Inhibition of Inflammation Mediated Through the Tumor Necrosis Factor α Biochemical Pathway Can Lead to Favorable Outcomes in Alzheimer Disease

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ABSTRACT: Tumor necrosis factor α (TNF-α) inhibitors have long been used as disease-modifying agents in immune disorders. Recently, research has shown a role of chronic neuroinflammation in the pathophysiology of neurodegenerative diseases such as Alzheimer disease, and interest has been generated in the use of anti-TNF agents and TNF-modulating agents for prevention and treatment. This article extensively reviewed literature on animal studies testing these agents. The results showed a role for direct and indirect TNF-α inhibition through agents such as thalidomide, 3,6-dithiothalidomide, etanercept, infliximab, exendin-4, sodium hydrosulfide, minocycline, imipramine, and atorvastatin. Studies were performed on mice, rats, and monkeys, with induction of neurodegenerative physiology either through the use of chemical agents or through the use of transgenic animals. Most of these agents showed an improvement in cognitive function as tested with the Morris water maze, and immunohistochemical and histopathological staining studies consistently showed better outcomes with these agents. Brains of treated animals showed significant reduction in pro-inflammatory TNF-α and reduced the burden of neurofibrillary tangles, amyloid precursor protein, and β-amyloid plaques. Also, recruitment of microglial cells in the central nervous system was significantly reduced through these drugs. These studies provide a clearer mechanistic understanding of the role of TNF-α modulation in Alzheimer disease. All studies in this review explored the use of these drugs as prophylactic agents to prevent Alzheimer disease through immune modulation of the TNF inflammatory pathway, and their success highlights the need for further research of these drugs as therapeutic agents.

KEYWORDS: Alzheimer disease, inflammation, TNF inhibition

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Introduction

Alzheimer disease (AD) is a neurodegenerative disorder characterized by progressive and irreversible memory impairment that starts with biochemical changes in the brain and ends with the destruction of neurons critical to the memory system.[1] Since its discovery more than a century ago, our understanding of the disease process has come a long way. Once thought to be synonymous with aging, it is now known to have a distinct underlying pathology that is independent of simply aging.

Alzheimer disease is a multifactorial disease with a complex interplay of genetics and environmental factors which helps explain its variable clinical presentation.[2] Traditionally, AD has been classified into hereditary and sporadic forms. The hereditary component is linked to a number of genes such as apolipoprotein E (*APOE*) and α-secretase.[3] Moreover, the hereditary form typically presents with an earlier age of onset, whereas the sporadic form has a later age of onset and a stronger association with factors such as neuroinflammation, vascular compromise, and free radical damage.[4] Regardless of the cause, we now know that these factors lead to a common end product, which is the abnormal accumulation of Aβ peptide that leads to neuronal dysregulation.[4]

The β-amyloid hypothesis states that the improperly cleaved β-amyloid precursor protein (βAPP) forms insoluble Aβ peptide aggregates in the brain, disrupting calcium homeostasis in neuronal cholinergic synapses, inducing apoptosis.[5] This theory explains the observed efficacy of both memantine and acetylcholinesterase inhibitors in the treatment of AD.[6,7] Memantine is a glutamate receptor blocker which prevents the intracellular accumulation of calcium in the neuron, delaying cytotoxicity, whereas acetylcholinesterase inhibitors increase the level of acetylcholine in the synapse, improving cognition.[8]

The efficacy of both these medications (current gold standard) is moderate because they target the pathology after it has occurred and at best offer symptomatic and temporary relief from cognitive impairment without affecting the formation of Aβ peptide aggregates. Current research is geared toward increasing efficacy of treatment by a disease-modifying approach that would target more upstream processes to slow down the formation of βAPP and insoluble aggregates that lead to neurodegeneration.[9–16] Current therapies targeting the formation of Aβ are failing, pushing the community to rethink targeted therapies for AD such as therapy that could potentially decrease neuroinflammation.

Pushed by this need for a new target, a theoretical framework that has recently gained attention is the critical role of neuroinflammation in the formation of βAPP.[9,10,12,17–20] According to this view, inflammatory processes are initiated in the central nervous system (CNS) by microglial cells through the release of cytokines in response to the βAPP resulting in a

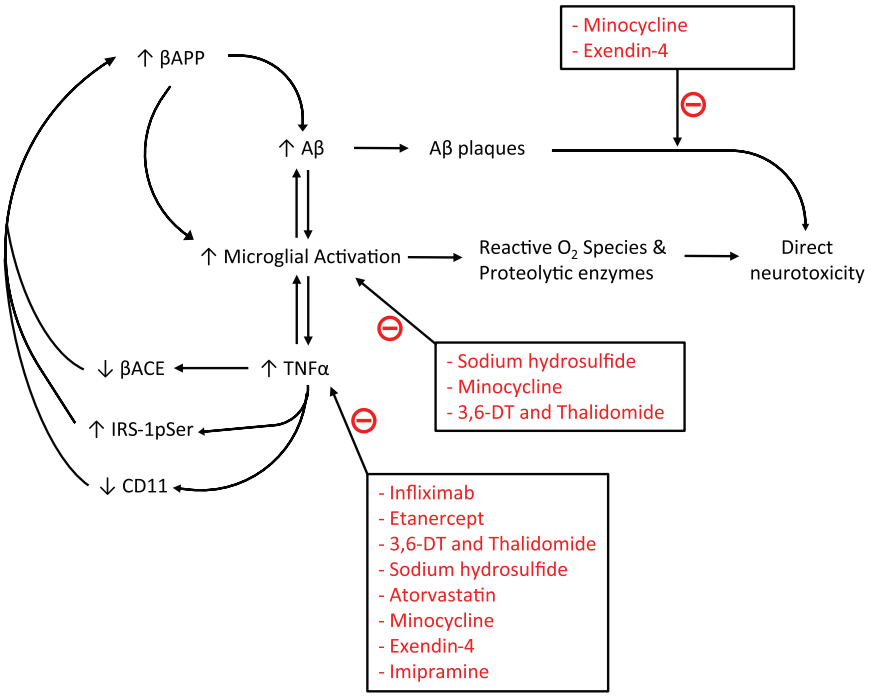

Figure 1. Neuroinflammatory pathways in Alzheimer disease pathogenesis and potential sites of therapeutic intervention.

chronic state of inflammation that worsens neural plaque load and accelerates disease progression.[21] A vicious cycle is thus installed between Aβ and inflammation.

The effect of inflammation in the CNS is 2-fold. First, activated microglial cells kill adjacent neurons by the release of toxic products such as reactive oxygen species and proteolytic enzymes.[22] Second, these inflammatory mediators enhance βAPP production and speed up the processing of βAPP into the insoluble Aβ peptide.[23] This insoluble peptide binds to microglial cell surface receptors and stimulates nuclear factor κB (NF-κB), further enhancing the production of cytokines,[24] leading to a downward spiral of chronic inflammation.

In addition to microglial cells, another cell type implicated in the pathogenesis of AD is the astrocyte. When astrocytes are stimulated by pro-inflammatory cytokines such as IL-1 and IL-6, they become activated (reactive astrocytes) and promote inflammation through the secretion of cytokines such as tumor necrosis factor α (TNF-α) and IL-6.[25,26]

Interestingly, microglial cells may play a protective role in the early stages of the disease process. Microglia have been shown to increase the phagocytic clearance of βAPP when they are moderately activated through scavenger receptor binding.[27] It is only when microglia are strongly activated that their phagocytic role is reversed and they start to increase their production of pro-inflammatory cytokines. In addition to having a direct cytotoxic effect on the adjacent neurons, these cytokines result in a decrease in scavenger receptors and Aβ-degrading enzymes in the microglia, canceling their neuroprotective role as the disease progresses.

This decrease in degrading enzyme activity and receptor expression on the microglia cell surface membrane is thought to coincide with an increase in both IL-1 and TNF-α.[28] The exact role of IL-1 is unclear and an extensive review of literature performed by Shaftel et al[29] reported both detrimental and beneficial effects of this cytokine in AD pathology. Similarly, IL-6 may also have a dual role in AD pathology, being both helpful and harmful depending on the disease stage.[30]

Of the cytokines involved in the pathogenesis of AD, TNF-α plays a central role in directing the inflammatory state in the brain and is the only cytokine shown to be consistently implicated as detrimental in AD.[31] The levels of TNF-α in the healthy brain are low and its role is unclear under physiological conditions. In chronic inflammation, levels of TNF-α are upregulated.[32] This makes TNF-α a valuable disease-modifying target for the treatment of AD, especially if used early in the disease process. It will be the focus of this review to describe the beneficial effect of TNF-α inhibitors on cognitive function correlated with the underlying biochemical pathology (Figure 1).

Methods

An extensive literature search was performed to identify relevant studies for the purpose of this review, using PubMed as the primary search tool. Search was performed until August 2016, and search terms included various combinations of the following: Alzheimer's, Alzheimer's disease, neurocognitive defects, dementia, neuro-inflammation, TNFα, TNF alpha, immunomodulation, and inhibitors. The search methodology was purposefully broad to ensure that the maximum number of studies was identified.

Prospective experimental studies and trials were included in this review. Studies that tested specific therapeutic agents in

animal models and showed a direct or indirect inhibiting action of treatment on TNF-α were of primary interest. Observational studies, systematic reviews, and meta-analyses were excluded, as were studies performed on human subjects and studies that did not explore any therapeutic agents. We also excluded in vitro studies due to our interest in studies that could evaluate cognitive outcomes. Studies that evaluated cognition without measuring TNF-α were excluded from this review. In addition, studies that measured TNF-α without the underlying biochemical changes in AD such as Aβ load, tau phosphorylation, and microglial activation were excluded.

Results

Thalidomide and thalidomide analogue 3,6-dithiothalidomide

Thalidomide is a drug that has been shown to reduce TNF-α synthesis by enhancing the rate of messenger RNA (mRNA) transcript degradation.[33,34] Its use as a TNF-α inhibitor has been reported in the treatment of multiple myeloma and erythema nodosum leprosum.[35,36] Given the inflammatory hypothesis, researchers wanted to investigate whether this drug could be used as a treatment option in AD through inhibiting the TNF-α pathway.

Belarbi et al[37] explored the effects of 3,6-dithiothalidomide (3,6-DT), a more potent analogue of thalidomide, on neuronal function in a mouse model of lipopolysaccharide (LPS)-induced neuroinflammation. This is an established murine model of AD that produces brain microglial activation and Aβ generation leading to cognitive impairment.[38,39] The authors infused either LPS or artificial cerebrospinal fluid (aCSF) into the fourth ventricle of 3-month-old mice for 28 days. Starting on day 29, the mice received intraperitoneal injections of 3,6-DT or vehicle for 14 days. After this, cognitive function was assessed by novel object recognition, novel place recognition, and the Morris water maze task (MWMT) to assess acquisition and consolidation of spatial memory.

Treatment with 3,6-DT led to a normalization of TNF-α levels. Levels were significantly elevated in the LPS-vehicle rats as compared with the aCSF-vehicle group. Treatment with 3,6-DT returned TNF-α levels back to control levels. There was no significant difference between the aCSF-vehicle and the LPS-DT groups. The authors also assessed the gene expression of factors involved in toll-like receptor (TLR)–mediated signaling pathways which are indicative of microglial activation. They found that TLR2 and TLR4 expressions were increased in the hippocampus of LPS-vehicle rats, but treatment with 3,6-DT normalized expressions of both.

Cognitive function was assessed with a novel object place recognition task. Animals from the LPS-vehicle group showed no preference to novel or familiar place. However, LPS-DT animals displayed a preference for the novel location which is more similar to the behavior of control animals. The MWMT showed a delayed learning of LPS-vehicle group which was significant on the second day. The LPS-DT rats showed an improved learning that was significant on the third day of training.

The work of Tweedie et al[40] extensively studied the biochemical actions of 3,6-DT and reported success with AD and cognitive outcomes by modulating the TNF-α pathway. They first assessed 3,6-DT action in vitro, by treating RAW 264.7 cells (macrophage-like cell line) with increasing concentrations of LPS to induce a dose-dependent generation of TNF-α protein and βAPP in the culture media. Pretreatment of cells with 3,6-DT prior to LPS administration reduced the synthesis of each factor in a dose-dependent fashion. 3,6-Dithiothalidomide was then studied in an in vivo rodent model. Rats were administered intraperitoneal LPS and displayed a marked increase in plasma TNF-α protein, but this increase was significantly attenuated by pretreatment with 3,6-DT. Similarly, hippocampal TNF-α mRNA levels and TNF-α protein levels were elevated by LPS and markedly suppressed by drug treatment. The 3,6-DT treatment was also found to reduce LPS-induced chronic neuroinflammation.

Finally, the authors assessed the effect of 3,6-DT in a 3xTg-AD mouse model (mice containing 3 transgenes associated with AD). 3,6-Dithiothalidomide was administered daily for 6 weeks, to adult and old mice, and resulted in a decrease in total and soluble βAPP levels in treated mice compared with vehicle-treated mice. Level of phosphorylated tau protein also presented a strong age-associated rise in old mice, and similar to soluble βAPP, it was attenuated by drug treatment in the older group. Aβ plaque formation in the old 3xTg-AD mice was found to be dramatically reduced by 3,6-DT. The authors also found 3,6-DT to improve age-associated memory deficits in the MWMT, with treated mice performing on par with vehicle control animals without AD.

Gabbita et al[41] also used a triple transgenic mouse model of AD to explore the beneficial effects of thalidomide and 3,6-DT. Transgenic mice were pretreated daily with 3,6-DT, thalidomide, or saline via intraperitoneal injections starting at 4 months of age and underwent cognitive and histopathological testing at 6 months of age.

Cognitive testing was performed using a radial arm maze task and a behavioral procedure task and showed a statistically significant effect of treatment on working memory errors. Transgenic mice that received saline injections performed significantly worse when compared with nontransgenic mice, thus establishing the integrity of the transgenic AD model. Further testing revealed that transgenic mice receiving 3,6-DT had better cognitive outcomes when compared with the control group.

Next, the authors quantified TNF-α and its expression within the brain. After 2 months of pretreatment with 3,6-DT, thalidomide, or saline, mouse brain tissue was harvested. A significant decrease was observed in brain TNF-α gene

expression in transgenic mice that received 3,6-DT, but similar effect was not observed with thalidomide administration. Administration of 3,6-DT also produced a significant decrease in TNF-α protein expression in the brain tissue, almost to near normal levels (no significant difference between 3,6-DT and non-AD mice).

Finally, treatment with both 3,6-DT and thalidomide produced a significant decrease in total, resting, and activated microglial cells. In addition, among the 3,6-DT–treated mice, the ratio of resting to activated microglial cells was visibly increased in the hippocampus, a morphological profile that is more similar to the nontransgenic hippocampus.

To summarize, thalidomide and its more potent analogue 3,6-DT brought about a favorable biochemical profile in the brain by decreasing TNF-α levels, soluble βAPP, phosphorylated tau, Aβ plaque formation, and a reduction in total, resting, and activated microglial cells. These biochemical changes translated into significantly improved cognition and memory in the treatment groups.

Minocycline

Minocycline is a tetracycline derivative with anti-inflammatory properties that rapidly crosses the blood-brain barrier and inhibits the microglial cells. Biscaro et al[42] investigated the role of minocycline in inhibiting microglial activation and increasing hippocampus-dependant performance. This was performed in a doubly transgenic (APP and mutant human presenilin [PS1]) mouse model of AD. Eleven-week-old transgenic and nontransgenic mice received minocycline, whereas control groups received only vehicle twice daily for first 2 days and once daily for the next 5 days until retroviral vector infusion. After this, they received a half dose of minocycline or vehicle once daily for the next 5 weeks. The retroviral infusion served to label the new hippocampal neurons.

The authors then performed cognitive testing; spatial memory was assessed in a Y-maze task, and no significant difference was found between the treated and control groups. Recognition memory was tested using a hippocampus-dependant object recognition task and found that nontransgenic mice and minocycline-treated mice performed significantly better than vehicle-treated mice. After the conclusion of cognition testing, cortical βAPP concentrations and hippocampal cytokine concentrations were measured. The transgenic mice started to show deposits of βAPP at 2 to 3 months of age in the hippocampus, and by 4 months of age, the deposits were scattered throughout the cortex. Treatment of 3-month-old mice with minocycline for 6 weeks reduced the total numbers of microglial cells in the dentate gyrus by 43%, reaching numbers observed in age-matched wild-type mice. Treatment attenuated microglial activation, thereby protecting neurogenesis. Minocycline was also shown to normalize the increased hippocampal levels of IL-6, but TNF-α inhibition did not reach significance within this time frame. Treatment with minocycline, however, did increase the concentration of IL-10, an anti-inflammatory cytokine that is a known downregulator of TNF-α. Thus, when taken in context of the literature reviewed, it can be extrapolated that TNF-α depression likely would have reached significance in a longer time frame of the study.

In summary, minocycline treatment reduced the total and activated number of microglial cells and this had a protective effect on neurogenesis. Treatment did not normalize TNF-α but increased levels of anti-inflammatory IL-10 which is a known inhibitor of TNF-α. This suggests that minocycline may indirectly decrease TNF-α concentration through decreasing microglial activation and increasing IL-10. Despite TNF-α levels not reaching statistical significance, cognitive benefits were still observed, with minocycline-treated mice performing at par to healthy mice.

Sodium hydrosulfide (NaHS)

Hydrogen sulfide (H2S) is an endogenous anti-inflammatory and neuroprotective agent with antioxidant and antiapoptotic effects in neuronal and glial cells.[43–45] Furthermore, the levels of *S*-adenosylmethionine, an activator of cystathionine β-synthase (main H2S-producing enzyme in the brain), have been found to be lower in AD brains compared with healthy brains.[46] Xuan et al[47] wanted to investigate whether H2S-releasing drugs (NaHS), through their anti-inflammatory action, may inhibit TNF-α and thus be beneficial in AD pathology.

They investigated this relationship by injecting βAPP into the rat hippocampus, and the MWMT was used to assess cognitive function. Immunohistochemistry was used to look at glial response and terminal deoxynucleotidyl tranferase–mediated deoxyuridine triphosphate nick end labeling (TUNEL) was performed to detect neuronal apoptosis. The rats were divided into 4 groups: control, control+ NaHS, Aβ, and Aβ+ NaHS. NaHS was administered intraperitoneally once daily for 3 days before surgery and continually for 9 days thereafter the injection of Aβ into the dentate gyrus.

Results showed that the learning and memory abilities of Aβ rats were significantly impaired compared with the control group. The TUNEL assay demonstrated that treatment with NaHS suppressed Aβ-induced neuronal apoptosis and lowered plaque load by approximately 31% compared with the untreated group. NaHS was also shown to decrease microglial activation and it attenuated the βAPP-induced increase in TNF-α content in the hippocampus.

Atorvastatin

In disease states such as arthritis, the lipid-lowering drug atorvastatin has been shown to decrease LPS-induced TNF-α expression in macrophages.[48–50] Zhang et al[51] evaluated whether

this agent would have a beneficial effect in AD treatment as well. Alzheimer disease was induced by injection of Aβ into the ventricle of adult rats, and their spatial learning and memory ability was then determined using the MWMT. Escape latency of the atorvastatin-treated AD mice was found to be significantly shorter than control AD group on day 3 and day 4. Similarly, the atorvastatin-treated AD rats showed a significant decrease in mean escape latency compared with the AD group. Both findings suggest improved spatial learning and memory in AD rat models that were treated with atorvastatin.

Hematoxylin and eosin staining of the hippocampus showed neurodegenerative changes such as sparsely formed cells and deeply stained nuclei in the AD group. However, in the atorvastatin-treated AD group, the neurons and the nuclei appeared similar to the control group without Aβ injection. However, it is important to note that this finding was based on subjective observation by the investigators and not quantified through statistical methods. Immunohistochemical testing was also performed and showed that staining of IL-1β, IL-6, and TNF-α was significantly decreased in the atorvastatin-treated AD group when compared with the vehicle-treated AD group, suggesting that atorvastatin inhibits expression of pro-inflammatory cytokines within the hippocampus.[51]

In summary, both NaHS and atorvastatin were observed to decrease levels of TNF-α and bring about cognitive improvement in the treatment groups. Atorvastatin also improved the morphological appearance of the neurons to that in healthy state, whereas NaHS was shown to inhibit Aβ-induced neuronal apoptosis, decrease microglial activation, and decrease Aβ plaque load.

Etanercept and infliximab

Kubra Elcioglu et al[52] conducted studies with etanercept, an anti-TNF agent that functions as a decoy receptor that binds and inhibits the actions of TNF-α, and infliximab, a monoclonal antibody against TNF. For their experiments, they used a rat model of dementia that was induced by intraventricular injection of streptozotocin which is widely used to mimic an AD-like condition in animal models.

Using the MWMT for cognitive assessment, they determined that the performance of the rats receiving etanercept or infliximab was significantly better than the control group. They also performed hematoxylin and eosin staining for general evaluation of the tissue and Bielschowsky staining for visualization of nerve fibers, neurofibrillary tangles, and senile plaques. Frontal, parietal, and temporal regions in neocortex and hippocampus were examined, and semiquantitative histologic evaluation was scored blindly by the investigators. Semiquantitative analyses were performed based on the number of plaques found on Bielschowsky staining of the neocortex and hippocampus. The numbers of plaques were rated as follows: +, 1 to 7; ++, 8 to 14; and +++, ≥15. Quantitative analysis showed the number of neurofibrillary tangles and plaques in the etanercept and infliximab groups (++) to be less than the control groups (+++).

Electron microscopy was also performed, and prominent changes such as swelling, vacuoles, and numerous lipofuscin granules were detected in all animals, but less so in the etanercept-treated or infliximab-treated rats. Neuronal degeneration with multivesicular inclusions was clearly visible in the untreated group, confirming the benefits of etanercept and infliximab that can be exploited for treating AD through TNF-α immunomodulation.

To summarize, etanercept and infliximab, which are direct inhibitors of TNF-α, improve cognitive outcomes in rats with AD. This benefit is likely explained by the resulting decrease in the number of plaques and neurofibrillary tangles observed and confirmed by favorable electron microscopy changes such as decrease in cellular swelling and vacuole formation.

Infliximab and exendin-4

Bomfim et al[53] investigated the mechanism by which antidiabetic agents ameliorate AD pathology. They hypothesized that TNF-α modulation through exendin-4 may be central to bringing about the observed benefits in cognition, with c-Jun N-terminal kinase (JNK) of the MAP kinase pathway being the critical factor. They also argued that brain levels of insulin and insulin receptors are lower in AD, suggesting that insulin resistance likely contributes to cognitive deficits.

They first measured the level of serine phosphorylation of insulin receptor substrate 1 (IRS-1pSer) in transgenic AD mice (APP/PS1) compared with wild type and showed a significantly higher density of IRS-1pSer neurons in their hippocampal CA1 regions. Similar findings were also demonstrated in cynomolgus monkeys, with intraventricular injection of Aβ oligomers triggering hippocampal IRS-1pSer. The authors also quantified TNF-α expression in AD mice and found TNF-α levels to be significantly increased with βAPP.

The authors then attempted to block TNF-α through administration of infliximab and found that abnormal IRS-1pSer triggered by βAPP was blocked by treatment with infliximab. The authors also investigated the effect of βAPP on JNK and found that JNK was significantly elevated in the βAPP group compared with vehicle. The JNK levels were also found to be significantly elevated in transgenic AD mice compared with wild type. Taken together, these results indicate that Aβ peptide induced elevation in TNF-α triggers abnormal activation of JNK which leads to abnormal serine phosphorylation of IRS-1.

The authors hypothesized that exendin-4 may prevent this abnormal activation. Pretreatment of transgenic mice with exendin-4 prevented Aβ-induced neuronal pathologies observed ex vivo, including impaired axonal transport, and significantly improved behavioral measures of cognition, as measured by MWMT. Exendin-4 was also associated with significantly

decreased plaque load, decreased levels of hippocampal IRS-1pSer, and decreased activation of JNK, suggesting that immunomodulation of the JNK/TNF pathway might explain the beneficial effects of exendin-4.

In summary, the above study demonstrated that the abnormal IRS-1pSer triggered by βAPP was inhibited by infliximab, showing that TNF-α plays a role in the abnormal phosphorylation of serine. Exendin-4 was shown to improve cognition by decreasing the activation of the JNK/TNF pathway which led to decreased IRS-1pSer and decreased plaque load.

Imipramine

Imipramine is a tricyclic antidepressant with potential immunomodulatory role in AD pathophysiology. Existing evidence from in vitro studies shows that imipramine inhibits both LPS-induced production of TNF-α and its gene expression in microglia, thereby protecting against cell death in a microglia/neuron coculture.[54] Desipramine, an active metabolite of imipramine, has also been shown to decrease TNF-α production in an animal model of LPS-induced sepsis.[55] Chavant et al[56] investigated the effect of a pharmacologic strategy targeting TNF-α with imipramine and its effect on Aβ-induced memory impairment, TNF-α expression, and βAPP processing. Alzheimer disease was induced in 4-week-old male mice by injecting $A\beta_{25-35}$ intracerebroventricularly. Mice in treatment group received intraperitoneal injections of imipramine for 14 days following $A\beta_{25-35}$ administration.

Short-term memory impairment was tested with the Y-maze test and showed that treatment with imipramine significantly prevented this Aβ-induced memory impairment. Similarly, testing for long-term memory impairment with the MWMT showed that imipramine facilitated learning capabilities of Aβ-impaired mice and produced decreased escape latencies.

Next, they performed enzyme-linked immunosorbent assay and Western blot studies on supernatants extracted from homogenized brain tissues harvested from mice 14 days after $A\beta_{25-35}$ administration. Enzyme-linked immunosorbent assays revealed a significant increase in TNF-α expression in the frontal cortex and hippocampus of Aβ-injected mice compared with controls, but this increase was significantly attenuated with imipramine treatment. Similarly, Western blot assays showed an enhancement in the level of βAPP in the hippocampus following $A\beta_{25-35}$ injection, and imipramine treatment significantly counteracted this increase. Neuronal Aβ immunoreactivity was also analyzed by immunohistochemistry, and a decrease in the number of the Aβ immunopositive cells was observed in imipramine-treated mice, reaching approximately 52% (compared with untreated mice) in cortex.

To summarize, treatment with the tricyclic antidepressant imipramine in a mouse model of Aβ-induced memory impairment showed significant improvement in short-term and long-term impairment. This improvement was likely linked to the significantly reduced levels of TNF-α expression and beneficial effect of imipramine on βAPP processing (Table 1).

Discussion

Chronic neuroinflammation is an important component of AD pathogenesis and plays a crucial role early on in the disease process. Tumor necrosis factor α has been shown to regulate numerous cellular processes such as cellular differentiation and survival, in addition to inflammation and cell death.[57] Inflammation and cell death are mediated by the TNFR1 receptor, whereas signaling through TNFR2 is associated with cell survival. The engagement of TNF-α with its receptor can activate 1 of 3 pathways: an apoptotic pathway mediated through a TNF-associated intracellular death domain, a NF-κB prosurvival pathway, and a JAK pathway involved in cellular differentiation that is proapoptotic. The levels of soluble TNF-α, receptor subtype activation, levels of expression on neurons, and the duration of neuroinflammation determine the consequence of TNF-α signaling and whether a prosurvival or proapoptotic response is favored.[58]

Modulation of the TNF-α pathway led to favorable outcomes in cognitive ability in animal models. In addition, the biochemical hallmarks of AD pathology such as extracellular plaque load, intracellular tau phosphorylation, and microglial and astrocyte activation were all shown to be decreased through the inhibition of the TNF-α pathway. It is likely that the inhibition of this signaling pathway prevents the strong activation of microglial cells, keeping them in a state of moderate activation where they play a neuroprotective role by enhancing the clearance of βAPP. With this moderate activation, a delicate balance of proapoptotic and prosurvival signaling is brought about that has the potential to delay the destruction of neurons in AD.

It is this need for a balance between microglial activation and TNF-α signaling that might explain why randomized controlled trials with a general inhibition of the inflammatory response through corticosteroids[59] and nonsteroidal anti-inflammatory drugs (NSAIDs)[49] did not reveal statistically significant outcomes in AD. Perhaps, the general inhibition of the inflammatory response brought about by steroids and NSAIDs nullifies the neuroprotective effect conferred by a balanced inflammatory system.

Data from transgenic animal models have suggested a clear involvement of cytokines, particularly TNF-α, at presymptomatic stages of AD pathology.[60] This implies that the dysfunction of viable neurons during early stages of AD may not represent a permanent state and may thus be amenable to rescue. An important consideration with respect to the efficacy of targeting TNF-α is the time line of the disease progression. Tumor necrosis factor α inhibitors may have more efficacy early on in the disease process when the integrity of the CNS

Table 1. Studies investigating neuroprotective benefits of anti-TNF drugs.

AUTHOR (YEAR)	STUDY MODELS	THERAPEUTIC INTERVENTION	EXPERIMENTS PERFORMED	RESULTS
Tweedie et al (2012)[40]	In vitro experiments with cell cultures LPS administration in rats Transgenic mice	3,6-DT	Cognitive testing with MWMT Immunohistochemical testing for TNF-α, Aβ, and βAPP levels, as well as tau phosphorylation	Treatment with 3,6-DT decreased TNF-α Elevation of βAPP reversed by 3,6-DT Improved memory testing in MWMT
Belarbi et al (2012)[37]	LPS administration in mice	3,6-DT	Cognitive testing with novel object and place recognition task, and MWMT Immunohistochemical testing for TNF-α and TLR expression	Treatment restored TNF-α to normal level Decreased TLR2 and TLR4 expression with treatment Improved cognitive function in 3,6-DT–treated mice
Gabbita et al (2012)[41]	Transgenic mice	3,6-DT and thalidomide	Cognitive testing with radial arm maze task and behavioral procedure task Immunohistochemical testing for TNF-α expression and microglial activation	Working memory errors reduced with therapy Reduction in TNF-α with 3,6-DT but not with thalidomide Decreased the number of total, resting, and activated microglial cells noted Treatment with 3,6-DT increased the ratio of resting to reactive microglia
Biscaro et al (2012)[42]	Transgenic mice	Minocycline	Cognitive testing with a Y-maze task and object recognition task Immunohistochemical testing for TNF-α, βAPP, and cytokine concentrations, as well as microglial activation	Recognition memory was improved in treated mice Minocycline reduced microglial cell activation Treatment led to normalized IL-6 and increased IL-10 concentration
Xuan et al (2012)[47]	βAPP infusion in rats	Sodium hydrosulfide (NaHS)	Cognitive testing with MWMT Immunohistochemical testing for microglial activation TUNEL to evaluate for Aβ plaque load and Aβ-induced neuronal apoptosis	Improved learning and memory abilities with treatment NaHS suppressed Aβ-induced neuronal apoptosis and lowered Aβ load Treatment reduced TNF and decreased microglial activation
Zhang et al (2013)[51]	Aβ infusion in rats	Atorvastatin	Cognitive testing with MWMT Hematoxylin and eosin staining of the hippocampus Immunohistochemical testing for TNF-α and cytokine levels	Restored cognitive function in Aβ-infused mice Hematoxylin and eosin staining showed attenuation of neurodegenerative changes Atorvastatin decreased IL-6, TNF-α, and IL-1β
Kubra Elcioglu et al (2015)[52]	Streptozotocin infusion in rats	Etanercept	Cognitive testing with MWMT Semiquantitative histologic evaluation after hematoxylin and eosin and Bielschowsky staining Electron microscopy	Improved cognitive performance following etanercept Deceased Aβ plaques and neurofibrillary tangles Decreased neuronal degeneration in treated rats
Bomfim et al (2012)[53]	Aβ administration in monkeys Transgenic mice	Infliximab and exendin-4	Cognitive testing with MWMT Quantification of IRS-1pSer and JNK levels	Infliximab blocked abnormal IRS-1pSer triggered by βAPP Exendin-4 decreased JNK/TNF-α activation and hippocampal IRS-1pSer Exendin-4 decreased plaque load and improved cognition
Chavant et al (2010)[56]	Aβ administration in mice	Imipramine	Cognitive testing with MWMT and the Y-maze test ELISA and Western blot to assess TNF-α expression and βAPP processing Immunohistochemistry to analyze neuronal Aβ immunoreactivity	Improvement in short-term and long-term memory testing Imipramine led to reduced levels of TNF-α expression and beneficial effects on βAPP processing Decrease in the number of the Aβ immunopositive cells observed in treated mice

Abbreviations: 3,6-DT, 3,6-dithiothalidomide; βAPP, β-amyloid precursor protein; ELISA, enzyme-linked immunosorbent assay; IRS-1pSer, insulin receptor substrate 1; LPS, lipopolysaccharide; MWMT, Morris water maze task; TLR, toll-like receptor; TNF-α, tumor necrosis factor α; TUNEL, terminal deoxynucleotidyl tranferase–mediated deoxyuridine triphosphate nick end labeling.

is relatively preserved. The process can be compared with a snowball effect where the longer it is allowed to run, the more the accumulating damage that becomes irreversible at some critical point of disease progression. With this in mind, a limitation of the above studies is that the treatment was started relatively early in the disease process. In practicality, by the time most patients with AD come to clinical attention, the disease is already at an advanced stage when the damage may be irreversible. In future research, it would be interesting to see how TNF-α inhibitors affect the different biochemical stages of the disease process. This could also have implications for early onset or genetic forms of AD where prophylactic treatment through TNF-α modulation may be a viable option.

Immunomodulation of the TNF-α pathway also alters AD pathophysiology through increased activation of CD11c-positive dendritic-like cells which are known to help clear plaques in the AD mouse model. Shi et al[61] demonstrated this by treating aged APP/PS1 double transgenic mice with intraventricular injections of infliximab, a monoclonal antibody against TNF-α. Using immunohistochemical staining and Western blot, they showed a marked increase in the CD11c-positive dendritic-like cells in mice brains after infliximab administration. In addition, fluorescent staining of brain sections revealed numerous βAPP in old transgenic mice, which was significantly reduced following intracerebroventricular injection of infliximab. Using an antibody specific to phosphorylated tau, they also found that infliximab administration significantly reduced tau hyperphosphorylation by up to 70%.

As discussed in the studies above, thalidomide and thalidomide analogues (3,6-DT) are promising treatment approaches to reduce the levels of TNF-α synthesis. The benefits of thalidomide might also be explained by decreased activity of β amyloid cleavage enzyme (BACE1/α-secretase) activity with long-term thalidomide treatment. He et al[62] studied APP23 transgenic mice that were administered thalidomide intraperitoneally from the age of 9 months when the Aβ plaques started to appear in the brain, until 12 months. Similar to the above discussed studies, they found that microglial activity, plaque number, and total amyloid protein were subjectively depressed in the thalidomide-treated mice in comparison with the control group. In addition, they also observed a significant decrease in BACE1 level expression in the treatment group compared with the age-matched mice treated with vehicle alone. Moreover, a significant reduction in c99 density, a cleavage product of BACE1, was noted in transgenic mice treated with thalidomide vs the vehicle group.

In the above studies with these drugs, appropriate control groups were used allowing the simultaneous evaluation of the adverse outcomes of treatment. Of particular concern was the fact that thalidomide has known side effect of causing locomotor problems. However, the use of the MWMT also allowed the evaluation of locomotor ability, and no adverse motor outcomes were observed in the treatment groups. Although thalidomide is a category X teratogen, it can be argued that its use in the treatment of AD is relatively safe because the disease mostly affects older people who are past their reproductive years. Similarly, imipramine has known anticholinergic side effects that can potentially worsen cognition; however, the limited available evidence suggests that low doses of imipramine are well tolerated by patients with coexisting AD and depression.[63]

It was interesting to note that a metabolic agent such as atorvastatin showed benefit in AD pathophysiology through TNF modulation. In addition, epidemiologic data from various populations have shown that statin users have a low incidence of AD.[64–67] Historically, this was believed to be secondary to the cholesterol-lowering activity of statins, but accumulating evidence of the anti-inflammatory action of statins[68] has led to the speculation that this anti-inflammatory activity might partly contribute to the low incidence of AD in statin users. As discussed above, Zhang et al[51] lend support to this hypothesis by demonstrating the anti-inflammatory actions of atorvastatin and the resultant improvement in learning and memory ability in an AD rat model induced by $A\beta_{1-42}$.

A major practical advantage of TNF-α inhibitors such as thalidomide, minocycline, exendin-4, imipramine, and atorvastatin is the ease of administration because these agents can be taken orally. Conversely, a disadvantage of direct anti–TNF-α drugs such as etanercept and infliximab is the fact that they can only be administered as intravenous infusions or injections. For central delivery, these agents must be injected perispinally into the CSF, and even then, there are concerns about drug concentrations reaching therapeutic levels in the brain.[69]

It is important to keep in mind that AD is a disease of the elderly, a segment of the population that is highly vulnerable to opportunistic infections such as fungi, toxoplasmosis, and reactivation of latent tuberculosis. An important adverse effect of any TNF-α inhibitor is the accompanying immunosuppression that would put this population at an even higher risk of opportunistic diseases. These are issues that would need to be addressed, such as through the use of prophylactic antibiotics in the management of the patient with AD on this treatment regime.

The major limitation of the above studies is that they were done on animal models of AD. The results are promising, but it remains to be seen whether the benefits observed in animal studies would show similar outcomes in humans. If efficacy is proven in human studies, an important question to ask is how this treatment would affect patients in different stages of the disease process. These are questions that were not answered by the animal studies discussed above and provide potential direction for future studies.

The strength of the studies was that they included both cognitive outcomes and measurement of the underlying immunochemistry such as TNF-α expression, β-amyloid plaque burden, and microglial activation. This allowed a mechanistic understanding of these agents and provided a clear explanation of how TNF-α modulation altered AD pathophysiology. Another strength was that outcomes were measured using

standardized tests such as the MWMT that are reliably known to test cognitive processes in mice models. Finally, most of the above studies used models of AD (transgenic, intracerebroventricular injection of Aβ) that have been established in the literature to be a close approximation of AD in the human brain.

The logical next steps would be to conduct human studies to build on the theoretical platform laid down using AD animal models. An advantage of the drug agents discussed above is that they have been used in humans for other disease processes, and thus, safety profiles and drug dosages are well established in the population. Future animal studies could explore the role of these agents during later stages of the disease process, and once this has been established, clinical trials in the patients with AD can be conducted to test efficacy. At that stage of human testing, it would also be worthwhile to study these agents in their prophylactic capacity (before AD symptoms manifest) versus therapeutic capacity (after symptoms appear).

Conclusions

The above studies provide valuable insight into the crucial role of TNF-α in AD progression. In summary, Aβ plaques in the CNS strongly activate macrophages which then lose their normal ability to phagocytose and clear this protein. Instead, they adopt a pro-inflammatory role where a state of chronic inflammation is brought about by the release of cytokines, TNF-α being the major culprit in this downward spiral of neuron damage.

Taken together, the above studies indicate that the inhibition of TNF-α has the potential to slow down this vicious cycle, leading to improved clearance of βAPP and Aβ through CD11c dendritic-like cells, lessening βAPP formation through decreased BACE activity and decreasing microglial activation to levels that are neuroprotective. It remains to be seen how much these individual factors such as BACE activity and IRS-1pSer affect each other, and whether their relationship can be understood as linear or parallel in the chain of events that lead to βAPP accumulation. Further research exploring how these various biomolecular factors may be related, or other factors that may independently influence this cycle, will help put together the pieces of the puzzle to create a unifying theory of this highly complex disease process.

This article highlights the various biochemical pathways that can be used to inhibit TNF-α before the escalation of immunomodulatory events that lead to the formation of insoluble plaques and then the destruction of neurons. Different methods of TNF-α inhibition were reviewed: drugs that inhibited TNF-α synthesis through mRNA degradation (thalidomide and 3,6-DT), direct TNF-α antagonists (etanercept and infliximab), and indirect TNF-α immunomodulating agents (atorvastatin, hydrogen sulfide, minocycline, imipramine, and exendin-4). The agents discussed in this article show significant promise in the treatment and prevention of AD pathophysiology. Although limited to animal models only, all agents improved cognitive outcomes (learning and memory) and produced favorable biochemical and histopathological changes in the brain. Treated animals demonstrated significant reduction in neuroinflammation and decreased neuronal degeneration. This was evident by the decreased production of pro-inflammatory TNF-α and the reduced burden of neurofibrillary tangles, βAPP, and Aβ plaques. Most importantly, the recruitment of microglial cells in the CNS was significantly reduced through these drugs, thus suppressing chronic neuroinflammation.

A point to be noted is that current data only support the use of these agents during early stages of disease, and future animal studies need to explore their efficacy at the different stages of the disease process. This would provide a more comprehensive and thorough understanding of the therapeutic potential of these drugs for AD and perhaps allow for treatment options to be tailored according to the biochemical stage of the disease progression.

Author Contributions

DS and ML conceived the idea for this review. DS developed the methodology, performed the literature review and compiled the data. DS and ML jointly wrote the manuscript and approved of the final version for publication.

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