In search of a treatment for Alzheimer's disease and potential immunonosuppresive therapeutic interventions

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REVIEW

Abstract

Alzheimer's disease (AD) is a serious neurodegenerative disease of aging. Recent projections of the dramatic increase in AD incidence worldwide by 2050 reveal its magnitude as a world-wide health crisis and underscore the urgent need to understand the etiology of AD in order to develop therapeutic interventions. A popular debate among scientists has traditionally pitted those in support of Beta amyloid protein as a causative factor ("Baptists") against others who implicate tau hyperphosphorylation ("Tauists"). Considering the significance of Beta amyloid protein and hyperphosphorlyated tau protein aggregates in AD pathology, this article delves into the nature of inflammation associated with these aggregates. Aspects of inflammation focus on microglia, resident immune cells of the CNS that are activated during AD inflammation and are known to play a significant role in pathogenesis. This article discusses the role of microglia, inflammation, and the immune response as a middle ground in the debate between the "Tauists" and the "Baptists" respective positions. It explores recent advances in immunotherapy and supports continued research in and use of immunosuppressive regimens as potential therapeutic interventions for AD.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is part of a broad set of dementias. AD is characterized by loss of memory and impaired cognitive function and is grouped as either Familial Alzheimer's Diseases (FAD), which shows an inheritance pattern, or Sporadic Alzheimer's Disease (SAD) that does not exhibit any such pattern. It has been noted as the most commonly occurring dementia among the elderly. Recent reports indicate that the rate of AD worldwide will quadruple by 2050. AD is marked by significant degeneration in the limbic and cortical structures of the brain. While the exact etiology of AD is not properly understood, several popular theories abound regarding its main causative factors. Although distinct characteristics in AD have been identified, the entire pathology of the disease is not completely delineated.

The onset of AD results in loss of synaptic connections, marked inflammation, depletion of neurotransmitter networks, and significant cell loss. On a pathological level, AD is also particularly marked by extraneuronal amyloid plaques and neurofibrillary tangles, which consist of hyperphosphorylated tau protein. Amyloid plaques and tau protein are believed to play a substantial role in the degeneration that characterizes AD patients. Yet, the mechanism by which protein aggregates act as causative agents of neurodegeneration has not been completely elucidated.

A popular debate has abounded for some time among those researching the causative agent of AD. One camp holds the plaques as responsible for direct neuronal deterioration. These proponents ("Baptists") believe that beta-amyloid protein forms plaques around brain neurons and can potentially cause tau tangles that can destroy neurons. An opposing hypothesis holds that tau protein cause neuronal death, and proponents of this idea are known as "Tauists".

Several studies have attempted to predict the onset of AD or develop an outline of pattern for its progression. For instance, some studies in human subjects have shown that depending on the genetic risk of AD, patterns of brain activation can predict a progressive decline in memory [1]. While such attempts have important ramifications for prevention, the most promising investigations remain those geared towards developing a curative and therapeutic intervention. In this regard, research has mainly focused on the underlying pathophysiological characteristics of AD (i.e. amyloid protein, tau, cerebral apolipoprotein E metabolism) or neurodegenerative processes (i.e. inflammation, oxidation, apoptosis), leading to neuroprotective approaches in clinical trials of estrogen, antioxidants, and anti-inflammatory agents in AD patients. In addition, mechanisms that target amyloid protein have been developed to delay disease progression or to prevent AD [2].

"TAUIST" (TAU PROTEIN) HYPOTHESIS

Neurofibrillary tangles, one of the hallmarks of AD pathology, are mainly composed of hyperphosphorylated tau protein aggregates that assemble themselves into intricate tangles through a complex mechanism. The tau protein is essential in providing structural support for microtubules and its abnormal phosphorylation results in the aggregation that is found in AD. The Tau hypothesis claims that the tau protein of the neurofibrillary tangles is the major source of degeneration.

The Tau hypothesis holds great merit. Autopsies of patient with early AD reveal the presence of tangles prior to plaques, lending credence to this hypothesis. Furthermore, amyloid protein is found in almost all old-aged brains, including those that show no signs of AD. Yet, the role of tau phophorylation is not completely understood. The general notion of tau phosphorylation as being central to AD pathogenesis has been challenged by a group of researchers who proposed a potential compensatory and neuroprotective mechanism against oxidative stress for tau phosphorylation [3]. It is clear that while neurofibrillary tangles are associated with the progression of AD, we are not sure whether these are a result of the disease or whether they actually cause it.

Neurofibrillary tangles composed of aggregated phosphorylated tau protein are observed in other diseases besides AD including Down's syndrome, corticobasal degeneration, Pick's disease, and progressive supranuclear palsy [3]. Such aggregations have been labeled "tauopathies" and while aggregated tau is phosphorylated at serine, threonine, and tyrosine residues, it has been determined that tyrosine phosphorylation is a particularly critical element in "tauopathies" [4].

While tau aggregation is commonly associated with abnormal tau phosphorylation, the exact role of phosphorylation as the initiating factor in neurodegeneration is poorly defined. Yet in recent years, data from animal models indicate that abnormal tau phosphorylation can cause neurodegeneration similar to that found in human patients [5]. The significance of tau protein is underscored by recent evidence suggesting that excessive Abeta initiates a cascade leading to loss of neurons partly through the post-translational modification of tau [6]. Roberson *et al.* [7] recently demonstrated that a reduction in endogenous tau protein prevented behavioral deficiencies in transgenic mice expressing APP and excitotoxic effects in transgenic and nontransgenic mice, suggesting tau reduction as a potential therapeutic strategy for the treatment of AD. Determining whether hyperphosphorylated tau is the cause or a reaction of AD pathogenesis is significant in determining the course of action for treatment. The role of these tangles and their association with inflammation in the course of AD is a significant area that has promising curative potential.

"BAPTIST" (AMYLOID PLAQUE) HYPOTHESIS

Amyloid plaques that result from AD primarily consist of insoluble Beta amyloid peptide fragments and are generally found in the association areas of the cerebral cortex, in contrast to neurofibrillary tangles that appear in the entorhinal cortex. Beta amyloid peptides are produced through the proteolysis of amyloid-precursor protein (APP). For a while now, Beta amyloid has been a primary focus of study in determining AD pathogenesis. While traditionally it has been known that Beta amyloid accumulates in the extracellular region, evidence in recent years has suggested that it can also deposit intracellularly as well [8]. Interestingly, features of AD always include Beta amyloid, yet neurofibrillary tangles are not always associated with AD. The

amyloid hypothesis asserts that Beta amyloid is the central mechanism of neurodegeneration in AD patients.

The amyloid hypothesis has been supported by a study of the APP, PS1, and PS2 genes that are responsible for Familial Alzheimer's Disease (FAD). This study revealed modulation of Beta amyloid metabolism, leading to Beta amyloid aggregation [9]. This finding is further corroborated by the amyloid-cascade hypothesis proposed by Hardy and Selkoe, who asserted that Beta amyloid acts as a trigger for all existing cases of AD [10]. This hypothesis also stipulates that the tau pathologies are simply consequences of Beta amyloid pathogenesis [10].

According to proponents of the Beta amyloid hypothesis, amyloid plaque formation is followed by inflammation and neurofibrillary tangles, both of which lead to neuronal death. Thus, this hypothesis brings to light the importance of inflammation in AD pathology. The wide range of mechanisms whereby Beta amyloid activates other inflammatory agents warrant attention in developing a treatment for AD. A growing body of evidence in recent years suggests that amyloid-beta peptides (Abeta) are indeed a causative factor of AD.

INFLAMMATION: LINK BETWEEN TWO HYPOTHESES

While both hypotheses wield respective merit, it is important to consider any common elements that may be indicative of key processes in AD pathology. Both hypothetical paradigms contribute to our current knowledge of the characteristics of AD. A potential middle ground in the two major camps on the debate regarding the etiology of AD may be the focus on the inflammation process, a feature that characterizes both amyloid plaques and tau protein. In recent years, research has pointed to the notion that primary inflammation may be directly responsible for AD pathology [11].

The two major groups of cells that contribute to inflammation are astrocytes and microglia. Microglia are resident immune cells in the CNS that are concentrated around amyloid plaques in AD patients. Cagnin *et al.* [12] provided *in vivo* evidence supporting the role of microglial activation in the progression of AD. While the role of microglia in AD pathology has also been supported by the presence of antimicroglial antibodies in CSF of AD brains, the nature of this role is poorly understood [13]. Microglia and astrocytes produce several neurotoxic molecules including hydrogen peroxide, prostanoids, and glutamate [11].

Microglia serve as a link between the two hallmarks of AD pathology since the activation of microglia triggers formation of Beta amyloid plaques and the subsequent release of cytokines from microglia induces signaling pathways for tau hyperphosphorylation. The formation of extraneuronal Amyloid plaques triggers the activation of microglia, which is then followed by the release of cytokines. AD is characterized by the alterations in the local environment surrounding microglia, inducing their activation and subsequent release of soluble factors, and modulating astrocytic glutamate uptake [14]. In the normal brain, microglia do not produce any toxic substances and are ramified in their resting state [15]. Yet in the AD brain, when activated by Beta amyloid, microglia take on an ameboid shape and increase in size while producing the inflammatory cytokines InterLeukin-1B (IL-1B) and Tumor Necrosis Factor alpha (TNF-alpha). These cytokines then stimulate signaling pathways for the formation of Neurofibrillary tangles in the neurons as well as for tau hyperphosphorylation. There is no apparent vasodilation or extravasation of neutrophils that occurs during this cytokine cascade [11].

Thus, a form of positive feedback occurs as a result of Beta amyloid. It is believed that this positive feedback mechanism of inflammatory responses may be the main cause of the degeneration of AD. Griffin *et al.* [16] have elucidated the possible role of a potential "cytokine cycle" by which the degeneration in AD brains occurs. Microglia that have been activated by Beta amyloid also produce and release reactive oxygen and nitrogen species in order to eliminate foreign substances [15]. Thus, oxidative stress due to microglial activity has also been suggested as a stimulator of the neurodegenerative pathology of AD.

Extensive evidence implicates amyloid deposition as a major trigger of microglial-mediated inflammatory response that results in cell loss and cognitive decline in the AD brain [17]. Yet, while reactive microglia are associated with Beta amyloid plaques in AD brains, whether they cause cell loss is still speculative [18]. Several studies have shown that microglia-induced phagocytosis of Beta amyloid helps in clearing amyloid plaques [11]. Majumdar *et al.* [19] recently showed that activated microglia acidify lysosomes and potentiate the degradation of amyloid fibirils in AD brains. Researchers have suggested that since proinflammatory cytokine stimulation of microglia may suppress the activation of phagocytosis, anti-inflammatory interventions can remove Beta amyloid in the AD brain [20].

Recently, therapeutic options have been suggested that inhibit the activation of microglia, thereby decreasing neuronal injury [21]. An interesting aspect of microglia is their reported beneficial effect in the brain. A potential protective role of the chemokine receptor Ccr-2 dependent microglia was suggested by a recent study in which Ccr2 deficiency in mice disrupted the accumulation of microglia and promoted AD progression [22]. Experiments in recent years suggest that activated microglia are capable of removing Beta amyloid plaques and that this clearance serves a neuroprotective role. This stems from the fact that in both *in vivo* and *in vitro* settings, microglia are able to partially degrade and phagocytose Beta amyloid deposits. Yet, during these scenarios Beta amyloid plaques still persist and

are not completely eliminated. This may suggest that microglia are rendered ineffective at some point during the course of AD neurodegeneration [11].

Thus, ambivalence exists for microglial activity and significance, and this has been reflected in the differing results of recent studies; some studies suggest that the activation of microglia by Beta amyloid triggers events of AD pathology, yet others have pointed to the beneficial effects of microglia. While some researchers have viewed inflammation as a process secondary to the degeneration in AD, recent evidence has shown that the mediatory molecules of inflammation may stimulate the processing of amyloid precursor protein (APP) [23] and inflammation can impair the scavenger function of microglia as initial steps in the neurodegeneration in AD brains [24]. Such intricacies allow for therapeutic interventions that can deal with either role of microglia in inflammation. The therapeutic potential of exogenous microglia was suggested by Takata *et al*. [25] after transplantation of rat microglia demonstrated migration to and subsequent clearance of Beta amyloid. The complex nature of neuroinflammation is further underscored by an unanticipated finding of a recent study by Shaftel *et al*. [26] that reported a transgenic AD mouse model that showed a reduction in amyloid as a result of the proinflammatory molecule IL-1beta overexpression when triggered in the hippocampus. This result suggests a possible beneficial role for proinflammatory molecules such as IL-1 beta in AD [27]. Yet, further studies are needed to fully elucidate the potentially protective role of such agents.

The exact mechanism of tau hyperphosphorylation has yet to be determined, but microglia may possibly play a role in tau pathology in AD as well. Studies have reported various kinases (cdk5, GSK-3B, p38-MAPK) as potential causes of tau hyperphosphorylation. It is believed that cytokines released by microglia help initiate this kinase cascade. Other reports point to the inhibition of phosphatase activity as causing this phenomenon.

The neurodegenerative consequences of chronic activation and overexpression of microglia and astrocytes have been clearly established by investigators [28]. Yet, the role of inflammation as part of AD pathology is not completely understood. Craft *et al*. [29] demonstrated that Beta amyloid induced inflammation is an early aspect of neuroinflammation, thus providing support for a causative link between neuroinflammation and neurodegeneration through administering a glial activation inhibitor that suppressed the neuroinflammation in mice. Yet, it is not entirely clear whether inflammation is a primary feature of the etiology of AD or whether it is secondary to AD pathology. The fact that microglia and astrocytes are believed to exhibit both neuroprotection and neurodegeneration activity further complicates the picture.

HOW THE DEBATE INFLUENCES DEVELOPMENT OF POTENTIAL TREATMENTS: TARGETING THE IMMUNE SYSTEM

Treatments for AD have traditionally focused on alleviating symptoms presented in patients. Yet, they fell short of targeting the mechanisms that may cause AD. Having established some main causative features of AD pathology and a link through inflammation, it is important to consider the related paradigms underlying potential treatments for AD.

One approach to reversing AD pathology is to target inflammation. This method relies on the notion that inflammation causes Beta amyloid plaques and tau hyperphosphorylated aggregates. It has been established that the accumulation of hyperphosphorylated tau protein aggregates can cause neuronal loss. It may then be possible to inhibit the inflammatory processes in AD to prevent this neuronal loss. The adverse consequences of microglial activity have been mentioned. Inhibiting the activity of microglia and the reactive oxygen species (ROS) that they release may be a major therapeutic strategy for slowing progression of AD. The combined use of immunosuppressive therapies that target lymphocyte immunoglobulin synthesis and immunomodulatory therapies that inhibit antigen presenting cells has been suggested to be particularly effective in the downregulation of activated microglia or macrophages [30].

Another viable method is to remedy the effects of the inflammation. Several treatments currently exist that counter the inflammatory processes in AD brains. These interventions focus on the role of inflammation, which is generally followed by microglia and astrocyte migration, and the mechanism of action of these agents work accordingly in the course of AD.

ANTIOXIDANT TREATMENTS

An early hallmark of AD is oxidative stress resulting from the presence of free radicals. This oxidative stress and subsequent damage is known to lead to cognitive deficits. Several sources of free radicals have been determined, including Beta amyloid regulated processes, accumulation of transition metals, dysfunction of mitochondria, and apolipoprotien E. Much of the free radicals present in AD brains stem from mitochondrial abnormalities. Since hyperphosphorylated tau and Beta amyloid deposition are two main features of AD that result from this oxidative stress, one potential therapeutic approach is to modify this oxidative stress. Moreira *et al*. [31] proposed that the oxidative modifications that occur in early stages of AD might act as a homeostatic response for compensatory mechanism against stressful agents rendering neurons to change priorities from maintaining normal function to ensuring their survival.

Microglia that are activated by Beta amyloid also release oxidative agents such as ROS and RNS. Thus it has been determined that one way to attenuate the progression of AD might be to utilize antioxidants. The wide range of antioxidants currently in use to counter AD include aromatic amine/imines, tetrahydracannabinol, and estradiol-17B. Several studies have even reported the role of polyphenolic compounds, antioxidants derived from fruits and vegetables, in blocking neuronal death in AD [32].

Vitamin E is known to have several protective and beneficial effects for the body including prevention against cancer, protection against heart abnormalities, slowing of age, and improvement in circulation. Vitamin E also plays a major role in suppressing the synthesis of prostaglandin E2. Several studies have stated a significant role of Vitamin E in conferring neuroprotection in the AD brain [33]. Other *in vivo* studies in transgenic mice have been also promising. In one study, researchers found that a Vitamin E-supplemented diet decreased Beta amyloid levels in transgenic mice, suggesting that Vitamin E may have a strong role in countering AD pathology [34]. Epidemiologic studies also suggest a positive role for antioxidants in attenuating AD neurodegeneration. One study conducted involving over 5,000 subjects described a correlation between dietary consumption of Vitamin E or Vitamin C and a decreased risk of developing AD [35].

Antioxidants serve as a viable treatment due to their broad-spectrum approach in down-regulating oxidative stress. Furthermore, the absence of side effects and general affordability of antioxidants have been noted as favorable aspects of such treatments. Studies that have highlighted the therapeutic potential of oxidant mechanisms in AD have stressed the limitations of immunotherapy as a method of eliminating the production of Beta amyloid [31]. Yet, any novel therapy must be based on targeting the exact source of free radicals, which has proven to be challenging.

ANTI-INFLAMMATORY TREATMENTS

Inflammation in the CNS is considered to act as a primary agent of neurodegeneration in AD. With respect to microglial involvement in inflammatory processes, several studies have pointed to a strong association with AD. These studies are also supported by *in vivo* studies in transgenic animals. Yet several aspects of inflammation still need to be properly elucidated. A major question is whether inflammation is a result of AD pathology or if it is a reactive process and secondary to AD progression. Further complicating the picture of inflammation and AD pathogenesis is the fact that microglia and astrocytes may have both neuroprotective and neurodegenerative roles. Baron *et al.* [36] have pointed to evidence that autoimmune mechanisms can clear Beta amyloid and participate in repair pathways in order to suggest boosting the immune system as opposed to suppressing the immune mechanisms as a therapeutic intervention. Yet, despite these shortcomings and unanswered questions, enough evidence has been put forth to suggest a positive role for anti-inflammatory agents in countering AD. Various anti-inflammatory drugs have demonstrated the ability to repress microglial activation and thereby exert a neuroprotective role in the CNS following injuries [21]. In particular, results of clinical studies provide support for the efficacy of three major anti-inflammatory agents: NSAIDs, glucocorticoid steroids, and cannabinoids.

NSAIDS

Non-steroid anti-inflammatory drugs (NSAIDs) are a group of agents that include acetic acid, salicylate, and COX-2 inhibitor classes of drugs. They exhibit analgesic and anti-inflammatory effects and work by inhibiting the cyclooxygenase (COX) enzyme that serves to catalyze the conversion of arachidonic acid into various eicosanoids. The significance of targeting the COX-2 enzyme is underscored by a recent study conducted in C57B16 mice. Cakala *et al*. [37] demonstrated that the COX-2 inhibitor NS-398 protects against memory disturbances induced by Abeta.

Several studies in recent years have indicated that NSAIDs may decrease the likelihood of AD onset [38].

Other researchers have demonstrated that NSAIDs may lower the likelihood of AD onset and slow cognitive decline [39]. Most of the studies corroborating this finding are epidemiologic and focus on arthritis patients who also suffer from AD, since arthritis is also treated with NSAIDs. These studies have shown an inverse relationship between treatment with NSAIDs while experiencing arthritis and AD [38]. Several epidemiological studies have also demonstrated a significant reduction of the risk of developing AD in long term users of NSAIDs, while studies in AD transgenic mice indicate a reduction in pathology depending of the dosage [40].

One method in which NSAIDs may be particularly effective is in countering the inflammatory nature of AD pathology due to their indicated role of regulating microglia. In one study, brain tissue derived from a normal individual with a history of NSAID use was contrasted with a group that had no history of NSAID use and no differences in Neurofibrillary tangles were shown [41]. However, four times as many activated microglia were found in the control group compared to the normal brain tissue [41]. This demonstrates the potential of NSAIDs in regulating the number of activated microglia, and thus has potential in countering AD pathology.

NSAIDs have also been shown to be effective in altering the production of Beta amyloid through various mechanisms. In one study, drugs such as ibuprofen and

indomethacin decreased the Beta amyloid 42 peptide by 80% in cells that were cultured [42]. Importantly, only select NSAIDs were shown to exhibit the effect. The researchers reported an increase in the release of the Beta amyloid 38 isoform, indicating an increase in production of this isoform, reducing the levels of Beta amyloid 42. It was also shown that NSAIDs regulate the ability of *y*-secretase to suppress Beta amyloid 42 production. In a separate study, it was demonstrated that NSAIDs are able to decrease Beta amyloid levels by inhibiting the action of the Rho protein, a member of the GTP class that regulate cell activity [43]. Again, only certain NSAIDs such as ibuprofen and indomethacin were shown to block Rho activity and thereby lower Beta amyloid levels. Thus, instead of acting through the COX pathway, there may be an alternate mechanism of action through which NSAIDs exert their effects in AD brains. Mohri *et al*. [44] recently reported that Prostaglandin (PG) D-2 that is produced in microglia acts as a mediator of inflammation in AD brains, shedding light on the mechanisms of action of NSAIDs in countering the effects of AD.

While NSAIDs are proven to be effective in countering AD through the stated mechanisms, their known side effects hinder any practical widespread use. NSAIDs are known to exhibit renal, cardiovascular, and CNS toxicity that are particularly detrimental when considering that AD is found in an elderly population that has existing problems in these areas. Due to these toxic side effects, they may not be the best immunosuppressive option for therapy. Current efforts are underway in the development and use of less toxic NSAIDs such as (R)-flurbiprofen, the less toxic enantiomer of fluribiprofen developed by Myriad Genetics, Inc., and while phase II trials show promise in lowering Abeta levels without significant neuro deficits, results of current phase III trials have yet to be reported [45].

GLUCOCORTIOCOID STEROIDS

Steroids are widely known to exhibit powerful anti-inflammatory effects. Several studies have demonstrated varied results in terms of glucocorticoid steroid use and potential for inhibiting inflammation. One study has shown that glucocorticoids can inhibit the induction of cytokines and chemokines in AD brains [46]. A trial geared towards determining whether treatment of prednisone slowed cognitive decline in AD patients revealed no marked difference in the rate of cognitive decline in the treated group when compared to the control group [47]. The fact that one study showed glucocorticoid levels were markedly increased in the CSF and blood serum of patients with AD compared to a control group with no dementia suggests that there may be a correlation between AD and elevated levels of steroids [48]. This finding underscores the complex nature of inflammation in AD pathology.

Glucocorticoid steroids have a potentially powerful effect. Yet, without any solid proof of their efficacy, such prescriptions are not as promising as other anti-inflammatory agents. Furthermore, there are a wide range of adverse effects linked with the use of steroids. Further studies need to be conducted in order to determine whether these steroids have an effect on AD pathology. Such studies would potentially involve measuring levels of glucocorticoid levels at different stages of AD and determining how they relate to the pathology.

While glucocortiocoid steroids have promise in their anti-inflammatory role, studies have suggested the role of glucocorticoids in AD pathology. In one study, researchers used APP transgenic mouse model of AD and demonstrated that transgenic mice showed andrenocorticol hyperactivity, suggesting that the changes cause alterations in the negative feedback regulation of the hypothalamic-pituitary-adrenocortical (HPA) axis, thereby resulting in the increased secretion of glucocortiocoids [49]. The promise of glucocorticoids as anti-inflammatory therapy is thus challenged by these studies suggesting that they have a key role in AD pathology. Since glucocorticoids have been reported to have primary initiation effects in AD pathology, the glucocorticoid antagonist mifepristone has been suggested as a useful drug to counter AD [50].

CANNABINOIDS

Cannabinoids are agents currently used for medical treatments that target the CB1 and CB2 receptors. The CB1 receptors exist in astrocytes and it has been determined that the stimulation of this cannabinoid receptor induces expression in human astrocytoma cells [51]. The CB2 receptor differs in that it is expressed exclusively in cells of the immune system and has no relation to cannabinoid psychoactive effects. Cannabinoids display neuroprotective effects in part because the activation of these cannabinoid receptors protects hippocampal cerebellar neurons from excitotoxic effects [52].

Researchers, intrigued by these protective effects, have been particularly interested in studying the localization of cannabinoid receptors in the AD brain and any relation to microglial activity. One group of researchers reported that CB2 receptors were selectively over-expressed in neuritic glia in the AD brain [53]. Even more promising was a study reporting that cannabinoids are able to prevent microglial activation while decreasing the production of NO in the rat brain [54]. In a separate study, researchers studied the activation of cannabinoid receptors in an animal model of AD *in vivo* in conjunction with a model that displayed microglial activation *in vitro*. [55] These researchers demonstrated a potential neuroprotective role of cannabinoids by concluding that the administration of cannabinoids prevented loss of neuronal markers and impairment of cognitive functions in rats [55]. These studies suggest the potential

utility of using cannabinoids as a preventive measure against the pathology of AD.

Several studies have aimed to elucidate the intracellular mechanisms of cannabinoids and AD pathology. Eubanks *et al*. [56] described a molecular mechanism by which Delta9-tetrahydrocannabinol (THC) inhibits acetylcholinestrase-induced amyloid beta aggregation by binding to a key region of amyloidgenesis.

The potential of cannabinoids in countering oxidative stress and repelling the inflammatory process is compelling. Cannabinoids are considered particularly potent due to the combination of both anti-inflammatory and neuroprotective mechanisms that they exhibit in degenerative disorders [55]. Thus, cannabinoids should be viewed with some caution. While preliminary data appear promising, more work is needed to establish a complete correlation between the activity of cannabinoids and the slowing of neurodegeneration.

IMMUNOTHERAPY

As previously mentioned, the Beta amyloid hypothesis has introduced new insights into the importance and significance of Beta amyloid plaque presence in AD pathology. Accordingly, current interventions have been developed to target Beta amyloid through immunotherapy. Beta amyloid immunotherapy aims at generating a response in the body against the existing Beta amyloid through a vaccine that contains Beta amyloid fragments. The idea entails the stimulation of the immune system to clear the remaining Beta amyloid by introducing these fragments into the AD brain. Anti-amyloid immunotherapy is believed to serve as the initial test of the Beta amyloid hypothesis for treating AD [57]. *In vivo* imaging has recently confirmed the progressive clearance of Beta amyloid in vessel walls of brain arteries (cerebral amyloid angiopathy) [58]. Immunotherapy approaches have primarily focused on Beta amyloid instead of tau protein. Rosenmann *et al*. [59] demonstrated the dangers of tau as a method of immunotherapy by utilizing tau protein in subjects to test tau-related autoimmune effects in mice.

Based on the notion that antibodies directed toward the N-terminal region of Beta amyloid peptides suppress *in vitro* production of toxic Beta amyloid, researchers investigated this idea with *in vivo* experiments. Researchers localized the epitope of anti-aggregating antibodies and after injecting phage that displayed the epitope, found the induction of antibodies against the anti-Amyloid peptide, preventing Beta amyloid formation [60]. Such experiments have provided optimism for scientists in developing a potential vaccine to counter the production of Beta amyloid in AD brains. One remaining challenge, however, is the development of an immunization technique that is suitable in humans.

Immunotherapy that removes Beta amyloid has been shown to contribute to the removal of early tau pathology. Oddo *et al*. [61] showed that the degradation of an injected Beta amyloid antibody is followed by Beta amyloid pathology prior to tau pathology. The fact that the clearance of Beta amyloid occurs before the removal of early tau pathology further bolsters the merits of the amyloid cascade hypothesis [62]. Investigations in transgenic mice have shown that Beta amyloid immunotherapy decreases the levels of soluble tau protein and improves cognitive function [63].

While these immunization strategies have proven to be successful in mouse models of AD, the exact mechanism of how antibodies render their effect has not been entirely delineated. There may be several mechanisms simultaneously working and factors including characteristics of epitope, amyloid burden, and isotype may all contribute to the clearance of amyloid [64]. Recent evidence has shed light on the mechanism by which Abeta immunization decreases Abeta plaques and ameliorates cognitive function. One study reported that levels of intracellular Abeta are decreased as a result of the treatment of neurons with Abeta antibodies [65]. Researchers identified antibody light-chain fragments that display proteolytic activity and that can hydrolyze Beta amyloid *in vitro*. [66] This provides a potential form of therapy if the antibody can be engineered to target the Beta amyloid found in AD brains. Frankel *et al*. [67] suggested an alternate approach of targeting Beta amyloid with a site-directed single-chain antibody prior to its release from the cell. A proposed immunologic approach is the development of antibodies towards an important region in the modulation of Abeta, the EFRH sequence between amino acids 3-6 of the N-terminal region of Abeta, to prevent plaque formation and to dissolve existing amyloid plaques [68]. These experiments are promising and are geared in a promising direction for developing an effective treatment for AD.

Alternative approaches have also been explored for developing viable interventions. Tseng *et al*. [69] have suggested that since amyloid beta inhibits proteasome function, thereby resulting in the accumulation of Abeta and tau, proteasome activity may be a key target for interventions. Thus, immunotherapy that targets proteasome activity may also hold promise. Several new therapeutic strategies in recent years have focused on hindering amyloidosis. In addition, an FDA-approved immunoglobulin fraction derived from human blood, intravenous immunoglobulin (IVIg), has reported promise in targeting Abeta [70]. Yet, more studies are needed to further elucidate its potential.

Considerable world-wide interest was generated in 1999, when scientists at Elan Pharmaceuticals developed an effective vaccine labeled AN-1792 to attack the amyloid plaques in mice AD brains. In one of the experiments, mice were genetically altered to develop AD and were then immunized with the AN-1792 vaccine prior to any signs of plaque formation. These mice

showed no pathological signs of AD one year later. As part of a separate experiment, elder mice that displayed key features of AD neurodegeneration were treated with the vaccine for a period of seven months. It was reported that in this duration, the mice did not exhibit amyloid plaque formation.

During the following year, in 2000, researchers at Brigham and Water's Hospital were successful in developing a nasal vaccine for AD administered in genetically engineered mice. This vaccine comprised of Beta amyloid fragments and when administered intranasally, reduced the presence of plaque in mice hippocampus by 50–60%. Interestingly, these effects were not evident through oral administration of the vaccine. This study corroborated the results of the Elan study by suggesting that an immunological intervention may be effective in countering AD pathology and neurodegeneration.

Studies that seemed to suggest the effectiveness of immunization with Beta amyloid as a strategy to reduce AD pathology and restore cognitive deficits in transgenic mice were subsequently attempted in humans. While the phase I human trials had positive results, phase IIA trials that utilized an active immunization approach were thwarted in humans due to the occurrence of aseptic meningoencephalitis in 6% of human subjects [71]. Yet the progress of the study was both astounding and promising. The amyloid cascade hypothesis lends credence to several immunotherapeutic interventions that have effective results contingent upon the fact that Beta amyloid can be attacked or suppressed.

Thus, a major challenge was developing a vaccine in humans that does not exhibit the adverse effects of encephalitis [72] in addition to microhemmorrhaging. Several studies in recent years have demonstrated great promise in this regard. Due to the suspension of the active amyloid beta (A beta) vaccine trial, passive immunization was recommended as a safer method although there was a reported increased risk of microhemorrhages in transgenic mice [73]. A primary problem associated with the introduction of active Beta amyloid as part of immunization strategies in hindering amyloidosis are the side effects. In order to address this problem, Nikolic *et al.* [74] suggested a novel beta immunization approach by introducing Beta amyloid transcutaneously in mice and demonstrated lack of deleterious effects of cerebral microhemorrhage and the infiltration of T cells. Asuni *et al.* [75] utilized a novel immunization approach of using Beta amyloid derivatives in adjuvants that promote humoral immunity as opposed to the cell-mediated adjuvants used in previous trials that resulted in meningoencephalitis, and demonstrated a lack of microhemmorhaging or increase of Beta amyloid deposits. In order to employ an immunization therapeutic strategy for AD without the adverse reactions, Rakover *et al.* [76] proposed an approach to inhibit the production of Beta amyloid through anti-APP antibodies directed against the beta-secretase cleavage site in Tg2576 transgenic mice and reported an improvement of cognitive function with a reduction in inflammation and microhemmorrhage. Recently, Mouri *et al.* [77] devised and tested an oral vaccine with a recombinant viral vector carrying Abeta, demonstrating an attenuation of Abeta and alleviation of cognitive impairment without resulting in inflammation in a mouse AD brain. Researchers also recently developed a UBITh AD vaccine by utilizing a Beta amyloid immunogen designed to elicit anti-N termal Beta amyloid antibodies that minimized inflammation, which was reported to be safe in animal models [78].

Several other advances have been made in the techniques of anti-Beta amyloid immunization. While most studies have utilized the N-terminal-specific antibody to target Beta amyloid, Gray *et al.* [79] recently demonstrated that C-terminal antibodies may have potential in Beta amyloid sequestration therapeutic approaches for AD. Yet, immunotherapy is still limited in its scope for targeting the mechanistic details of AD pathology [80]. Moretto *et al.* [81] demonstrated positive results of injection of the Trsx(Abeta15)4 antigen combined with the adjuvant alum that elicited a favorable antibody response against Abeta. Lambert *et al.* [82] introduced a novel strategy using amyloid beta-derived diffusible ligands (ADDLs) as an antigen for the generation of monoclonal antibodies. Recent studies have also suggested that anti-amyloid immunotherapy has the potential to induce recovery from AD through the restoration of neurons and cognitive function in AD patients [83].

The results of the immunization trials display great promise to the research community. Immunotherapy is the most viable and proven technique for treatment since it targets Beta amyloid that is the most likely causative agent of neuronal loss and cognitive deficits in AD brains. It has been proposed that active immunization strategies have been successful in the reduction of Beta amyloid levels due to microglial phagocytosis, while passive immunization strategies have resulted in antibodies in the periphery enhancing the efflux of Beta amyloid [79]. Yet, immunotherapy is still limited in its scope of targeting the mechanistic details of AD pathology. The other limitations of immunotherapy include developing a safe and effective regimen. One problematic aspect of a potential vaccine for human administration lies in how the medical community will determine who may require the regimen. Since most AD patients do not display detectable genetic markers linked to AD, it may be challenging to select proper candidates for the vaccine. While it may take several years before an effective and safe vaccine is developed for use in humans, these studies still present great potential for a possible cure.

CONCLUSION

Alzheimer's disease is a debilitating dementia that requires serious attention on the part of the medical community. The popular debate on AD has traditionally been split between those who view tau phosphorylation as the cause of the pathology and the proponents of the Beta amyloid protein as a causative agent. Both camps on the Alzheimer's debate have contributed to our understanding of AD by introducing alternate paradigms to its pathology. The current debate on AD provides us with groundwork upon which to build therapeutic strategies and treatments. A middle ground is the immune system, since inflammation is known to be associated with Beta amyloid plaques and tau-hyperphosphorylated protein aggregates. Further exploration of the intricacies of the immune system and how they relate to AD pathology can help us combat this disease. The two differing paradigms regarding AD pathology have thus pointed in a promising direction: inflammation and its possible role in AD. As discussed, microglial activity and other inflammatory agents may serve as the bridge that links these two camps.

Considering the potential role of inflammation in AD pathology, effective measures of treating this dementia must be developed that act upon a wide range of mechanisms of the immune system. While current therapies aim at slowing the cognitive decline in AD brains and in treating the behavioral effects of disease progression, immunosuppressive treatments target the underlying etiology of AD. A potential therapeutic intervention for AD patients is to reverse inflammation. While it remains unclear as to whether inflammation is a cause of or is secondary to pathology in AD brains, the association between this inflammation and the Beta amyloid plaques and neurofibrillary tangles is compelling. Therefore, targeting the inflammatory process serves as a method with great potential for slowing the neurodegenerative effects of AD.

Currently, antioxidants, anti-inflammatory treatments, and immunotherapy are three major treatments of equal importance. Antioxidants are known to have some effective results. Anti-inflammatory treatments are proven to be effective, yet still display several side effects. Yet, anti-oxidants are more promising than anti-inflammatory treatments, which are known to have side effects. Since activated microglia release factors contributing to oxidative stress, countering the oxidative agents holds promise in curbing neurodegeneration.

More promising and comprehensive studies support the therapeutic role of these agents over anti-inflammatory agents. The problem with antioxidants, however, is that they target only one manifestation of AD pathogenesis. A truly viable alternative must be one that is able to handle all the various facets of the pathology. Thus, an even more effective strategy may be to target the activity of Beta amyloid first, since this could potentially reverse not only the oxidative stress but also all of the effects of the plaques. It is clear that attacking Beta amyloid activity remains the best option for countering the effects of AD.

Immunotherapy remains the most promising and effective therapeutic intervention for AD. Results from the studies of immunization in transgenic mouse models of AD are compelling. Immunotherapy through vaccines designed to target Beta amyloid using both active and passive immunization strategies have proven to be successful. The effects of immune therapy lend credence to the amyloid cascade hypothesis since plaque obliteration in animals contributes to improvement of memory and cognitive deficits. Further clinical trials and vaccination can potentially generate an effective response against Beta amyloid plaques. Considering the negative effects associated with active immunization, passive immune strategies will take on greater importance given their potential lack of adverse effects. Alternative future strategies for combating AD progression include targeting other key agents of inflammation. For instance, a key facet of potential therapy in the future may also include targeting microglial activity through a potential vaccine.

This article calls for an emphasis on the immune system and inflammation in developing a viable treatment for AD. While several therapeutic interventions have been suggested, a greater understanding of microglia and the workings of inflammation in the CNS may shed light on other aspects of AD that have not yet been explored. Further advances in therapeutic interventions designed to disrupt the mechanisms of inflammation (i.e. immunotherapy) still hold the most promise for slowing disease progression or reversing the adverse effects of AD.

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