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Current Thinking on the Mechanistic Basis of Alzheimer's and Implications for Drug Development

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Alzheimer's disease (AD) is the most common cause of dementia and is characterized by the aggregation and accumulation of two proteins in the brain, amyloid-β (Aβ) and tau. Aβ and tau begin to buildup 15-20 years before the clinical onset of AD dementia. Increasing evidence suggests that preventing or decreasing the amount of aggregated forms of both Aβ and tau in the brain can serve as potential disease-modifying treatments for AD.

Alzheimer disease (AD) is a neurodegenerative disorder primarily characterized by accumulation of the amyloid-β (Aβ) in amyloid plaques in the extracellular space of the brain and tau in neurofibrillary tangles inside neurons. Aggregation of Aβ and tau seems to lead to neurotoxicity. Therefore, much of drug development has focused on decreasing these protein aggregates using approaches such as immunotherapy or inhibiting Aβ or tau generation. To complement these approaches, the identification of AD risk genes, such as apolipoprotein E (*APOE*) and others, will hopefully lead to a better understanding of the mechanisms underlying protein aggregation and pave the way for a personalized medicine approach for AD in the future.

Hallmarks of Alzheimer disease

AD, first described in 1906 by Alois Alzheimer, is the most common cause of dementia. To date, about 35 million people worldwide are affected and the prevalence is rising because of an increased life expectancy in our aging society. Alarmingly, this number is expected to triple by 2050.

AD is characterized by neurodegeneration in specific brain regions leading to decline in memory and other cognitive abilities. Accumulation of amyloid-β (Aβ) in extracellular amyloid plaques and hyperphosphorylated tau in intraneuronal neurofibrillary tangles are the pathological hallmarks of the disease, ultimately leading to neuronal dysfunction and loss. Autosomal dominant forms of AD are primarily caused by mutations in genes important for the generation of Aβ (particular longer forms of Aβ, such as Aβ42) from amyloid precursor protein (APP), whereas the main risk factors for late-onset AD (age >60) are age and genetics. Two distinct pathways for cleavage of APP exist. In the nonamyloidogenic pathway, alpha-secretase cleaves APP, which is then further processed by γ-secretase. In the amyloidogenic pathway, APP is first cleaved by β-secretase before γ-secretase generates amyloidogenic Aβ (mostly $A\beta_{38}$, $A\beta_{40}$, and $A\beta_{42}$ peptides).

To date, no cure or therapeutic intervention is available for AD that can slow disease progression. Therefore, treatments are limited to modest alleviation of symptoms by using drugs, such as acetylcholinesterase inhibitors and N-methyl-D-aspartic acid glutamate receptor antagonists, which do not seem to affect the underlying cause of the disease. Because aggregation and accumulation of Aβ and tau play an essential role in the development of AD, therapeutic approaches have predominantly focused on preventing or decreasing the accumulation of these potentially toxic agents. However, the identification of several other genes, which are associated with an increased risk to develop late-onset AD, may provide us with additional insights in the mechanism of the disease and open the door for much needed new treatment strategies.

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doi:10.1002/cpt.200

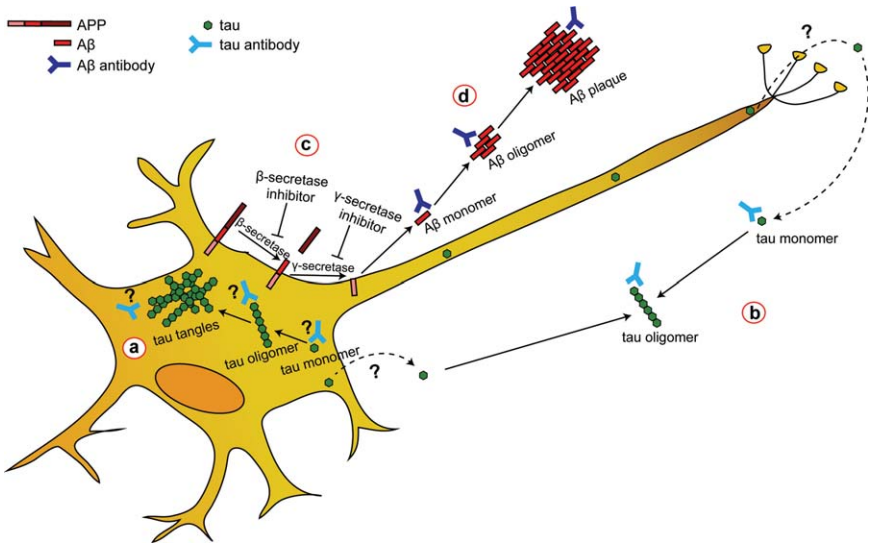

Figure 1 Therapeutic approaches targeting amyloid-β (Aβ) and tau directly. (**a**) Anti-tau antibodies targeting different multimerization states (and also conformation and phosphorylation states) are currently being tested, but, so far, it is not clear whether these antibodies can enter neuronal cells to act on intracellularly localized tau. (**b**) Although the mechanism as to how tau is released into the extracellular space is unknown, the extracellular tau pool is a potential target for tau-directed immunotherapy, especially because increasing evidence points to the mechanism of tau spreading being from cell-to-cell via the extracellular space. (**c**) The β-secretase and γ-secretase inhibitors/modulators could be useful to prevent generation of Aβ from amyloid precursor protein (APP) in the amyloidogenic pathway. (**d**) Anti-Aβ-immunotherapy can be designed to target Aβ monomers, oligomers, and/or plaques.

Therapeutic approaches targeting Aβ and tau directly

Interestingly, in both early and late-onset AD, Aβ aggregation precedes the development of neocortical tau pathology. This, together with the observation that autosomal dominant forms of AD are primarily caused by mutations in genes important for the generation of Aβ, led to the development of the "amyloid cascade hypothesis," which proposes that deposition of Aβ in the brain is the initial event that ultimately leads to the development of AD.[1] Therefore, most initial treatment strategies focused on reducing Aβ burden, predominantly by active and passive immunization targeting Aβ, but also by targeting the β-secretase and γ-secretase complexes (**Figure 1**). Active immunization strategies with Aβ have been among the first approaches tested in clinical trials. Although the initial study using full length $Aβ_{42}$ (AN1792, ClinicalTrials.gov identifier NCT00021723) had to be suspended in Phase IIa when four patients developed meningoencephalitis, progress with regard to safety of vaccination has been made by using shorter Aβ peptides and different adjuvants. Despite the significant side effects in the initial study, further analysis of several study participants revealed reduced Aβ plaque burdens in patients who showed an antibody response.[2] This gave hope for future studies. So far, three Phase II trials using different Aβ peptides have been completed (CAD106, ACC-001, AFFITOPE AD02; ClinicalTrials.gov identifier NCT01097096, NCT01284387, and NCT01117818), but results have not yet been reported. In parallel, efforts have been made to translate results from passive immunization studies in mice to the clinic. The most advanced clinical trials with antibodies targeting different epitopes within Aβ include bapineuzumab, solanezumab, gantenerumab, crenezumab, and aducanumab. Two large Phase III trials with bapineuzumab (ClinicalTrials.gov identifiers NCT00575055 and NCT00574132) and solanezumab (ClinicalTrials.gov identifiers NCT00905372 and NCT00904683) did not show benefit with respect to primary clinical outcomes in patients, although there were several statistically significant secondary outcomes favoring solanezumab over placebo. This has led to three additional ongoing clinical trials with solanezumab in both preclinical and mild AD (ClinicalTrials.gov identifiers NCT01900665, NCT01760005, and NCT02008357). A recent Phase Ib trial with aducanumab (ClinicalTrials.gov identifier NCT01677572) also reported very promising results in regard to plaque removal and reduction of cognitive decline and is moving to a Phase III trial. Aβ oligomers have not yet specifically been targeted in humans. Because soluble Aβ oligomers may represent a particularly toxic form of Aβ,[3] future trials targeting these species may be warranted.

Interestingly, evidence suggests that accumulation of tau but not Aβ correlates with clinical symptoms in patients with AD. This suggests that tau could be a suitable therapeutic target. Under normal conditions, tau is a highly soluble and natively unfolded microtubule-associated protein. However, under disease conditions, tau becomes hyperphosphorylated, leading to dissociation from microtubules and accumulation into intracellularly localized insoluble neurofibrillary tangles. Recent evidence suggests that tau aggregates can spread from cell to cell by a so far unknown mechanism, thereby rendering extracellular tau a potential target for therapies (**Figure 1**). A large body of literature describes promising results with active as well as passive immunization directed against tau in tau transgenic mice. In these studies, different tau conformations, phosphorylation states, as well as multimerization states were targeted. So far, only one tau peptide fragment

(AADvac1) has entered clinical trials in a Phase I study evaluating safety, tolerability, and efficacy in an active immunization approach (ClinicalTrials.gov identifier NCT02031198), and passive immunotherapy approaches targeting tau are underway (ClinicalTrials.gov identifiers NCT0249 4024 and NCT02460094).

While immunotherapy will hopefully be useful in reducing existent neurofibrillary tangles and Aβ plaques, depending on the target the antibodies recognize, they could also help to prevent accumulation by binding to free, soluble Aβ and tau species. To enhance the prevention effect, it might be helpful to combine immunotherapy with other approaches that aim at reducing the initial production of toxic agents. Here, β-secretase and γ-secretase inhibitors/modulators, which target the generation of Aβ at the level of APP cleavage in the amyloidogenic pathway, are two of the most promising tools for future therapeutics (**Figure 1**). Several β-secretase inhibitors show encouraging results in mouse models and also in Phase I clinical trials, and they are now being tested further for potential side effects and efficacy in Phase II/III clinical trials (ClinicalTrials.gov identifiers NCT02322021, NCT01739348, and NCT02245737). Research targeting γ-secretase has already completed a Phase III clinical trial with the γ-secretase inhibitor semagacestat (ClinicalTrials.gov identifier NCT00762411). It failed to meet clinical outcomes and showed significant side effects, such as worsening cognition, probably because of inhibition of notch signaling. The γ-secretase modulators, which more specifically inhibit APP cleavage over other targets of γ-secretase, such as Notch, are promising as they should not have these side effects.

Role of genetic risk factors for Alzheimer disease

Although lifestyle and environmental risk factors may influence AD pathology, this does not explain all differences seen within patients with AD. Genome-wide association studies have identified several risk genes associated with the development of late-onset AD. So far, the mechanisms remain somewhat elusive, although some clues have emerged. These new findings will hopefully help to better understand AD and to identify new therapeutic approaches for patients with late-onset AD. Specific risk factors might modulate responses to AD therapies and their genetic status should therefore be taken into consideration when clinical outcomes in patients are analyzed. One example in this regard is the *APOE* gene, which is thought to predominantly influence AD risk by acting on Aβ clearance and aggregation. In addition to its role in influencing Aβ pathology, apoE may also influence AD by its involvement in other processes, such as synaptic plasticity, cell signaling, metabolism, and neuroinflammation. Following up on its interaction with Aβ in plaques, recent research in the past years on immunotherapeutic approaches targeting apoE has shown beneficial effects on Aβ plaque burden in APP transgenic mice.[4] Three different alleles of the *APOE* gene–ε2, ε3 and ε4–exist. The most common allele, ε3, is considered to be neutral with regard to AD development. However, the presence of the ε2 allele is protective, whereas ε4 is associated with an increased risk to develop AD. Although the ε4 allele is neither sufficient nor necessary for development of AD, understanding its role in more detail might prove important when different treatment strategies are considered.

Another example for a genetic risk factor is *TREM2*. TREM2 is an innate immune receptor expressed on the surface of microglia in the brain. *TREM2* mutations, especially the R47H mutation, have been associated with an increased risk to develop AD. Although the mechanism by which TREM2 mutations leads to increased risk for AD is not known, one possibility is that the mutations lead to less effective lipid signaling and microglial activation.[5] Data from several mouse studies also suggest a role for TREM2 in the microglia response to Aβ plaques. In addition to TREM2, additional evidence of the role of the brain's innate immune response in AD is the fact that *CD33*, another microglial-specific gene, is an AD risk factor.

CONCLUSION

To date, immunotherapies targeting either Aβ or tau seem to be promising approaches to prevent and possibly treat AD. However, given the fact that the most advanced approaches targeting Aβ failed to meet expected clinical outcomes, earlier treatment intervention before the onset of significant neurodegeneration as well as more aggressive treatment strategies with agents that actively remove amyloid plaques may be necessary in the future. This will likely include identification of patients with biomarkers before clinical onset, modification of drug doses, and combination therapies to better target Aβ, tau, and both molecules. In addition, further elucidating the role of *APOE*, the innate immune system, and other genetic risk factors for AD will hopefully provide more insight into the mechanisms that lead to AD development and help to tailor treatments more specific to different patient groups.Additional Supporting Information may be found in the online version of this article.

ACKNOWLEDGMENTS

This work was supported by grants from The JPB Foundation and Cure Alzheimer's Fund to DMH and by a National Science Foundation graduate research fellowship (DGE-1143954).

CONFLICT OF INTEREST

D.M.H. co-founded and is on the scientific advisory board of C2N Diagnostics. C2N Diagnostics has licensed certain anti-tau antibodies to AbbVie for therapeutic development. D.M.H. is on the scientific advisory board of Neurophage and consults for Genentech, Lilly, AbbVie, and AstraZeneca. D.M.H. receives research grants from the NIH, Tau Consortium, Cure Alzheimer's Fund, the JPB Foundation, Eli Lilly, and C2N Diagnostics. The other authors have nothing to disclose.

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