Alzheimer's Disease: A Central Role for Amyloid

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Describing the neuropathology of his first reported case, Alois Alzheimer wrote in 1907: "Scattered through the entire cortex, especially in the upper layers, one found military foci that were caused by the deposition of a peculiar substance in the cerebral cortex" (1). Almost 9 decades later, students of Alzheimer's disease (AD) are in the midst of a debate about whether the Bavarian psychiatrist was right in thinking of the "peculiar substance"—now known to be β-amyloid—as a causative feature of the syndrome which bears his name. As perhaps never before, this debate should be earnestly pursued, with accrual of rigorous evidence on either side, because amyloid-inhibiting therapeutic compounds are actively being sought and are likely to emerge before long. It is important to confirm or deny the hypothesis that β-amyloid deposition plays a central and sometimes causative role in the disease and is thus a worthy therapeutic target. In the following pages, the arguments for and against an early and critical role for β-amyloid deposition are reviewed, and a way to incorporate amyloid plaque formation into the complex pathogenic cascade of the disease is proposed.

Why Has β-Amyloid Received So Much Attention in Recent AD Research?

There can be no doubt that characterization of the 40–43 residue amyloid β-protein (Aβ) and its large precursor, amyloid β-protein precursor (BPP), has received intense scientific scrutiny both from investigators traditionally working on AD and those in other disciplines who have increasingly chosen to focus their research on these molecules. Although numerous reasons for this level of interest could be suggested, four explanations are particularly compelling. First, cerebral Aβ deposition is an invariant feature of AD, and that form of Aβ deposit which is intimately associated with dystrophic dendrites, axons, microglia and astrocytes (the neuritic plaques) (2) is substantially more abundant in the limbic and association cortices of AD than age-matched control brains in virtually all cases. (This is not always the case for the "diffuse" Aβ plaques, which largely lack associated neuritic and glial alteration, as will be discussed below.) Second, at least two genetically defined forms of AD appear to be caused by altered BPP metabolism or expression and subsequent accelerated Aβ deposition: families bearing BPP missense mutations (3) and patients with trisomy 21 (4). Importantly, the neuropathological phenotype in these forms is usually highly similar to, if not indistinguishable from, that of far more common sporadic and familial forms of the disease. Third, several other amyloid deposition diseases in humans have been elucidated at the molecular level, and in most of these, altered structure and metabolism of an amyloidogenic precursor protein (e.g. transthyretin, cystatin C, gelsolin) have been shown to be the molecular cause of the respective disease (e.g. 5). Fourth, other than β-amyloidosis, no compelling pathogenetic agent that can be said to be etiologic for AD has emerged after years of intensive research. The lack of rigorous evidence that a specific viral or toxic agent, a primary loss of trophic factor(s), or a specific defect in intermediary metabolism can initiate the AD cascade has allowed the "amyloid hypothesis" to emerge as the etiopathogenetic mechanism with arguably the most extensive and compelling supporting data.

Concerns have been raised that the amyloid hypothesis has been "oversold," the implication being that β-amyloid has been proposed to explain most or all features of the disease. Although many investigators studying BPP and Aβ would argue that they have not claimed this, it should clearly be stated that cerebral deposition of Aβ cannot by itself explain the clinicopathological phenotype of AD. The very fact that diffuse Aβ deposits may occur in substantial numbers in the brains of aged non-demented humans and lower mammals in the absence of surrounding neuritic/glial alteration suggests that Aβ can serve as an initiator and/or early pathological product in a slow, multi-step cascade that ultimately produces neuronal, and thus clinical, dysfunction. Disruption of synapses (6), formation of neurofibrillary tangles (NFT) composed of hyperphosphorylated tau protein (e.g. 7) and widespread neuritic dystrophy are more proximate and quantitatively meaningful markers of the AD clinical syndrome than is amyloid deposition. The central thesis of this review is that Aβ deposition is a necessary but not sufficient factor for the pathogenesis of AD. Deposits of Aβ are almost always abundantly present in clinically relevant regions of the AD brain and are the earliest known histological alteration to occur, but there must be numerous additional molecular and cellular changes accompanying Aβ deposition before neuronal/synaptic dysfunction and thus cognitive impairment can be expected to occur. Furthermore, a central question of current AD

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research—whether Aβ deposition is a cause or an effect of the disease—is increasingly likely to be answered "both." In at least some cases, DNA point mutations leading to βPP mismetabolism and heightened Aβ deposition have virtually been proven to be the molecular cause of the disease, whereas in other cases, excess Aβ deposition appears to be an early response to an alteration of another gene product (e.g. the as yet undefined gene on chromosome 14). A plausible explanation for this complexity is that AD is actually a polygenic syndrome in which an array of distinct genetic alterations will ultimately be found to underlie many or most AD cases, and that these act through a common pathogenetic mechanism that serves to alter the balance between Aβ production and clearance in the brain. Those choosing to focus on βPP metabolism as a route to the mechanism and treatment of AD should emphasize that there are many other features of the disease that need to be intensively studied and understood before a complete and balanced picture of pathogenesis can emerge.

Salient Characteristics of Aβ and its Large Membrane-anchored Precursor

The proteaceous, ~6-10 nm extracellular filaments (amyloid fibrils) that form innumerable spherical plaques and accumulate in cerebral and meningeal microvessels in AD patients are composed of a hydrophobic peptide designated Aβ (8-12). Aβ is a proteolytic fragment of βPP that comprises the 28 amino acids immediately amino-terminal to the single transmembrane domain of the precursor plus the first 12-15 residues of that domain (13). The term βPP actually designates a complex group of type I integral membrane glycoproteins, 100-140 kDa in relative molecular mass, that undergo N- and O-linked glycosylation, phosphorylation, sulfation and proteolytic cleavage and secretion of the large extramembrane region (14-17). The heterogeneity of βPP molecules also arises in part from alternative splicing of a single gene, which is located in humans on the long arm of chromosome 21 (13, 18-21). βPP is expressed in virtually all mammalian cells, and it shows considerable evolutionary conservation (e.g. 22, 23). Indeed, recent descriptions of other mammalian proteins with extensive sequence similarity (albeit largely distinct in the Aβ region) (24-26) confirm that βPP is one member of a family of closely related transmembrane polypeptides.

The intracellular processing of βPP and the pathways by which Aβ could either be generated or degraded have received considerable attention. An as yet unidentified protease (dubbed α-secretase) can cleave mature, N- plus O-glycosylated βPP at residue 687 (βPP70, numbering) just outside the transmembrane region (i.e. residue 16 of Aβ) to release the soluble ectodomain (βP70) into the extracellular fluid (27). Those βPP molecules cleaved at this site obviously cannot release intact Aβ, but the portion of molecules undergoing such secretion varies considerably and may only approximate 30% even in robustly secreting cells (16). Primary neurons, astrocytes and microglia all express βPP abundantly but show low levels of α-secretase cleavage of βPP in culture and instead appear to process most of the precursor via an alternative mechanism (28). Initial evidence that one such pathway might involve trafficking to endosomes and lysosomes included the immunochemical detection of βPP epitopes in lysosomes (29) and the accumulation of various carboxy-terminal fragments in cells treated with agents that inhibit lysosomal function (30, 31). Some of these fragments are potentially amyloidogenic, that is, their size and Aβ immunoreactivity indicate that they contain the intact Aβ region (31). This indirect evidence of a lysosomal processing pathway for βPP has subsequently been confirmed by the direct demonstration that cell-surface βPP, labeled with either antibodies or biotin, can be transported to late endosomes and lysosomes, within which an array of membrane-retained, Aβ-bearing fragments can be found (32). The reinternalization of cell-surface βPP probably involves a consensus sequence (asn-pro-xxx-tyr) in its cytoplasmic tail known to mediate the endocytosis of certain membrane-spanning receptors via clathrin-coated pits. Some cell-surface βPP molecules can alternatively serve as a substrate for α-secretase cleavage, releasing βP70 into the medium (32, 33). There is evidence that βPP, can also be generated intracellularly (34, 35). In summary, two general routes for intracellular βPP trafficking—exocytotic (secretory) and endocytotic (reinternalization and lysosomal targeting)—have been identified to date, although other routes are likely to be found.

Aβ is a Soluble Product of the Normal Cellular Processing of βPP

It had been generally assumed that the Aβ fragment, containing as it does part of the membrane-anchoring domain of βPP, would not be released from cells without prior membrane injury and thus must derive from aberrant processing of βPP. This concept, as well as the highly hydrophobic composition of Aβ, dissuaded investigators from searching for Aβ in normal biological fluids. However, metabolic labeling of primary cells or βPP-transfected cell lines followed by immunoprecipitation of their conditioned media with Aβ antibodies revealed the presence of a soluble 4 kDa peptide, which, upon sequencing, was confirmed to be Aβ (36-38). A related 3 kDa peptide (designated p3) was also precipitated from media by antibodies to the carboxy-terminus of Aβ; sequencing showed that this fragment began at or adjacent to the α-secretase cleavage site (Aβ residue 17) (36). Aβ was also found to be present in normal and AD cerebrospinal fluid (CSF) (37, 38) and perhaps in plasma (37). Trials to determine whether CSF or plasma Aβ levels are sig-
nificantly altered in AD patients are underway. ELISA measurements have shown that the physiological levels of soluble Aβ in culture medium and human CSF are in the high picomolar to low nanomolar range (37, 39).

A wide variety of βPP-expressing primary cells and transformed cell lines have now been shown to secrete Aβ continuously under normal culture conditions (36–38, 40). Experiments using cell biological toxins or mutagenesis of βPP suggest that Aβ is generated from mature, membrane-inserted βPP in an acidic compartment other than the lysosome; for example, in the late Golgi or in early endosomes (e.g. 41). Sequencing of secreted Aβ reveals considerable amino-terminal heterogeneity; although the major species usually begins at asp₁₅, minor peptides begin at residues ile₁₄, val₁₃, phe₁₂, and glu₁₁ (Aβ numbering) (36–38). Some of these species apparently accumulate together with the major asp₁₅ peptide in AD senile plaques (9, 42). As regards the carboxy terminus of soluble Aβ, the major species appears to end at val₃₀ (37), but longer and shorter peptides are found both in culture medium (43) and human CSF (39). That in vitro Aβ production is directly relevant to the situation in vivo is strongly supported by the occurrence of the same Aβ peptides in both fluids.

To summarize available information on βPP metabolism, both neural and non-neural cells can process some precursor molecules in a regulated manner by at least two alternative but normal proteolytic events. One of these involves α-secretase cleavage of mature, N- plus O-glycosylated βPP at lys₂₀, within the Aβ region, probably during as well as after its transport from the Golgi to the cell surface. The second involves cleavage of mature βPP at met₃₇, creating the amino terminus of Aβ (asp₆₂). One subcellular localization of this cleavage event appears to be the early endosome, because iodination of cell-surface βPP in living cells has recently been shown to lead directly to secretion of iodinated Aβ into the medium (44). After either mode of βPP cleavage, portions of the resultant ~10 and ~12 kDa carboxy-terminal fragments undergo an additional cleavage in the region val₆₁⁻thr₇₁, creating the carboxy termini of the p3 and Aβ peptides, respectively. The latter cleavage seems to be associated with the rapid release of p3 and Aβ from the cell, as they appear to occur at very low levels intracellularly.

In Vitro Studies Suggest Several Putative Functions for βPP and Aβ

Given the widespread cellular expression of βPP and its homologues, it is important to determine the functional properties of the molecule and its secreted derivatives, regardless of their importance to the mechanism of AD. Those secreted forms of βPP, which contain a Kunitz-like protease inhibitor (KPI) motif (referred to as protease nexin 2 [PN2]) have been shown to inhibit trypsin, chymotrypsin, the y subunit of nerve growth factor and other serine proteases in vitro (45–47). A previously identified serine protease inhibitor involved in coagulation, factor XIIa inhibitor, has been found to be identical to PN2, suggesting one potential physiological role for the major secreted βPP derivative (48). Because PN2 is stored in the α-granules of platelets and released upon platelet activation, it has been postulated that this secreted derivative may be involved in the repair process of vascular injury and in wound healing (48, 49). There is also evidence that βPP may serve as an extracellular matrix molecule which can mediate the adhesion and growth of neural and non-neural cells (50–54). Because endothelial cells express βPP and polarized epithelial cells have been shown to secrete βPP, (and also Aβ) preferentially from their basolateral surface (55), such cells could serve as a source of βPP, in the basement membrane of blood vessels. Besides a possible role in cell adhesion, βPP (both KPI+ and KPI− forms) has been shown to have a growth-promoting activity on non-neural and neural cells (56, 57). βPP has also been shown to provide neuroprotective properties for cultured primary neurons, in part by lowering intracellular calcium levels (58). In addition to demonstrating these putative activities of secreted βPP, cell culture studies have also suggested that full-length βPP expressed at the cell surface confers a neuronal attachment and neurite-promoting activity (59).

The recognition that Aβ is also a constitutively secreted product of βPP metabolism has raised the question of whether it may have a physiological function throughout life. Alternatively, it may simply represent a semi-stable intermediate during βPP catabolism, one that accumulates in part because of its hydrophobic sequence. Soluble Aβ and its fragments have been shown to produce trophic effects on neurons and their processes when added to culture medium (60–62). Aβ is also capable of interacting with extracellular matrix components such as laminin or fibronectin to promote neurite outgrowth, suggesting that Aβ could function in part as an immobilized substrate rather than a diffusible ligand (63). The hypothesis that Aβ might act as a ligand for a neuronal receptor (e.g. as a receptor for tachykinin peptides, with which Aβ shares limited homology [61]) has not yet been confirmed by showing that Aβ specifically binds to neuronal membranes at physiological doses and with saturable and displaceable kinetics (see e.g. 64). Establishing a clear functional role for Aβ at physiological concentrations in neural and non-neural tissues will require much further study. Even if a normal function is confirmed, this would not preclude the therapeutic use of Aβ inhibitors (see below), because such molecules can be designed to produce only partial inhibition.

The Molecular Genetics of Familial AD Implicates βPP Mismetabolism

Not surprisingly, the clearest insights into the actual causes of AD have come from the application of genetic
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linkage analysis to families with autosomal dominant forms of the disease. The identification of a glu → gln substitution at residue 693 of BPP

_\text{trans} (residue 22 of Aβ) established the molecular basis of hereditary cerebral hemorrhage with amyloidosis of the Dutch type (65), a disease that produces an amyloid phenotype clearly related to that of AD, including widespread cortical Aβ deposition in the form of diffuse plaques. Closely following upon this observation was the discovery of first one and then several other BPP missense mutations within or flanking the Aβ region in various families with early-onset AD (reviewed in 3). The strong linkage of these BPP missense mutations to the clinical-pathological phenotype has provided unequivocal and mechanistically important evidence that disordered BPP metabolism is capable of initiating the complex neuropathology of AD. Moreover, the biochemical mechanisms by which these several mutations lead to the AD phenotype are being steadily identified, and the results to date point clearly to quantitatively or qualitatively abnormal Aβ production rather than to dysfunction of BPP as the responsible event (66–69). Perhaps the single form of familial AD whose mechanism has been most clearly elucidated is that of the double mutation lys_{69} → asn/met_{69} → leu found in a Swedish kindred (70). Transfection of this mutant form of BPP into cells induces a 5–8-fold augmentation of Aβ production (66, 67). Moreover, blinded analyses of Aβ levels in the media of primary skin fibroblasts cultured from Swedish gene carriers vs their unaffected relatives confirms a several-fold increase in Aβ secretion (71). Importantly, this increased Aβ production by cells from a phenotypically normal tissue was found even in a presymptomatic subject more than a decade prior to the expected age of onset of AD in this family. This observation provides incontrovertible evidence that excessive Aβ production can arise prior to or in the absence of local cellular pathology rather than as a secondary effect thereof.

Another type of BPP missense mutation, i.e. the three substitutions that have been found at codon 717 (3), has also been modeled _in vitro_ recently. The results suggest that these mutations lead to an increased portion of secreted Aβ peptides ending at residue 42 rather than 40 (68). As there is evidence that this slightly longer and more hydrophobic peptide can serve at low concentrations as a "seed" for enhanced aggregation of Aβ_{42} and other shorter peptides (72), these findings provide a plausible mechanism for the premature and severe Aβ plaque formation which is found in patients with the codon 717 mutations. Studies of yet another missense mutation (ala_{69} → gly) suggest that this substitution alters the amino-terminal microheterogeneity of secreted Aβ, including the appearance of more hydrophobic species (69). This mutation might also lead to enhanced amyloid deposition because the sequence switch within the β-peptide increases its potential for fibrillogenesis, a mechanism suggested by studies of the adjacent Dutch (glu_{693} → gln) mutation (73).

While rare, BPP missense mutations demonstrate clearly that the full-blown familial AD phenotype can begin with quantitative or qualitative changes in the Aβ fragment of BPP that lead to early and accelerated amyloid deposition. In trisomy 21, the overexpression of structurally normal BPP molecules (74) presumably underlies the development of a histopathological phenotype essentially indistinguishable from that of AD, since diffuse Aβ deposits begin to accumulate progressively early in life (sometimes in the teens or younger), prior to the development of any other cytopathological features of AD (4, 75). The recent discovery that a large percentage of families with early-onset AD is linked to a locus on chromosome 14 (76–79) confirms that AD is genetically heterogeneous, a conclusion that is not surprising in view of the remarkable commonness of the syndrome. Because these families experience an even earlier onset than the BPP-linked families of an otherwise indistinguishable severe β-amylodotic phenotype (80), the defective gene on chromosome 14 may well turn out to have a direct or indirect role in the expression or processing of BPP or in the generation, stability or clearance of Aβ itself. The existence of familial AD cases caused by genes on chromosome 14 and on other autosomes not yet identified indicates that a typical β-amylodotic phenotype can arise secondarily from different genetic alterations. In all of these situations, the progressive cerebral deposition of Aβ appears to be an early and necessary, but not sufficient, event in the pathogenesis of AD. The opposing concept, that the same β-amylodosis can sometimes be pathogenetically critical (as in the BPP mutation cases) and at other times be a clinically unimportant byproduct of the disease process, seems highly implausible. Further, such a tenet flies in the face of knowledge about other progressive amyloidoses and age-linked disorders such as atherosclerosis, which often have a common and critical pathological cascade that can arise from numerous distinct molecular etiologies.

Apolipoprotein E and the Pathogenesis of AD

The most recent genetic trait to be implicated in AD is the normal polymorphism of the apolipoprotein E (ApoE) gene on chromosome 19. The exciting discovery that the ε4 allele of ApoE can segregate with both sporadic and familial forms of AD (81–83) has been widely confirmed and has sparked interest in how this gene product may enhance both the likelihood of developing clinical AD and the rate at which it occurs. The latest developments in this rapidly evolving aspect of AD research are reviewed by Roses in this issue (84).

The first evidence that ApoE might be involved in AD was the report by Namba et al (85) that antibodies to
ApoE label vascular and plaque amyloid deposits and some NFT. It was subsequently pointed out that ApoE, like certain other circulating proteins, can associate not only with β-amyloid but also with numerous other types of amyloid, including those found in non-neural tissues (86). Strittmatter and colleagues (81) then showed that synthetic Aβ peptide immobilized on filters could bind ApoE present in human CSF. This finding, coupled with the known localization of the ApoE gene on chromosome 19q13.2, led these investigators to determine whether ApoE could be the gene responsible for their earlier observation that markers in this region showed apparent linkage to late-onset familial AD kindreds (87). They found that certain subjects with a family history of late-onset AD had a 2–3-fold increased likelihood of developing clinical AD if they had one e4 allele and an 8-fold increase with two e4 alleles (83). This group also reported a striking and consistent increase in both plaque and vascular Aβ deposits in e4 vs e3 homozygotes, with e4 heterozygotes having an intermediate rise (88). This result has been confirmed and extended by Rebeck and colleagues (89). Importantly, these two studies found no significant increase in the number of NFT in patients with one or two e4 alleles. Both studies concluded that the expression of ApoE4 substantially increases the tissue burden of β-amyloid, and Rebeck and colleagues (89) further suggested that an alteration of Aβ clearance might be involved, in view of their finding that a known receptor for ApoE complexes, the LDL receptor-related protein (LRP), showed enhanced immunochemical reactivity within senile plaques.

These findings raise two important questions. First, is ApoE itself the responsible gene on chromosome 19 or, less likely, is it in linkage disequilibrium with an adjacent gene that actually confers these phenotypic characteristics? Even if another gene were responsible, this would not obviate the possible utility of ApoE genotyping as a risk factor for the development of the disease and a way to help support a probable clinical diagnosis. Second, assuming that the ApoE gene itself is biologically implicated, what is the biochemical mechanism of its effect? The findings of Schmechel et al (88) and Rebeck et al (89) lead to the plausible but as yet unproven model that ApoE4 (or the absence of ApoE3) leads to decreased clearance, increased extracellular deposition or some other change in Aβ economy that augments the tissue burden of the peptide. This testable hypothesis now deserves further experimental attention. However, Roses, Strittmatter and their colleagues recently proposed, and voiced their strong support for, an alternate hypothesis: that the ApoE3 protein normally allows tau protein to stabilize microtubules and that its decrease or lack in patients with one or two e4 alleles leads to a dissociation of tau from microtubules and its enhanced phosphorylation and polymerization into the pathological paired helical filaments (PHF) of the NFT. They based this proposal on experimental evidence, not yet published, that purified ApoE3 can bind to recombinant tau in vitro whereas purified ApoE4 cannot. While all hypotheses regarding the role of ApoE in AD pathology should be addressed, there are numerous major issues that need to be explained before such a disease mechanism can be considered credible.

1) The only evidence that ApoE and tau might specifically interact appears to be based on in vitro mixing experiments using relatively high doses of each purified protein. The kinetics of this interaction have not been reported, and consequently, it is unclear whether this occurs in a physiological range. One needs to establish whether ApoE and tau can interact at normal concentrations and under conditions present in living cell systems. This could either be studied in cell culture or in experimental animals. It is known that the interaction of two purified proteins demonstrated in vitro may not occur in the same fashion or at all in the complex environment of the cell. The presence of a free cysteine on ApoE3 but not E4 that could potentially form a disulfide bond with cysteines on tau might explain the in vitro interaction; however, a variety of other proteins with exposed SH groups might also interact selectively with E3 and not E4 under similar conditions. In short, the specificity and physiological relevance of the ApoE3–tau binding observed in vitro needs to be firmly established.

2) It is unclear whether or how ApoE would come into contact with tau, which is a cytosolic protein believed to be restricted to the neuronal cytoplasm. In the nervous system, ApoE has been found to be expressed and secreted by astrocytes, and it is present in the CSF and presumably in the extracellular space of brain. Because neurons express LRP and perhaps other ApoE-binding receptors on their cell surface (89), extracellular ApoE could be internalized by neurons. However, it would presumably be confined to the endosomal/lysosomal system; a novel mechanism for the escape of the endocytosed, membrane-surrounded ApoE molecule from lysosomal degradation and its transport into the cytoplasm would need to be found. Immunochemical demonstration of ApoE staining of neurons by both tau and ApoE antibodies will not be sufficient, particularly in postmortem human brain. Even in optimally preserved rodent brain or cultured neurons, the actual coexistence and association of ApoE and tau as soluble molecules in the neuronal cytoplasm will require careful immunoelectron microscopy.

3) There has been no previous evidence that tau requires ApoE in order to perform its normal function as a microtubule-associated protein. Tau by itself or with other microtubule-associated proteins (in the absence...
of any ApoE) has been shown in many studies to bind to tubulin and to associate with microtubules. Careful kinetic studies under physiological conditions will now be required to prove the hypothesis that ApoE3 is critically necessary for tau to perform its normal functions of promoting the assembly of and stabilizing microtubules in intact neurons.

4) Roses' hypothesis places emphasis on a critical permissive role for ApoE4 in AD tangle formation. However, NFT are known to occur abundantly in a diverse array of etiologically distinct neurological disorders (e.g., 90), and some of these have no apparent relationship to ApoE genotype (e.g., Parkinson's disease, in which NFT indistinguishable from those that develop in AD often occur). Indeed, Roses and colleagues have specifically reported that Down's syndrome shows no segregation with ApoE genotype (82), and yet robust NFT formation occurs in virtually all cases of this disorder. Perhaps the most cogent example is the occurrence of NFT (and indeed the full-blown AD histological phenotype) in patients with AD who are ApoE3 homozygous. It is difficult to understand how the absence of ApoE3 plays a critical role in driving the pathogenesis of AD when many AD patients have two e3 alleles.

5) Although there can be little doubt that the altered phosphorylation state of tau which leads to widespread NFT formation is an important pathological feature that is likely to relate to neuronal/neuritic dysfunction and cell death in the disease, there is currently no rigorous evidence that tangle formation precedes amyloid plaque formation in AD. Indeed, as reviewed above, there is evidence from numerous laboratories that diffuse Aβ deposits predate, by many years or decades, tangle formation in trisomy 21 and normal human brain aging (and, by implication, in AD). Thus, a primary pathogenic role for PHF formation that is in turn driven by ApoE genotype does not fit with known neuropathological information.

6) Whereas Aβ deposition and neuritic plaque formation are seen in all cases of AD, some 10–15% of cases have been reported to show very little or no neocortical NFT formation (91, 92). Again, a primary role for NFT formation driven by ApoE is improbable in such cases, which otherwise have the usual clinical and pathological features of AD (91). (One would like to know whether these largely tangle-free cases always represent ApoE3 homozygotes.)

7) Importantly, the new hypothesis by Roses and colleagues does not incorporate the role of BPP metabolism and Aβ deposition in the disease mechanism, despite the fact that the authors themselves showed a substantial and statistically significant increase in amyloid plaque formation in e4 vs e3 gene carriers (88).

This last point raises the issue that any hypothesis about the fundamental mechanism of AD should strive to include most or all of the known observations about the disease. The most compelling model will be one that can incorporate the diverse cellular and molecular alterations documented in AD brain tissue in a rational cascade. The role of progressive Aβ deposition must be addressed by any credible model, particularly in view of the strong genetic evidence implicating it in the disease.

A particular concern about analyzing the strengths and weaknesses of the disease mechanism proposed by Roses and colleagues is that it is apparently based almost exclusively on in vitro protein–protein binding data, and this data was not published and therefore unavailable to other workers in the field at the time the theory was promulgated. The public proposal of a broad and far-reaching scientific hypothesis, particularly one that is proposed to change radically the scientific community's view of the mechanism of a major human disease, should be based on as much peer-reviewed, published experimental data as possible, so that it can be carefully analyzed, and experiments to confirm or deny the hypothesis can quickly be undertaken. In few situations is this more important than AD, in which there is intense interest in understanding the correct mechanism of the disease by both the scientific and lay public and in which even minor advances in research on the disease are dramatically magnified by the press. From this viewpoint, and with the severe caveats listed above, the question can be raised as to whether the widespread and intensive discussion of the ApoE/tau hypothesis substantially precedes the scientific data required to rigorously assess it.

The Relationship of β-Amyloid Deposition to Other Features of AD Pathogenesis

In constructing a disease model which is likely to stand the test of time, one must begin with what has already been proven. In at least a few familial AD families, βPP missense mutations and premature and progressive deposition of quantitatively or qualitatively altered Aβ is very likely to represent the initial event. Trisomy 21 is similarly likely to involve such a mechanism, based on the lifelong increase in βPP expression. In the vast majority of familial and sporadic AD cases, we do not have a clear understanding of the initiating molecular event(s). However, the fact that very few important differences in neuropathological phenotype have been found among various familial and sporadic forms of the disease suggests that it is reasonable to consider mechanisms analogous to those in the chromosome 21-linked cases for other forms of the disease. Therefore, the hypothesis put forward here emphasizes that AD is a polygenic syndrome in which several (perhaps many) distinct gene defects lead, directly or indirectly, to altered βPP expression, processing or degradative metabolism or changes in
Aβ aggregation or binding that ultimately result in a chronic imbalance between Aβ production and clearance. This imbalance leads to the gradual accumulation over years or decades of widespread Aβ deposits, far in excess of those produced during aging. Why most of the parenchymal deposits assume a roughly spherical form remains an enigma and probably relates in large part to the unresolved question of the precise cellular source of Aβ in plaques. While most Aβ deposits appear to remain largely non-fibrillar and unassociated with surrounding neuritic and glial dystrophy, some deposits in association cortex, limbic structures and certain other regions become increasingly dense and fibrillar. During this evolution, numerous "β-amylloid-associated proteins" (e.g. heparan sulfate proteoglycan, α-antichymotrypsin and certain complement components) become associated with most cortical plaques, but less so with plaques in "non-symptomatic" brain regions such as cerebellum. Slow accrual of more Aβ and the accumulation of the associated proteins may in part be responsible for the "maturity" of cortical plaques. An additional gradual alteration is the local appearance of activated microglia and reactive astrocytes in and around the developing plaque. It is likely that a specialized inflammatory process occurs in many of the amyloid-bearing plaques in cortex.

Either in concert with or following the microglial and astrocytic responses, Aβ itself and/or its closely associated proteins gradually induce a mixture of trophic and toxic effects on immediately surrounding neurites. Evidence that Aβ may have toxic effects on cultured neurons has been provided by numerous recent studies and is reviewed elsewhere (93). One emerging principle about Aβ toxicity is that it depends in part on the aggregation state of the peptide, with insoluble aggregates having β-sheet conformation being more likely to induce cytotoxicity. The centrifugal involvement of many neurites in the vicinity of maturing Aβ plaques could account for the fact that neurites of varying transmitter specificities are found in any one plaque. Nevertheless, it is likely that certain neuronal populations are more or less resistant to the trophic and toxic influences of the plaque. One major result of the progressive β-amyloidotic process on surrounding neurites is predicted to be the induction of cytoskeletal alterations, including alterations of tau phosphorylation and subsequent PHF formation. How extracellular Aβ accumulation and plaque maturation might trigger alteration of tau and other intraneuronal proteins remains an active issue for further study.

The result of the multiple cellular and molecular changes that are hypothesized to follow early Aβ deposition and plaque maturation is the progressive dysfunction and death of many neurons, including profound synaptic loss (6), both within association cortex and in subcortical regions that project to cortical and limbic areas rich in amyloid plaques. The synaptic loss would not have to occur solely in the immediate vicinity of the mature plaque. The basis of the selective vulnerability of neurons to this process and the curious failure of diffuse Aβ deposits in certain brain regions (e.g. cerebellum, striatum) to "mature" remains unknown. The possibility that amyloid-bearing neuritic plaques never derive from diffuse Aβ deposits but rather are an entirely distinct type of lesion cannot be dismissed, although this seems unlikely. The model proposed here can nicely incorporate the effects of ApoE genotype, in that ApoE4 may modify importantly the β-amyloidotic process in a way that alters the balance between Aβ deposition and clearance in favor of increased plaque and vascular amyloid (88).

Perhaps the most frequently voiced objection to this amyloid cascade hypothesis is that some, and often many, Aβ plaques are found in the brains of aged humans who were cognitively normal. However, these limbic and cortical Aβ deposits are almost entirely of the diffuse type. Stains which sensitively recognize dystrophic neurites, such as the Bodian silver stain or various immunocytochemical markers (tau, ubiquitin, etc.), show little reaction in most of these cases. Indeed, the absence of peri-plaque neuritic alteration and associated astrocytosis, microgliosis and tangle formation represent the neuropathological basis on which we distinguish AD from "normal aged" brains at autopsy. The presence of diffuse deposits of Aβ, which have indeed been shown to contain no significant decrease in synaptic density (in contrast to the synaptic loss within mature neuritic plaques) (94), should not, in my view, be construed as evidence that Aβ deposition is essentially innocuous and plays no dynamic role in the cytotoxicity which surrounds dense fibrillar deposits of Aβ. In this regard, a reasonable analogy can be made to fatty cholesterol streaks as a very early lesion in the pathogenetic cascade that leads to atherosclerosis. While the total amount of fatty streaks in the vasculature as a whole is not a simple and direct correlate of the degree of symptomatic cardiovascular or cerebrovascular disease, the number and location of mature, advanced cholesterol plaques with their numerous other molecular and cellular constituents can be correlated with clinical disease, particularly when such plaques occur at critical sites in the vascular tree. In the same way, while total Aβ burden is indeed increased in the large majority of AD cases compared to age-matched controls, it is the mature amyloid-rich plaques with their surrounding neuritic and glial alteration that should be considered a partial correlate of the clinical state, not the total number of immunoreactive Aβ deposits, many of which remain diffuse even at the end of the patient’s life.

The fact that Aβ deposition by itself does not always show a simple, uniform relationship to the clinicopathological phenotype should not dissuade us from pursuing strategies to reduce the cerebral burden of Aβ deposits, particularly of those that are associated with surrounding
glial activation and neuronal dystrophy. The steadily mounting evidence that BPP metabolism and Aβ aggregation are pathogenetically critical features of the disease has now led to widespread attempts to identify molecules which can interfere with one or more steps in this complex cascade. Among these steps, inhibition of the proenzymes which generate Aβ, retardation of the conversion of soluble monomeric Aβ to insoluble aggregated Aβ, and interference with the toxic response of neurons and their processes to Aβ or closely associated proteins are among the strategies being most actively explored at present. Numerous other approaches, including, for example, inhibition of the peri-plaque inflammatory response and interference with tau hyperphosphorylation, will receive increasing attention in the near future. This multi-pronged attack on the disease process envisages a future in which several distinct pharmacological therapies will be developed to address both different stages in its evolution and some of the distinct molecular causes of the AD syndrome.

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