

# The Biochemical Foundations of Alzheimer's Disease and Potential for Immune Therapies

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## Alzheimer's Disease

Alzheimer's disease (AD) is characterized by progressive neurocognitive decline associated with widespread propagation of amyloid-beta and tau protein fibrils. Early stages are asymptomatic though the onset of cognitive debility and subsequent dementia emerges with the prion-like propagation of amyloid deposits and tau neurofibrillary tangles, resulting in pervasive neuronal death and white matter atrophy.

## The Biochemical Foundations of Alzheimer's Disease

Multiple theories have been established to explain the physiological cascade involved in the onset of AD. Oldest among these theories is the cholinergic hypothesis, which arose during a particularly research-intensive era in the field of neurochemistry and anatomy (1). Findings from this two-decade period from the mid-1960s to the mid-1980s established a foundation upon which the molecular basis of neurodegenerative diseases could be closely examined. Chief among these neurophysiological mediators are cholinergic receptors, which play an important role in a wide spectrum of homeostatic functions. Consequently, the manifold nature of these receptors gives way too broad a range of neurological disease states upon their dysfunction (2-4), including those found in AD (5). Amyloid beta deposits have been found to form extracellular amyloid clumps known as plaques, leading to neuromodulating effects that can occur at picomolar concentrations, irrespective of the neurotoxic state of amyloid beta (6).

The role of acetylcholine in memory recall was



Elana Cohn: *Sagittal*

demonstrated by the use of receptor antagonists in monkeys and rats. Subjects receiving infusions in the peripheral cortex showed marked decline in the ability to recognize stimuli (7, 8). Subsequent studies demonstrated that various degrees of cognitive impairment arise from region-specific application of receptor antagonists (9, 10). Post-mortem examinations of AD brains revealed depleted levels of cholinergic activity, particularly choline acetyltransferase, a transferase responsible for acetylcholine synthesis, and acetylcholinesterase, a hydrolase that breaks down acetylcholine, in the neuromuscular junction and neural synapses located within the cerebral cortex (11). In Alzheimer's patients, frontal and temporal regions of the brain responsible for memory and cognition were especially depleted with respect to cholinergic receptors (12, 13).

Much of the criticism levied against this hypothesis stems from confounding factors that show a natural decline of cholinergic activity in healthy

rat brains (14, 15), as well as a broad spectrum of neurodegenerative disease (16). These revelations point to the more general phenomenon of cholinergic decline as a symptom, rather than the impetus of neurodegeneration.

## Amyloid-Beta

The pivotal role of amyloid-beta in the progression of AD pins the peptide as the central tenet of the amyloid cascade hypothesis. Upon observation of Auguste Deter's brain (who would later become the first patient to be formally diagnosed with AD) Alois Alzheimer, the physician credited with the first published clinical observation of AD dementia, noted "numerous small miliary foci are found in the superior layers...[that] are determined by the storage of a peculiar material in the cortex". Indeed, Alzheimer would go on to conflate these plaques with "the most serious form of dementia", adding that "the plaques were excessively numerous and almost one-third of the [patient's] cortical cells had died off" (16). These extracellular plaques would eventually come to be known as abnormal accumulations of peptide amyloid-beta. The description of amyloid-beta pathology as a "cascade" implies its central role as a vanguard of AD progression, postulating the formation of amyloid plaques as the prerequisite for neurofibrillary tangle formations of tau protein.

Amyloid-beta's precursor, amyloid precursor protein (APP), is a transmembrane protein which has been found to influence synaptogenesis and, most recently, protein synthesis in dividing human cells (17), among other processes. Its abundance in interneuronal ER and Golgi (18) membranes contributes to its involvement in AD pathogenesis, whereby the sequential cleavage of APP by either  $\alpha$  or  $\beta$  (BACE-1) and  $\gamma$ -secretase enzymes, respectively, produces plaque forming and non-plaque forming variants of free-floating amyloid-beta peptide in the neuronal interstitium. In the event of primary cleavage by  $\alpha$ -secretase, soluble APP (sAPP $\alpha$ ) is secreted, leaving behind an 83- residue membrane-bound fragment (CTF $\alpha$ ) (19). Conversely, initial cleavage with  $\beta$ -secretase produces a 99 amino-acid transmembrane peptide (CTF $\beta$ ). In both instances, the membrane bound peptides are next cleaved by  $\gamma$ -secretase to yield amyloid-beta from CTF $\beta$  and a small protein

### Key Point: Alzheimer's Symptoms

Generally symptoms first appear in patients in their mid-60s. Three stages : (1) early, preclinical stage with no symptoms (2.) Mild Cognitive Impairment (MCI) and (3) Alzheimer's Disease.

- Early symptoms most often include memory problems, but may also include other forms of cognitive difficulties including difficulty finding words, visual or spatial problems, or impaired reasoning and judgment.
- Mild AD is the stage at which AD is most commonly diagnosed. Patients may wander or get lost, lose the ability to handle money or pay bills, repeat questions, spend longer to complete normal daily tasks, or misplace items. Behavior and personality changes may also be seen.
- Moderate AD advances memory loss and confusion. Patients may have problems recognizing familiar faces, difficulty carrying out multistep tasks, hallucinations, delusions, and paranoia. They may have an inability to learn new things, problems coping with new situations, and impulsive behavior.
- Severe AD is the stage at which patients lose the ability to communicate and take care of themselves. They may experience weight loss, seizures, skin infections, and difficulty swallowing. They may sleep more, groan, moan, or grunt, and lose control of bowel and bladder.

Retrieved from <https://www.nia.nih.gov/alzheimers/topics/symptoms>.

(P3) from CTF $\alpha$ . The amyloidogenic potential of cleavage products is determined by the location of  $\gamma$ -secretase proteolysis; in the event of cleavage of amyloid-beta valine-40, Ab-40, a 40 amino-

acid variant, is secreted. In the event of cleavage at alanine-42, ab 42, the 42 amino-acid variant, is secreted. While Ab-40 has been determined to be a natural component of cerebrospinal fluid and plasma (20), even potentially possessing neuroprotective properties, its counterpart, Ab-42 has been implicated as the pathological trigger of plaque formation (21).

## The Tau Hypothesis

In post-mortem examination of AD patients, Alois Alzheimer's also described "peculiar, deeply stained bundles of neurofibrils" colocalized with dead cortical cells. Unbeknownst to the physician, he was describing one of the two neuropathological findings consistent with AD — tau neurofibrillary tangles. Distinguished in its ubiquity across a spectrum of neurodegenerative disorders, tauopathies are not unique to AD, however tau fibrillation subsequent to amyloidosis is a hallmark sign.

As a major microtubule stabilizing protein in the central nervous system (CNS), tau maintains cytoskeletal stability through polymerizing and depolymerization of tubulin subunits (22). Its affinity for tubulin is modulated by kinases and phosphatases (23). In the event of hyperphosphorylation, tau dissociates from its cytoskeletal origin in the form of free-floating tau monomers. Consequently, these monomers self-assemble to form oligomeric structures which serve as scaffolds for the development of larger, pathogenic neurofibrillary tangles capable of propagating interneuronally, whereupon exogenous tau fibrils can induce tauopathies in neighboring cells in a prion-like manner known as seeding (24).

The duality of amyloid plaques and tau fibrils in the pathophysiology of AD lend credence to two of the later aforementioned theories. AD-associated tauopathies can seldom form without the presence of amyloid plaques (25), however extracellular amyloid deposition is not sufficient to elicit neurodegeneration (26, 27). The tau hypothesis is therefore the most concise understanding of the biochemical underpinnings of AD (28).

The molecular intersect between the two processes remains unclear; however, recent studies have shown that oxidative stress stemming from the presence

of toxic amyloid-beta upregulates a regulator (RCAN1) of calcineurine, a phosphatase of tau, and glycogen-synthase kinase-3 $\beta$  (GSK3 $\beta$ ), a tau kinase. Concomitantly, the imbalance between an increase in phosphorylation activity and a decrease in dephosphorylation of tau results in the formation of tau fibrils, thus providing a coherent link between amyloidosis and fibrillation (29). This link implies that the mitochondria invariably plays a part in AD etiology, giving birth to a relatively novel theory in which the cellular powerhouse forms the crux of the disease. The mitochondrial cascade hypothesis posits the formation of amyloid plaques on the genetic resiliency of the mitochondrial electron transport chain. Over time, the propensity of the mitochondria to regulate damage via reactive oxygen intermediates, along with its ability to generate ATP via oxidative phosphorylation, declines (30). Age-related physiological changes in mitochondrial function result in compensatory responses, among them the secretion of amyloid-beta. Indeed, studies have found an association between mitochondrial amyloid-beta levels and the degree of cognitive impairment in transgenic mice (31). Moreover, rat neurons treated with electron transport chain inhibitors have been found to enhance tau pathology (32, 33) while cytochrome oxidase inhibitors, which function to impede the reduction potential of the final link in the ETC, cause substantial alterations in the cleavage of APP towards its toxic amyloid-beta descendant (34). This theory helps bridge the discrepancy between genetics and sporadic onset of AD otherwise not explained by allelic variants that induce amyloidosis.

## Structural Characteristics of Tau Protein

Microtubule associated protein tau is a seminal component in the maintenance of structural integrity of neurons. Located on the 17th chromosome, tau transcripts in the CNS are composed of 16 exons, three of which (2, 3, and 10) are alternatively spliced to produce six potential isoforms expressed differentially throughout development. These isoforms are characterized by the presence of three or four repeat tubulin binding regions at the C- terminus and the presence, or lack thereof, of additional inserts at the N-terminus. The presence and absence of exon 10 in the modified tau transcript gives rise to four and three repeat regions, respectively. Irrespective of the

presence of exon 10, the repeat regions 3R (R1-R3) or 4R (R1-R4) are also encoded by exons 9,11, and 12 (35). The largest of these isoforms contains exon 4A (an intermediate region between exon 4 and 5) and is unique in its localization to regions of the peripheral nervous system such as the spinal cord and the retina.

The importance of the N-terminus as a projection domain is maintained by a highly acidic character capable of interacting with cellular components such as the plasma membrane (36), mitochondria and serving as a key intermediate in the maintenance of structural rigidity (37), axonal growth (38) and diameter (39). Conversely, the C-terminus is characterized as a positively charged, basic region connected to the N-terminus via a proline-rich mediator (39). This region is directly bound to cytoskeletal tubulin and facilitates polymerization events conducive to cytoskeletal alterations. It is important to note that while 4R and 3R variants of tau bind microtubules, additional repeat regions have been shown to enhance binding affinity while simultaneously contributing to nucleation rates among dissociated tau (39).

### Post-Translational Modification of Tau

Post-translational modifications of tau have been proposed as key drivers of Alzheimer's pathology, among them glycosylation (40), acetylation (41) and phosphorylation (42).

The hyperphosphorylation of tau protein is a common factor among all aforementioned scenarios (42). As such, the phosphorylation state of tau has thus far been the main determinant of tau pathology and the balance between kinase/phosphatase activity takes center stage. Full length tau (441 aa) has been found to have a total of 80 serine/threonine, along with 5 threonine phosphorylation sites (46), each corresponding to various severities of cytopathology in AD (47). Most of these phosphorylation sites lie in the proline-rich region connecting the projecting N-terminus with the microtubule binding C-terminal region (39). Similarly, tau serves as an intermediary between phosphatases, enzymes that dephosphorylate targeted substrates, and microtubule stability (48).

### Structural and Mechanistic Features of Tau Fibril Formation

Dissociation of protein tau from microtubule binding sites is the neuropathogenic foundation of tauopathy in AD. Subsequent to detachment, monomeric tau assumes an unstructured configuration, which can be attributed to its positive charge low hydrophobic character at physiological pH levels and (49). The lack of hydrophobic residues precludes sufficient hydrophobic forces to sustain a secondary structure, and phosphorylation events contribute to a change in electrostatic character, disassociation and self-assembly (50). These amyloid regions, narrowed down to hexapeptide sequences <sup>275</sup>(VQIINK)<sup>280</sup> and <sup>306</sup>(VQIVYK)<sup>311</sup> are sufficient for the growth and propagation of tau fibrils, among other amyloid derivatives (51,52). While a significant portion of tau retains its random-coil structure even within fibrils, constituent regions of the amyloid core retaining the beta-sheet rich motifs remain (53). This is also demonstrated by the aggregation of tau in the presence of anionic compounds such as heparin (53) and arachidonic acid (54). Spectroscopic studies using FRET and hydrogen/deuterium mass spec examinations have proposed an 'S' shaped model for monomeric tau, whereby contact is maintained between the N-terminus and the proline-rich region and the C-terminus and amyloidogenic regions of tau (55). Interactions between tau hydrophobic regions or polyanionic substances results in a conformational change from unstructured random-coils to beta-sheets, a pervasive feature of amyloids (51).

Tau monomer interactions result in the formation of parallel "stacks" of tau beta-strands connected via intermolecular hydrogen bonds, similar to structures of amyloid-beta (56) and alpha-synuclein deposits (57) in Parkinson's disease. Outer regions of tau filaments exhibit exposed hydrogen bond donors and acceptors (58), features that promote further aggregation and are absent in natural beta-sheet proteins to avoid aggregation (59). In this way, tau dimers are able to attract proximal monomers and grow in an unimpeded stacking fashion.

**Key Point: Tauopathies**

Tau protein's normal function: microtubule assembly and stabilization

**Progressive supranuclear palsy (PSP):** onset over 40 years of age, falls within first 12 months of disease, vertical supranuclear gaze palsy, pseudobulbar palsy, axial dystonia, and dementia

**Pick's disease (PiD):** aphasia, behavioral changes (apathy, disinhibition, alteration in food preference, poor self care), and dementia (poor planning, reasoning and organization)

**Frontotemporal dementia with parkinsonism (FTDP-17):** familial syndromes in which there are personality and behavioral changes early or motor difficulties often mistaken for Parkinson's or PSP

**Corticobasal degeneration (CBD):** movement disorder and dementia (non-fluent aphasia, behavioral changes, apraxia)

**Parkinson's disease complex of Guam (PDC Guam):** Parkinsonism and dementia found in the fifth and six decades of life in the Chamorro people of Guam

**Dementia associated with Down's syndrome:** similar presentation to Alzheimer's but occurring 10-20 years earlier

Williams, D. R. (2006). Tauopathies: classification and clinical update on neurodegenerative diseases associated with microtubule-associated protein tau. *Intern Med J*, 36(10), 652-60.

**Seeding and Intercellular Propagation of Tau**

The presence of preformed tau aggregates potentiates fibrillation of endogenous tau by recruiting of dissociated monomers and oligomers (60). This facet of tauopathies allows tau fibrils to propagate in a pathogenic, prion-like fashion whereby exogenous fibrils or oligomers serve as "seeds", or molecular scaffolds, for monomeric tau in adjacent cells. Indeed, transgenic mice expressing P301L human mutant tau localized to the entorhinal cortex demonstrated propagation of fibrils to adjacent regions (61). Cultured cell experiments demonstrate cellular ability to uptake tau oligomers, but not monomers, via endocytosis (62). This seeding potential is determined by its structural conformation. In these instances, deletion of motifs <sup>275</sup>(VQIINK)<sup>280</sup> and <sup>306</sup>(VQIVYK)<sup>311</sup> eliminates the capacity of full-length tau to seed (63). Currently, there are two potential models to explain seeding, the oligomer-nucleated conformation induction and template-assisted growth (64). The major difference between these two models is the structural component(s) of tau that influence fibril formation. Oligomer-nucleated conformational induction establishes a high-energy scaffold which attracts monomeric tau that binds in succession to lower energy and form oligomers (65). Unlike the template-assisted growth model, fibrils do not integrate dissociated monomers, but are rather formed only after the formation of oligomers (66). Dimeric, trimeric and oligomeric intermediates between monomer and fibril formation have been established in aggregation studies involving other fibrillation prone agents (67) and AD peptide amyloid-beta (68). Toxicology comparisons between neurofibrillary tangles and tau oligomers injected into mouse brains found that oligomer-infused brains showed diffuse tau pathology into neighboring brain regions, whereas NFT-treated cells displayed localized deposits, implicating oligomers as the component most responsible for intercellular tauopathies (69).

**Braak Staging and the Prion-like Propagation of Tau**

The entorhinal region receives input from the neocortex and is involved in higher cognitive functions and the limbic system, as well as in the

formation of memories and emotions. Intracellular tau deposits first appear in an area adjacent to the entorhinal region called the transentorhinal region, which functions as a relay between the neocortex and the entorhinal region. The manner of neurofibrillary tangle propagation is closely associated with the degree of cognitive decline (70). The limbic stages consist of minimal NFT presence in the neocortex, with the fibrils concentration localized to the entorhinal and transentorhinal regions, concomitant with noticeable cognitive impairment. End stage AD presents with widespread damage to the neocortical areas, resulting in extensive cognitive impairment and advanced dementia.

### Origins of Immunotherapy Against Alzheimer's

As one of the hallmark pathologies of AD, aggregates of amyloid beta have been one of the primary immune targets of AD therapies for quite some time. Mice immunization with A $\beta$ <sub>1-42</sub>, an alloform associated with toxic oligomers, showed reduced plaque burdens and retained cognitive functions relative to their non-immunized counterparts (71). Subsequent human trials were halted after a subset of patients developed encephalitis post-immunization, likely due to the extensive activation of CD8+ cytotoxicity (72, 73). Nevertheless, post-mortem autopsies indicated clearance of amyloid but retention of Tau pathology (74). This efficacy of this active vaccine, known as AN-1792, was undermined by a dearth of clinical effectiveness in rescuing cognitive decline (75). In another study on animal models, the clearance of extracellular amyloid plaques was accompanied by the reduction of early tau pathology but retention of hyperphosphorylated neurofibrillary tangles (76, 77). Conversely, tau antibody treatment did not affect amyloid load, indicating that amyloid deposits serve as a precursor to tauopathy, though analysis of normal brained individuals has shown tangle formation in the temporal lobe without the presence of amyloid plaques.

Passive immunizations with monoclonal antibodies against A $\beta$  epitopes have proven effective in phase II and III clinical trials. CSF analysis in patients immunized with bapineuzumab showed a significant decline in phosphorylated tau (78). Nevertheless,

phase III trials were discontinued when 6% of subjects developed aseptic meningitis.

Passive and active immunization of targeting Tau fibrils have also become a mainstay in AD immunotherapy. Studies exhibiting clearance after antibody treatment were either targeted at tau phospho-epitopes or fibril specific conformations. In these cases, phosphorylation of tau was reduced and fibril load significantly decreased (79), establishing a correlation between tau antibody titer count, fibril load and cognitive performance (80). In other cases, passive immunization of phosphorylated tau was found to have significantly decreased NFT burden while increasing microglial activity (81).

### Mechanism of Antibody Mediated Therapy

Although the efficacy of tau antibodies against pathogenic aggregates has been well documented, the mechanism by which this phenomenon occurs is obscure. Chief among several theories is that antibodies directly inhibit the fibrillation or even work to reverse the process altogether (82). This theory is corroborated by the clearance amyloid-beta aggregates in in-vitro studies. Indeed, studies have found that, similar to their amyloid-beta counterparts, tau antibodies cross the neuronal membranes via clathrin-mediated endocytosis and co-localize with intercellular fibrils (83). Additionally, antibodies have been found to interfere with the prion-like interneuronal propagation of tau by directly interacting with extracellular tau seeds (84).

Due to the neuroinflammatory nature of tauopathies, microglial clearance has been found to be a major form of fibril clearance (85). However, studies using mouse models for anti-amyloid-beta antibodies have also shown that clearance can occur in a non-Fc-mediated fashion with the use of antibodies lacking fragment crystallizable regions essential for the interaction of immune system components, such as microglia, with pathogens (86).

### The Blood-Brain Barrier, An Obstinate Foe

One of the major obstacles to immunotherapy against neurodegenerative disorders is the human blood-brain barrier (BBB), a restrictive vasculature

of endothelial cells exhibiting high electrical resistance. In healthy individuals, the BBB functions as a selective safeguard against potential antigens and neurotoxins, impeding the entry of large or hydrophilic molecules, while facilitating transport of metabolically essential nutrients and molecules. The innate bulkiness of immunoglobulins poses a major obstacle to developing effective therapeutic measures for combating neurodegenerative disorders. Indeed, radioimmunoassays have found that approximately 0.1% of circulating IgGs, the most common of the 5 immunoglobulin classes (A,G,M,E, and D), can be detected in the CNS (87). However, the efficacy of the BBB can be severely compromised during neurological disorders such as multiple sclerosis, viral meningitis and tumors (88). Inflammatory events in AD have also contributed to increased BBB permeability and the pathological spread of amyloid plaques. Given the rapid turnover of cerebrospinal fluid (CSF) into the bloodstream, intrathecal injections directly into the CSF are equivalent to prolonged intravenous injections, amounting to limited therapeutic efficacy (89). Moreover, a logarithmic decrease in drug distribution throughout the brain has been shown in bulk-flow delivery of drugs directly into brain tissue (90). As such, antibody delivery for neuro-immune therapy is a popular research topic. Three major approaches to this problem include the application of lipid-mediator molecules, which can passively diffuse through the BBB; carrier mediated transport (CMT) of small water-soluble molecules; and the exploitation of receptor-mediated transport (RMT). Theranostics, the use of molecular platforms for drug delivery and diagnostics, relies on lipid or water-based carriers to transport antibodies across the membrane (91). These platforms have been used in the delivery of AB-antibody fragments via synthetic liposomal elements such as polyethylene glycol polymer chains (PEG) (92). Advances in RMT take advantage of metabolic receptors mediating BBB access to transport bound antibodies into the brain parenchyma. Insulin and transferrin receptor ligand-bound AB-antibodies have been shown to effectively cross the BBB through receptor-mediated transcytosis, enhancing brain exposure 55-fold in some instances (93, 94).

### Additional Obstacles in Immunotherapy

Differences in the neurophysiology of animal models

and human patients is an obstacle. Transgenic animal studies utilizing exogenous tau may find considerable discrepancies in phosphorylation patterns and epitope sites in patient counterparts. The lingering possibility of cytotoxicity from T-cell mediated responses has been a repetitive theme in human studies, all of which have been failed due to recurrent instances of neuroinflammation. In animal studies, the use of Freund adjuvant to stimulate cell-mediated immunity actually exacerbated tau pathology and neuroinflammation (95, 96). Subsequent commentary on these outcomes suggests that adjuvants eliciting a Th2 response, which triggers humoral immunity and the production of antibodies, could potentially help mitigate these outcomes (97).

### The Future of Immunological Therapy

Immunological therapy was the paragon of medical discoveries in the 20th century. Priming the host immune system against external pathogens has been a mainstay of western medicine for centuries and continues to be at the forefront of preventative medicine. However, physiological diversity in disease states such as Alzheimer's make this task more challenging as epitopes, adverse reactions and physiological barriers represent barriers to developing clinically effective therapies.

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