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Amyloid-β in Alzheimer's Disease: Effects on Disease Pathogenesis and Recent Advances in Clinical Trials

Anya Gurudatt[1] and Alicia Van Enoo[2]

[1]San Mateo High School, 506 N Delaware St, San Mateo, CA 94401, USA.
[2]Department of Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0634, USA.

Correspondence: Anya Gurudatt,

Abstract:- This paper will be a review of current and ongoing literature surrounding how the aggregation of amyloid-β can induce cognitive impairment, neuronal loss, and the overall pathogenesis of Alzheimer's disease through its interactions with glutamate receptors, microglia, tau proteins, and lipid rafts. Through these mechanisms of action, amyloid-β can induce synaptic dysfunction, neuroinflammation, neurotoxicity, impaired synaptic plasticity, and other issues that worsen AD-associated neurodegeneration. Multiple preclinical studies have been conducted to better understand these mechanisms as potential targets for therapeutic intervention. Unfortunately, when tested in clinical trials, drugs targeting amyloid-β have consistently failed. However, there are still futures for various ongoing clinical trials targeted at dissolving soluble and insoluble amyloid-β deposits, reducing the production of amyloid-β, and preventing amyloid-β aggregation, specifically: passive vaccination, monoclonal antibodies, and γ-secretase inhibitors. Monoclonal antibodies, in particular, have proven to be most promising. This paper reviews some of the most novel and recent findings and their implications for the future of the field. This paper will also evaluate the most promising forms of therapeutic intervention that have been and are currently being investigated, as well as limitations and future prospects of targeting amyloid-β to slow the progression of Alzheimer's disease.

I. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease associated with memory loss and other such cognitive and behavioral deficits ('2021 Alzheimer's disease facts and figures', 2021). The start of AD has been found to precede symptoms by decades (Gordon et al., 2018; Reiman et al., 2012), making it a difficult disease to treat before damage occurs, and there is currently no cure for AD. Symptoms begin with mild cognitive impairment and then progress to memory loss and cognitive impairment, with late stages of AD entailing physical damage and inability to communicate or go about daily tasks ('What Are the Signs of Alzheimer's Disease?', 2021). It currently affects an estimated 6.2 million Americans, a number expected to grow to 13.8 million by 2060 ('2021 Alzheimer's disease facts and figures', 2021). As of 2021, health care and hospice services for AD cost an estimated $355 billion ('2021 Alzheimer's disease facts and figures', 2021), making effective prevention and treatment of the disease more needed than ever in multiple respects. One highly implicated pathology in the progression of AD is amyloid-β (Aβ), a short protein fragment that forms when amyloid precursor protein (APP) is cleaved (Chen et al., 2017). APP is a membrane protein that plays a vital role in neuronal development, but when it is split by the enzymes β-secretase and γ-secretase, it can form neurotoxic fragments such as Aβ. The cleavage of APP entails these enzymes snipping off a portion of the protein. This fragment, Aβ, is typically cleaved into either 40 (Aβ40) or 42 (Aβ42) amino acid peptides (Chen et al., 2017). Aβ42 is thought to be especially neurotoxic ('What Happens to the Brain in Alzheimer's Disease?', 2021). Certain anti-Aβ antibodies meant to slow the progression of AD target specific peptides of Aβ.

Aβ contributes to AD-associated neurodegeneration in multiple ways, a phenomenon described by the amyloid cascade hypothesis (Barage and Sonawane, 2015). One mechanism is through the abnormal accumulation of Aβ into extracellular plaques. Aβ plaques are insoluble aggregates of misfolded Aβ protein fragments that clump together due to their sticky texture ('Amyloid Plaque - an overview | ScienceDirect Topics', 2021). Aβ aggregates lead to neurodegeneration by inducing neuroinflammatory microglia and neuronal death. They can also obstruct intracellular transport, impair synaptic plasticity, and cause further deterioration by interacting with other mechanisms such as lipid rafts and tau proteins. Tau is a protein that stabilizes microtubules (Medeiros et al., 2011), a series of rigid 'tracks' in neurons that help transport molecules and communicate (Cooper, 2000). Physiologically, tau proteins help preserve neuronal structure, but in an AD brain, tau proteins are chemically changed and hyperphosphorylated. They become misshapen and get tangled up with other tau, causing the microtubules to fall apart and forming aggregates of tau called neurofibrillary tangles (NFTs) (Medeiros et al., 2011). Like plaques, tangles are another highly implicated pathology in the progression of AD.

There are many studies, both completed and ongoing, in regard to Aβ's mechanisms of action, as well as many clinical trials evaluating the efficacy and safety of drugs targeting Aβ in order to slow and prevent the progression of AD. Unfortunately, many clinical trials aimed at targeting Aβ have been unsuccessful (Orgogozo et al., 2003; Salloway et al., 2014; Landen et al., 2017a), despite Aβ's pivotal role in AD-associated neurodegeneration. Preclinical studies in mice using therapies such as active immunization, and γ-secretase inhibitors have found promising results (Mangialasche et al., 2010; 'Long-Term Potentiation - an overview | ScienceDirect Topics', 2021; Blanke and VanDongen, 2009), while clinical trials have fallen short (Mangialasche et al., 2010). The most promising therapies targeting Aβ are currently monoclonal antibodies (mAbs), a type of passive immunization, although the recent approval of Aducanumab has sparked controversy. This paper reviews the most recent studies and clinical trials in the field that have allowed scientists to identify novel therapeutic strategies to target Aβ. While there are other factors that contribute to the pathogenesis of AD, this paper focuses on the role of Aβ, specifically why it is key to preventing the progression of the disease and what the future of novel anti-Aβ therapy looks like.

II. AMYLOID-B: MECHANISMS OF ACTION

- *Aβ Interactions with Receptors and Synaptic Dysfunction*

Interactions between Aβ and certain neurotransmitter receptors have been shown to negatively impact synaptic transmission and long-term potentiation (LTP), leading to the progression of AD. LTP is a process that entails strengthening of neuroplasticity, thereby increasing neuronal signal transmission and improving memory function and learning ('Long-Term Potentiation - an overview | ScienceDirect Topics', 2021). Aβ also acts to disrupt synapses, particularly N-methyl D-aspartate receptor (NMDAr), a glutamate-gated ion channel with underlying roles in neuroplasticity and memory (Blanke and VanDongen, 2009). Researchers have previously demonstrated that soluble Aβ aggregates strongly inhibit NMDAr-dependent long-term potentiation in the dentate gyrus of rat hippocampal slices (Wang et al., 2002). As research evolved, scientists accounted for species differences to validate this finding by using Aβ derived from human AD patients. Shankar et al. (2008) conducted a study in which they extracted cerebrocortical Aβ dimers from human patients and put it into rat hippocampus. It was found that long-term depression (LTD) was enhanced, and metabotropic glutamate receptors were necessary for this to occur. Furthermore, NMDAr was required for the facilitation of dendritic spine loss stemming from the Aβ dimers. Interestingly, in a study using rat hippocampal slices, Aβ's negative effect on dendritic spine loss was found to be completely preventable through the use of an NMDAr antagonist, supporting the idea that neurotransmitter receptors play a vital role in mediating certain neurotoxic mechanisms of Aβ (Shankar et al., 2007) (Figure 1a).

While many studies have shown that Aβ oligomers can cause synaptic dysfunction (Wang et al., 2002; Shankar et al., 2008; Rui et al., 2010), few have analyzed underlying mechanisms fueling these effects. Rui et al. (2010) conducted a study using cultured hippocampal neurons, in which they determined why Aβ1-42 oligomers significantly reduced the number of postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAr), a glutamate receptor that plays an essential role in synaptic plasticity ('AMPA Receptor - an overview | ScienceDirect Topics', 2021). The study found that these Aβ oligomers inhibited mitochondrial transport to dendritic spines, causing a significant reduction in levels of AMPAr at the postsynaptic membrane. These Aβ-induced trafficking defects resulted in deficits in LTP induction and overall synaptic dysfunction.

Together, these studies suggest that soluble Aβ oligomers contribute to the pathogenesis of AD by impairing synaptic plasticity. Future biological and clinical research into Aβ's interactions with neurotransmitter receptors is needed to understand the exact mechanisms of action responsible for Aβ-induced synaptotoxicity in AD. It is important to note that although most studies have focused on the deleterious effects of Aβ on neurotransmission, some studies have demonstrated a neuroprotective effect of Aβ. Niidome et al. (2009) conducted a study using a thioflavin T assay, in which they discovered that synthetic Aβ1-42 monomers reduced NMDAr-induced Ca2+ influxes and neuronal death. Expanding research into the potential neuroprotective effects of Aβ is important when considering potential therapies because targeting Aβ could potentially inhibit its protective properties, rendering therapies as less effective.

- *Aβ-Induced Microglia and Inflammation*

While the exact pathophysiological causes of AD remain unclear, microglia activation, and the consequent release of proinflammatory cytokines, is a largely implicated factor in the pathogenesis of AD. Microglia, the brain's immune cells, play an important role in maintaining brain homeostasis, including fighting off infections and phagocytosing cellular debris ('Microglial cells - Latest research and news | Nature', 2021). In fact, microglia have been found to phagocytose fibrillar Aβ as a neuroprotective immune response (Ries and Sastre, 2016). However, many studies have shown that microglial degradation of Aβ is very minimal, and it has been shown to actually lead to the onset of pathogenic processes that ultimately result in increased Aβ production, suggesting that it may play a more harmful than helpful role in the progression of AD (Stalder et al., 2001; Chung et al., 1999; Frackowiak et al., 1992). Soluble forms of Aβ have been known to trigger the chronic activation of microglia, resulting in inflammation, neuronal damage, and neurotoxicity (Katsumoto et al., 2018) (Figure 1b). Additionally, microglia express genes such as triggering receptor expressed on myeloid cells 2 (TREM2), a protein-coding gene that supports the immune response to pathology induced by Aβ (Ulland and Colonna, 2018). Various forms of soluble TREM2 (sTREM2)

in microglial cell cultures and Trem2-KO mice have been found to contribute to the pathogenesis of AD by significantly increasing the production and expression of proinflammatory cytokines (Zhong et al., 2017), which are signaling molecules that exacerbate disease progression and inflammation (Dinarello, 2000). Furthermore, the overexpression of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-18 contribute to neurodegeneration in AD in multiple ways (McAlpine et al., 2009; Dugan et al., 2009; Pickering and O'Connor, 2007). Previous studies have linked the upregulation of proinflammatory cytokines to neuronal death in 3xTg-AD transgenic mice (McAlpine et al., 2009) and have found impaired cognition and spatial learning in wild-type C57BL6 mice (Dugan et al., 2009). Furthermore, inhibition of LTP and memory formation was observed in a variety of in vivo AD models (Pickering and O'Connor, 2007).

Another pathogenic process that occurs in AD is microgliosis, an intense microglial response to pathogenic damage (Li and Zhang, 2016), at the core of Aβ deposits. A recent study conducted by Zhang et al. (2021) using the 5xFAD mouse model of AD, discovered a significant increase in neuronal cell death restricted to the core of Aβ deposits. Interestingly, they found that size of the deposit correlated with and the extent of neuronal loss. Authors hypothesized that this was because microglial activation occured only at the core of Aβ deposits, thereby only causing microgliosis-induced neuronal loss in those areas. It is important to note that in the previously cited Shankar et al. (2008) murine study, one conclusion drawn was that Aβ plaque cores are primarily inactive. However, the recent Zhang et al. (2021) study demonstrates that in fact, it is at the core of plaques in which microgliosis and neuronal loss are induced most, disproving this older, outdated interpretation. Furthermore, many researchers in the past have challenged the belief that neurodegeneration in AD is dependent on Aβ, attributing the main progression of the disease to interactions with other pathologies, such as tau (Chételat, 2013; Herrup, 2015; Reitz, 2012). However, while it is important to acknowledge other pathologies when addressing neurodegeneration in AD, the amyloid cascade hypothesis and important role of Aβ early must be taken into consideration with these, given that Aβ has been found to precede and give way to other pathologies such as tau (Bilousova et al., 2016). Zhang et al. (2021) support this idea, given that they employed a mouse model with no deposition of pathologic tau in the brain, yet cellular death was still observed in core Aβ deposit areas. Corroborating the importance of Aβ-induced microglial activation, Hu et al. (2021) found that in an APP/PS1 mouse model, the inhibition of early, sustained microglial proliferation prevented senescence and the development of disease-associated microglia, which in turn directly impaired the pathology of Aβ and consequential synaptic dysfunction. Understanding the interactions between microglia and Aβ in AD is crucial to advancing our understanding of the innate mechanisms driving neurodegeneration in AD. Future research should address the regulation of Aβ-induced chronic microglial activity, as that is key to preventing the subsequent inflammation. Additionally, identifying ways to reduce neurotoxic effects while enhancing the neuroprotective effects of microglia activation is a promising future area of research as it can slow the progression of AD.

- *Aβ Enhancement of Tau and Neurofibrillary Tangles*

While Aβ alone can fuel the pathogenesis of AD in multiple ways, it is worth noting how Aβ synergistically interacts with and enhances other pathologies that, in turn, can also worsen neurodegeneration. The relationship between Aβ plaques and tau, the protein that makes up NFTs implicated in the pathology of AD, has been widely investigated (Busche and Hyman, 2020). Various studies have demonstrated an effect of Aβ on the propagation of tau and the resulting consequences on cognition, memory decline, and the progression of AD. For example, researchers discovered that, γ- and β-secretase inhibitors that prevented the development of Aβ also prevented tau propagation in a 3D human cell model of of AD (Lee et al., 2016). Furthermore, it was recently reported that the injection of paired helical filaments (PHFs) in an Aβ mouse model (5xFAD) promoted tau pathology more so than in wild-type mice without amyloid precursor proteins (APP), suggesting that the presence of APP plays a role in the propagation of endogenous tau (Vergara et al., 2019). In support of this idea, a study using a combined APP-Tau mouse model (APPOSK-tau264) revealed that Aβ oligomers that have not yet formed plaques can still trigger neurofibrillary tangle formation, causing impaired spatial sense and memory, as well as synaptic loss in the hippocampus (Umeda et al., 2014). Thus far, the studies discussed have investigated indirect interactions between Aβ or APP and tau and their consequences. However, a more recent study discovered evidence of a direct interaction between Aβ and tau. Using a cell-free thioflavin T assay, researchers discovered that Aβ can directly trigger the aggregation of tau through cross-seeding (Vasconcelos et al., 2016) (Figure 1c), a mechanism through which misfolded protein oligomers can promote the aggregation of another protein (Morales et al., 2009). This prion-like mechanism of action of Aβ could be a significant contributor to disease progression in AD and warrants further investigation in the human AD brain.

Beyond mouse models, Aβ's effect on the development of tau has also been demonstrated in human patients. Timmers et al. (2019) conducted a study in patients with normal cognition, mild cognitive impairment, preclinical AD, and prodromal AD, in which a significant Aβ-dependent correlation between cerebrospinal fluid (CSF) tau levels and cognitive impairment was found. Furthermore, Hanseeuw et al. (2017) conducted a study in patients with preclinical AD, in which it was found that Aβ and tau in the neocortex predicted memory decline, but not Aβ-independent tau in the entorhinal cortex. Although murine studies investigating Aβ-tau synergy have considerably advanced the field of AD research, it is important to note certain limitations. The wild-

type mouse tau used in the referenced studies contains certain structural differences from human tau, and the two only share 88% sequence homology (Drummond and Wisniewski, 2017). Tau in mice do not form NFTs, thus tau mouse models must express mutated human tau protein. However, these mutations do not occur in humans, and so the interaction between Aβ and these mutated tau proteins found in mouse models may not accurately depict the interactions that occur in human AD patients. Future studies in mice regarding Aβ-tau interactions must be conducted such that they account for interspecies differences; this can better translate findings in animals to a clinical context, which in turn can help future therapies target Aβ-tau synergy and more effectively slow the progression of AD.

- *Pathogenesis of Aβ-Lipid Interactions*

One of the newest avenues of research in the AD field aims to investigate the interaction between Aβ and lipid rafts, which are microdomains located in external membrane layers ('Lipid Raft - an overview | ScienceDirect Topics', 2021). Lipid rafts are important for membrane fluidity, receptor trafficking, neurotransmission (Korade and Kenworthy, 2008), synaptic plasticity, and neuronal development (Sezgin et al., 2017). Recently, Poejo et al. (2021) cited them as a mechanism that increases cellular levels of neurotoxic Aβ due to Aβ's high affinity for binding to lipid rafts on the neuronal membrane. However, as noted in a recent review by Levental et al. (2020), there is still much controversy surrounding whether or not lipid rafts have meaningful physiological roles, given that they have only been indirectly measured in isolated membranes using in vitro studies (Raghupathy et al., 2015; Kinoshita et al., 2017). Despite a lack of definitive evidence on their biological significance, the impact of lipid rafts and prefibrillar Aβ oligomers on neurotoxicity was recently examined (Diociaiuti et al., 2019). They found that in a lipid raft model, salmon calcitonin solutions containing Aβ oligomers induced an increase in intracellular calcium ion (Ca2+) levels (Figure 1d). The resulting Ca2+ dyshomeostasis has been shown in vivo to cause dendritic spine loss and impair synaptic plasticity mechanisms vital to learning and memory (Kuchibhotla et al., 2008). Furthermore, Ca2+ dysregulation promotes neurotoxicity by inducing neuronal oxidative stress (Gibson and Thakkar, 2017), an imbalance in the amount of reactive oxygen species and corresponding antioxidants (Betteridge, 2000). Investigating Aβ's interactions with lipids is a promising new direction in the discovery of underlying factors in the pathogenesis of AD. Further research aimed at understanding this interaction may allow researchers to identify methods of reducing Ca2+ overloads, a highly implicated pathological mechanism in the neurotoxicity associated with AD.

III. DISCUSSION

Collectively, the effects of Aβ on glutamate receptors, synaptic transmission, microglia, tau, and lipid rafts lead to cognitive and behavioral deficits implicated in AD, such as neuroinflammation and toxicity, impaired synaptic plasticity, neurodegeneration, and eventual loss of memory, physical capabilities, and even death. Experimental data continues to support the amyloid cascade hypothesis, and it is therefore crucial that the prevention and slowing of the progression of AD addresses Aβ-mediated effects. Further preclinical research still needs to be conducted on the potentially neuroprotective effects of Aβ on glutamate receptors and cellular homeostasis; the failure of many clinical trials could possibly be due to the elimination of Aβ also countering its protective properties, thus further study is required to better understand neuroprotective mechanisms of action. In addition, studying means of reducing the neuroinflammatory effects of microglia in AD while enhancing their neuroprotective physiological purpose could be a promising future area; enhancing their ability to phagocytose Aβ could prevent the Aβ deposition that even precedes other neurotoxic pathologies such as tau, thereby slowing neurodegeneration. Currently, some studies covered in this paper are limited by difficulty in applying findings in murine research to that of humans. In order to better understand the molecular biology of pathologies such as tau, researchers must better be able to translate animal phenotypes to clinical contexts, especially given that this can improve the future efficacy of drugs. Future preclinical research must also explore the novelty of lipid rafts and their interactions with Aβ. Given that the physiological significance of lipid rafts remains unproven, further investigation and clarification could potentially help reduce Ca2+ influxes and neurotoxicity, and targeting Aβ-lipid raft interactions could even lay the foundation for a therapeutic intervention.

Multiple studies and clinical trials over the years have demonstrated the crucial role played by Aβ and Aβ-induced mechanisms in AD. However, while this paper focuses on Aβ, it must be noted that Aβ is not the only important component in disease pathogenesis. Given Aβ's synergistic interactions with tau, both of which have been proven to be critical factors in the progression of AD, the unsuccess of clinical trials could be due to the failure to target both Aβ and tau. Future trials and research should explore this idea in order to gain a better understanding of Aβ-tau synergy and apply that to a more effective therapy. Ultimately, Aβ is a pathology that continues to largely influence the pathogenesis of AD, and the most recent and promising developments made in the field warrant further investigation of Aβ, its pathologies, and future Aβ-targeted therapies.

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