Inflammation In Relation to Alzheimer's Disease and Potential Treatments

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Abstract. Alzheimer's disease (AD) affected millions of people. As it is widely associated with its two hallmarks, Aβ and NFT, its relationship with inflammation is less studied, but gaining more attention in recent years as more experiments and researches provide sufficient results to prove the close relationship between the two. By collecting and covering a variety of experiments and reports conducted over the years, this review projects to include an in-depth analysis of pathogenesis of Alzheimer's disease, discussing its connection with inflammation in its formation and development, while seeking for treatments targeting inflammation as a potential solution to prevent, reduce and reverse Alzheimer's disease in the future. From researches done up to date, there are numerous failures regarding anti-inflammatory treatment of Alzheimer's, but some early studies show positive results of new drugs targeting different inflammatory mechanisms which are able to attenuate the disease. More of relevant studies can be done in the future as a potential solution to AD.

Keywords: Inflammation; Alzheimer's disease; cytokines; neuroinflammation; microglia.

1. Introduction

Alzheimer's disease (AD) was first discovered in 1906 by German psychiatrist and neuroanatomist, Alois Alzheimer, who revealed its abnormal protein conformability, it has been over a century since humans' encounters with this unique form of dementia. The symptoms that Alzheimer's manifests are distinctive, which might include frequent forgetfulness in the early stages, and severe cognitive and linguistic dysfunction or behavioural disorders as the condition exacerbates.

As a multifactorial disorder, the formation of AD is linked with numerous risk factors. One of the most significant risk factors is one's age. The prevalence of AD rises with age, reaching about 19% in people aged 75 to 84 and 30–35%, possibly even up to 50%, in people over 85 years old. On an annual basis, the occurrence of AD is only around 1% for age group of 65 to 70 years old, while six to eight times more for people older than 85 years old [1]. Besides age, multiple risk factors including natural risk factors such as genetics, arguably making up 70% of all risk factors attributed, or acquired risk factors, including gender, lifestyle and diets can also play a role in triggering and progressing neuropathological developments of Alzheimer's. Research has indicated that smoking will increase the risk of AD 2 to 4 times, while it is estimated that a total of 12 (obesity, diabetes, etc.) of the main risk factors contribute to 40% of total dementia cases [2].

Alzheimer's disease contributing to roughly 60% to 70% of dementia. AD is prevalent among seniors aged 65 or over at a rate of around 1 in 9 people (10.7%) in the United States [3], and according to estimations, the number will double every 20 years. AD has a very high mortality rate at the same time, ranked as the sixth leading cause of death in the US [4]. Such features of Alzheimer's cause huge financial burdens on individual families and society. In 2020 alone, the total expenses for the treatments of Alzheimer's were estimated to be around $305 billion, and a further $1 trillion are predicted to be invested in healthcare of AD in the future caused by aging of population. Furthermore, in 2021 alone, roughly 16 billion hours are devoted to caring for them by 11 million family members and volunteers [4]. Consequently, finding a cure for Alzheimer's is necessary.

Due to its complex nature, numerous mechanisms in the pathways remained to be unclear. Nevertheless, the presence of Alzheimer's has been recognized by two representative biological hallmarks: extracellular aggregates of insoluble β-amyloid peptide(Aβ), and neurofibrillary tangles (NFT) which are composed of hyperphosphorylated tau protein (P-tau) in the neuronal cytoplasm in

cortical and limbic areas of the brain. However, the two substances had been proven to be not the cause of AD, and therapeutic treatments directly aiming at these two markers were continuously failing whilst growing pieces of evidence of inflammation as a core mechanism to AD were unearthed in recent years' studies. Thus, by using complete second-hand data, this review will introduce the different pathways of Alzheimer's, elucidate inflammation's role and pathways in the development of AD, and discuss the current progress of finding a cure for Alzheimer's and, particularly, from the aspect of curing inflammation.

2. Etiology of AD

Alzheimer's will cause significant changes in the brain. The main observable change comes with the size of the brain, significantly shrinking as AD aggravates. This is caused by the deaths of neurons as their communication, metabolism and repair functions are damaged. Thus, when areas linked to memories and language shrink, typical symptoms of Alzheimer's will take place. It has been confirmed since the first discovery that the levels of Aβ plaques and neurofibrillary tangles (NFT) are directly related to Alzheimer's. Increased levels of Aβ, p-tau protein is observed in a study on rats with significant cognitive impairment. Similar to this, repeated systemic LPS injections of Aβ lead to prolonged cognitive deficits in mice models [5]. And thus, the classical Aβ pathway and p-tau pathway are to be brought up first.

3. Aβ pathway

One conventional theory of initiation of AD is the Aβ hypothesis. In this theory, pathogenesis of Aβ starts with amyloid precursor protein (APP), an integral transmembrane protein which has an essential role in regulating survival and growth of neuronal cells in the brain [6]. Human APP gene was first found on chromosome 21 in 1987, and in 1991, a locus related to early onset genetically inherited AD was discovered close to APP gene, implying the relationship of the two for the first time. Since then, more than 25 mutations in relation to APP have been identified [7], including point mutation of STM2 discovered on chromosome 1, and locus AD3, related to the susceptibility of AD inclusive of a gene resembling APP.

Researchers found two pathways of APP cascade, nonamyloidogenic and amyloidogenic pathways (figure 1), which in both APP undergoes several cleavages. For nonamyloidogenic pathway, APP is primarily split by α-secretase enzymes, majorly by ADAM (A Disintegrin and Metalloproteinase) 10 and ADAM17, both highly expressed in the brain. Then APP is processed by γ-secretases, resulting in the production of large soluble peptide APPα [8], which functions mostly as a neuroprotective factor and assists cell-substrate adhesion, and p3. For amyloidogenic pathway, it is cleaved in an altered way. β-secretase, mainly of BACE-1, first cleaves APP to form the sAPPβ and CTFβ. The latter give rise to the neurotoxic amyloid beta peptides. Aβ 40, Aβ 42, and Aβ-43 are produced due to different cutting sites by γ-secretase. While more of Aβ 40 are present, Aβ 42, the key component of Aβ plaques, and Aβ-43, has higher neurotoxicity. The hydrophobic Aβ peptides will naturally further oligomerize, then form aggregated plaques. By blocking the calcium ion channels, Aβ plaques will then also alteration of calcium homeostasis, increasing oxidative stress and hence inducing neuronal apoptosis.

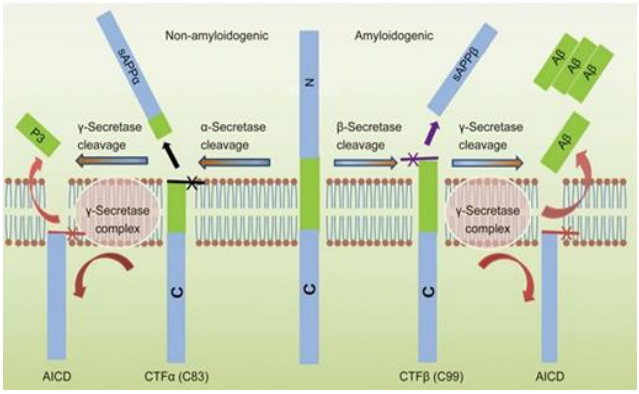

Figure. 1 Amyloidogenic and Nonamyloidogenic pathways of APP processing [8].

4. Hyperphosphorylated tau protein (P-tau) pathway

Neurofibrillary tangles (NFT), another characteristic of Alzheimer's, are formed by intertwined filaments of hyperphosphorylated tau protein. Tau proteins normally cofunction with tubulin to form and stabilize microtubules while forming a network between them. It is crucial for cytoskeletal plasticity during embryogenesis and early development [9]. During the development of AD, however, by abundances of Aβ plaques, specific kinases including CDK5 and GSK3β activated [1] will cause hyperphosphorylation of tau protein, which will undergo further oligomerization as this process increases its resilience to breaking down by proteasome. As a result, the microtubules begin to disassemble and accumulate together with p-tau proteins to form NFTs. As the microtubule network disintegrates, loss of communication and signal transduction occurs between neurons, which finally leads to apoptosis.

5. Neuroinflammation's in AD

In recent years, however, the inflammation hypothesis had been an emerging theory of the fundamental cause of AD. Evidence has been found in Alzheimer's cases and animal models to prove this hypothesis. In AD cases that also had short-term peripheral infections, the cognitive ability of patients had a sudden decline, while returning to previous level after recoveries. It was also found that the level of inflammatory cytokines increased after clinical onset of AD, and strong cytotoxic effects were observed on neurons after peripheral inflammatory reactions. In another research, elevations in inflammatory proteins during midlife are indicated to link with smaller brain volumes and defective white matter microstructural integrity in later stages of life [10], suggesting that inflammation is directly associated with neurodegeneration.

Neuroinflammation is closely interconnected with Aβ pathway and P-tau pathway: that Aβ amyloid within the CNS will bring out activation of microglia, which then cause a pro-inflammatory cascade to produce a variety of substances and finally lead to deaths of neurons, while also producing kinases and cause the fibrillation of tau proteins to form NFTs.

6. Microglia and inflammation pathways on aβ

Microglia are primary response to infections and injuries in CNS. Normally, microglial cells remain inactive in the brain and only carry out surveillance by various signaling mechanisms, including receptors for numerous cytokines and chemokines and regular neurotransmitters. When a threat to CNS is recognized, microglia will become active, and undergo morphism causing them to be enlarged and migrate to the site of threat. Altered signaling mechanisms are thus induced. In the case of AD, the reactive oxidative stress caused by Aβ plaques can result in microglial infiltration around the plaque areas. Then, protein accumulation behaves as a pathogenic trigger to activate toll-like receptors (TLRs) on microglia, which prompts the release of inflammatory cytokines. Also, cell-

surface receptors such as CD14, α6β1integrin, CD47, and TLRs will bind to Aβ and NFTs after recognition by microglia, leading to phagocytosis by microglia degrading enzymes like neprilysin to remove them. As the microglia cells are involved in the clearance, the pro-inflammatory cytokines they release will bring additional microglia to plaques. So, in earlier stages of Alzheimer's, inflammation plays a positive role, as proven in animal models such as in mice [11]. However, when microglia are enlarged for extended periods, they will be unable to process the Aβ plaques anymore. The overproduction of pro-inflammatory mediators will cause age-related microglial priming, meaning that baseline level of inflammation will be raised upon repetitive inflammatory stimulations, making further inflammatory responses inefficient and microglia becoming insensitive. This will cause an exacerbation of AD pathology, which the Aβ plaques and pro-inflammatory cytokines begin to accumulate, known as reactive microgliosis. This will result, in turn, downregulation of the expression of Aβ-phagocytosis receptors, and thus decreasing ability of Aβ degrading enzyme activity to perform phagocytosis and clearance, and Aβ plaques will further sustain. In addition, neurotoxins are produced in this process which degrades the neurons. Membrane attack complex C5b-9, for example, was found to be highly related to synaptic loss [12], through the production of a variety of cytokines and elements of complement system. Neuroinflammatory processes [13], in fact, can enhance the oxidative stress caused by Aβ plaques on neurons (figure 2).

Microglia cells will also undergo substantial phenotypic changes caused by aging, which might be the key reason of why AD is age-related. Microglia that were isolated from post-mortem brain tissue displayed significant genetic differences from their younger, healthier counterparts, including genes involved in cell adhesion, expression of cell surface receptors, et cetera. Higher levels of CD11c and CD14 are expressed in aged microglia, implying more phagocytotic responses, and higher levels of reactive oxidative species and inflammatory cytokines are also produced.

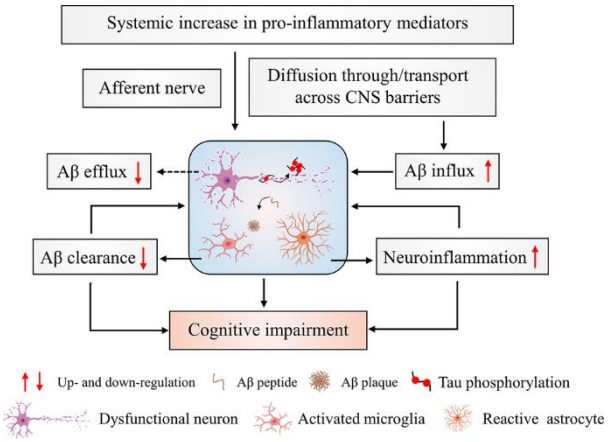

Figure. 2 Relation of neuroinflammation and Aβ in causing cognitive impairment [13].

7. Prepare Your Paper Before Styling

Researches have shown that the inflammatory cytokines IL-1β in chronic inflammation will cause activation of kinases CDK5, GSK3β, the factor which triggers hyperphosphorylation of tau protein. As aging will cause higher production of IL-1β, a higher level of p-tau protein and hence NFTs are produced over time and cause the disease to progress and more severe cognitive deficits (figure 2). Phosphorylation of tau is also indicated by stimulation of TLR4 in mouse models with microglial inflammation [14].

8. Neuroinflammation and genetically related receptor factors

ApoE was thought as the genetic risk factor for AD. It is a member of apolipoproteins with the primary function of regulating the delivery of lipids and steroids including cholesterol through corresponding receptors. The gene is located on chromosome 19. Three isoforms of it are apoE2,

apoE3, and apoE4. ApoE4 is thought to confer the highest risks of AD. ApoE4 usually acts as a mediator, maintaining synaptic connections and can repair damaged neurons. In the pathology of AD, however, the elimination of Aβ peptide is more effectively facilitated by the apoE2 and apoE3 variants, whereas apoE4 isoform binds to Aβ plaques to promote its polymerization. ApoE4 also promotes the strongest pro-inflammatory effects [15] and worsens the pathology.

The genetic modification of the TREM2 is one of the primary drivers of microglial AD clearance. It has been demonstrated that TREM2 genetic variation will increase the likelihood of developing late-onset AD (LOAD) [16]. TREM2, a highly expressed cell-surface Ig superfamily receptor on microglia, participates in the phagocytosis of neuronal debris and aβ. It controls the central nervous system's phagocytic and inflammatory responses. Through interactions of TREM2 and DAP12, CCL19 and CCL21 chemokines are given rise to and thus phagocytosis is carried out by microglia. The point mutation on TREM2 will cause the receptors of microglia unable to clear Aβ from the CNS, hence intensifying the progression of AD. A study by Lamb and Holtzman groups shows this genetic variant will worsen cortical tau pathology in h-tau mice too [17]. Morphologically dystrophic microglia and broad neuronal stress hyperactivation, particularly in pathways connected to ERK, JNK, and GSK3, were present along with this deterioration.

Other than TREM2 there are also a number of microglia-specific receptors crucial in the immune responses of AD. CX3CR1 is a surface receptor highly expressed on microglia. Its exclusive binding ligand, chemokine CX3CL1 (fractalkine) is one of the representative inhibitory factors released by neurons, affecting migration and proliferation of microglia after injuries or infections. Studies have shown that CX3CL1 is able to reduce the expression of IL-6 and TNFα with LPS stimulation, and therefore can reduce neuronal death caused by microglia. The role of CX3CL1 in the pathology of AD, however, is still mostly unclear. However, the deletion of CX3CR1 in a tau transgenic mouse results in increased microglial activation and increased tau phosphorylation [18], though in β-amyloid mice models the deletion caused less neuronal loss and microglial activation. Another study using APP/PS1 mouse model showed similar results with the further reduction of Aβ deposition.

Some other genetic changes of receptors in the neuroimmune system can also change the pathological condition of microglia and AD in the brain. As an example, the genetic deletion of the inflammatory enzyme's caspase-1 or NLRP3, will cause improvement of efficiency of clearance of Aβ microglia [19]. Genetic deletion of tau can also cause inflammation to be alleviated, with lower levels of IL-1β and lower neurotoxicity.

Overall, there are two stages of inflammation involved in development of AD. First, there is a non-specific response of microglia and astrocytes such as microglia to a stimulus such as infection or stress, including Aβ plaques. Signaling via cytokines and complement system is activated to cause inflammation and draw the immune cells to the site of stimuli. In this stage, inflammation is usually beneficial and will allow Aβ plaques to be cleared up. Then, in the second stage, due to accumulation of stimuli and impaired adaptive immune responses including phagocytosis, low-level continuous inflammation is present with AD's progression, known as chronic inflammation. The long-lasting inflammation will cause CNS invasion by peripheral monocytes and full microglial activation, releasing excessive inflammatory cytokines and neurotoxins [20], eventually eliminating neurons (figure 3). Thus, brain inflammation appears to be a double-edged sword, playing a neuroprotective role during the acute-phase response and is destructive at the stage of chronic inflammation.

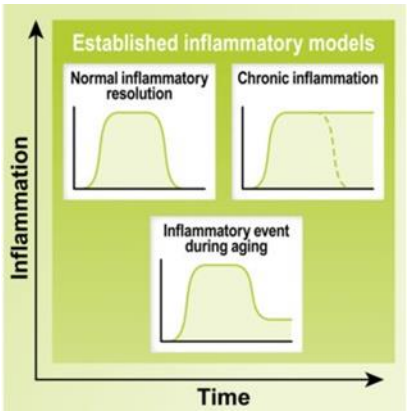

Figure. 3 Timeline of normal inflammation, chronic inflammation and inflammation in AD [20].

9. Treatments of Alzheimer's

AD is still considered as uncurable and irreversible. Until June 7, 2021, there is only one drug, aducanumab. As Aβ plaques are visibly removed under treatment of aducanumab, there is no visible alleviation of symptoms of the condition. There has been no significant progress in medications targeting Aβ, the hallmark of AD. While phase 3 trials of monoclonal antibodies have obtained unfavourable results, BACE-1 inhibitors, such as verubecestat, failed to diminish cognitive decline of tested patients statistically as well. idalopirdine, intepirdine, and latrepirdine, and dihydropyridine (DHP) calcium channel blockers, such as Nilvadipine,did not express clinical efficacy in experiments. These failures are produced by a variety of reasons, influenced by the complex nature of Alzheimer's and incorrect pharmacokinetics or pharmacodynamics as well.

Driven by observations of microglia, another theory of treatment, immunotherapy, has emerged in recent years. In animal models, it has been reported to prevent the formation of and assist in clearing existing Aβ deposits while removing neuronal debris. In human trials, initial Aβ immunization clinical trials had mixed results, and large phase III clinical trials are already proceeding for antibodies targeting mid-region of Aβ and the terminus, with earlier results suggesting efficacies.

Treating Alzheimer's from the aspect of inflammation, however, has been discussed for decades already. NSAIDs have been concluded to decrease relative risks of AD, providing various beneficial effects on attenuating dementia. Nevertheless, in years of later stages of clinical placebo-controlled trials, the results for different NSAIDs are almost completely statistically negative. Moreover, effects in some experiments by NSAIDs are becoming non-negligible. In fact, primary prevention research with naproxen and celecoxib was halted as a result of the medications' severe negative effects. This is probably due to the fact that different types of inflammation occur in different stages of Alzheimer's. and although it is undeniable that NSAIDs will alleviate inflammation concurrent with Alzheimer's years before the usual onset age according to some experiments, it may even raise the likelihood of the disease later.

Even so, the work on developing drugs targeting neuroinflammation to treat AD ensues, and some of them are already undergoing clinical trials. XPro1595 is an artificial variant of TNF-α which blocks the natural TNF-α pathway in inflammatory responses. Different from other TNF-α inhibitors, XPro1595 does not subdue immune responses nor affect neuronal connections, and did not show neurogenesis, learning, and memory deterioration like other NSAIDs in mice models [21]. Another potential NSAID that is still in earlier stage testing is the GC 021109, a ligand that binds to the G-protein-coupled receptor (GPCR) P2Y6. By substituting the natural ligand of P2Y6 receptor, adenosine diphosphate, phenotype shifting of microglia is reduced, and microglial responses, namely phagocytosis and release of proinflammatory cytokines [22]. NP001, a form of purified sodium chlorite, can function as a downregulator of NF-κB to decrease the IL-1β, and lower the level of inflammation. However, little trial has been done on this NSAID, though current studies showed that

it remains to be "safe and well-tolerated". In general, the development of drugs targeting inflammation has acquired preliminary positive results and has great potential to be tested in the future.

10. Conclusion

AD has very complicated formation and progression pathways, which are not fully understood until today. Inflammation, including neuroinflammation and peripheral inflammation, has played a key role in this process. The two distinctive hypothesized pathways based on two hallmarks of AD, the amyloid cascade and the p-tau cascade are linked closely with inflammation, which controls the advancement of both to an extent. It has the ability to degrade amyloid protein at earlier phases. or can promote the formation of Aβ plaques and NFTs. Surprisingly, little study had been done on treatment of Alzheimer's from this perspective compared to the two biological hallmarks of AD mentioned above. This can be attributed to the unsuccessful development of NSAID treatment in earlier decades by a variety of reasons, most importantly its dangerous side effects. In recent years, as more researches and reports are made to elucidate further closeness of inflammation as a core mechanism in AD, interest in anti-inflammatory drugs are reignited. Currently, there are multiple new NSAIDs under development, and although only rudimentary results are achieved, they exhibited fewer side effects with more potency. Whether this is a future final solution to AD to save millions of people is still unclear, but a bright future is ahead.

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