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β-Amyloid peptide and the Alzheimer's disease

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SUMMARY

The formation and deposition of the -amyloid peptide (A) are largely thought to be responsible for the development of Alzheimer's disease (AD). Researchers have been perplexed by the sluggish to nonexistent association between the degree of clinical dementia and the amount of neuritic plaque disease in the human brain for a long time. This question has been resolved thanks to recent developments in our knowledge of the origins of amyloid disease. The solubility of A and the amount of A in various pools may now be more directly tied to disease state, according to substantial evidence. The make-up of these pools of A reflects various populations of amyloid deposits and is clearly correlated with the patient's clinical condition. Using imaging methods, such as novel amyloid imaging agents based on the chemical makeup of histology dyes, it is now possible to monitor the development of the disease in a living patient while also monitoring the amyloid pathology. It's interesting that these methods show that the A deposited in AD differs from that discovered in animal models. In general, deposited A does not exhibit the same physical and biochemical properties as the amyloid observed in AD and is more readily removed from the brain in animal studies. Important questions about the creation and evaluation of potential medicinal agents are brought up by this.

Keywords: Amyloid; β-Amyloid Precursor Protein; Aβ; Oligomer; Fibril

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INTRODUCTION

The extracellular plaque deposits of the β-amyloid peptide (Aβ) and the flame-shaped neurofibrillary tangles of the microtubule binding protein tau are the two hallmark diseases necessary for a diagnosis of Alzheimer's disease (AD). Mutations in presenilin-1 (PS1), presenilin-2, or the -amyloid precursor protein (APP), which is the precursor protein for A, are linked to familial early onset types of AD (PS2). The catalytic subunit of -secretase, the last endoprotease in the pathway that produces the peptide, can be either PS1 or PS2. The amounts and distribution of Aβ deposition are only weakly correlated with the clinical manifestation of the disease, despite this genetic evidence and the demonstrated involvement of Aβ in inducing synaptic dysfunction, disrupting neural connectivity, and association with neuronal death in a brain region-specific manner. Clinically, Alzheimer's disease is indicated by a slow and steady decline in cognitive function, and neuropathologically, the condition is indicated by the presence of neuropil threads, loss of specific neurons, and loss of synapses in addition to the classic signs of neurofibrillary tangles and senile plaques. For the benefit of doctors, the techniques for evaluating the pathology and categorizing the stages of AD have been standardised and codified. The density of tau protein neurofibrillary tangles and neuritic amyloid plaques in the affected brain areas is the standard indicator of pathology. The presence of neuritic plaques composed (in large part) of highly insoluble Aβ 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].

The process of creating a disease stage categorization for AD has not been straightforward, and the existing system(s) are not entirely accepted. The final decision about the staging of a disease state is still made by clinicians, neuropsychologists, and pathologists in clinicopathological conferences. The staging system's significant drawback is that it can only be applied roughly to living subjects. It is necessary to use a clinical diagnosis of probable AD because the pathology of AD is only discovered at autopsies. Research on disease causes is severely impacted by the absence of an in-life diagnostic test, which is especially problematic for clinical trials because it adds further heterogeneity to the subject population. In particular, if the stage of the disease is ambiguous or the patient population

is not well characterized, therapeutics cannot be fully tested if they must be delivered before the disease has advanced past a certain stage.

Why Aβ deposition is only tangentially connected to the severity of dementia has long perplexed researchers studying AD. The amount of Aβ deposition may have potential floor or ceiling effects that could contribute, but it's also possible that Aβ exerts its main effects sooner by starting a chain of events that, once started, continue without Aβ. The human Aβ vaccination trial may provide some evidence to back up this claim (AN-1792). The brain Aβ deposition in these cases was far lower than might be anticipated based on historical levels for a particular clinical stage, despite the fact that the number of people who have undergone autopsy is still incredibly tiny. Despite this noticeably smaller level of Aβ, which was probably brought about by the immunotherapy, the participants' cognitive loss persisted to the point of end-stage dementia, which was clinically identical to untreated AD [2]. Since we have no way of knowing the pre-treatment amyloid load and the number of instances is too low for a true cross sectional comparison, this is not absolute proof that the elimination of Aβ was successful. It is alluring to assume that these findings indicate that Aβ works as a catalyst for a degenerative process that persists even after it is withdrawn [3]. Although the most likely cause of this ongoing degeneration is an ongoing build-up of misfolded hyperphosphorylated tau that directly contributes to the loss of more neurons, the exact mechanism is unknown. This is a challenging theory to verify, though, as it calls for the accurate diagnosis of AD in patients at a very early, preclinical stage-a feat that is now impossible even with the most accurate and sensitive methods of disease diagnosis.

Another explanation is that the disease's accompanying enormous neuronal loss is caused by a particular form or kinds of Aβ. The ability of the techniques used to quantify Aβ to differentiate between disease-related Aβ and less important forms weakens the link with clinical stage. In prion illnesses, where the same protein sequence can take on various disease-causing conformations, each of which causes neuro-degeneration in a specific distribution of brain regions and yields a different clinical appearance [4,5], we find a parallel to this situation. In this review, we propose that Aβ is polymorphic, producing conformational forms or distinct pools of Aβ, some of which are more relevant to disease than others. Methods and systems to identify these pertinent types are improving, which will allow this theory to be tested.

An overview of Aβ metabolism, catabolism, and clearance

The primary component of amyloid plaques in the brain and cerebrovasculature of AD and Down's Syndrome patients was first identified as the ~4 kDa Aβ peptide, which was produced from the bigger APP [6-8]. Although the exact role of APP has not been determined, substantial study has enhanced our understanding of how the Aβ peptide is synthesized and then either transported outside the brain or destroyed there. The interaction of these elements determines the final amount of Aβ that forms amyloid plaques in the brain. The age of disease onset and disease duration may change as the disease progresses. The enzymatic processes responsible for the metabolism of APP to Aβ 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, sAPPβ. A fragment of 99 amino acids (CTFβ, which begins with the N-terminal aspartyl residue of Aβ) remains membrane bound, and is in turn rapidly cleaved by γ-secretase to generate Aβ. Cleavage by γ-secretase is somewhat imprecise, resulting in a C-terminal heterogeneity of the resulting peptide population. Hence, numerous different Aβ species exist, but those ending at position 40 (Aβ40) are the most abundant (~80-90%), followed by 42 (Aβ42, ~5-10%). The slightly longer forms of Aβ, particularly Aβ42, 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 consecutively cleaved by α-secretase (a collection of metalloprotease enzymes), generating sAPPα and the 83 amino acid CTFα. The subsequent γ-secretase cleavage of CTFα produces the more benign p3 fragment instead of Aβ. γ-Secretase cleavage of either membrane bound CTF also generates a cytosolic element, AICD (APP intracellular domain, sometimes referred to as CTFγ), which may play a role in signal transduction [10-13]. Because of their essential role in the generation of Aβ, 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 multi-subunit 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 Aβ 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 Aβ 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 (Aβ and CTFβ) 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 Aβ 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 Aβ degradation. 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. IDE has approximately a 20-fold higher affinity for insulin compared to Aβ, but hydrolyzes insulin at a much slower rate. Thus, insulin acts as an effective inhibitor of the IDE-dependent cleavage of Aβ, which may form the basis for a link between type II diabetes, hyperinsulinemia, and AD. In the case of AD, both NEP and IDE decrease in normal aging and in disease-affected regions. Further, NEP has been shown to decrease in the CSF in early AD. Although most Aβ 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.

In spite of substantial catabolism within the brain, a significant amount of Aβ remains undegraded. As with other metabolites, mechanisms exist to transport Aβ across the blood brain barrier (BBB) and out into the circulation. Interfering with this mechanism causes a large increase in the amount of Aβ that remains in the brain, leading to its ultimate accumulation. Soluble Aβ is exchanged across the BBB by two principle mechanisms, the low-density lipoprotein receptor-related protein (LRP) on the abluminal (brain) side, and the receptor for advanced glycation end products (RAGE) on the luminal (blood) side. The net efflux of Aβ across the BBB can predict the degree of cerebral amyloid burden. It is unclear why a bidirectional mechanism exists for the transport of Aβ, 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.

The concept of Aβ pools

The variety of the pathology is instantly apparent when examining a silver-stained sample of AD brain tissue under a microscope. The evaluation of brain pathology using silver impregnation techniques is a time-honored method, and the methods employed now hardly differ from those utilised by Alois Alzheimer to first define the illness. A classification system has developed over the course of more than a century to make sense of the disease's evolution, thanks to the labour of many doctors and pathologists. However, some information was averaged out and lost in the process. In the past, simplification was required to enhance comprehension and offer a foundation for the testing of disease mechanism hypotheses. Re-evaluating the "lumping" procedure used to distinguish between sub-populations of disease cases and various subtypes of plaques and tangles is necessary in light of contemporary technological advancements, new methods of examining the relationship between the pathology and brain biology, and our improved understanding of the biochemical basis of the disease. The clinical development of the disease will be closely associated with particular sub-populations if these misfolded protein aggregates are polymorphic like prions. The pathological components of the disease stage could thus be assessed antemortem using imaging agents based on analogues of histology dyes that are more discriminating among conformational states.

Microscopists can distinguish between various kinds of the deposits by looking at the staining traits and shape of the lesions in AD. On the other hand, biochemists search for further strategies to distinguish between these forms and ascertain their makeup. The AD brain's silver-stained structures that also exhibit standard histologic stains like Congo Thioflavins S and T, as well as red, are fibrillar proteinaceous structures that are often very insoluble. Although a primary important protein (Aβor tau) makes up these structures, other proteins and specific glycolipids are also linked to them but are not actual components of the fibril formations. These elements are not typically regarded as pathogenic because they are also present in normal brain tissue (although, their presence can occasionally obstruct some A detection techniques, especially antibody-based ones). It is possible to separate and purify neurotic plaques (Aβ), cerebrovascular amyloid (Aβ), and neurofibrillary tangles (tau) from other insoluble components. They are resistant to solubilization, even by abrasive detergents such sodium dodecyl sulphate (SDS) and Sarkosyl, and must be depolymerized with concentrated formic acid. The fibrils

spontaneously rearrange into their original form when the denaturants are withdrawn.

A series of more precisely graded extraction techniques have been used to investigate the transition of the Aβ peptide from soluble monomer through diffuse plaques and deposits into neuritic plaques. The original efforts were intended to identify the components of the prominent plaques and tangles in the brain. A fraction of Aβ with intermediate solubility can be extracted using low ionic strength alkaline solutions (like low salt diethylamine) and SDS treatment. The remaining fraction (mostly neuritic plaques) is then dissolved in 70% formic acid, leaving lipofuscin granules but no Aβ in the small amount of residue. The Sarkosyl-soluble and -insoluble pools of tau protein in the AD brain are assumed to reflect neurofibrillary tangles, which must be broken down by strong acid to form monomeric tau.

Aqueous buffer or diluted alkali released soluble and adsorbed Aβ species, SDS-extraction removed diffuse deposits, and formic acid extraction was necessary to solubilize the neuritic plaques and cerebrovascular amyloid, according to studies of the extractability of Aβ at different stages of AD progression compared to the histology.

Analogous extraction profiles were produced by brain tissue from APP/PS1 knock-in mice at similar phases of Aβ deposition. Therefore, the Aβ-peptide creates structures with identical physical-chemical properties in AD and in animal models of brain Aβ pathology at this degree of resolution, which is more distinct than staining morphology. We still need to characterize tau-containing structures in this way. Immunohistochemical staining of tissue sections reveals numerous deposits of Aβ peptide with few or no fibrils that stain poorly or not at all with amyloid dyes. 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 Aβ peptide by sequence-specific immunostaining. The absence of a regular fibril structure is the cause of the lack of birefringence. There have been described conformation-dependent antibodies that identify synthetic fibrils and neuritic plaques but not diffuse amyloid deposits. It is possible that some anti-fibril monoclonal antibodies can distinguish between different forms of polymorphic Aβ fibrils because not all A fibril forms respond with them equally effectively. However, this ability has to be definitively proven. Individual neuritic plaques or sections within a single plaque may be differently arranged, according to a thorough analysis of neuritic plaques stained with a variety of fluorescent polythiophene derivatives whose emission spectra are sensitive to the amount of order in an amyloid fibril.

With these probes, synthetic Aβ-peptide fibrils made under various conditions (agitation or quiescence) stain differently, demonstrating the ease with which Aβ-fibrillar polymorphism can develop. Solid state NMR has also detected synthetic fibril structural polymorphism under various fibril-forming circumstances. Clinicians have long wished to measure the progression of AD disease in the living human brain. The ability to investigate AD *in vivo* can at the very least be used as a proxy for measuring disease progression and, ideally, as a diagnostic tool to identify the disease's early, preclinical stages. It has taken a lot of work to create noninvasive, sensitive, and specific biomarkers for AD in blood or cerebrospinal fluid that could detect the illness in its early stages or predict AD progression before clinical symptoms occur. This has been extremely challenging to understand and is still a hot topic of research. Exciting results have been obtained utilizing a different method that images and measures the amyloid pathology in patients' brains. In the process, it may have revealed distinctions between the human disease and the systems used to simulate the process in animal models. While oligomeric Aβ is likely present at concentrations far below the detection limits of current imaging technology, other pools of Aβ may be more suitable for imaging. The SDS-insoluble Aβ isolated from AD brain has shown to have a distinct fibril molecular structure by solid state 13C NMR and could be a target for development of an imaging ligand.

A derivative of the amyloid dye, Thioflavin T, Pittsburgh Compound 1 (PIB, 6-OH-[2, 4-N dimethyl-phenyl benzathiazole]) was prepared with physicochemical properties that made it a good brain imaging ligand. After labeling with 11C for PET imaging, increasing amounts of deposition in specific brain regions could be detected in parallel with decreased glucose metabolism (18F-fluorodeoxyglucose) in those same regions following the disease progression in AD patients. The utility of this and other probes for detecting MCI and predicting which of those subjects will progress to AD is being evaluated. However, PIB has already, from the point of view of this review, provided a valuable perspective on potential Aβ polymorphism and a possible explanation for why our animal models are Aβ 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 Aβ fibrils. 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 Aβ fibrils, aged transgenic mouse Aβ brain as well as the brains of aged non-human primates (squirrel monkey, macaques, chimpanzee), all with human sequence Aβ 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. Although other explanations are currently being tested, one possibility is that the polymorphic form of Aβ fibril or Aβ-containing complex in AD is enriched in high affinity PIB sites.

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

Multimerization, nucleation and deposition

The biological impact of those species is largely dependent on the assembling of Aβ into multimeric complexes. There are two assembly steps, each of which produces an assembly with a unique set of biological traits. The initial research with Aβ was focused on the fibrillar and amorphous peptide deposits, which are the histological signs of AD. The earlier stage of Aβ assembly, which involves soluble multimers of the peptide, is currently the focus of attention. These structures cause a different set of hazardous events than fibrils and are orders of magnitude more toxic to cells of various types. Additionally, they differ morphologically and conformationally. Monomer or fibril-specific antibodies do not detect soluble oligomers, while fibril-specific antibodies do not recognize oligomer-specific conformational antibodies. The PIB tale mentioned earlier and the potential for plaque engagement with oligomer populations make it likely that a complete abandonment of fibril involvement in AD at this time is premature, although the emphasis of the Aβ field has switched to soluble oligomers.

Oligomers form readily from the Aβ(1-42) peptide, less well so from the more abundant Aβ(1-40). There is a close correlation between the ratio of 42/40 and age of disease onset in familial AD. The C-terminus of Aβ(1-42) is critical for oligomer formation. Bitan, et al. 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. Aβ oligomers with SDS-stable substructures as small as dimers isolated from AD brain and CSF have been shown to disrupt synaptic electrophysiology. Whether these intermediates arise during assembly, or after disassembly *in vivo*, remains to be determined. Soluble synthetic Aβ oligomers are highly polymorphic with stable sizes that depend on the method of preparation. 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 as well as direct activity of oligomers on the membrane bilayer, especially at high (μM) concentrations which may stem from their surface activity. The concentration of soluble oligomers in CSF or brain interstitial fluid is in the pM range.

Fibril formation is studied at high micromolar concentrations of monomeric synthetic peptide to increase the probability of a fibril nucleus forming. There is evidence that soluble oligomer formation and fibril formation may be different pathways, although mechanistically both processes have to pass through multimeric stages. Since Aβ 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 Aβ monomer concentration and is highly specific for the form of amyloid fibril. The process is reversible *in vivo* in transgenic mouse models monitored by multiphoton microscopy and is surprisingly rapid in that system and includes vascular amyloid. Although this process has not been documented in the live human brain, the effects of active and passive antibody administration in animal models and human immunization trials suggest that Aβ deposits in the brain will be in equilibrium with the interstitial Aβ. There is also evidence consistent with insoluble Aβ deposits serving as a reservoir for soluble oligomers. Not all pools of Aβ 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 Aβ and AD

Alzheimer's disease is a condition that only affects people. Even the closest primate ancestors of ours do not exhibit pathology, much less a clinical outcome that may be regarded as true AD. However, research using a number of animal models has contributed much to our understanding of how amyloid disease arises. Although there are numerous models of Aβ deposition, no animal model completely captures every aspect of AD. In a very broad sense, these can be classified into two categories: genetically modified mice that express mutant variants of APP, and models where amyloid disease develops spontaneously with age. The researcher is not saddled with the multiple limitations that come with genetically modified mice, such as separating the contributions of over expression and added mutations to the model phenotype, which makes naturally occurring amyloid accumulation in animals appealing. Although they live much longer than rats (some nonhuman primates live very long lives), animals in whom amyloid deposition happens as a result of natural ageing can be difficult to use because of their high expense.

Nonhuman primates (NHPs) have many characteristics with key human biochemical pathways and have nearly identical Aβ and APP sequences to humans, but they acquire surprisingly little AD-like neuropathology as they age. While older NHPs frequently have some amyloid deposition, this is rather minimal in comparison to AD patients. Neurofibrillary tangle pathology is not a typical

characteristic of disease in NHPs, but chimpanzees may be an exception. However, aberrant neurofilaments can frequently be detected with certain amyloid deposits. There hasn't been much biochemical research done on amyloid isolated from NHPs, but it's noteworthy to note that a recent study suggested that soluble Aβ-peptide levels may be higher in chimpanzees than in AD. Canines that are becoming older accumulate a lot of amyloid. Canines begin to accumulate significant amounts of amyloid at the age of 10, whereas older NHPs may take decades. Although there is little neuronal loss seen, amyloid accumulation in canines is associated with age-related cognitive impairment. Similar to AD, canine Aβ deposition is fueled by Aβ42; however, the deposition occurs nearly entirely in diffuse deposits with little neurofibrillary tangle disease or neuritic plaques.

However, the amount of deposited Aβ is comparable to AD cases when compared to NHPs, and a sizable percentage is very insoluble. This would seem to imply that the amyloid in the ageing dog and the disease-causing amyloid in humans are numerically similar. Therefore, dogs might serve as a helpful bridge between genetically altered mice models and AD. Although there are still few human studies, it is important to keep in mind that immunization with fibrillar Aβ in aged canines may be a more accurate representation of human trials with this therapeutic approach than preclinical mouse models. Understanding of amyloid deposition *in vivo* has been made possible thanks in large part to insights from mice models. Reviewing the comprehensive research on mice models of Aβ deposition and AD pathology would be outside the scope of this article, although there are already several outstanding reviews. There are few things worth noting. First off, all models require the insertion of some combination of familial AD mutations into APP or PS1 or both, despite the fact that mice models offer significant advantages in terms of speed and economy, with some acquiring amyloid deposition before birth. Generally speaking, adding more mutations speeds up the disease. The unintended consequence of this project is that significant changes are being made to proteins (especially APP) whose functions are unknown, and it is unknown what will happen to these changes once they have caused amyloid buildup. Second, in most cases, in order to enhance the deposition of Aβ, it is required to overexpress APP carrying human sequence Aβ at relatively high levels (using an ectopic promoter). Similar warnings apply to this as they do to the introduction of mutations. Another issue is that rat Aβ, which can influence the assembly of the human sequence peptide both *in vitro* and *in vivo*, is present in the majority of these models.

Despite certain cautions, the knowledge gained from genetically altered mice has been very beneficial. Research in transgenic mice demonstrated that Aβ42, and not Aβ40, is virtually exclusively responsible for amyloid deposition. Transgenic mice have been used to demonstrate convincingly that Aβ increases the rate of neurofibrillary tangle pathology in mice which also express mutant tau protein, placing Aβ pathology firmly upstream of tangle pathology in the hierarchy of disease progression. Higher order, soluble oligomeric forms of Aβ are toxic to neurons and cause deficits in long term potentiation, providing crucial evidence that a soluble intermediate form of Aβ 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. 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. 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 Aβ 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 may be a further reflection of the differences between polymorphic forms or complexes of the Aβ 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-Aβ targeted therapeutics.

CONCLUSION

Understanding the condition of Aβ is important for reasons other than intellectual ones. Clinical conceptions still strongly rely on classical pathology since they have not kept up with molecular knowledge. Given the difficulty in identifying clinically relevant biomarkers of AD progression, novel criteria, such as specific molecular polymorphic forms of Aβ, need to be examined.

The creation and application of imaging markers is one area where it is crucial to take various states or pools of Aβ into account. The experiments with PIB and related benzothiazole radio ligands have shown that Aβ fibrils have several binding sites with various molecular specificities. On fibrils, the sites' ligand stoichiometries vary, and some of them seem to partially overlap. The problem then becomes one of pharmacology. It turns discovered that PIB binds with a high affinity to a site that is different from the site(s) where Congo Red and DDNP derivatives bind. In the afflicted areas of AD brain, this high affinity PIB site is present at high stoichiometry with respect to Aβ. Only very low stoichiometry PIB binding is seen in unaffected regions of AD brain or any region of the plaque-rich transgenic mouse brain or in non-human primate temporal or parietal cortex containing similar amounts of insoluble Aβ. 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 Aβ, 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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CONFLICTS OF INTEREST

The author declares there is no any conflict of interests.

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