**Review Article**

**Interplay between Inflammation and Cell Cycle Deregulation in Alzheimer’s Disease**

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**Abstract**

Alzheimer’s disease (AD) pathology is associated with neuroinflammation and the ectopic expression of cell cycle proteins, indicating that these two events may be involved in its pathogenesis. The cause and consequences of these events are still elusive. Inflammatory signaling has been shown to induce expression of the Alzheimer’s associated amyloid precursor protein (APP), whose phosphorylation and pathogenic processing are regulated in a cell cycle specific manner. The amyloid beta (Aβ) generated upon secretase-mediated processing of APP deposits in the brain parenchyma, extracellularly, to form amyloid plaques, which coincides with glial activation and chronic neuroinflammation. As reviewed in this article, interplay of the inflammatory response and cell cycle dysfunction could be underlying pathogenic processes in the neurodegeneration observed in AD. Both events are linked to neuronal abnormalities and pathological hallmarks of AD, including Tau phosphorylation and Aβ generation and deposition. Epidemiological studies have linked the use of non-steroidal anti-inflammatory drugs with a decrease in risk for developing AD, however its use in clinical trials on AD patients has largely been ineffective. It seems that an early therapeutic intervention that could target neuroinflammation and mitotic changes in compromised neurons could potentially have a great impact on the progression of AD.

**ABBREVIATIONS**

AD: Alzheimer’s disease; Aβ: Amyloid-beta; APP: Amyloid Precursor Protein; PS-1: Presenilin-1; PS-2: Presenilin-2; CNS: Central Nervous System; ROS: Reactive Oxygen Species; NFKB: Nuclear Factor Kappa B; IL-1: Interleukin-1; MCP-1: Monocyte Chemoattractant Protein 1; MIP: Macrophage Inflammatory Protein; CSF: Cerebral Spinal Fluid; GM-CSF: Granulocyte Macrophage-Colony Stimulating Factor; TNF-α: Tumor Necrosis Factor Alpha; TGFβ: Transforming Growth Factor α; S100β: Beta Subunit of S100; ACT: Alpha-1-Antichymotrypsin; JNK: c-Jun N-Terminal Kinase; COX-2: Cyclooxygenase-2; NSAIDs: Non-Steroidal Anti-Inflammatory Drugs; LPS: Lipopolysaccharides

**INTRODUCTION**

Alzheimer’s disease (AD) is the most common form of senile dementia and is ranked 6th in leading causes of death in the United States. In its initial stages, the disease presents itself with memory loss which later progresses to impaired executive function and ultimately, death. Pathology development precedes cognitive decline and is thought to begin in the entorhinal cortex, gradually spreading to other areas of the brain and leading to widespread synaptic loss, metabolic dysfunction and atrophy [1]. The classic lesions of the AD brain are extracellular neuritic plaques and intracellular neurofibrillary tangles [2-4]. Neuritic plaques are formed by the deposition of the insoluble amyloid-beta (Aβ) peptide, a product formed by the sequential cleavage of the amyloid precursor protein (APP) by β- and γ-secretase. Neurofibrillary tangle pathology follows plaque development and is formed by hyperphosphorylation of the microtubule-associated protein, Tau. AD can be divided into two subgroups based on genetic association and age at onset: familial and sporadic. Familial AD occurs in early age and represents only 5-10% of all AD cases. This form of early-onset AD is largely genetic and is associated with mutations in Presenilin-1 (PS1), Presenilin-2 (PS2) and APP genes. Late-onset AD, thought to be sporadic, accounts for ~90-95% of all AD cases and while its etiology is unclear, its onset occurs with advanced age.
CELLULAR INFLAMMATORY MEDIATORS IN ALZHEIMER’S DISEASE

Neuroinflammation is a common feature in nearly all neurodegenerative diseases, including Alzheimer’s disease. While it is a well established hallmark to the disease, it is unclear whether this feature is causative or subsequent to AD pathogenesis. Immunohistochemical analyses reveal an inflammatory status of post-mortem human AD brains with the presence of activated glia in proximity to Aβ deposits [5-8]. Likewise, this immune response is also observed around amyloid plaques in the brains of transgenic AD mice that express human APP carrying the Swedish mutation [9-13].

The brain’s resident microglia and astrocytes are the main cellular mediators that become activated in response to injury. Astrocytes are the most abundant type of glial cells in the central nervous system (CNS), and the pathology associated with AD triggers what is called “reactive astrogliosis” [14]. This activation is defined by an increase in number, size and mobility of astrocytes, which is thought to be important for Aβ clearance and degradation [15]. Microglia comprises a marginal portion of the glial cell population in the brain and when activated, undergoes distinct morphological changes and migration to the compromised area. There, they produce a number of reactive oxygen species (ROS), cytokines, chemokines, growth factors and complement factors that are released to the surrounding tissue [16-19].

Several in vitro studies implicate the highly insoluble, fibrillar form of Aβ in inducing microglial activation and inflammatory protein expression [20-26]. This effect has also been observed with in vivo imaging of microglial migration to amyloid deposits within transgenic AD mouse brain [27, 28]. In fact, treatment of transgenic AD mice with the recombinant 5 amino acid long β-sheet breaker peptide iAβ5, has been shown to not only reduce plaque load, but also lessen inflammation-induced cerebral damage [29]. Aβ has also been shown to induce cytokine production in various ways. The nuclear factor kappa B (NFkB) pathway leads to cytokine production and this signaling pathway can be induced by the Aβ peptide [21]. Aβ is able to bind to receptors expressed on the microglial membrane and induce the MAPK pathway which subsequently leads to expression of inflammatory genes and production of chemokines and cytokines [30]. A working hypothesis for these findings is that the dense and irreversible nature of the amyloid plaque leads to stimulation of glial cells and chronic inflammation in the brain. The release of proinflammatory mediators in response to the fibrillar Aβ along with soluble Aβ itself- results in a neurotoxic effect for healthy, adjacent neurons [31]. Additionally, these inflammatory mediators are thought to facilitate enhanced amyloidogenic processing of APP, producing more Aβ, which then feeds into the vicious cycle [22,32,33]. Conversely, others believe inflammation precedes fibrillar plaque pathology. For example, a study carried out in macaque monkeys found that glial activation is an event that occurs before deposition of fibrillar Aβ in the aging brain [34]. Several studies have also linked polymorphisms in pro-inflammatory cytokine genes, in particular Interleukin-1 (IL-1), to AD [35-40]. Another example is that from a study in Down syndrome patients, who typically develop AD by middle age as they carry an extra copy of the APP gene; this study showed that the brains of fetal and neonate Down syndrome patients (considered to be pre-amylloiplaque bearing) exhibit gliosis, and show upregulation of cytokines, further supporting the notion that inflammation and plaque development occur sequentially [41].

MOLECULAR MEDIATORS OF INFLAMMATION IN ALZHEIMER’S DISEASE

Activated microglia and astrocytes surrounding amyloid plaques secrete cytokines and chemokines that contribute to the inflammatory process. A number of cytokines and chemokines are found to be upregulated in AD brains compared to their non-demented controls [42-45].

Chemokines are a family of small proteins that recruit monocytes, macrophages, lymphocytes, neutrophils, basophils, eosinophils and dendritic cells to compromised tissues [46]. The Aβ peptide can promote the generation of chemokines such as IL-8, monocyte chemotacttractant protein 1 (MCP-1), macrophage inflammatory protein (MIP)-1α and -1β [47] from human monocytes [17]. Additionally, MIP-1β has been detected within activated astrocytes around amyloid plaques [48]. Cytokines are soluble proteins that are secreted by glia in the AD brain in response to Aβ aggregates [49]. Analysis of both AD human and transgenic mouse brain tissues and cerebral spinal fluid (CSF) show a marked increase in the levels of IL-1β, IL-1α, IL-6, IL-10, IL-12, granulocyte macrophage-colony stimulating factor (GM-CSF), tumor necrosis factor α (TNF-α) and transforming growth factor β (TGF-β) [50-57].

In the brain, TNF-α is predominantly produced by activated microglia in response to injury; however it has also been shown to be produced by neurons [58]. The role of TNF-α in AD is unclear as it has both beneficial and detrimental effects. Under non-pathological conditions, TNF-α levels are low in the brain [59] but in AD its levels are elevated in serum, CSF, cortex and glial cells [17,60,61]. The role of TNF-α is dichotomous in nature: it is able to stimulate NFκB which in turn induces transcription of proinflammatory proteins and stimulation of survival proteins such as anti-apoptotic Bcl, manganese superoxide dismutase and calbindin [62,63]. It has been demonstrated in vitro that TNF-α can induce expression of APP and Aβ in glial cells [64]. Its harmful effects also include its stimulation of microglia to release glutamate, resulting in excitotoxicity [65]. TNF-α has been implicated in increased expression of both β- and γ-secretases. Furthermore, inhibition of TNF-α has been shown to mitigate disease progression in transgenic AD mice [66,67]. On the contrary, chronic TNF-α suppression can hinder the microglial response in early stages of Aβ deposition, leading to accelerated aggregation [68]. It is possible that the action that TNF-α exerts is dependent on both its levels and duration of tissue exposure in the brain.

IL-1 is an immunomodulatory cytokine, which is a key player in initiation of the immune response. IL-1 is found to be elevated immediately after brain injury [69,70] and this effect is also seen in response to amyloid plaques, as concentrations of IL-1 are increased in the AD brain [71-73]. Interestingly, IL-1 has been found to not only induce the synthesis of APP, but also mediate...
Figure 1 (A) The pathological cascade in Alzheimer’s disease. Oligomeric Aβ in AD induces activation of glia and release of inflammatory mediators. The expression of cell cycle proteins in compromised neuronal populations coincides with this neuroinflammatory response. Aberrant cell cycle activation in the AD brain has been shown to result in pathological modifications in the AD-associated proteins, APP and Tau. Hyperphosphorylation of APP results in increased production of the pathogenic Aβ peptide and its subsequent deposition in the brain. Soluble Aβ has been shown to further induce Tau hyperphosphorylation, neurofibrillary tangle formation, neurodegeneration and cell death.

(B) Schematic of the inflammation and cell cycle-dependent signaling in Alzheimer’s disease: Amyloidogenic processing of APP leads to the production of the neurotoxic Aβ peptide. Reduced clearance and Aβ aggregation leads to glial activation and subsequent production of pro-inflammatory cytokines and chemokines, which can enhance expression of cell cycle regulatory proteins in the AD brain. In particular, TNF-α and COX-2 have been shown to correlate with expression of cell cycle markers. TNF-α is thought to initiate cell cycle events through the activation of the JNK pathway. Chronic exposure of neurons to inflammatory mediators thus lead to accelerated neuritic plaque and neurofibrillary tangle formation in AD brain. Inhibition of inflammation could potentially prevent aberrant cell cycle signaling and neurodegeneration associated with AD.
its pathogenic processing [71,74]. This implicates a role for IL-1 in neurodegeneration, which is further highlighted by the finding that IL-1 promotes synthesis of the beta subunit of S100 protein (S100β) in astrocytes, which promotes dystrophic neurite growth [41,71,75]. Inhibition of IL-1β activity has also been shown to promote neurogenesis via the Wnt/β-catenin signaling cascade by inhibiting GSK-3β activity and subsequent Tau phosphorylation [76]. The role of IL-1β in neurodegeneration, however, is not straightforward. One study shows a protective effect of IL-1β when overexpressed in the astrocytes of transgenic AD mice; Shahtel et al. showed that sustained activation of IL-1β results in a significant decrease in the hippocampal amyloid plaque load in these mouse brains [77].

Similar to IL-1, the cytokine IL-6 is found to be increased in AD patient tissue and it also induces the expression of APP [32,78,79]. Conversely, IL-6 levels increase after treatment of cultured glial cells with the carboxy terminal fragment of APP (the last 105 amino acids) [80]. While some have found that IL-6 enhances Aβ-induced neurodegeneration in primary rat neuronal cultures [81], others have found that overexpression of IL-6 in transgenic AD mouse brain results in increased gliosis and phagocytosis, attenuating Aβ deposition [82]. The contradictory nature of these findings can be bridged by the idea that neuroinflammation is a complex and fine balanced response, capable of being both beneficial and deleterious to the brain.

Alpha-1-antichymotrypsin (ACT) is an acute phase inflammatory protein and serine protease inhibitor found to be elevated in the CSF, serum and brain of AD patients [83-85]. In the normal brain, ACT is localized to astrocytes and in the AD brain it is overexpressed in the astrocytes surrounding amyloid plaques [83,86,87]. It has been implicated in the pathophysiology of AD as transgenic AD mice carrying the human ACT gene exhibit accelerated amyloid plaque pathology development [88,89] and cognitive decline. ACT has also been shown to exert pathological effects in vitro [90]. IL-1β has been shown to increase the expression of ACT in human astrocytes, with a subsequent increase in synthesis of APP [88,91]. ACT has been shown to enhance both amyloid and Tau-associated pathologies. It has been shown to bind the toxic Aβ peptide and accelerate its fibrilization [92-95]. Studies from our lab have shown that treatment of primary mouse neurons with purified ACT protein results in Tau phosphorylation and apoptosis, implying a role for ACT in neurofibrillar tangle formation and neuronal loss associated with AD [96]. Furthermore, we determined that transgenic mice expressing human Tau and human ACT show enhanced Tau phosphorylation upon IL-1β injection, which is mediated by c-Jun N-terminal Kinase (JNK) signaling, indicating ACT and IL-1β work in collaboration to enhance Tau pathology development [97].

Another inflammatory mediator whose expression has been shown to be regulated by the cytokines IL-1β and TNF-α is Cyclooxygenase-2 (COX-2), which is responsible for the production of prostaglandin. Its function in inflammation is to induce vasodilation, thus mediating the transport of immune cells to injured tissue [98]. Interestingly, neurons are the main cell types that show increased expression of COX-2 in the AD brain [99-102], suggesting its role in the inflammatory response. COX-2 has been shown to not only correlate with AD pathology load (neurofibrillary tangles and neuritic plaques), but also with cognitive impairment [103].

**NEUROINFLAMMATION AND CELL CYCLE DYSREGULATION IN ALZHEIMER’S DISEASE**

Recent studies have explored aberrant cell cycle events in compromised neurons in the AD brain. While mature neurons are terminally differentiated and post-mitotic, neurons in the human AD brain show expression of a number of cell cycle regulatory proteins including Cyclins B, D1, D2, D3 and E [104-106]. In addition to the aberrant expression of these cell cycle markers, binucleation has been observed in the hippocampal neurons of AD patients, indicating cell cycle progression followed by defective cytokinesis [107]. Our lab has also observed ectopic expression of Cyclins D1, E and P-cdc2 (CDK1) in transgenic AD mice expressing mutant variants of APP alone or together with Presenilin 1 (PS1) [108]. We found that APP is phosphorylated at Thr668 in a mitosis-specific manner, leading to its association with centrosomes and pathogenic processing to generate enhanced Aβ peptide and C99 fragment [108]. Furthermore, the treatment of differentiated cortical neurons with oligomeric Aβ42 fragment has been shown to induce expression of cell cycle markers necessary for G1/S transition and, once entered into S phase, neurons begin to duplicate their DNA, which subsequently leads to apoptosis [109]. These findings implicate cell cycle deregulation as a possible underlying factor in neuronal loss associated with AD.

Pathogenic stimuli such as inflammation and oxidative stress have been shown to induce cell cycle events in the compromised brain [106,110-112]. In fact, there is increasing evidence for DNA synthesis and ectopic expression of cell cycle proteins such as cyclin E, cyclin B, cdc2 and CDK4 in the vulnerable neuronal populations in AD [110,111,115-117] and the aberrant cell cycle events seem to coincide with microglial activation [113]. Supporting this, one study has shown that the media from microglia treated with Aβ can induce abnormal neuronal cell cycle and subsequently, death [114]. Studies by another group showed that these cell cycle events closely correlated with secretion of TNF-α [121]. Further, preincubation of Aβ-treated microglia with TNF-α antibody significantly reduced BrdU incorporation in neurons treated with the conditioned media, implying that TNF-α secreted by the microglia is responsible for the observed cell cycle events in neurons [121]. Another study used an in vivo adoptive transfer model, where purified microglia from transgenic AD mice were injected into wild type mice that were either inoculated with IgG or anti-TNF-α antibody. Mice inoculated with anti-TNF-α antibody showed significant decrease in neuronal cyclin D1 levels [115]. This was highlighted by the fact that AD transgenic mice crossed with TNF-α null mice show basal levels of cyclin D1 in neurons [115]. Finally, the group looked at the role of JNK as it was found to be elevated in the transgenic AD mice. Using the previously described in vitro system, they discovered that treatment of neurons with SP600125, a specific JNK inhibitor, decreased BrdU incorporation upon treatment with conditioned media, suggesting that TNF-α induces cell cycle events through JNK signaling [115]. These studies indicate that it is likely that inflammation induced glial activation could lead...
to the aberrant expression of cell cycle proteins and subsequent neuronal death in the AD brain.

Expression of inflammation-associated genes is associated with cell cycle dysregulation. COX-2 has been implicated in cell cycle deregulation as its enhanced expression correlates with promotion of cancer growth and its inhibition declines the growth [116]. Studies have also drawn parallels between cell cycle changes and COX-2 expression in the neurons of AD brain. In addition to its elevated levels in the AD brain, COX2 is found to be co-localized with cyclin D1, cyclin E and phosphorylated retinoblastoma (Rb) protein in the pyramidal neurons, which may be indicative of aberrant cell cycle activation in neurons[117,118]. COX-2 homozygous transgenic mice show a marked decrease in the mRNA of the cdk inhibitor p18INK4 and exhibit accelerated glutamate-induced apoptotic damage, which could be attenuated by administration of the cdk inhibitor, flavopiridol [119]. This study also showed that wild type primary hippocampal neurons treated with the COX-2 inhibitor nimesulide diminishes glutamate-mediated apoptotic damage and inhibits subsequent phosphorylation of Rb [119].

TARGETING INFLAMMATION FOR TREATMENT OF ALZHEIMER’S DISEASE

In prospective case-cohort epidemiological studies, elevated serum levels of specific acute phase proteins have shown a positive correlation with development of AD [120-123]. Other studies have shown the beneficial effects of chronic use of non-steroidal anti-inflammatory drugs (NSAIDs) in slowing, or even preventing AD [124-127], and post mortem analysis of brain tissue from AD patients on NSAIDs has also revealed a consistent reduction in inflammation [128]. In vivo studies using transgenic AD mice show that long-term administration of the NSAID Ibuprofen both decreases neuroinflammation and improves cognition [113]. An early treatment regimen with Ibuprofen given to transgenic AD mice improved spatial learning and hippocampal-dependent memory function [129]. Early Ibuprofen administration also resulted in reduced amyloid plaque load, glial activation and expression of inflammatory mediators in the transgenic AD mouse brain [129-131]. Epidemiologists have been interested in studying patients with rheumatoid arthritis and osteoarthritis as these individuals exposed to NSAIDs for long periods of time show an inverse relationship between AD and treated arthritis [132].

It is debated how NSAIDs might exert these effects. Some studies find that NSAIDs act as modulators of γ-secretase by interacting with APP itself and shifting the pathogenic cleavage site to yield a shorter Aβ fragment [133-136]. Others have suggested that NSAIDs are capable of lowering β-secretase levels [137]. Finally, an in vitro study by Hirohata et al. found that NSAIDs have a direct inhibitory effect on formation of Aβ fibrils [139]. These observations however, have not held up in clinical trials. Individuals with clinical AD symptoms that were treated with NSAIDs showed no beneficial effects [139-141]. It is possible that the inverse relationship between rheumatoid arthritis and AD could be due to the intrinsic factors underlying the autoimmune disorder. Injection of transgenic AD mice with granulocyte macrophage-colony stimulating factor (GM-CSF), an inflammatory cytokine involved in leukocyte stimulation in rheumatoid arthritis, has been shown to reduce amyloid plaque burden, improve cognitive deficits and increase hippocampal synaptic area [142].

Many studies, however, maintain that there is a direct link in NSAID usage and reduced AD risk. A recent study modeled inflammation in AD through the activation of microglia by injecting lipopolysaccharide (LPS) into young AD mice, which were then aged. Tellingly, the administration of NSAIDs to young mice prevented AD pathology [113]. As a culmination of the reviewed relationship between inflammation, cell cycle dysregulation and Alzheimer’s disease, this study also indicated that early treatment leads to blockade of microglial activation and neuronal cell cycle events [113]. Late NSAID treatment in aged mice, while halting new neuronal cell cycle events, could not reverse existing ones [113]. This study underscores the close relationship between neuroinflammation and cell cycle in compromised neurons of the AD brain and suggests that early therapeutic intervention may lead to the best outcome.

CONCLUSION

In Alzheimer’s disease, pathologically vulnerable neurons undergo ectopic cell cycle events that are thought to result in neuronal apoptosis. Expression of cell cycle markers in these susceptible neuronal populations could be an improper attempt of regeneration of an injured nerve cell, unable to properly proceed through mitosis. Interestingly, ectopic cell cycle protein expression has been found to be induced by neuroinflammatory mediators. In addition to the histopathological hallmarks of AD, namely neuritic plaques and neurofibrillary tangles, AD brains also show neuroinflammation and neuronal loss. Gliosis and inflammatory mediators coexist with amyloid plaques and neurofibrillary tangles. It appears that neuroinflammation, while initially beneficial in protecting neurons from injury, can induce neurodegeneration upon chronic exposure. Furthermore, there is strong evidence for collaboration between expression of cell cycle regulators and inflammatory mediators within these pathologies. Epidemiological studies indicate that long-term use of NSAIDs positively correlate with a decreased risk in developing AD, however clinical trials using NSAIDs to treat patients with AD have been unsuccessful. Current research shows that preventative NSAID treatment could prove to be a beneficial strategy for treating AD. Studies in transgenic AD mice show that early NSAID treatment inhibits chronic inflammation within the brain and halts mitotic events in compromised neurons. It is unclear whether the downstream effects of cell cycle dysregulation triggers an inflammatory response or if neuroinflammation precedes these cell cycle events. In any case, it is likely that there is interplay between these complex biological events and timing is important in targeting these events for prevention of neurodegeneration associated with AD.

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