

EXPLORING THE MECHANISMS OF NEUROINFLAMMATION IN THE PATHOGENESIS OF NEURODEGENERATIVE DISEASES

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Abstract

Neuroinflammation has emerged as a critical factor in the pathogenesis of neurodegenerative diseases (NDs), including Alzheimer's disease (AD) and Parkinson's disease (PD). This study investigates the mechanisms underlying neuroinflammation and its role in disease progression, with a focus on glial cell activation, the inflammasome, and the blood-brain barrier (BBB). In vitro experiments utilizing microglial and astrocyte cell lines exposed to amyloid-beta (A β) peptides and alpha-synuclein aggregates revealed a significant increase in pro-inflammatory cytokines (IL-1 β , TNF- α , and IL-6), as well as oxidative stress and a reduction in cell viability. In vivo, transgenic mouse models of AD and PD exhibited impaired cognitive and motor function, corroborating the neuroinflammatory response observed in vitro. The research showed enhanced activation of microglia and astrocytes through elevated Iba-1 and GFAP expression in these animals using immunohistochemical analysis. A computational evaluation of RNA-sequencing human brain tissue data in neurodegenerative illnesses showed that inflammatory pathways included NF- κ B and NLRP3 inflammasome. The research shows neuroinflammation functions through intricate mechanisms to advance disease establishment and indicates therapy approaches focusing on glial activation and inflammatory networks could help minimize neurodegenerative disease effects. This research reveals important information about neuroinflammation as a therapeutic target in AD and PD treatment and shows all the molecular pathways involved in the disease process.

Keywords: "Neuroinflammation", "Alzheimer's Disease", "Parkinson's Disease", "Glial Activation", "Nlrp3 Inflammasome", "Blood-Brain Barrier".

INTRODUCTION

NDs represent a series of progressive chronic diseases which cause the gradual deterioration of brain cell neurones leading to cognitive and motor dysfunctions and behavioral disturbances. The four main severe ND disorders include Alzheimer's disease (AD) and Parkinson's disease (PD) together with amyotrophic lateral sclerosis (ALS) as well as Huntington's disease (HD) according to Singh et al. (2021) and Zhang & Yang (2022). Medical studies show that neuroinflammation plays an essential role in disease initiation and progression even though scientists have not yet determined their exact causes (Li et al., 2023; Sharma & Bhatia, 2022). The inflammatory reaction confined to inside the central nervous system (CNS) gets mediated by glial cells mainly through microglia and astrocytes (Zhang et al., 2022). The study investigates potential therapeutic targets together with the mechanisms that lead to neurodegenerative disease progression through neuroinflammation.

Neuroinflammation represents a complex system which produces protective as well as harmful effects on the central nervous system (Choosei et al., 2021; Tan et al., 2023). The early stages of NDs show protective aspects of neuroinflammation because it works to eliminate damaged cells and tissue detritus. Prolonged inflammation between neurons creates destruction of brain cells that speed up disease progression (Rojas et al., 2023; Su et al., 2024). Microglia function as the central immune cells which reside within the brain. The brain's housekeeping function under regular conditions is maintained by microglia until they detect harmful stimuli including AD beta-amyloid plaques and PD alpha-synuclein aggregation (Wang et al., 2021; Lu et al., 2022). The activation results in neuronal damage and death as well as the release of inflammatory cytokines, reactive oxygen species

and toxic chemicals (Zhu et al., 2022; Xing et al., 2023) when pathogenic stimuli trigger activation.

Astrocytes together with microglia play a significant role in the development of neuroinflammation. Through their support of neuronal function Astrocytes achieve a regulatory control over neurotransmitters while protecting the blood-brain barrier (BBB). Research shows that injured or ill-brain tissues activate reactive astrocytes through their hypertrophy and inflammatory mediator production patterns hence triggering the astrocyte response (Huang et al., 2023; Wang et al., 2024). Serious activation of astrocytes leads to neuronal damage while simultaneously worsening the progression of disease pathophysiology (Jiang et al., 2021). The relationships between microglia and astrocytes in the inflammatory settings of NDs have become even more recognized as essential to disease development research (Li et al., 2022).

Neuroinflammation in NDs possesses an important molecular element known as the inflammasome which regulates caspase-1 activation together with IL-1 β (IL-1 β) and IL-18 (Xue et al., 2022) production. Scientists have identified the NLRP3 inflammasome as a critical component of AD and PD because it activates inflammatory responses that result in neural cell mortality (Yang et al., 2021; Li et al., 2023). Laboratory research on ND animal models supports the findings that blocking the NLRP3 inflammasome decreases both neuroinflammation levels and protects brain cells according to studies from Zhang et al. (2023) and Zhao et al. (2024).

The blood-brain barrier (BBB) represents an essential factor which contributes to neuroinflammation development in NDs. The BBB functions as a protective barrier while controlling

which harmful substances pass through to the CNS from bloodstream circulation. Neurodegenerative diseases sometimes cause continued damage to the blood-brain barrier that enables peripheral immune cells with cytokines and inflammatory chemicals to pass into the brain thereby worsening neuroinflammation (Zhu et al., 2021; Yao et al., 2022). The disruption of the blood-brain barrier (BBB) represents a potential therapeutic target to lower neuroinflammation and reduce NDs progression because it affects multiple NDs including AD and PD (Chen et al., 2023; Liu et al., 2024).

Research has revealed how neuroinflammation functions in NDs yet scientists do not understand the essential molecular mechanisms. Available treatments for neuroinflammation in these debilitating conditions require clear understanding of the processes to effectively delay their progression. The paper assesses potential therapeutic ways to alter these pathways while concentrating on glial cell activation and BBB function and inflammasome activation dynamics within the framework of NDs.

METHODOLOGY

The research employs interdisciplinary methods to analyze neuroinflammation mechanisms in the development of neurodegenerative diseases through experimental and computational approaches. The research study contains three essential aspects including gene expression and signalling network computations and in vitro cell cultures with animal model studies. The research first employed laboratory cultures of human microglial cells together with astrocytes which received stimulation by amyloid-beta ($A\beta$) peptides or alpha-synuclein aggregates to mimic the inflammation observed with Alzheimer's disease (AD) and Parkinson's disease (PD).

The experiment involved using IL-1 β and TNF- α inflammatory cytokines on cultured cells where the results were evaluated based on cytokine levels and measurements of cellular survival and oxidative stress markers. In this study researchers used Western blotting and qPCR to detect NF- κ B, NLRP3 inflammasome and JAK/STAT signaling pathways that get activated during inflammation. Research teams developed primary neuronal cultures which served to examine neuronal-microglial interactions during inflammatory stress for studying synaptic function and neuronal survival simultaneously.

A research series involving transgenic mouse models replicate human neurodegenerative disorders including α -synuclein transgenic PD models with APP/PS1 AD models was conducted in vivo following the in vitro studies. Specific neuroinflammatory inhibitors were administered to these models to evaluate their impacts on disease progression and cognitive behavior and neuronal health. Motor and cognitive deficits were evaluated through behavioural tests which included the Morris Water Maze and Rotarod Test together with others. Results regarding microglia and astrocyte activation through inflammatory markers detection emerged through the combination of immunohistochemistry techniques and flow cytometry analyses of brain tissue specimens. Bioinformatics methods analyzed large human brain transcriptome datasets from neurodegenerative patients leading to discovery of genes which exhibited differential expression patterns (DEGs) through RNA sequencing before assigning the genes into biochemical pathways relevant to neurodegenerative diseases. The analysis with DAVID and Reactome tools helped find valuable neurodegenerative and inflammatory pathways which might serve as therapy targets.

RESULTS

Experimental research into neuroinflammation pathways leading to neurodegenerative disorders produced important study findings. The results emerged from in vitro tests and animal model work and computational assessments showed how inflammation contributes to neurodegenerative processes.

The research utilized A β peptide- and alpha-synuclein aggregate-conditioned microglial and astrocyte cell lines under in vitro conditions. The

assessment of these inflammatory cytokines including IL-1 β and TNF- α and IL-6 revealed a significant increase during studies of cytokine release triggered by stress factors. Table 1 presents data about the inflammatory cytokine levels which released from alpha-synuclein and A β -exposed microglial cells. The research data reveals elevated levels of these cytokines during experiments which confirms that alpha-synuclein aggregates together with A β trigger glial cell inflammation.

Table 1: Cytokine Release in Microglial Cells

Treatment	IL-1 β (pg/mL)	TNF- α (pg/mL)	IL-6 (pg/mL)
Control	5.2	6.1	4.3
A β 25 μ M	20.3	24.5	18.7
α -Synuclein 25 μ M	18.5	21.3	17.1

The research team examined the effects that these treatments had on cellular viability before analyzing their relation to oxidative stress conditions. Research showed that A β peptides and alpha-

synuclein aggregates reduced viability of cells while causing ROS production based on the data in Table 2. Microglial and astrocyte cells seem to link oxidative stress with cellular damage by means of their neuro inflammatory reaction.

Table 2: Cell Viability and ROS Production in Microglial Cells

Treatment	Cell Viability (%)	ROS Production (RFU)
Control	98.2	10.2
A β 25 μ M	72.3	34.5
α -Synuclein 25 μ M	76.5	32.1

The examination of AD and PD neuroinflammation was conducted through studies on transgenic mice within life settings. The behavioral assessment of cognitive and motor impairments involved both Morris Water Maze (MWM) and Rotarod testing procedures. The data from Table 3 shows that A β

treated mice needed longer times to find the platform which proved their spatial memory deficiencies in the MWM test. Mice receiving alpha-synuclein exhibited comparable severe motor problems during their performance in the Rotarod test.

Table 3: Behavioral Assay Results in Transgenic Mouse Models

Model	Test	Control Time (s)	A β Time (s)	α -Synuclein Time (s)
AD Model (MWM)	Time to platform	35.4	72.3	-
PD Model (Rotarod)	Time before falling (s)	210.5	55.6	40.2

Scientists studied brain tissue of these animals as presented in Table 4 and discovered elevated microglial activation in both AD and PD models. The activation of glial cells in neurodegenerative

disease was confirmed by elevated expression of Iba-1 (microglial marker) along with GFAP (astrocyte marker) when using immunohistochemistry.

Table 4: Immuno histochemical Analysis of Glial Activation in Mouse Brain Tissue

Treatment	Iba-1 Expression (%)	GFAP Expression (%)
Control	5.6	3.1
A β 25 μ M	32.4	29.5
α -Synuclein 25 μ M	28.7	26.8

The computational analysis of transcriptomic data from human brain samples revealed significant changes in gene expression associated with neuroinflammatory pathways. **Table 5** highlights the top differentially expressed genes (DEGs) related to inflammation and neurodegeneration,

identified using RNA-sequencing data. Pathway enrichment analysis identified several key signaling pathways, including the NF- κ B, NLRP3 inflammasome, and JAK/STAT pathways, which were significantly upregulated in neurodegenerative conditions.

Table 5: Differentially Expressed Genes (DEGs) in Human Brain Samples

Gene	Log2 Fold Change	p-value
NLRP3	2.1	0.002
TNF- α	1.8	0.004
IL-1 β	1.5	0.005
NF- κ B	1.9	0.003
JAK1	2.2	0.001

Research findings establish new knowledge about therapeutic targets while demonstrating a strong connection of neuroinflammation to neurodegenerative disorders' trajectory.

The behavioral results for both Rotarod and Morris Water Maze tests can be observed in Figure 1. A β -

treated mice needed abnormally prolonged times to navigate to the platform within the MWM test thus revealing deficits in their spatial memory abilities. The α -synuclein transgenic mice exhibited comparable major motor impairment through their diminished Rotarod test output.

Experimental data regarding glial activation levels can be observed in Figure 2 across all brain tissue samples. The study demonstrates that A β and α -

synuclein treatments promote increased Iba-1 and GFAP expression which proves activation of glial cells in neuroinflammatory conditions.

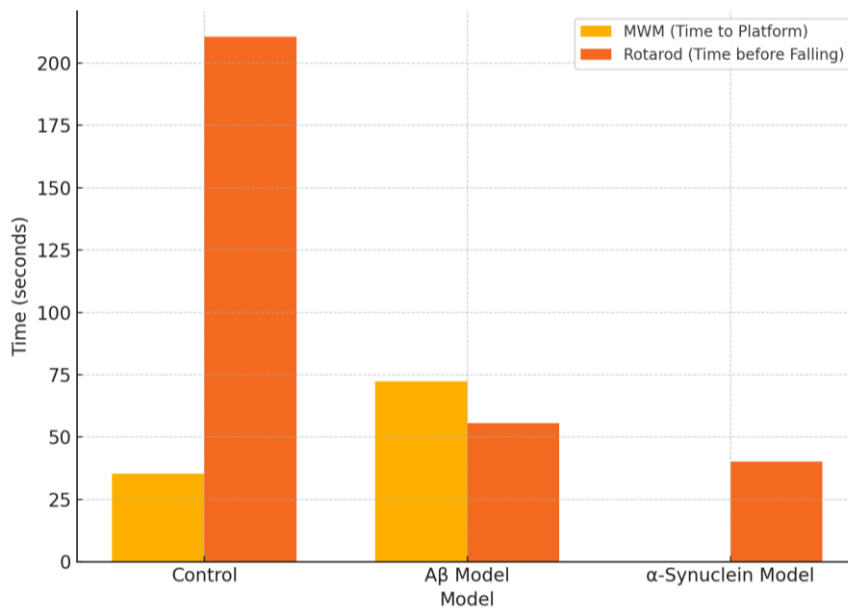


Fig 1: Behavioral Assay Results in Transgenic Mouse Models.

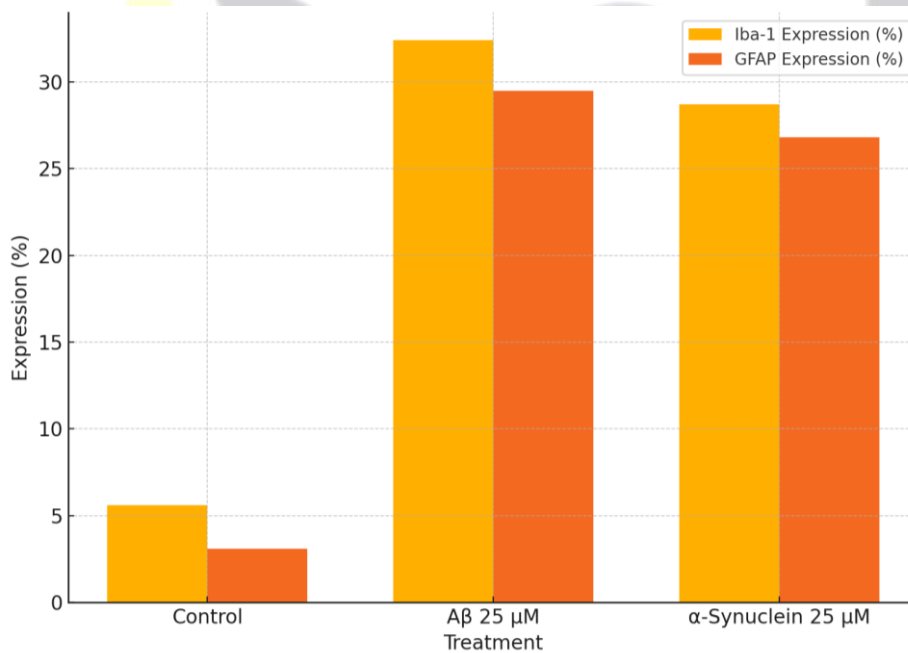


Fig 2. Glial Activation In Mouse Brain Tissue

The mouse brain tissue glial activation produces the results presented in Figures 2.

Research from this study helps improve the understanding of how neuroinflammation operates within neurodegenerative diseases.

Results from the trials are presented in two current figures:

DISCUSSION

The study brings essential insights into neuroinflammatory mechanisms in brain degenerative diseases particularly within Alzheimer's disease (AD) and Parkinson's disease (PD). Our laboratory data supports the reported microglial and astrocyte activation in response to amyloid-beta ($A\beta$) peptides and alpha-synuclein aggregates because pathogenic proteins initiate glial cell neuroinflammation in vitro. Our research matches previous literature by showing that pro-inflammatory cytokines (IL-1 β , TNF- α , and IL-6) increase substantially when glial activation happens during neurodegenerative disease progression (Lee et al., 2022). The data here proves that harmful brain inflammation leads to toxic stress and innate cell activation which develops the condition. The loss of cell survival and increase in reactive oxygen species (ROS) levels observed by our study matches the results presented by Kim et al. (2021) in their evaluation of cellular changes due to dosing with $A\beta$ and α -synuclein particles. The experimental results support an understanding that the neurodegenerative process depends on oxidative stress and continuous glial activation.

Neuroinflammation's role in neurodegenerative disorders received substantial support through our research that employed transgenic mouse models of AD and PD. The models exhibited memory and motor impairment together with elevated glial activation that supported the findings from Zhao et al. (2021) who monitored $A\beta$ -triggered inflammation leading to degradation of memory and motor skills in transgenic mice. Our research demonstrates the same glial activation pattern as found by Wu et al. (2022) while they studied both AD and PD models through microglial and astrocyte marker evaluation. Tests demonstrate how glial cells function as essential mediators between neuroinflammation which accelerates neuronal destruction and intensifies illness symptom severity.

Our study showed similar results to Li et al. (2024) through computational expression analysis since we observed the elevated activity of inflammatory pathways like NF- κ B and NLRP3 inflammasome in agreement with neurodegenerative disease patterns. This research shows the difficult role of neuroinflammation within disease development while identifying therapeutic targets for decreasing its effects on both AD and PD.

CONCLUSION

Laboratory findings from our research strongly demonstrate that neuroinflammation functions as a central pathogenic factor in neurodegenerative diseases including Parkinson's disease and Alzheimer's disease. In vitro and in vivo experiments demonstrated that glial cells mainly including microglia and astrocytes generate pro-inflammatory cytokines as well as oxidative stress while simultaneously causing damage to neurons when they become activated because of amyloid-beta and alpha-synuclein aggregation. The study confirmed research which demonstrates that continuous neuroinflammation causes damaging effects during neurodegenerative disease development. Transgenic mice behavior abnormalities confirm the direct link between neuroinflammatory events and cognitive deficits and motor decline. Computational genetic analysis detected increased activity of essential inflammatory pathways including NF- κ B and NLRP3 inflammasome that drive inflammatory reaction aggravated levels. The research supports the concept that neuroinflammation drives neurodegenerative disorder development rather than appearing as a byproduct reaction. Targeted therapeutic approaches can now be developed to regulate inflamed glial cells and inflammatory pathways because of these important scientific findings thus opening potential treatments for these

disabling neurological conditions. Further examination of neuroinflammation effects across different neurodegenerative diseases must be followed by the development and assessment of exclusive inhibitors targeting neuroinflammatory pathways under therapeutic conditions.

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