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Alzheimer's Disease and the β -Amyloid Peptide

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Abstract

Alzheimer's disease (AD) pathogenesis is widely believed to be driven by the production and deposition of the β -amyloid peptide (A β). For many years, investigators have been puzzled by the weak to nonexistent correlation between the amount of neuritic plaque pathology in the human brain and the degree of clinical dementia. Recent advances in our understanding of the development of amyloid pathology have helped solve this mystery. Substantial evidence now indicates that the solubility of A β , and the quantity of A β in different pools, may be more closely related to disease state. The composition of these pools of A β reflects different populations of amyloid deposits, and has definite correlates with the clinical status of the patient. Imaging technologies, including new amyloid imaging agents based on the chemical structure of histologic dyes, are now making it possible to track amyloid pathology along with disease progression in the living patient. Interestingly, these approaches indicate that the A β deposited in AD is different from that found in animal models. In general, deposited A β is more easily cleared from the brain in animal models, and does not show the same physical and biochemical characteristics as the amyloid found in AD. This raises important issues regarding the development and testing of future therapeutic agents.

Keywords

Amyloid; β -Amyloid Precursor Protein; A β ; oligomer; fibril

The two hallmark pathologies required for a diagnosis of Alzheimer's disease (AD) are the extracellular plaque deposits of the β -amyloid peptide (A β) and the flame-shaped neurofibrillary tangles of the microtubule binding protein tau. Familial early onset forms of AD are associated with mutations either in the precursor protein for A β (the β -amyloid precursor protein, APP) or in presenilin-1 (PS1) or presenilin-2 (PS2). Either PS1 or PS2 can be the catalytic subunit of γ -secretase, which is the final endoprotease in the pathway that generates the peptide. Despite this genetic evidence and the demonstrated involvement of A β in inducing synaptic dysfunction, disrupting neural connectivity, and association with neuronal death in a brain region-specific manner, the amounts and distribution of A β deposition are only weakly correlated with the clinical expression of the disease.

Alzheimer's disease is characterized clinically by a progressive and gradual decline in cognitive function and neuropathologically by the presence of neuropil threads, specific neuron loss, and synapse loss in addition to the hallmark findings of neurofibrillary tangles and senile plaques. The methods used to assess the pathology and classify stages of AD have been standardized and codified to provide a guide for clinicians. Standard measures of pathology refer to the density of neuritic amyloid plaques and neurofibrillary tangles of tau protein in affected brain regions. The presence of neuritic plaques composed (in large part) of highly

insoluble A β in the brain parenchyma is required for a diagnosis of AD. Deposits of tau protein are also present, although they are also found in a number of less common neurodegenerative diseases, notably in the absence of neuritic plaques. The neurofibrillary tangles in the different diseases have some distinctive morphological features and may exhibit a distinct composition of tau isoforms that differs from AD [1].

Development of a disease stage classification for AD has not been a simple process, nor is there complete consensus with the system(s) that are in place. Definitive staging of disease state remains a judgment call decided in clinicopathological conferences between clinicians, neuropsychologists, and pathologists. A major deficiency in the staging system is that it can only be approximately applied in the living subject. Since AD pathology is determined at autopsy, a clinical diagnosis of probable AD has to be used instead. The lack of an in-life diagnostic test greatly hampers research efforts on disease mechanisms, and is a particular problem for clinical trials as it introduces additional heterogeneity into the subject population. Therapeutics cannot be properly tested if they need to be administered before the disease progresses past a certain stage, especially if this stage is nebulous or the patient population is poorly defined.

Why A β deposition is only weakly related to the degree of dementia has been an enduring puzzle in the AD field. While potential floor or ceiling effects in the amount of A β deposition could contribute, there is also the possibility that A β exerts its major effects early by triggering a cascade of processes that, once begun, proceed independently of A β . Some support for this argument might be found in the human A β immunization trial (AN-1792). Although the numbers of individuals to come to autopsy is still very small, the brain A β deposition in these cases was far lower than might be expected based on historical levels for a given clinical stage. In spite of this markedly lower amount of A β , presumably caused by the immunotherapy, the subjects continued to decline cognitively to an end stage dementia that was clinically indistinguishable from untreated AD [2]. This is not iron-clad proof that the removal of A β succeeded, since we have no way of knowing the pre-treatment amyloid load, and the number of cases is too small for a true cross sectional comparison. It is tempting to speculate that the implication of these results is that A β acts as a trigger for a degenerative process that continues even if it is removed [3]. It is not clear what the mechanism might be for this continued degeneration, although a continued accumulation of misfolded hyperphosphorylated tau, leading directly to further neuron loss, is perhaps the most likely candidate. However, this is a difficult hypothesis to test because it requires the reliable identification of subjects with AD at a very early, preclinical stage, a feat that is currently not possible even with the most sensitive and dependable means of diagnosing the disease.

Another possible explanation is that a specific form or forms of A β are responsible for the massive neuronal death that accompanies the disease. The tools used to quantify A β are not able to distinguish the disease-related A β from less relevant forms which weaken the correlation with clinical stage. An analogy of this situation is found in prion diseases in which the same protein sequence can assume multiple disease-causing conformations, each causing neurodegeneration in a distinct distribution of brain regions resulting in different clinical presentations [4,5]. In this review we suggest that A β is also polymorphic, producing conformational form(s) or specific pool(s) of A β that are disease-relevant while others are less so. Progress is being made in methods and systems to delineate these relevant forms, which will allow testing of this hypothesis.

A β Metabolism, Catabolism and Clearance

The ~4 kDa A β peptide, derived from the larger APP, was first isolated as the principal component of amyloid deposits in the brain and cerebrovasculature of AD and Down's

Syndrome patients [6-8]. Although the function of APP itself has not been resolved, extensive research has advanced our knowledge of how the A β peptide is produced, and how it is subsequently degraded within the brain, or transported out into the periphery. The final amount of A β that accumulates as amyloid deposits within the brain is determined by the interplay of these factors. Changes with disease progression could contribute to the age of disease onset and disease duration.

The enzymatic processes responsible for the metabolism of APP to A β are now reasonably well understood. APP is sequentially cleaved by two membrane-bound endoprotease activities, β - and γ -secretase. β -secretase first cleaves APP to release a large secreted derivative, sAPP β . A fragment of 99 amino acids (CTF β , which begins with the N-terminal aspartyl residue of A β) remains membrane bound, and is in turn rapidly cleaved by γ -secretase to generate A β . Cleavage by γ -secretase is somewhat imprecise, resulting in a C-terminal heterogeneity of the resulting peptide population. Hence, numerous different A β species exist, but those ending at position 40 (A β 40) are the most abundant (~80-90%), followed by 42 (A β 42, ~5-10%). The slightly longer forms of A β , particularly A β 42, are more hydrophobic and fibrillogenic, and are the principal species deposited in the brain [9].

β -secretase activity is believed to be the rate limiting step in the amyloidogenic pathway, and processes ~10% of the total cellular APP. The remaining APP, close to 90%, is constitutively cleaved by α -secretase (a collection of metalloprotease enzymes), generating sAPP α and the 83 amino acid CTF α . The subsequent γ -secretase cleavage of CTF α produces the more benign p3 fragment instead of A β . γ -Secretase cleavage of either membrane bound CTF also generates a cytosolic element, AICD (APP intracellular domain, sometimes referred to as CTF γ), which may play a role in signal transduction [10-13]. Because of their essential role in the generation of A β , both β - and γ -secretase are considered to be prime targets for the development of anti-AD pharmaceuticals [9,14].

γ -Secretase is now known to be a multisubunit enzyme composed of the proteins APH1, PEN2, nicastrin, and presenilin (PS1 or PS2). The enzyme complex likely contains one copy of each subunit [15], and is responsible for the cleavage of multiple membrane proteins in addition to APP. Although the exact functional roles of each component have yet to be fully elucidated, presenilin is believed to form the active site of the aspartyl protease [16,17], and nicastrin likely serves as a substrate docking subunit [18]. All four components are necessary for γ -secretase to mature and function correctly [19,20]. γ -Secretase has a relatively novel mechanism in that it cleaves within the lipid bilayer and can only process substrates that are first cleaved by another protease to remove a large ectodomain region [21]. The enzyme does not have identified specific sequence requirements for substrate recognition, and cleavage within the membrane is instead controlled by a variety of other factors, such as the length of the transmembrane domain [22,23]. Although the amount of γ -secretase activity does not appear to increase in AD, alterations in γ -secretase activity leading to the production of longer forms of A β are the major genetic cause of early onset, familial AD [24,25], an effect that can be mimicked with a variety of allosteric γ -secretase modulating agents [26].

β -Secretase is a membrane-bound aspartyl protease, but one that cleaves APP and its other substrates outside of the bilayer [27-31]. There are two major forms of the enzyme, BACE1 and BACE2, which are >65% homologous [32,33]. The major form of the enzyme responsible for A β production, BACE1, is highly expressed in brain, but is also found at lower levels in other organs [27,34]. In contrast, the second form of the enzyme, BACE2, is low in the brain but is present in most peripheral tissues at higher levels [33]. The knockout of BACE1 in mice leads to a massive reduction in the levels of the downstream products of the enzyme (A β and CTF β) in brain [35-37]. Although these studies indicate that BACE1 is the major β -secretase activity in brain, some residual activity might be attributable to BACE2 [38], and both forms

of BACE can compete for substrate [39-43]. β -Secretase activity and protein are both significantly increased in sporadic AD [44-46]. This effect shows a brain regional selectivity that roughly parallels disease affected regions, and is related to both plaque burden and disease duration [45-47]. β -secretase activity has also been seen to increase with age in rodents and nonhuman primates [48], although these species do not develop AD. Recently, evidence has emerged that cathepsin B [49] or cathepsin D [50] may also be able to serve as β -secretase-like enzymes under some circumstances, although this view is controversial.

Although much emphasis has been placed on understanding the production of A β from APP, in recent years some attention has been shifted to the processes responsible for peptide degradation. Two major enzymes, neprilysin (NEP) and insulin degrading enzyme (also known as insulysin; IDE), are believed responsible for most A β degradation [51-55]. Neprilysin is a plasma membrane bound type II metalloprotease that is responsible for the extracellular degradation of a variety of peptides; IDE, also a metalloprotease, is active both intra- and extracellularly [56-58]. IDE has approximately a 20-fold higher affinity for insulin compared to A β , but hydrolyzes insulin at a much slower rate. Thus, insulin acts as an effective inhibitor of the IDE-dependent cleavage of A β , which may form the basis for a link between type II diabetes, hyperinsulinemia, and AD [58,59]. In the case of AD, both NEP and IDE decrease in normal aging and in disease-affected regions [60,61]. Further, NEP has been shown to decrease in the CSF in early AD [62]. Although most A β degradation can be attributed to NEP and IDE, a substantial body of evidence indicates a likely role for lysosomal degradation, by enzymes such as cathepsin B [63].

In spite of substantial catabolism within the brain, a significant amount of A β remains undegraded. As with other metabolites, mechanisms exist to transport A β across the blood brain barrier (BBB) and out into the circulation. Interfering with this mechanism causes a large increase in the amount of A β that remains in the brain, leading to its ultimate accumulation [64]. Soluble A β is exchanged across the BBB by two principle mechanisms, the low-density lipoprotein receptor-related protein (LRP) on the abluminal (brain) side [65], and the receptor for advanced glycation end products (RAGE) on the luminal (blood) side [66]. The net efflux of A β across the BBB can predict the degree of cerebral amyloid burden [67]. It is unclear why a bidirectional mechanism exists for the transport of A β , or if this transport has an important physiological role that is unrelated to AD. However, it is possible that the disruption of these mechanisms, coupled with other extensive co-morbid vascular abnormalities within the AD brain, contribute significantly to and are affected by the development of amyloid pathology [68].

The Concept of A β pools

A look through a microscope at a silver-stained section of AD brain tissue immediately shows the heterogeneity of the pathology. The use of silver impregnation techniques to evaluate brain pathology is an old approach, and the ones in use today differ little from those used by Alois Alzheimer when he initially described the disease. Over more than a century, a classification system has evolved to make sense of the progression of the disease, a tour-de-force effort of countless clinicians and pathologists. However, in the process a certain amount of information was averaged out and lost. In the past, simplification was necessary to improve understanding and provide a basis for testing hypotheses of the disease mechanism. Modern advances in technology, as well as new ways of studying the relationship of the pathology to brain biology and our improved understanding of the biochemical basis of the disease, require a re-evaluation of the 'lumping' procedure used to discriminate among subpopulations of disease cases and different subtypes of plaques and tangles. If these misfolded protein structures are indeed polymorphic like prions, specific subpopulations will be strongly correlated with the clinical progression of the disease. Imaging agents based on analogs of histological dyes which are

more discriminating among conformational states could then be used antemortem to evaluate the pathological components of the disease stage.

Microscopists use the staining characteristics and morphology of the lesions in AD to define different forms of the deposits. On the other hand, biochemists look for other ways to differentiate these forms and to determine their composition. The silver-stained structures in AD brain that also stain with the classic histologic dyes Congo Red, and Thioflavins S and T are fibrillar proteinaceous structures that are highly insoluble under most conditions. Although these structures are composed of a major key protein (A β or tau), additional proteins and certain glycolipids are also associated with them while not being part of the fibril structures themselves. Since these components are also found in normal brain they are generally not considered to be pathologic (however, their presence can occasionally interfere with various methods of A β detection, particularly those that are antibody dependent). Neuritic plaques (A β), cerebrovascular amyloid (A β) and neurofibrillary tangles (tau) can be purified away from other insoluble components and separated from each other [69]. They resist solubilization even with harsh detergents such as sodium dodecyl sulfate (SDS) and Sarkosyl, requiring concentrated formic acid for depolymerization. When the denaturants are removed, the fibrils spontaneously reassemble into their previous form.

While original efforts were designed to identify the components of the prominent plaques and tangles in the brain, a series of more finely graded extraction procedures have been applied to investigate the transition of the A β peptide from soluble monomer through diffuse plaques and deposits into neuritic plaques. Low ionic strength alkaline solutions (such as low salt diethylamine) and SDS-treatment can extract fractions of A β of intermediate solubility, and 70% formic acid dissolves the remainder (mostly neuritic plaques), leaving lipofuscin granules but no A β in the small amount of residue [70]. Tau protein in the AD brain is characterized as pools of Sarkosyl-soluble and -insoluble material, with the latter thought to represent the neurofibrillary tangles which require strong acid for dissociation into monomeric tau [71].

From studies of the extractability of A β at different stages of AD progression compared to the histology, aqueous buffer or dilute alkali released soluble and adsorbed A β species, SDS-extraction removed diffuse deposits, and formic acid extraction was required to solubilize the neuritic plaques and cerebrovascular amyloid [72,73]. Brain tissue from APP/PS1 knock-in mice at corresponding stages of A β deposition produced analogous extraction profiles. Hence, at this level of resolution, more distinct than staining morphology, the A β peptide forms structures with similar physical-chemical properties in AD and in mouse models of brain A β pathology. Tau-containing structures remain to be characterized in this way.

Immunohistochemical staining of tissue sections reveals numerous deposits of A β peptide with few or no fibrils that stain poorly or not at all with amyloid dyes [74]. Although this diffuse amyloid was once thought to be an early stage of neuritic plaque development, this has proven difficult to resolve conclusively. The amyloid dyes (Congo Red and the Thioflavins) light up neuritic plaques but only weakly react with other deposits that can be silver stained in brain sections. Congo red birefringence reveals oriented periodic organization in neuritic plaques but not in these other structures, and this diffuse material has been shown to be A β peptide by sequence-specific immunostaining. The lack of birefringence is due to a lack of regular fibril structure. Conformation-dependent antibodies that do not recognize diffuse amyloid deposits, but which recognize synthetic fibrils and neuritic plaques have been described [75,76]. Not all forms of A β fibrils react equally well with all anti-fibril monoclonal antibodies, suggestive of the ability of such reagents to discriminate polymorphic fibril forms, but this property remains to be conclusively demonstrated.

Detailed study of neuritic plaques stained with a series of fluorescent polythiophene derivatives whose emission spectra are sensitive to the amount of order in an amyloid fibril revealed that individual plaques or regions within a single plaque could be differently organized [77]. Synthetic A β peptide fibrils prepared under different conditions (agitation or quiescence) stain differentially with these probes indicating that A β fibril polymorphism can readily occur. Synthetic fibril structural polymorphism under different fibril-forming conditions is also observed by solid state NMR [78].

Assessing the development of AD pathology in the living human brain has been a dream of clinicians. A means to study AD *in vivo* serves minimally as a surrogate measure of disease progression, and hopefully as a diagnostic tool to detect an early, preclinical stage of the disease. A great deal of effort has gone into the development of noninvasive, sensitive and specific biomarkers in the blood or cerebrospinal fluid for AD that could pick up early stage disease or predict progression to AD before clinical symptoms appear. This has proven very difficult to sort out, and remains an area of intense investigation [79]. An alternative approach using analogs of amyloid dyes to image and quantify amyloid pathology in the brain of subjects has produced exciting results and in the process perhaps has provided insight into differences between the human disease and the systems used to recapitulate the process in animal models. While oligomeric A β is likely present at concentrations far below the detection limits of current imaging technology, other pools of A β may be more suitable for imaging. The SDS-insoluble A β isolated from AD brain has shown to have a distinct fibril molecular structure by solid state ^{13}C NMR and could be a target for development of an imaging ligand [80].

A derivative of the amyloid dye, Thioflavin T, Pittsburgh Compound 1 (PIB, 6-OH-[2, 4-N dimethyl-phenyl benzothiazole]) was prepared with physicochemical properties that made it a good brain imaging ligand [81]. After labeling with ^{11}C for PET imaging, increasing amounts of deposition in specific brain regions could be detected in parallel with decreased glucose metabolism (^{18}F -fluorodeoxyglucose) in those same regions following the disease progression in AD patients [82]. The utility of this and other probes for detecting MCI and predicting which of those subjects will progress to AD is being evaluated [82,83]. However, PIB has already, from the point of view of this review, provided a valuable perspective on potential A β polymorphism and a possible explanation for why our animal models are A β pathology models but fail to recapitulate the full spectrum of AD pathology with its massive cell death.

Histological staining with dyes takes place at high concentrations and then the tissue is washed (differentiated) to remove excess dye. In brain imaging, for technical and toxicology reasons, only nanomolar concentrations of ligand can be used. PIB is employed similar to a pharmacological ligand in these studies. There are both high (nM) and low (μM) affinity PIB binding sites on synthetic and biological A β fibrils [84]. Only the high affinity binding site is significantly occupied under imaging conditions. When binding studies of PIB to AD brain fibrils are performed a large proportion of the binding is due to the high affinity site. By contrast, synthetic A β fibrils, aged transgenic mouse A β brain as well as the brains of aged non-human primates (squirrel monkey, macaques, chimpanzee) [85], all with human sequence A β peptide in similar amounts, have the low affinity site in overwhelming proportion. Images can only be obtained in transgenic mice when PIB with ten-fold higher specific radioactivity is used to detect the very small amount of high affinity binding site [86]. Although other explanations are currently being tested, one possibility is that the polymorphic form of A β fibril or A β -containing complex in AD is enriched in high affinity PIB sites.

Another indication of the unique polymorphic structure of AD brain A β is that it is much more efficient at seeding A β fibril formation when injected into the brains of transgenic mice producing A β peptide than are equivalent amounts of synthetic A β fibrils or A β extracts from plaque-containing transgenic mouse brains [87-89].

Multimerization, Nucleation and Deposition

The assembly of A β into multimeric structures is key to the biological effect of those species. There are two phases of assembly which have different characteristics and lead to assemblies with different biological properties. Initial work with A β focused on the histological hallmarks of AD, the amorphous and fibrillar deposits of the peptide. Current focus is on the earlier phase of A β assembly which involves soluble multimers of the peptide. These structures are orders of magnitude more toxic to cells of different types than are the fibrils and trigger a different set of toxic events [90]. They are also morphologically and conformationally distinct. Oligomer-specific conformational antibodies do not recognize monomer or fibrils, and fibril-specific antibodies do not recognize soluble oligomers. While total abandonment of fibril involvement in AD at this time is probably premature given the PIB story discussed earlier and the potential for plaque involvement with oligomer populations [91], the focus of the A β field has shifted to soluble oligomers.

Oligomers form readily from the A β (1-42) peptide, less well so from the more abundant A β (1-40) [92]. There is a close correlation between the ratio of 42/40 and age of disease onset in familial AD [93]. The C-terminus of A β (1-42) is critical for oligomer formation. Bitan *et al.* [94] defined some of the structural parameters for the different steps of *in vitro* oligomer assembly with synthetic peptides mutated in that region. While the early intermediates during oligomerization of synthetic peptide are unstable and require photochemical trapping of the intermediates, stable small oligomers can be isolated from biological systems. The explanation for this difference in stability is unknown. A β oligomers with SDS-stable substructures as small as dimers isolated from AD brain and CSF have been shown to disrupt synaptic electrophysiology [95,96]. Whether these intermediates arise during assembly, or after disassembly *in vivo*, remains to be determined. Soluble synthetic A β oligomers are highly polymorphic with stable sizes that depend on the method of preparation [97]. While investigators agree that soluble oligomers are biologically active and can cause cell death under some conditions, the mode of action of soluble oligomers also remains to be settled. Receptor-mediated effects are noted [98,99] as well as direct activity of oligomers on the membrane bilayer, especially at high (μ M) concentrations which may stem from their surface activity. The concentration of soluble oligomers in CSF or brain interstitial fluid is in the pM range [100].

Fibril formation is studied at high micromolar concentrations of monomeric synthetic peptide to increase the probability of a fibril nucleus forming. There is evidence [101] that soluble oligomer formation and fibril formation may be different pathways, although mechanistically both processes have to pass through multimeric stages. Since A β concentrations in brain interstitial fluid are at least three-orders of magnitude lower than in fibril forming assays, it is likely that fibril formation is nucleated on extracellular matrix or cell surfaces. Fibril growth by extension on both synthetic and AD brain pre-existing fibrils is linearly dependent on the A β monomer concentration [102] and is highly specific for the form of amyloid fibril [103]. The process is reversible *in vivo* in transgenic mouse models monitored by multiphoton microscopy [104] and is surprisingly rapid in that system and includes vascular amyloid [105]. Although this process has not been documented in the live human brain, the effects of active and passive antibody administration in animal models [106] and human immunization trials [2] suggest that A β deposits in the brain will be in equilibrium with the interstitial A β . There is also evidence consistent with insoluble A β deposits serving as a reservoir for soluble oligomers [91]. Not all pools of A β in the human brain may participate in this rapid exchange of monomer and the relative involvement of the different pools in the pathologic process is unknown.

Modeling States of A β and AD

Alzheimer's disease is a human specific disorder. Even our most closely related primate relatives do not develop pathology, much less the clinical outcome that can be considered to be actual AD. Still, much of our understanding of how amyloid pathology develops has been driven by studies in a variety of animal models. Although there is no animal model that accurately reflects every facet of AD, there are many models of A β deposition. In a very broad sense, these can be subdivided into models where amyloid pathology develops naturally with age, and genetically modified mice that express mutant forms of APP. Animals in which amyloid deposition occurs naturally is attractive in that the researcher is not burdened with the numerous caveats that accompany genetically modified mice, such as separating the contributions of over expression and introduced mutations to the model phenotype. However, animals in which amyloid deposition occurs as a consequence of normal aging have a substantial longer lifespan than rodents (very long in the case of some nonhuman primates), and their use can be difficult to justify based purely on their associated cost.

Although nonhuman primates (NHPs) have identical A β sequence to humans and a near identical APP sequence, and they overlap in many aspects with relevant human biochemical pathways, they develop surprisingly little AD-like neuropathology with age. While older NHPs typically show small amounts of amyloid deposition, this is quite modest compared to cases of AD [107-109]. Although abnormal neurofilaments can frequently be identified with some amyloid deposits, neurofibrillary tangle pathology is not a typical feature of pathology in NHPs [108], although chimpanzees may be an exception [110]. Only a small amount of biochemical work has been performed on amyloid isolated from NHPs, although interestingly, a recent study indicated that there may be more soluble A β peptide in the chimpanzee than in AD [110].

Aged canines also develop substantial amyloid deposition with age. Unlike aged NHPs, which may require several decades, canines show substantial amyloid deposition from approximately ten years of age [111]. Amyloid deposition in canines is also correlated with age-related cognitive dysfunction [112], although little neuronal loss is observed. Similar to AD, A β deposition in canines is driven by A β 42; however, the deposition occurs almost exclusively in diffuse deposits, with no neuritic plaques or neurofibrillary tangle pathology [113]. Nonetheless, compared to NHPs, the quantity of deposited A β is comparable to AD cases, and a substantial proportion is highly insoluble [114]. This would seem to suggest that the amyloid in the aged canine quantitatively overlaps with the amount of amyloid in human disease. Thus, canines may represent a useful intermediate between genetically modified mouse models and AD. In this regard it is worth noting that immunization with fibrillar A β in aged canines may be a better parallel to human trials with this therapeutic approach than preclinical mouse models, although data from humans are still sparse [2,114].

Insights from mouse models have been indispensable for our understanding of amyloid deposition *in vivo*. It is beyond the scope of this article to review the extensive literature on mouse models of A β deposition and AD pathology, and several excellent reviews exist [115-117]. There are a number of points worth mentioning. First, although mouse models have economics and speed as a major advantage, with some models developing amyloid deposition at birth [118], all models require the introduction of some combination of familial AD mutations into APP or PS1 or both. As a general rule, incorporating more mutations accelerates the pathology. The hidden cost in this endeavor is that substantial alterations are being introduced into proteins (particularly APP) with unknown function, and the consequences of these mutations beyond driving amyloid deposition are unknown. Second, with few exceptions [73,119], it is necessary to over express APP containing human sequence A β at relatively high levels (using an ectopic promoter) to drive the deposition of A β . This carries with it the same

note of caution as does the introduction of mutations. An additional concern is that in the majority of these models the rodent A β is present which can affect the assembly of the human sequence peptide *in vitro* and *in vivo* [120-122].

Caveats aside, insights derived from genetically modified mice have been highly useful. Work in transgenic mice showed that amyloid deposition is driven almost entirely by A β 42, and not A β 40 [123]. Transgenic mice have been used to demonstrate convincingly that A β increases the rate of neurofibrillary tangle pathology in mice which also express mutant tau protein [124,125], placing A β pathology firmly upstream of tangle pathology in the hierarchy of disease progression. Higher order, soluble oligomeric forms of A β are toxic to neurons and cause deficits in long term potentiation [126,127], providing crucial evidence that a soluble intermediate form of A β may drive the early disease process rather than the amyloid deposits themselves. Recently, mice have also been used to demonstrate that amyloid can deposit with extraordinary rapidity in the brain [104]. What is also remarkable is the large number of preclinical interventions that have been shown to reverse amyloid deposition in mice. This may be a function of the state of the amyloid in mice which is consistently less crosslinked and chemically modified than in AD [128]. The amyloid deposited in the mouse brain may be considerably more plastic than human amyloid, possibly as a consequence of a far shorter *in vivo* dwell time in mice, thus avoiding the extensive A β modification and cross-linking observed in human material.

The observation that the stoichiometry of high affinity PIB-binding in AD brain is drastically reduced in the transgenic APP mouse model [84] may be a further reflection of the differences between polymorphic forms or complexes of the A β amyloid, some of which may be more related to disease pathology than others. As of yet, it is not known what significance these differences may entail for our understanding of the progression of the disease but they will likely complicate the development of anti-A β targeted therapeutics.

Conclusions

Understanding of the state of A β is of more than academic interest. Clinical concepts have not kept pace with molecular knowledge and remain heavily dependent on classical pathology. The difficulty in defining clinically useful biomarkers of AD progression indicates that new parameters need to be investigated, which could include particular molecular polymorphic forms of A β .

One area in which it is important to consider different states or pools of A β is in the development and use of imaging markers. What has become clear from the studies with PIB and similar benzothiazole radioligands is that there are multiple binding sites on A β fibrils that have different molecular specificities [129-131]. The sites have different stoichiometries on fibrils for their ligands and some appear to partially overlap. Thus, the issue becomes a pharmacological one. PIB turns out to bind with high affinity to a site distinct from binding site(s) for Congo Red derivatives and DDNP derivatives. This high affinity PIB site is found at high stoichiometry with respect to A β in affected regions of AD brain. Only very low stoichiometry PIB binding is seen in unaffected regions of AD brain or any region of the plaque-rich transgenic mouse brain (Klunk, Rosen, LeVine, and Walker, unpublished results) or in non-human primate temporal or parietal cortex containing similar amounts of insoluble A β [85]. The type of amyloid binding ligand used is important. The plethora of new imaging molecules with different structures proposed for clinical imaging studies may not be reporting the same form of A β , much less a disease-related one. The uncritical use of such agents could potentially generate further confusion in the literature. On the other hand, these ligands could be quite useful, provided that their binding site selectivity has been characterized under *in vivo* imaging-like conditions.

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