Review paper

Folia Neuropathologica

A critical analysis of the 'amyloid cascade hypothesis'

Richard A. Armstrong

Vision Sciences, Aston University, Birmingham, UK

Folia Neuropathol 2014; 52 (3): 211-225 DOI: 10.5114/fn.2014.45562

Abstract

The 'amyloid cascade hypothesis' (ACH) is the most influential model of the pathogenesis of Alzheimer's disease (AD). The hypothesis proposes that the deposition of β-amyloid (Aβ) is the initial pathological event in AD, leading to the formation of extracellular senile plaques (SP), tau-immunoreactive neurofibrillary tangles (NFT), neuronal loss, and ultimately, clinical dementia. Ever since the formulation of the ACH, however, there have been questions regarding whether it completely describes AD pathogenesis. This review critically examines various aspects of the ACH including its origin and development, the role of amyloid precursor protein (APP), whether SP and NFT are related to the development of clinical dementia, whether Aβ and tau are 'reactive' proteins, and whether there is a pathogenic relationship between SP and NFT. The results of transgenic experiments and treatments for AD designed on the basis of the ACH are also reviewed. It was concluded: (1) Aβ and tau could be the products rather than the cause of neurodegeneration in AD, (2) it is doubtful whether there is a direct causal link between Aβ and tau, and (3) SP and NFT may not be directly related to the development of dementia, (4) transgenic models involving APP alone do not completely replicate AD pathology, and (5) treatments based on the ACH have been unsuccessful. Hence, a modification of the ACH is proposed which may provide a more complete explanation of the pathogenesis of AD.

Key words: *Alzheimer's disease, amyloid cascade hypothesis, β-amyloid, neurofibrillary tangles, disease pathogenesis.*

Introduction

The 'amyloid cascade hypothesis' (ACH) [57] is the most influential model of the pathology of Alzheimer's disease (AD) proposed over the last 25 years. As a result, numerous studies of AD pathogenesis have been carried out [6,8] and potential treatments proposed and tested based on the ACH [69,113].

It was the discovery of β-amyloid (Aβ), the most important molecular constituent of senile plaques (SP) [49], and mutations of the amyloid precursor protein (APP) gene linked to early-onset familial AD (FAD) [27,50], which led to the original formulation of the hypothesis [57]. The ACH proposed first, a direct relationship between the development of Aβ, in the form of senile plaques (SP), and neurofibrillary tangles (NFT) and second, between these lesions and clinical dementia. Hence, deposition of Aβ is the initial pathological event in AD, leading to the formation of SP, tau-immunoreactive NFT, neuronal loss, and ultimately clinical dementia. The ACH is often regarded as the 'conclusive model' of the aetiology of early-onset FAD, and sporadic AD (SAD), a more complex disorder caused by a variety of factors, but resulting from essentially the same pathological cascade [42,127].

Communicating author:

Dr Richard A. Armstrong, Vision Sciences, Aston University, Birmingham B4 7ET, UK, phone: 0121-204-4102, fax: 0121-204-4048, e-mail: R.A.Armstrong@aston.ac.uk

Since its publication there have been observations difficult to reconcile with the ACH and concerns whether it completely describes AD pathogenesis [11,90]. First, transgenic mice, in which genes overexpress amyloid precursor protein (APP), do not reproduce the predicted cascade [39,90]. Second, SP and NFT are separated in brain both spatially [11] and temporally [39,82] questioning the pathogenic link between them. Third, Aβ and tau could be the reactive products of neurodegeneration rather than their cause, arising as a consequence of oxidative stress [126]. Fourth, study of various neuronal injury biomarkers does not support a central pathological role for Aβ, especially in late-onset SAD [28]. These observations suggest a more complex relationship between Aβ, tau, and AD pathogenesis than suggested by the ACH. In addition, concentration on the ACH may have been to the detriment to the study of other possible aetiologies including: (1) perturbation of vesicular trafficking, (2) disruption of the cytoskeletal network, and (3) disturbance of membrane cholesterol [37]. These views are supported by a critique of the ACH by Teplow [133] who argues that 'insufficient rigour' has been applied to studies of Aβ in AD based on the ACH and 'confusion not clarity' has been the result.

This review critically examines various aspects of the ACH including its origin and development, the role of amyloid precursor protein (APP), whether SP and NFT are related to the development of clinical dementia, whether Aβ and tau are 'reactive' peptides, and whether there is a pathogenic relationship between SP and NFT. The results of transgenic experiments and treatments for AD designed on the basis of the ACH are also reviewed. Finally, a modified scheme based on the ACH is proposed which may provide a more complete description of the pathogenesis of all forms of AD.

Key observations in the development of the amyloid cascade hypothesis

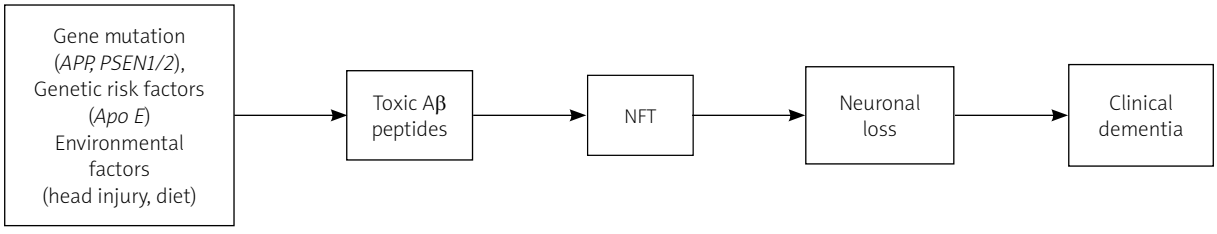
The original formulation of the ACH as proposed by Hardy and Higgins [57] is shown in Figure 1. A number of key observations resulted in the formulation and subsequent development of the hypothesis:

1. Aβ was discovered as the most important molecular constituent of SP [49], emphasising the importance of amyloid peptides in AD.

2. Altered proteolytic processing of APP and its accumulation were shown to be early events in AD and were followed by microglial activation, astrocytosis, and dystrophic neurites (DN) [105].

3. Mutations of *APP* gene [27,50] were linked to early-onset FAD, the presence of point mutations in *APP* evidence that amyloid deposition was the causative factor [56]. Three main groups of APP mutations have been identified, i.e., those which act at: (a) the beta APP cleaving enzyme 1 (βACE1) site, (b) the gamma APP cleaving enzyme site, and (c) the mid-domain of the Aβ region.

4. Formation of Aβ peptides was directly linked to APP processing with two events being necessary to generate Aβ [116]: (a) cleavage by βACE1 which cleaves APP on the amino side of Aβ resulting in a large secreted derivative and an Aβ membrane-associated C-terminal derivative (CTFβ) and (b) cleavage by γ-secretase which cleaves CTFβ to release the Aβ peptide [63]. A variety of Aβ peptides can be formed as a result of cleavage of APP [54], the most common being $Aβ_{42}$ [131], found largely in discrete Aβ deposits [139], and the more soluble $Aβ_{40}$, also found in association with blood vessels [86], and which may develop later in the disease [35]. In addition, $Aβ_{43}$ may be formed and has a particularly potent amyloidogenicity [115]. Mutations of *APP* within the Aβ coding region can also result in the deposition of $Aβ_{38}$ in vessel walls, especially in those



Aβ – β-amyloid, Apo E – apolipoprotein E, APP – amyloid precursor protein, PSEN1/2 – presenilin genes 1 and 2, NFT – neurofibrillary tangles

Fig. 1. The amyloid cascade hypothesis (ACH) in its original form.

cases with extensive cerebral amyloid angiopathy (CAA) [88]. Early toxic soluble oligomers could also be involved, which vary with a type of mutant, thus providing a genetic basis for variations in pathogenesis observed among FAD cases [46,48].

5. Subsequently, the most common form of early-onset FAD was linked to mutations of *presenilin* (*PSEN)* genes *PSEN1* [123] and *PSEN2* [78]. Full length PSEN protein is composed of nine trans-membrane domains located on the endoplasmic reticulum membrane. Endoproteolytic cleavage of PSEN and assembly into γ-secretase complex is followed by transport to the cell surface, thus potentially influencing APP processing [61]. Hence, mutant *PSEN1* could enhance 42-specific-γ-secretase cleavage of normal APP resulting in increased deposition of amyloid-forming species [129]. Alternatively, deficiency of PSEN1 may inhibit the normal cleavage of APP [34,141]. *PSEN1* also appears to alter the ratio of Aβ species, the ratio of $A\beta_{40/42}$ being lower and the ratio of $A\beta_{42/40}$ higher in *PSEN1* cases compared with SAD [60].

6. Studies of FAD cases caused by APP_{717} (valine–isoleucine) mutation showed them to have significant numbers of tau-immunoreactive NFT supporting a link between APP and degeneration of the cytoskeleton [75].

7. Early experiments using transgenic mice expressing high levels of APP resulted in Aβ deposition, synaptic loss, and gliosis [44].

8. In Down's syndrome (DS), which replicates many of the features of AD pathology [5,9], water soluble peptides ending in residue 42 precede the formation of SP and also increase with age [114].

9. Aβ peptides are toxic and can induce cell death depending on cell type [55], Aβ toxicity being mediated by the necrotic rather than the apoptotic pathway [20,76].

10. Aβ may induce the phosphorylation of tau by: (a) directly interacting with a domain of APP (714-723) [47,125], (b) by inducing tau protein kinase I and subsequently, tau proteins recognised by the antibody Alz-50 [132], (c) as a result of synergisms between Aβ and tau [100,101], or (d) by directly altering the phosphorylated state of tau [23].

11. Senile plaques and NFT acquire several 'secondary' constituents (Table I) which may be involved in the maturation of Aβ deposits into SP [7] including silicon and aluminium [82], acute-phase proteins such as α-antichymotrypsin and α_2-macroglobulin [40,81,136] and their mediator interleukin-6 (IL-6) [122], intercellular adhesion molecules such as cell adhesion molecule 1 (CAM1) [40], apolipoprotein E (Apo E) which is present in the earliest stages of SP formation [145], apolipoprotein D (Apo D) [36], the heterodimeric glycoprotein clusterin, vibronectin, the complement proteins C1q, C_4, and C_3 [137], blood proteins such as amyloid-P (especially in classic 'cored' SP) [67,68], cathepsins B/D [124], and the

Table I. Molecular composition of senile plaques (SP) and neurofibrillary tangles (NFT) in Alzheimer's disease (AD) (From: Armstrong [7])

Pathology	Molecular composition
Diffuse SP	APP (lacking C terminus), Aβ oligomers, especially $A\beta_{42/43}$, apolipoprotein E, α_1-antichymotrypsin, HSPG, complement proteins (C1q, C_3, C_4), amyloid-P, may contain neuronal and astrocytic markers
Primitive SP	APP (N & C-terminal), $A\beta_{42/43}$, free ubiquitin, conjugated ubiquitin, PHF-antigen, phosphorylated tau, chromogranin-A, bFGF, apolipoprotein E, interleukin-6, acetylcholinesterase, cholinergic, somatostatin, GABA, neuropeptide-Y, parvalbumin, and catecholamine immunoreactive neurites
Classic SP	$A\beta_{42/43}$ ('core'), a-synuclein ('ring'), $A\beta_{40}$, actin, tubulin, phosphorylated tau, NF-protein, CAM, chromogranin-A ('ring'), α_2-macroglobulin, complement proteins ('core'), immunoglobulins ('core'), amyloid-P, α_1-antichymotrypsin, antitrypsin, antithrombin III, apolipoprotein E and D ('core'), DOPPEL, bFGF, PrP, may contain acetylcholinesterase, cholinergic, somatostatin, GABA, neuropeptide-Y, parvalbumin, and catecholamine + neurites ('ring'), silicon/aluminium ('core'), interleukin-6 ('ring')
Intracellular NFT	Phosphorylated 3R/4R tau (C & N terminal), ubiquitin (C & N termini), MAP, NF-protein, apolipoprotein E, synaptophysin, bFGF, HSPG
Extracellular NFT	Degraded tau (lacking N/C termini), GFAP, Aβ, ubiquitin (lacking N-terminus), amyloid-P, Apo E

Aβ – β-amyloid, APP – amyloid precursor protein, bFGF – basic fibroblast growth factor, CAM – cell adhesion molecule, GFAP – glial fibrillary acidic protein, HSPG – heparan sulfate proteoglycan, MAP – microtubule associated proteins, NF-protein – neurofilament protein, PHF – paired helical filament, PrP – prion protein

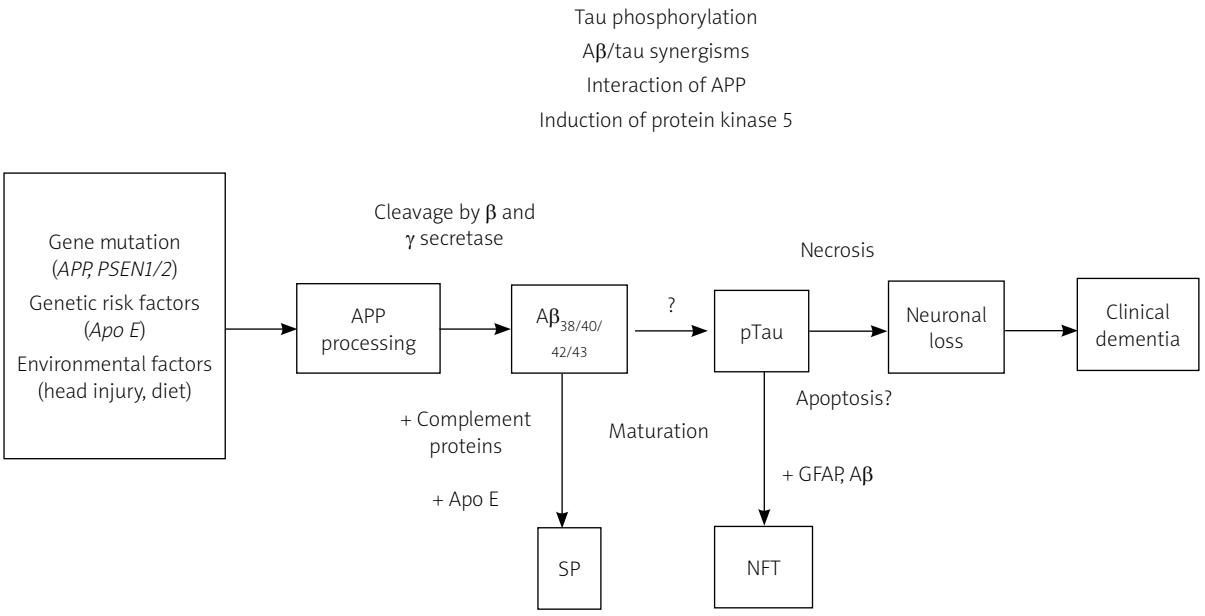
sulphated glycosaminoglycans such as heparan sulphate proteoglycan (HSPG). Many of these proteins may act as molecular 'chaperones' enhancing Aβ aggregation, ultimately forming a growing SP [7]. In addition, the molecular composition of NFT varies markedly depending on whether they are intracellular NFT (I-NFT) or extracellular NFT (E-NFT). Unlike I-NFT, E-NFT are immunoreactive for glial fibrillary acidic protein (GFAP) and Aβ [144], and also contain significant amounts of amyloid-P [104] and ubiquitin [17]; these proteins likely to have been acquired after degeneration of NFT-containing neurons.

These observations suggest that in early-onset FAD, Aβ within SP is the residue of the effects of a pathogenic gene mutation which, via the accumulation of toxic and insoluble Aβ oligomers, leads to the formation of NFT, cell death, and dementia. Since the pathological phenotype of FAD is similar, apart from age of onset, to that of the more common SAD [13,59,97], similar mechanisms, via additional genetic risk factors and/or environmental factors, are assumed to be involved in the pathogenesis of all types of AD [128]. A more fully developed version of the ACH incorporating these various features is shown in Figure 2.

Problems and limitations of the amyloid cascade hypothesis

Formation, structure and function of amyloid precursor protein

There are a number of questions regarding APP and Aβ which have implications for the ACH. First, little is known of the normal functioning of APP [37], which remains a 'current enigma' [93]. Amyloid precursor protein has been implicated in various brain functions including development, learning, memory, and synaptic plasticity, and it may also be secreted as a neuroprotectant [62,93]. Second, the detailed structure of APP does not appear to explain how aggregated peptides such as Aβ are formed [73]. One possibility is that excess APP overwhelms the normal degradation pathway leading to the formation of Aβ, as in DS. Third, some mutations of APP, e.g., amino acid changes at codon 717, appear to shed little light on its mechanism of action in AD [91]. Hence, amino acid substitutions at codon 717 in APP_{717} cases are not incorporated into amyloid deposits and hence, mutant Aβ may have additional metabolic effects which determine pathogenesis [79]. Fourth, secretion of Aβ requires cleavage at both the N and C-ter-



Aβ – β-amyloid, Apo E – apolipoprotein E, APP – amyloid precursor protein, GFAP – glial fibrillary acidic protein, PSEN1/2 – presenilin genes 1 and 2, NFT – neurofibrillary tangles, pTau – hyperphosphorylated tau, SP – senile plaques

Fig. 2. The amyloid cascade hypothesis (ACH) in a more fully developed form.

minal sites [83] and therefore, membrane damage may be necessary before Aβ can be formed [140], i.e., neuronal degeneration may precede Aβ formation. Fifth, Aβ toxicity requires micromolar concentrations of Aβ whereas levels in brain are frequently in the picomolar range [84]. Hence, AD may be caused by inflammatory reactions associated with activated microglia rather than by Aβ directly [84]. Moreover, in toxicity experiments, Aβ may not induce neuronal loss directly but may act to increase the risk of death of neurons from other causes [17,76,142].

Correlation of classical pathology with clinical dementia

If the ACH is an accurate description of AD pathology, then there should be a correlation between the abundance of SP and/or NFT and clinical dementia. However, the link between these lesions and cognitive impairment is often complex both in humans [106] and in rodent models of AD [44]. Several studies have attempted to correlate the numerical density of SP and NFT in AD brain with patient age and duration or degree of dementia [19,117,140]. Hence, significant correlations have been reported between: (1) the quantity of Aβ in entorhinal cortex and degree of cognition [30], (2) density and surface area of Aβ and months of severe dementia [19], (3) NFT counts and dementia in sectors CA1, CA4 and the subiculum [117], and (4) between neuritic SP and NFT and degree of dementia [140].

By contrast, Hyman *et al*. [65] found no quantitative measure of Aβ correlated with age in control patients, or with disease duration in AD, and suggested that Aβ did not accumulate in the brain throughout the course of the disease. That the relationship between SP and NFT and dementia is more complex was also emphasised by Bennett *et al*. [21]. No change in SP or NFT density was found from biopsy to autopsy suggesting that the pathology was well advanced before development of overt clinical symptoms. In a further study of 97 elderly cases with no symptoms of dementia, AD-type pathology was observed in 20-40% of cases, and although NFT were age-related there was no correlation between Aβ load and age [106]. Similarly, a significant correlation was observed between neuronal loss, NFT, and disease progression but not with Aβ [51]. In addition, some studies report that SP density declines with advancing AD, possibly as a result of the removal of Aβ deposits by glial cells [10]. A similar conclusion was reported by McKenzie *et al*. [85] who found that neither mean nor maximum SP density increased with age. Hence, SP may not progressively accumulate over the course of the disease but develop over a limited period of time and then stabilise to a constant level or even decline. These results question whether there is a direct or simple association between the formation and abundance of SP and NFT and developing dementia in AD as suggested by the ACH.

Is the formation of Aβ and tau a reactive process?

In survivors of head injury, enhanced levels of APP are found in neuronal perikarya and in DN surrounding Aβ deposits, similar pathological features to AD [45]. The processing of APP into Aβ in these cases occurs within the synaptic terminal fold of the axons, the presence of glial cells not being necessary for the conversion. Hence, APP formation may be a response to neuronal injury in AD [45]. Subsequently, it was shown that specific neurons in medial temporal lobe secreted large quantities of APP and that there were more APP-immunoreactive neurons in these areas in head injury patients [85]. Hence, increased expression of APP in head trauma may be an acute-phase response to neuronal injury [112], overexpression of APP leading to Aβ deposition. This conclusion is supported by the localisation of several acute-phase proteins within the different morphological types of Aβ deposit including diffuse, primitive, and classic deposits, e.g., amyloid-P, complement factors, and α-antichymotrypsin [7] (Table I). Furthermore, Regland and Gottfries [108] proposed that APP is involved in AD secondarily to maintain cell function. APP may therefore, maintain neuronal growth and survival and its putative neurotrophic action is also suggested by the observation that it shares structural features with the precursor for epidermal growth factor [108]. Furthermore, NFT may be part of the neurons response to injury [109].

Animal experiments also suggest that the formation of Aβ may be a reaction to brain damage. Hence, experimental lesions that damage the nucleus basalis in rat brain elevate APP synthesis in the cerebral cortex suggesting APP production is a specific response to loss of functional innervation of the cortex [138]. Chemically induced lesions of the brain

produce similar results. For example, lesions of the nucleus basalis using N-methyl D-aspartate (NMDA) elevate APP synthesis in cortical polysomes [138] and, in areas of brain damaged by kainite [70], APP was recorded in dystrophic neurites adjacent to the lesion. In addition, intrathecal or intraparenchymal injections of a toxin induced the formation of APP in hippocampal neurons subsequent to neuronal damage [68].

Lesion experiments can also induce pathological changes implicated in NFT formation. Hence, denervation of the dopamine pathways and septal lesions affecting both the cholinergic system and γ-aminobutyric acid (GABA) neurons which project to the dentate gyrus, result in a loss of dendritic microtubule associated protein 2 (MAP2) and the appearance of tau-immunoreactive dentate gyrus granule cells [134]. Hence, denervation of these pathways may cause transsynaptic changes in dentate gyrus neurons and these changes could represent a precursor stage to NFT formation.

These observations suggest that Aβ and tau may be the consequence of neuronal degeneration and not its cause and therefore, do not initiate the pathological cascade in AD. Hence, Aβ and NFT formation may occur 'downstream' of the 'real' causative events in AD but once formed, could initiate a secondary phase of degeneration.

Is the formation of neurofibrillary tangles related to Aβ?

Several attempts have been made to explain how Aβ may lead to NFT, as proposed by the ACH, but none have become universally accepted [23,47,100,101]. Alzheimer's disease was first recognised as a distinct disease entity in 1910 and was named after Alzheimer by Kraepelin based on the clinical and pathological description of the original cases [52]. Of the two original cases described by Alzheimer [2], however, both had numerous SP but only one of the cases had significant numbers of NFT [52], thus creating a controversy as to the relationship between the two 'signature' pathological features [58,72,87]. In addition, SP and NFT can develop alone in different disorders, e.g., NFT in tangle-only dementia [143] and Aβ in hereditary cerebral haemorrhage with amyloidosis of the Dutch type (HCHA-D) [64]. These observations suggest a more complex relationship between Aβ and tau formation than suggested by the ACH.

Studies also suggest that SP and NFT exhibit distinct but independently distributed topographic patterns in the cerebral cortex in AD [12,64]. Braak and Braak [22] showed that tau pathology occurred first in entorhinal cortex, often in the absence of SP, whereas the subsequent spread and distribution of Aβ was more variable. Studies of the spatial patterns of SP and NFT also show them to be clustered, the clusters often being regularly distributed relative to the pia mater [3]. Clusters of SP and NFT, however, are not spatially correlated, which would not support a direct pathogenic link between them. Perez *et al*. [103], however, showed that $A\beta_{25-35}$ could result in tau aggregation and that a decrease in Aβ aggregation was induced by tau peptides. Hence, aggregation of tau may be correlated with disassembly of Aβ which could explain the lack of spatial correlation [12]. In addition, SP and NFT may be temporally separated in the brain [82]. In entorhinal cortex [39] and in sector CA1 of the hippocampus [43], for example, NFT may precede the appearance of SP against the prediction of the ACH. In sector CA1, it is possible that Aβ is present in neurons before NFT are formed but not easily detectable by conventional methods [43].

Disconnection between the processes of Aβ and tau formation does not invalidate other features of the ACH. Hence, Aβ deposition may be the earliest lesion detectable in AD while tau formation occurs later by an independent process [66]. It is also possible that Aβ and tau formation are different consequences of degeneration of the same neurons, SP forming on the axonal terminals of NFT-containing neurons [3,31,102], which could also explain the spatial separation of SP and NFT within the brain.

Evidence from transgenic experiments

Based on the ACH, a variety of transgenic rodent models have been developed in an attempt to replicate AD pathology, including those based on single, double, and triple-mutation. Single mutation models usually involve various *APP* transgenes only. Hence, in the *APP:V642I* mouse, there was an increase in $A\beta_{42}$ deposition, but no mature SP or NFT were formed [71], this result being fairly typical of such experiments. In the 'double Swedish model' (*APP: K670N, M671L*), however, Aβ deposits similar to those found in AD were formed, the deposits being immunoreactive for Apo E, but no NFT were observed [120]. In the 'Indian/Swedish mutation model', both diffuse and compact-type Aβ deposits were observed

at 9-10 months of age, amyloid deposition increasing with age, and neocortex and amygdala being affected first followed by hippocampus and thalamus [38]. In addition, using the *APP:Tg2576* mouse, increased Aβ-induced oxidative stress was observed which encouraged increased activity of stress-related kinases and subsequently, tau phosphorylation in neurites surrounding SP [107].

Double mutation models usually include mutations of both *APP* and *PS1* transgenes. In one study (*APP: K670N, M671L; PS1: M146L*), long-term potentiation (LTP) of neurons was observed as early as three months, reduced LTP paralleling the appearance of SP [135]. In a similar model, which also incorporated an *APP V717I* mutation, there was age-related loss of pyramidal neurons in the hippocampus CA sectors including at sites devoid of Aβ deposition [119]. In a further *APP/PS* model, Aβ deposits were observed at eight weeks and hyperphosphorylated tau as punctate deposits at 24 weeks, consistent with the ACH, but DN were not as heavily labelled as those in AD [74].

A few transgenic experiments have involved triple mutation models (*APP*, *PS1* and *tau*). In one experiment (*APP:* Swedish mutation*, PS1: M146V, tau: P301L*), Aβ and tau pathology was present in temporal lobe with regional specificity similar to AD, Aβ deposition occurring before tau formation as predicted by the ACH [98]. In further experiments involving this model, AD immunotherapy was found to reduce Aβ and early tau epitopes also suggesting a direct pathogenic relationship between the two pathologies [99].

Hence, the presence of an *APP* mutation alone or in combination with *PSEN1* can result in Aβ deposition, but apart from evidence of hyperphosphorylated tau in neurites associated with SP, do not appear to induce NFT or a significant inflammatory response. The presence of tau transgenes in the form of a triple model appears to be necessary to more fully replicate AD pathology consistent with independent formation of Aβ and tau. However, in an APP-transgenic model, a reduction in endogenous tau ameliorated some of the effects mediated by Aβ which suggests a link between the two pathologies [110,111].

In conclusion, it is difficult to decide whether the presence of a specific mutation directly causes cell death as a result of Aβ-mediated toxicity or determines whether Aβ is secreted by neurons already damaged by a different process or from aging. A further problem is formation of Aβ may initiate a secondary phase of neurodegeneration that is difficult to distinguish from primary neuronal damage due to other causes. Nevertheless, a recent model, viz., *TgF344-AD* which incorporates mutant *APP* and *PS1* genes, exhibited age-dependent amyloidosis, tauopathy, gliosis, apoptotic loss of neurons in cortex and hippocampus, in addition to cognitive disturbance, and may offer a more complete model of AD [29].

Therapies for Alzheimer's disease based on the amyloid cascade hypothesis

There are many potential targets for AD therapy including APP cleavage, cytoskeletal destruction, neurotubule and ion homeostasis, metal ion accumulation, protein misfolding, oxidative stress, neuronal death, and gene mutation [24]. Therapies based on the ACH, however, have received considerable attention. Possible treatment strategies based on the ACH include inhibition of APP, inhibition of the proteases that liberate Aβ, and retarding maturation of Aβ into SP [121].

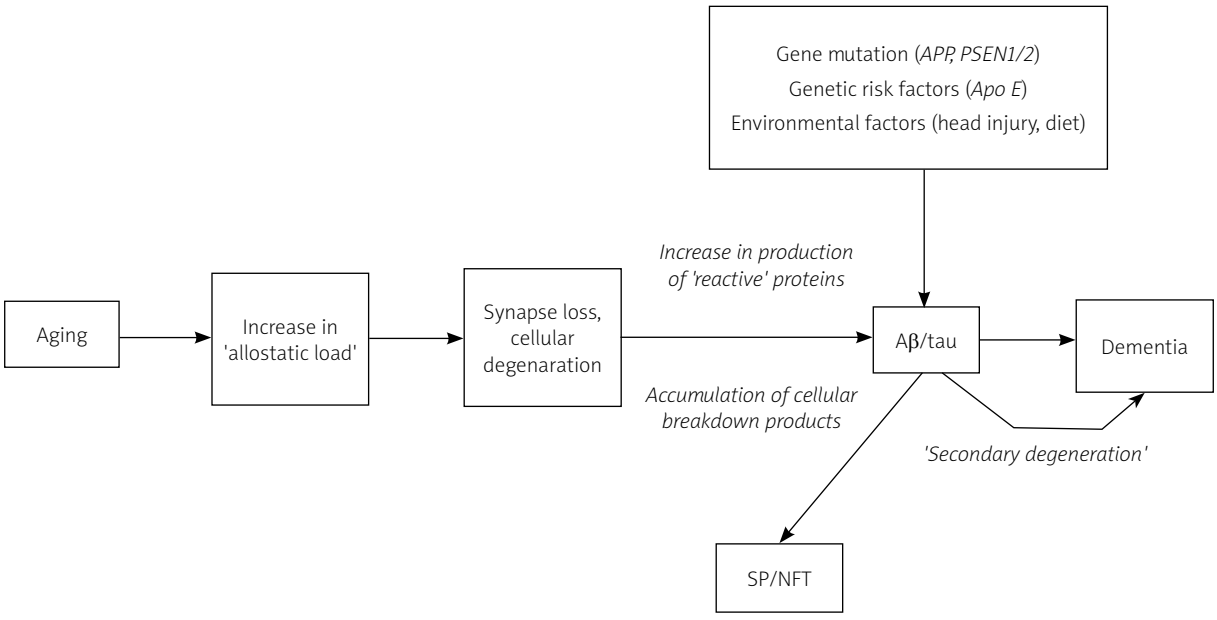
Some therapies have been based on targeting βACE1 [41]. In mice lacking this gene, there was a little apparent effect on behaviour and promising results were obtained in reducing the levels of SP. In humans, however, βACE1 also cleaves substrates involved in myelination, retinal homoeostasis, brain circuitry, and synaptic function, all of which could be affected if the enzyme was targeted [41]. In addition, treatment with γ-secretase inhibitors has been tested in rodent models and in humans. In rodents, early treatment with γ-secretase inhibitor can prevent or ameliorate Aβ deposition but established SP [1] and hyperphosphorylated tau aggregates [99] appear more resistant. In this regard, the failure of semagacestat in clinical trials, a well-characterised γ-secretase inhibitor, was particularly notable as Aβ deposition was reduced by this compound in both transgenic mice and humans [95].

There have also been clinical trials designed to remove or prevent Aβ formation using specific antibodies. Such antibodies can capture peripheral Aβ and prevent its re-entry from the periphery to the brain [32,33]. In addition, binding of specific antibodies to Aβ in deposited SP could activate microglia, thereby potentially clearing plaques by phagocytosis

[118]. Hence, a vaccine against Aβ was developed by activating microglia to remove SP [92]. Nevertheless, in clinical trials, some patients exhibited brain inflammation as a result of lymphocyte infiltration and elevated protein levels suggesting a breakdown of the blood brain barrier and entry of T-cells into the brain. Hence, Aβ-specific activated T-helper cells may amplify existing pro-inflammatory conditions present in AD. In addition, cytotoxic T-cells could attack APP present on the surface of many cells including neurons and release Aβ.

Treatment with bapineuzumab resulted in an overall Aβ deposit load and degree of CAA similar to controls [113]. Treatment, however, also altered the Aβ peptide profile of the patient, decreasing the proportion of $A\beta_{42}$ and increasing that of $A\beta_{40}$, but none of the treated patients exhibited improved cognition [113]. Subsequently, there has been a cessation of phase III clinical trials involving bapineuzumab in patients with mild to moderate levels of disease [26]. No treatment benefits have been established and several side effects reported. One possible explanation is that Aβ oligomers are pro-oxidants which combine with redox-active metals to form reactive species [130]. Hence, reducing Aβ may fail to halt cognitive decline because it is the effect of oxidative stress rather than Aβ directly which results in AD, Aβ and tau being upregulated to address the redox imbalance, possibly further accelerating the disease process [130].

As a result, after nearly 25 years of the theory, no new therapeutic agents for AD have reached the clinic based on the ACH [93]. Recent remarks by Castellani and Perry [26] that studies of Aβ metabolism have essentially been 'exhausted to no avail' reinforce this conclusion. Nevertheless, there are two questions raised by these trials. First, by how much should Aβ be reduced in brain to obtain a therapeutic effect [69]? Estimates of the total amount of Aβ deposited in an AD brain vary from approx. 4 mg [94] to 10 mg per brain [53]. In addition, it is difficult to quantify the rate of production of new Aβ in humans, which is often extrapolated from rodent cell culture models [18], thus making it difficult to establish treatment dosage. Second, are treatments applied to patients 'too late' in the disease process, i.e., after significant neuronal degeneration has already occurred, for trials to be effective? Recent criteria for the identification of pre-symptomatic AD using biomarkers and brain scan [89] may enable treatments to be tested at a much earlier stage of the disease and therefore, a more rigorous trial of the various treatments based on the ACH are possible in future.



Aβ – β-amyloid, Apo E – apolipoprotein E, APP – amyloid precursor protein, PSEN1/2 – presenilin genes 1 and 2, NFT – neurofibrillary tangles, SP – senile plaques

Fig. 3. A modified scheme based on the amyloid cascade hypothesis (ACH).

A modified scheme for Alzheimer's disease pathogenesis

These observations taken together suggest that the ACH may not provide a complete explanation of AD pathogenesis. There is doubt regarding the primary role of Aβ, whether Aβ leads to tau formation, and whether SP and NFT are directly related to clinical dementia. There are two possible directions of future research. First, to attempt a further understanding of the mechanisms of Aβ-mediated neuronal loss within the context of the ACH [69] or second, to modify the ACH itself.

This review advocates the second approach and proposes a modified scheme based on the ACH, which incorporates the various concerns and questions raised (Fig. 3). First, the primary factor in this scheme is age-dependent breakdown of anatomical systems and pathways within the brain and consequent loss of synapses [25]. The extent of this aging effect, which begins early in life, is mediated by the degree of lifetime stress (the 'allostatic load'). The brain is the ultimate mediator of stress-related mortality through hormonal changes resulting in hypertension, glucose intolerance, cardiovascular disease, and immunological problems [25]. Second, the consequence of increasing allostatic load is gradual synaptic disconnection, neuronal degeneration, and the upregulation of genes determining various reactive and breakdown products resulting in the formation of Aβ and tau [14,96,108,138]. Third, in small numbers of families, specific *APP* or *PSEN* mutations influence the outcome of this age-related degeneration by determining the quantity, solubility, and/or toxicity of the molecular product. Cells have mechanisms to protect against the accumulation of misfolded and aggregated proteins including the ubiquitin system and the phagosome-lysosome system. Neuronal degeneration in individuals with specific mutations results in the accelerated formation of Aβ and tau, and then a further phase of 'secondary' neurodegeneration, which overwhelms the protection systems. Early-onset FAD is the consequence of this process. Fourth, in individuals without a specific genetic mutation, and where more complex genetic and environmental risk factors are present, the outcome of age-related loss of synapses is mainly soluble and smaller quantities of insoluble proteins which are degraded by the cellular protection systems and do not significantly accumulate to form SP and NFT. With advancing age, however, the protective systems become less effective resulting in slowly accumulating quantities of Aβ and tau. The result of these insidious processes is that the cellular protection systems do not become overwhelmed until much later in life, the consequence being late-onset SAD.

This modified scheme may explain the similarities in the phenotypes of FAD and SAD, why SP and NFT often appear to be distributed independently, and reflects data suggesting that Aβ and tau may be formed as a response to cellular degeneration.

Predictions of the modified hypothesis

The modified hypothesis makes a number of predictions that could be usefully investigated to test its validity. First, significant signs of neuronal degeneration should precede Aβ deposition especially in late-onset SAD. Second, any toxic effects of Aβ will be secondary rather than primary. Third, SP and NFT are both the consequences of neuronal degeneration essentially arising independently as a result of age-specific synapse loss. Fourth, in transgenic experiments, the effect of the transgene will be age-dependent. Hence, in a model which incorporates an *APP*: *V717I* mutation, for example, there was an age-related loss of pyramidal neurons in the hippocampus CA sectors including at sites devoid of plaque deposition [119], consistent with this prediction.

Implications of the modified hypothesis

The modified scheme has a number of implications. First, that SAD is not a disease linked primarily to defective genes but a complex syndrome dependant on the rate of aging and indirectly influenced by numerous genetic and environmental risk factors acting over a lifetime. Second, the hypothesis questions whether the presence, distribution, and molecular determinants of SP and/or NFT should continue to play a primary role in the pathological diagnosis of AD. If SP and NFT are ultimately the products of brain degeneration and not its initial cause [14], they may represent relatively late stages of the disease. Hence, there could be cases of AD that are difficult to classify pathologically because they may have insufficient numbers of SP and NFT or exhibit early developmental stages of these pathologies. In addition, if SP and NFT represent the consequence of neurodegeneration affecting different parts of neurons rather than being characteristic of a particular disease, there are likely to

be many cases that show combinations of pathological features, i.e., there will be some overlap between different disorders. Numerous examples of such cases have been reported in the literature, e.g., dementia with Lewy bodies (DLB) with associated AD pathology, Creutzfeldt-Jakob disease (CJD) with AD, Pick's disease (PkD) with AD, and these cases are often difficult to classify within the existing system [15]. Finally, should significant efforts continue to be devoted to immunotherapy and other treatments designed to remove Aβ from the brain? Such treatments could be beneficial in limiting the degree of secondary degeneration caused by Aβ. Nevertheless, Aβ might also be beneficial to the nervous system by promoting neurogenesis [80] and having a range of other protective functions [77]. In addition, excessive removal of Aβ could potentially reduce chelation within the brain resulting in enhanced oxidative stress [16].

Conclusions

Since 1992, the ACH has played the most influential role in explaining the pathogenesis of AD. It proposes that the deposition of Aβ is the initial pathological event leading to the formation of SP and NFT, death of neurons, and ultimately dementia. A number of major limitations of the ACH have been identified: (1) SP and NFT may develop independently, (2) SP and NFT may be the products rather than the cause of neurodegeneration in AD, (3) SP and NFT may not be closely correlated with the onset, severity and development of clinical dementia, (4) transgenic rodent models do not generally replicate all aspects of AD pathology, and (5) treatments based on the ACH have not been successful. A modification to the ACH is proposed which may better explain the pathogenesis of AD, especially in late-onset cases of the disease. The modified scheme makes a number of predictions which could be usefully investigated.

Disclosure

Author reports no conflict of interest.

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