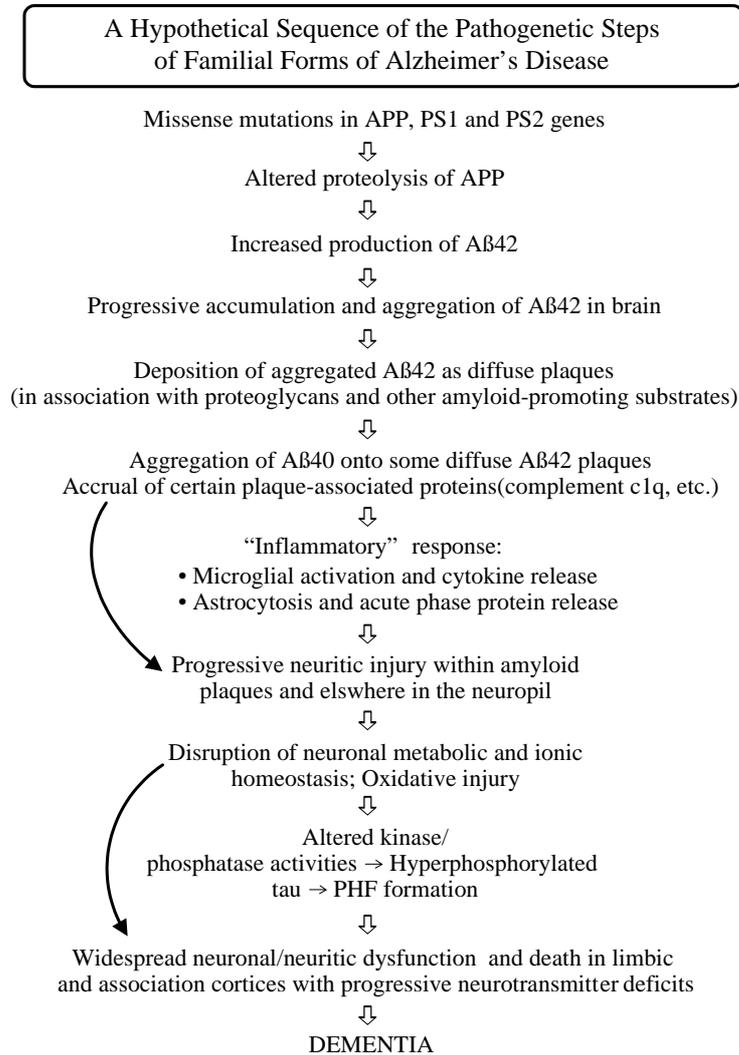


Fig. 1.

such precursor lesions, whether they are diffuse plaques in AD or fatty streaks in atherosclerosis, may advance or “mature” under the influence of numerous distinct genetic and/or environmental factors to accumulate in sufficient numbers and locations to contribute to local cytopathology. Again, it is not necessary to postulate that the  $A\beta_{42}$  diffuse deposit, or the mature  $A\beta_{42}$ - and  $A\beta_{40}$ -rich fibrillar amyloid plaque, is the sole or even major effector of cytopathology; these lesions probably constitute a reservoir for smaller, diffusible  $A\beta_{42}$  and  $A\beta_{40}$  oligomers that may induce early cellular changes and that exist in equilibrium with the light microscopically visible, particulate  $A\beta$  material (granules and fibrils) present in diffuse and fibrillar plaques. Because one generally sees considerable local microglial, astro-

cytic and neuritic alteration within and intimately surrounding fibril-rich  $A\beta$  deposits, it remains likely that development of more mature, fibrillar deposits (which could be associated with very high local concentrations of oligomers) contributes to progressive cellular dysfunction. Put another way, the rise in steady-state levels of dimers and/or oligomers in brain interstitial fluid (and perhaps intracellularly) in quantities insufficient to allow formation of light microscopically visible deposits would be expected to produce minimal, largely subclinical neuronal dysfunction, presumably with little or no progression to clinical symptoms. Only when cerebral  $A\beta$  accumulation is sufficient to also allow extensive diffuse plaques and, to at least some degree, more mature, fibril-rich plaques does one actually ob-

serve progressive symptoms of dementia.

### 3. Extracellular vs intracellular $A\beta$

Before we discuss how accumulation of  $A\beta$  may arise and how it may be responsible for the initiation of the disease cascade, we should discuss a question of rising interest, namely the pathogenic roles of intracellular vs extracellular  $A\beta$ . We have known since 1992 that, in contrast to previous postulates,  $A\beta$  is normally generated by intact, healthy cells and circulates in extracellular fluids in all humans throughout life. With this knowledge in hand, one no longer needed to postulate that prior membrane injury was needed to allow release of  $A\beta$  from its partially intramembranous position within APP. The realization that  $A\beta$  peptides (including  $A\beta_{40}$  and  $A\beta_{42}$ ) are normal metabolic products of intracellular APP processing allowed one to consider  $A\beta$  accumulation as a potentially primary event in AD pathogenesis. In other words, the presence of  $A\beta$  in the extracellular and intracellular spaces was normal, and anything that changed the balance between its arrival in and removal from these spaces could gradually increase its levels. In this sense, the earlier notion of  $A\beta$  accumulation as a necessarily secondary or tertiary process in AD pathogenesis was weakened, although a new question arose: what kinds of factors can lead to an increase in the levels of  $A\beta$  in the brain? Because  $A\beta$  is normally made by cells, it obviously arises intracellularly. Virginia Lee and colleagues have shown that  $A\beta$  can accumulate inside cultured neurons in a form that requires solubilization in formic acid, suggesting an aggregated state [18]. In our laboratory, Dominic Walsh and co-workers have recently been able to detect stable  $A\beta$  dimers ( $\sim 8$  kDa) inside cells that express APP, including in primary cortical neurons expressing endogenous levels of the precursor [20]. Therefore, we are moving to a conclusion that the first oligomerization of  $A\beta$  actually begins intracellularly. Whether such intracellular  $A\beta$  species have a principal pathogenic role in AD cytopathology or they must be accompanied by substantial extracellular  $A\beta$  accumulation remains unclear. At the late stages of the disease (i.e. in postmortem AD brains), when very large amounts of  $A\beta$  peptide in various forms are readily detectable extracellularly by sensitive antibodies, one cannot usually detect appreciable intraneuronal or other intracellular aggregates. Electron microscopic studies of post-mortem AD brain have generally failed to document definite intracellular  $A\beta$  fibrils. However, intracellu-

lar  $A\beta$  accumulation can apparently occur earlier in the course of AD-type pathology, as has been recently reported [2,7]. It is thus likely that  $A\beta$  begins to oligomerize intracellularly. Nonetheless, it is reasonable to speculate that subsequent extracellular accumulation of oligomers and higher polymers is needed to allow progressive glial alteration and neuronal/neuritic dystrophy.

### 4. Thirteen points that support the $A\beta$ hypothesis of AD

Let us now review the principal elements of support for the  $A\beta$  hypothesis of AD. *First*, all patients with Alzheimer's disease (as it is defined universally) accumulate some and usually many deposits of first  $A\beta_{42}$  and then  $A\beta_{40}$  in regions of the brain important for memory and cognition. *Second*,  $A\beta_{42}$  diffuse plaques occur increasingly with age in neurologically normal individuals (and also in several lower mammals, including virtually all primates), strongly suggesting that  $A\beta_{42}$  accumulation can precede all other cytopathological features of AD. *Third*, the APP gene is encoded on chromosome 21, and trisomy 21 (Down's syndrome) leads invariably to very early  $A\beta_{42}$  accumulation ( $\leq 12$  yrs of age) and subsequent accrual of more mature  $A\beta$  deposits, microgliosis, astrocytosis, neuritic dystrophy and neurofibrillary tangles, ultimately yielding a neuropathological phenotype essentially indistinguishable from that of AD. It is of special interest that translocation Down's patients whose translocation break point excludes the APP gene in the duplicated portion fail to develop the neuropathology of AD, thus clearly implicating duplication of the APP gene as the basis for the AD phenotype in Down's syndrome [14]. *Fourth*, three genes (APP, presenilin 1 and presenilin 2) have been implicated to date as the sites of dominantly transmitted mutations that can unequivocally be said to cause forms of AD, and extensive in vitro and in vivo modeling of these mutations has clearly shown that all of them increase the cellular production of  $A\beta_{42}$  and its subsequent extracellular accumulation. *Fifth*, expression of familial Alzheimer's disease (FAD)-linked mutations in APP and/or presenilins in transgenic mice leads to progressive accumulation of  $A\beta_{42}$ , first in a biochemically (but not yet morphologically) detectable form, and later as diffuse and mature  $A\beta$  plaques associated with microgliosis, astrocytosis, and neuritic/synaptic dystrophy resembling such changes in AD brain. *Sixth*, there is now compelling evidence that presenilin may

itself be  $\gamma$ -secretase, thus suggesting that the mutations which cause the most common and aggressive form of familial AD are in the very protease that generates  $A\beta$  [5,10,21]. Seventh, the ApoE4 polymorphism, the major known genetic risk factor for late onset AD, appears not to alter the cellular production of  $A\beta$  but rather changes its clearance and/or fibrillization in some way that leads to enhanced accumulation of diffuse and fibrillar  $A\beta$  deposits in the brain. Importantly, the  $A\beta$ -elevating effect of inheriting one or two ApoE4 alleles has been documented in the brains of clinically healthy aged humans free of (or prior to the development of the AD neuropathological syndrome [13]. *Eighth*, application of  $A\beta$  in aggregated form (but not in monomeric form at the same concentrations) to cultured neurons or microglia reproducibly causes morphological, biochemical and electrophysiological changes in these cells. *Ninth*, microinjection of aggregated  $A\beta$  in plaque-equivalent doses into aged primate, but not young primate or aged rodent, brain induces substantial local gliosis and neuronal loss, helping explain an apparent species barrier to  $A\beta$ -induced lesion formation in rodents vs primates [6]. *Tenth*, many symptomatic AD patients have significantly decreased CSF concentrations of  $A\beta_{42}$  without parallel decreases in  $A\beta_{40}$  [11], consistent with the principal accumulation of  $A\beta_{42}$  in myriad diffuse and mature deposits in brain parenchyma. *Eleventh*, elevation of  $A\beta_{42}$  levels in the plasma and in the conditioned media of biopsied skin fibroblasts can be seen in healthy subjects harboring APP or presenilin missense mutations long before the onset of clinical symptoms of AD [16], clearly indicating that  $A\beta$  elevation is not a secondary response to the disease process in this form of AD. *Twelfth*, an alternate hypothesis, that  $A\beta_{42}$  accumulation and deposition occurs secondary to a primary neuronal disturbance involving tau hyperphosphorylation/accumulation in neuronal somata (tangles) and neurites, is not supported by the recent discovery of pathogenic tau mutations in humans [9,19]; these produce a profound neuronal degeneration and ultimately death of the patient without any secondary  $A\beta$  accumulation or plaque formation. Thus,  $A\beta_{42}$  accumulation can lead to secondary neurofibrillary tangle formation, but the converse has not been clearly shown to date. *Thirteenth*, an etiopathogenic event or agent other than  $A\beta$  accumulation that could explain the initiation and progression of the disease has not emerged from more than two decades of intensive biological research on AD.

These 13 points, while not unequivocally proving the  $A\beta$  hypothesis of AD, provide very strong evidence in

favor of it. Final proof must now come from a) treating patients with mild AD with agents that chronically decrease  $A\beta$  production (e.g.,  $\gamma$ -secretase inhibitors [5, 10]) or  $A\beta$  accumulation (e.g., the  $A\beta$  vaccine [15]) and observing a slowing or stabilization of clinical progression; and/or b) treating presymptomatic individuals at high risk of imminent development of symptoms with such agents and delaying onset of clinical disease. Only with successful human trials can the  $A\beta$  hypothesis of AD ultimately be proven. Fortunately, we may not be more than a few years away from gathering such in vivo proof. Further pre-clinical research, including extensive trials of  $A\beta$ -lowering molecules in APP/PS transgenic mice, could clearly add fuel to the fire, but will not prove the case.

It is not the intention of this article to discuss in detail how  $A\beta_{42}$  accumulation, once it occurs, initiates and propagates the complex molecular and cellular changes of AD. The mechanism of neuronal dysfunction is an aspect of AD pathogenesis that remains unsettled and controversial. In my view, current evidence does not allow firm conclusions about the precise sequence of events that occur downstream of  $A\beta_{42}$  accumulation in the brain. Nevertheless, one may speculate about this cascade in a hypothetical sense, and I therefore provide Fig. 1 to convey my own views of a likely temporal sequence of the events in AD pathogenesis that follow  $A\beta_{42}$  elevation, at least in the familial forms of AD. It may ultimately be difficult or impossible to establish a definitive sequence of events, and indeed this sequence may well differ among humans with AD. Because we are unlikely to encounter human hosts that do not mount both a microglial/ astrocytic response and a neuritic/synaptic response to  $A\beta$  accumulation, I believe it will be difficult to separate these two responses in vivo in a clear temporal fashion. However, my current opinion is that microglia are present in the brain to respond sensitively to molecular alterations in the extracellular space and therefore may be well poised to initiate an inflammatory response prior to the development of synaptic/neuritic/neuronal alterations.

What is wrong with the  $A\beta$  hypothesis of AD put forward here? In my view, there are few if any critical defects in the hypothesis that make it unlikely to be validated in the long run. Nevertheless, the most nagging criticism that one hears repeatedly is that  $A\beta$  burden and plaque counts correlate poorly with the presence and/or the degree of clinical impairment. But recent studies using quantitative morphometry [3,4] or biochemical assays [12] have refuted this strongly held assumption, as indeed did some of the early plaque and

tangle quantitation studies of Blessed, Tomlinson and colleagues. There now exist compelling data that total A $\beta$ -immunoreactive deposits, neuritic plaques and/or tissue content of A $\beta$ , do show statistically significant and sometimes strong correlation coefficients with both the presence and the degree of clinical impairment. Importantly, the oft-cited neuropathological finding that neurofibrillary alteration in entorhinal cortex and hippocampus may be the earliest lesion of AD is based on elegant temporal studies of human brain specimens of increasing age [1]. However, observing dystrophic neurites prior to any recognized A $\beta$  deposits in such studies is difficult to interpret vis à vis the pathogenesis of AD per se. This is because one simply does not know that all of the middle-aged or elderly individuals who died having some dystrophic neurites in limbic structures would necessarily have developed clinical AD. Moreover, this semiquantitative neuropathological approach could not yet have included sensitive quantitative of A $\beta$  dimers/oligomers or very early, diffuse A $\beta$  deposits. In my opinion, attempts to obtain highly precise clinical-neuropathological correlations in AD, while interesting and technically elegant, tend to simplify an extremely complex and variable phenotype in the brain. Because diffuse plaques often occur in cognitively "normal" older individuals and these deposits are now widely believed to be precursor lesions, we should expect a complicated, non-linear relationship between A $\beta$  amount in the brain and degree of clinical impairment. Some hosts can apparently tolerate quite high cerebral burdens of monomeric and even oligomeric A $\beta$  with minimal, "sub-symptomatic" glial and neuritic alterations. This is what one would expect from considering the complex, multi-faceted relationship between blood lipid levels, vascular cholesterol deposition, and clinical vascular syndromes (angina, MI, CVA, etc.). Slow, chronically evolving pathologies in aged humans often do not have a simple, linear relationship to symptom burden. Given the enormous complexity of the cellular and biochemical changes that are being steadily identified in AD brain tissue, I find it remarkable that one has been able to obtain as robust a degree of correlation between lesion density (measured at death) and clinical impairment (measured during life) as has been reported in some studies. This "nagging issue" simply cannot, in my view, obviate the remarkably strong genetic, biochemical, histopathological and animal modeling data that support a central, initiating role for A $\beta$  accumulation in Alzheimer's disease.

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