International Journal of Innovative Technology and Interdisciplinary Sciences
https://journals.tultech.eu/index.php/ijitis
ISSN: 2613-7305
Volume 7, Issue 1
DOI: https://doi.org/10.15157/ijitis.2024.7.1.24-30
Received: 11.03.2024; Revised: 03.05.2024; Accepted: 11.05.2024

Research Article

Inflammation-induced Deficits to Learning and Memory in C. elegans Through Notch Dysregulation

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Abstract

Neurodegenerative diseases are increasingly common in the aging population. Recently, increased inflammation has been observed in the brains of individuals with neurodegenerative diseases. In this work, we examine the role of inflammation in the regulation of learning and memory in Caenorhabditis elegans. C. elegans exposed to pro-inflammatory cytokines were subjected to a chemotaxis learning and memory assay. A significant decrease in learning and memory was seen, with the greatest decrease observed in the IL-6 treatment group. Exposure to the pro-inflammatory cytokine IL-6 caused a decrease in overall Notch1 and regulator Adm-4 expression. Dysregulation of the Notch pathway may provide a mechanism for the observed decrease in learning and memory following IL-6 exposure.

Keywords: Neurodegeneration; Learning and Memory; Notch; Inflammatory Cytokines; C. elegans

INTRODUCTION

Neurodegenerative diseases are characterized by slow and progressive destruction of the central nervous system [1]. These disorders prevent normal functioning of the brain by inducing cell death, preventing axonal regeneration, and damaging neuronal structure. The causes of these conditions are only partially understood, but the effects are easily seen through the loss of sensory, motor, and cognitive function. In addition, neurodegenerative diseases are known to produce an abnormal buildup of proteins, such as Aβ, in the brain and tissues [2]. The discovery of cytokines and other related receptors in the brains of patients with Alzheimer's disease (AD) enhanced our understanding of neuroinflammation and suggested that immunological processes in the brain may be involved in the onset of these neurodegenerative diseases [3]. Rothwell and Strijbos [4] found that in vivo interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-a) were rapidly activated in response to both induced and clinical neurodegeneration. Increased levels of pro-inflammatory cytokines, such as IFN-gamma,

TNF-a, and IL-6, and their receptors have been observed in AD brains, and interactions between IFN-gamma and Aβ lead to increased TNF-a and neuronal toxicity [5]. IL-6 has been shown to have a significant regulatory role in the brain. Specifically, IL-6 is released by microglia, which can have a negative impact on neuron survival [6] and lead to reduced neurogenesis when overexpressed [7]. C. elegans has been shown to have cytokine-like molecules such as CLEC-47, which show similar responses to immune stimuli as cytokines in mammals [8]. When expressed in murine macrophage cells, CLEC-47 can induce the expression of inflammatory cytokines, including TNF-alpha and IL-6 [8]. Previous studies have shown the presence of a TNF receptor-associated factor gene in C. elegans [9]. Many cytokines, such as the IL-6 family, utilize gp130 and JAK/STAT signaling to carry out their cellular activities in mammalian cells; C. elegans has a similar pathway with transcription factor STA-1 and kinase SID-3 as key components of the response to viral infection [10–11].

Notch signaling, specifically Notch1, is a highly conserved regulator of neurogenesis in adults [12]. Previous studies have shown that mice with Notch1 mutations have impairments in learning and memory [13]. ADAM17 (TACE) is a mammalian gene that regulates lin-12/notch signaling, and C. elegans has the ortholog, adm-4 [14]. Because Notch1 is a crucial step in neurogenesis in adults, we hypothesize that altering adm-4 expression could negatively affect neurogenesis, thus affecting short- and long-term memory.

Further research into the underlying mechanisms leading to neurodegeneration and the resulting cognitive deficits can help provide advances in treatments and prevention. There are currently no cures for neurodegeneration but there are medications that can extend memory functions temporarily and be used to lessen the symptoms associated with degeneration [15]. Understanding the connection between neuroinflammation, learning and memory, and the regulation of neurogenesis can lead to advances in our understanding of neurodegenerative diseases and provide novel treatments in the future.

MATERIALS AND METHODS

C. elegans Strains and Culture Conditions

The wildtype and mutant strain, BC10699 dpy-5(e907) I, were obtained from the Caenorhabditis Genetics Center (CGC, University of Minnesota), and worms were cultivated on nematode growth medium plates (NGM) seeded with E. coli OP50 at 22°C.

Age-Match Bleaching Technique

C. elegans were collected using distilled H2O (1 mL) and pipetted into a centrifugation tube (1.5 mL). Two washes per plate were performed. A bleach solution (2:1 bleach to NaOH) was added to each centrifugation tube with worms (0.4 mL) and vigorously shaken every two minutes for a total of ten minutes. After ten minutes, the samples were centrifuged for 30 seconds at 22°C at 16.0 RPM. All liquid was removed without disturbing the pellet and 0.4 mL of DI was added to each tube before repeating centrifugation. The

pellets were washed and centrifuged a total of three times. Pellets were combined, plated, and incubated at 22°C for three days.

Cytokine Exposure

1 ug/ml IL-6, IFN-gamma, or TFN-alpha (Peprotech) was added to the OP50 food medium for 3 days post-bleaching for the experimental group.

Memory and Learning Assay

Adapted from the protocol from Kauffman et al. [16], a chemotaxis assay is utilized for short-term learning and a memory assay with 30-minute holding to obtain a learning index.

Quantifying Gene Expression

Three days post-bleaching, worms were collected using M9 buffer (1 mL) and allowed to settle to the bottom of the tubes before the liquid was removed, and they were washed with M9 buffer. Slides were allowed to dry overnight. Fluorescent microscopy was performed, and images were assessed via the Image J area tool. The nerve ring of the worm was circled from side to side of the worm, widthwise. The fluorescence intensity was standardized by dividing the value by the area.

Notch 1 Quantification

To assess total Notch1 levels, protein lysates from control and IL-6-treated worms were analyzed via an Invitrogen Notch1 ELISA kit.

RESULTS AND DISCUSSION

Learning Index Decreases After Exposure to Inflammatory Cytokines

To determine if inflammatory cytokines are directly involved in their diminished cognitive ability, C. elegans were exposed to the inflammatory cytokines IL-6, IFN-gamma, and TNF-alpha, and short-term memory was examined via a chemotaxis learning and memory assay. The learning index was standardized to 1 for the control and each cytokine was compared to the control. IFN-gamma led to a modest (0.72, $p = 0.066$) but not significant decrease in memory (Figure 1). TNF-alpha led to a significant decrease in learning and memory (0.42, $p = 0.001$); however, IL-6 had the most drastic impact on learning and memory (0.10, $p = 6.2E-05$) in C. elegans (Figure 1). This data indicates that exposure to inflammatory cytokines can result in a decreased ability to learn and retain short-term memory.

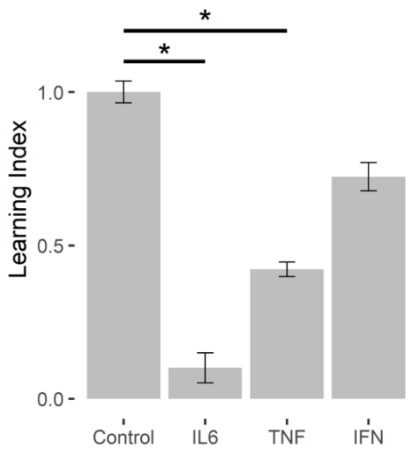

Figure 1. Exposure to inflammatory cytokines leads to decrease in learning and memory. *C. elegans* were exposed to inflammatory cytokines for 3 days and then subjected to a learning and memory assay to measure a learning index. In IL6 and TNF exposure the decline is significant. The learning index is standardized to control. P<0.05.

IL-6 Exposure Leads to Decrease in Notch Protein

Given that IL-6 had the most significant impact on learning and memory, we set out to examine potential mechanisms leading to this decrease. It is known that the Notch signaling pathway is essential to neurogenesis [12] and decreases in signaling lead to decreased memory in mice [13]. Whole protein lysates from control and IL-6-treated C. elegans were collected and Notch1 protein levels were assessed via ELISA. IL-6 exposure led to a significant decrease in total Notch1 protein (IL-6 116 pg/ml, Control 418.6 pg/ml, p = 0.02) (Figure 2).

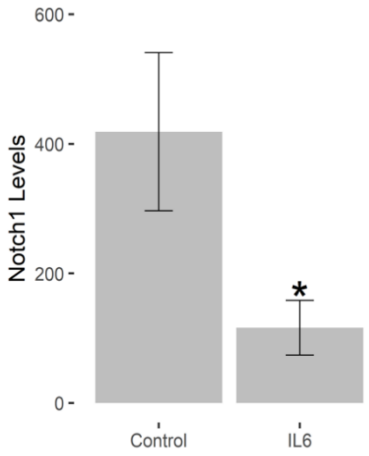

Figure 2. *C. elegans* were exposed to IL6 for 3 days and then whole worm lysate was collected and processed for ELISA. Following exposure to IL6, *C. elegans* show an overall reduction in total Notch1 (pg/ml) as measured via ELISA. p<0.05

Decrease in ADM-4 Expression Following IL6 Exposure

To further explore the mechanism leading to decreased learning and memory, a closer look at the regulation of Notch in neurons was explored. The nerve ring is a major site of neural control in C. elegans, so we examined the expression of the key regulatory protein, adm-4, in the nerve ring of control and IL-6-treated C. elegans. Utilizing worms that had been GFP-tagged at adm-4, we quantified the average fluorescence intensity at the nerve ring to measure average adm-4 expression. C. elegans exposed to IL-6 had significantly reduced average fluorescence intensity (arbitrary units) at the nerve ring compared to the control (IL-6 2.16, Control 2.97, $p = 0.01$) (Figure 3).

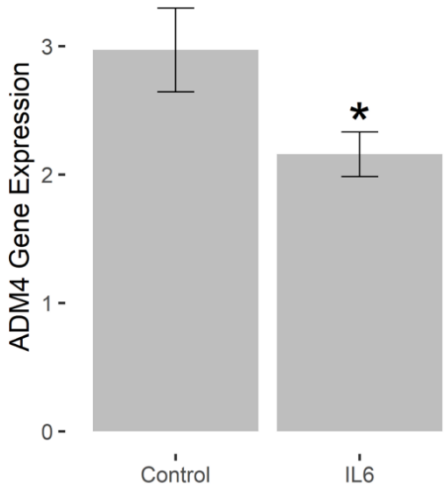

Figure 3. GFP tagged *C. elegans* were exposed to IL6 for 3 days and then the nerve ring was examined for average fluorescence. When *C. elegans* are exposed to IL6, there is a significant reduction in ADM-4 expression at the nerve ring, as measured by average GFP expression. $p<0.05$.

Implications of findings

This study suggests that chronic exposure to inflammatory cytokines can lead to a significant decrease in learning and memory, similar to what is seen in neurodegenerative diseases such as Alzheimer's disease in humans. It has been observed that increases in inflammation, specifically cytokines such as IL-6, are present in the brains of individuals with these diseases, which leads to the neuronal death, yet mechanisms for this death and therapeutic targets have yet to be identified [15]. With these findings shown here, a potential target in the Notch pathway has been identified. The Notch pathway is an important regulator of adult neurogenesis. A recent review indicated that neurogenesis is vital to normal cognition and impaired neurogenesis is in part responsible for the cognitive deficits seen in Alzheimer's disease mouse models, with Notch1 being one of the signaling pathways that is altered [17]. This work provides an exciting new potential model to

explore to further the understanding of the mechanism of inflammation induced neurodegeneration and potential therapeutic targets.

CONCLUSION

The significant decrease in learning index for cytokine-treated C. elegans indicates that inflammation can impact learning and memory ability through regulation of the Notch signaling pathway. These data suggest that neurodegeneration may involve an inflammatory immune response. Previous studies have shown that inhibition of ADAM17 (an ortholog of adm-4) activity can lead to significant reductions in Notch signaling [18] and ADAM17 is known to cleave several cytokine receptors, including IL6R [19]. Our results showed that inflammation induced by IL-6 in C. elegans led to decreased expression of adm-4 and disruption of Notch1. The loss of Notch1 signaling shown here likely prevented its normal role in promoting neurogenesis and if that impacted the hippocampus, it would lead to a disruption in the learning and memory processes.

Finally, IL-6 is currently of special interest due to its role in COVID-19 disease progression. Many individuals with long-term COVID following the SARS-CoV2 infection have lingering neurological symptoms, including fatigue and memory issues. Recent research has suggested that IL-6 may mediate the long-term COVID process [20]. Based on the data shown here, further investigation into IL-6 signaling in the brain could provide essential insights into neurological disease pathology and treatment.

CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

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