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A Microglia-Containing 3D Human Brain Organoid for Studying HSV-1-Induced Alzheimer's Disease

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Abstract:

The pathogenesis of Alzheimer's disease (AD) has been widely associated with amyloid-β (Aβ) plaques and neurofibrillary tangles (NFTs). Recent research suggests that herpes simplex virus type 1 (HSV-1) may play a role in the development of AD by triggering neuroinflammation. Microglia, as the immune cells of the central nervous system, contribute significantly to AD-related neuroinflammatory processes, but their precise role in HSV-1-induced AD is not fully understood. This study aimed to explore the relationship between HSV-1 infection and AD pathology using microglia-containing human brain organoids (MC-HBOs). We found that after infecting MC-HBOs with HSV-1, there was a significant increase in Aβ deposition, NFTs, and neuron loss. Microglia appeared to facilitate amyloid plaque formation and neurofibrillary tangle development, as well as participate in the clearance of Aβ. The activation of microglia also correlated with elevated levels of pro-inflammatory cytokines such as IL-6, enhancing neuroinflammation. Furthermore, inhibiting IL-6 expression in microglia reduced Aβ aggregation, suggesting that microglia may be a therapeutic target for preventing or slowing the progression of AD. This model highlights the important role of microglia in HSV-1-induced AD and offers potential strategies for future therapeutic interventions.

Keywords: Alzheimer's disease, microglia, HSV-1, amyloid-β, neuroinflammation

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that has a slow course, which results in a gradual deterioration of higher cognitive functions, memory, and behavior[1,2]. It is the leading cause of dementia in those who are 65 years of age and older. The pathogenesis of AD is a multifactorial process that includes several molecular and cellular changes. Another characteristic of AD is the formation of amyloid plaques which is caused by accumulation of extracellular deposits of Aβ peptides produced from the APP. Another hallmark of Alzheimer's disease is neurofibrillary tangle formation, which consists of

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abnormally phosphorylated forms of tau protein that interfere with the normal functioning of neurons. These tangles affect the transport within the neurons and thus cause neuronal dysfunction and cell death. Also, the disease is characterized by synaptic and neuronal losses, which are also the causes of cognitive impairments seen in the patients. Microglia which are the immune cells of the CNS are involved in the neuroinflammation process that characterizes AD[4]. To the Aβ and tau pathology, microglia become activated and release pro-inflammatory cytokines, which can enhance neuronal injury, and increase the deposition of Aβ.

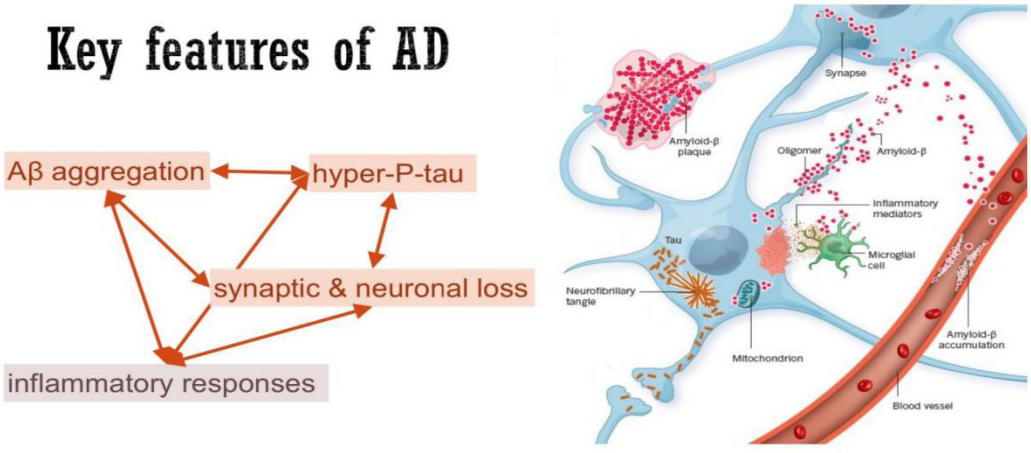

Figure 1: Key features of AD.

The present work seeks to establish the contribution of microglia in HSV-1-mediated AD pathology employing a 3D human brain organoid model that encompasses microglia (MC-HBOs). In this study, we will try to explore the process through which herpes simplex virus type 1 (HSV-1) infection, microglia activation, and Alzheimer's disease (AD)-related pathologies are related to one another to provide insights into how viral infection may lead to AD development and progression.

2. Methods

Generation of Microglia-Containing Human Brain Organoids (MC-HBOs)

Differentiation of Human Induced Pluripotent Stem Cells (hiPSCs) into Neural Precursors

To maintain the self-renewal of the cells we first cultured the human induced pluripotent stem cells (hiPSCs) in mTeSR medium[2]. On day 0 we created embryoid bodies (EBs) using a culture medium known as embryoid body formation medium (EBFM) supplemented with B27 and Y27.This brought about the process of changing the cells.

Neural Differentiation

On day 1, we treated the EBs with SB and LDN to stop certain signals that could interfere with turning into neural cells. From day 8 to day 14, we added FGF2 to help the cells become neural precursors.

Differentiation into Microglia

At the same time, we were turning another batch of hiPSCs into microglia. We used a medium with BMP4, IL3, and GM-CSF from day 30 to day 50. These microglia showed markers like CD11B, CD45, IBA1, CX3CR1, and P2RY12, proving they were microglia.

Formation of Human Brain Organoids (HBOs)

On day 14, we put the neural precursors into Matrigel, a gel-like substance that supports the cells to grow into 3D structures, making human brain organoids (HBOs).

Co-Culture of Microglia with Brain Organoids

By day 30, these HBOs were ready to mix with the microglia, forming MC-HBOs. The experimental group had these MC-HBOs, while the control group had HBOs without microglia. We did RNA-seq analysis on both groups on day 33 to study them.

Infection with HSV-1

Culture and Infection

We grew both the MC-HBOs and control HBOs for 33 days. After this period, we infected both groups with HSV-1 and grew them for an additional 3 days. This step was important to simulate the viral infection and see its impact on Alzheimer's disease features within the organoids.

Immunofluorescence and Antibody Staining

Assessment of Aβ Deposition

For the detection of Aβ, we employed the thioflavin T (ThT) staining, a dye that is known to bind to amyloid fibrils. We noticed that when the brain organoids were infected with HSV-1 there were more Aβ deposits in the presence of microglia. This was evident with ThT staining

whereby the amyloid fibrils were easily distinguishable within the organoids.

Engulfment of Aβ by Microglia

We also evaluated the relationship between microglia and Aβ. We used ThT to stain Aβ and TMEM119 antibodies to stain microglia. It was evident that there was an overlap of these labels to reveal that microglia were internalizing Aβ meaning that they are involved with the clearance of Aβ.

Neurofibrillary Tangle (NFT) Formation

Moving on, we considered the neurofibrillary tangles (NFTs) which are the result of tau protein over-phosphorylation. Hyperphosphorylated tau protein is known to cause the above changes and we detected it using phospho-tau antibodies. The results indicated that microglia promoted the generation of NFTs after HSV-1 infection, a hall mark of Alzheimer's disease.

Assessment of Neural Loss

We employed immunofluorescence to label these neuronal-specific proteins including TUJ and MAP2. The findings of the study revealed that the MC-HBOs were smaller than controls meaning that there was neural loss. This suggests that microglia may have a part in neuron death in this Alzheimer's disease model.

Evaluation of Gliosis and Neuroinflammation

To study gliosis and neuroinflammation, we did immunofluorescence staining for the glial marker GFAP and used PCR to measure pro-inflammatory and anti-inflammatory cytokines. Microglia presence led to more gliosis and neuroinflammation, both key features of Alzheimer's disease.

Inhibition of Cytokine Expression

Construction of IL-6 Knockdown Model

To inhibit cytokine expression, we made an shRNA sequence targeting IL-6 and put it into microglial cells using an HSV-1 vector. We confirmed the IL-6 knockout with Western blot.

Observing Aβ Distribution After IL-6 Knockdown

After stopping IL-6 expression, we used ThT staining to see Aβ distribution. Inhibiting IL-6 led to less Aβ aggregation in HSV-1 infected MC-HBOs.

Results

In this study, we investigated the role of microglia in HSV-1-induced Alzheimer's disease using microglia-containing human brain organoids (MC-HBOs). After infecting both the MC-HBOs and control HBOs with HSV-1, we used thioflavin T (ThT) staining to identify Aβ deposits. The findings pointed to the fact that the transplantation of microglia in the MC-HBOs caused a general increase in Aβ deposition in comparison to the control group (Figure 2). This implies that microglia are important in boosting amyloid pathology during HSV-1 infection hence supporting the hypothesis.

We extended our analysis of the relationship between microglia and Aβ by labeling Aβ with ThT and microglia with TMEM119 antibodies. The fluorescent signals were observed to overlap suggesting that microglia were in the process of phagocytosis of Aβ in the MC-HBOs (Figure 3). This finding is consistent with the role of microglia in affecting the clearance of amyloid plaques.

To evaluate the development of NFTs, we used the antibody staining of phospho-tau that recognizes the phosphorylated tau protein. Microglia were present in the MC-HBOs and this resulted in an enhanced formation of NFTs post HSV-1 infection than the control group (Figure 4). That is why it can be suggested that in this model of Alzheimer's disease, microglia are involved in tau pathology.

Immunofluorescence staining using neuronal-associated proteins TUJ and MAP2, we found that MC HBOs were significantly smaller than control HBOs. This finding suggests that there is a vast loss of neurons where microglia are present when infected with HSV-1 (Figure 5). Our finding that the sizes and neuron densities of the MC-HBOs are smaller than those of the MCs indicates that microglia may participate in neurodegenerative processes in this model.

To investigate the effects of microglia on gliosis and neuroinflammation, we performed immunofluorescence staining using the astrocytic marker, GFAP, and ELISA for cytokines. The analysis revealed that enhanced microglia activation led to elevated reactive gliosis as well as increased levels of pro-inflammatory cytokines including TNF-α and IL-6 whereas the levels of anti-inflammatory cytokines IL-10 and IL-4 were also decreased (Figure 6 and 7). This suggests that microglia cause an inflammatory profile in the MC-HBOs post HSV-1 infection.

To investigate the possibility of cytokines being a therapeutic target, we synthesized a shRNA sequence that targets IL-6 and transduced it into microglial cells by HSV-1. Using Western blot we confirmed the knockout of the IL-6. As a result of the down-regulation of IL-6, aggregation of Aβ was found to be reduced in the HSV.

3. Results

In this study, we attempt to define the function of microglia in the development of HSV-1-associated Alzheimer's disease using MC-HBOs. After exposing the MC-HBOs and the control HBOs to HSV-1, we performed ThT staining to detect Aβ aggregation. The findings presented herein revealed that the establishment of the MC-HBO model induced a robust infiltration of microglia associated with elevated levels of Aβ deposition compared to the control group (Figure 2)[1,4]. This indicates that microglia have an important function in aggravating amyloid pathology concerning HSV-1 infection. We then analyzed the co-lo-

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calization of ThT-labeled Aβ and TMEM119-antibody-labeled microglia. As the fluorescence signals overlapped with each other, the result suggested that the microglia in the MC-HBOs were engaged in the process of phagocytosis of Aβ. This finding also suggests the utilization of microglia in the removal of amyloid deposits. To examine the formation of NFTs, we used phospho-tau antibody staining since phospho-tau is a hyperphosphorylated tau protein. This was because of the activation of microglia in the MC-HBOs which enhanced the production of NFTs following HSV-1 infection as compared to the control group.

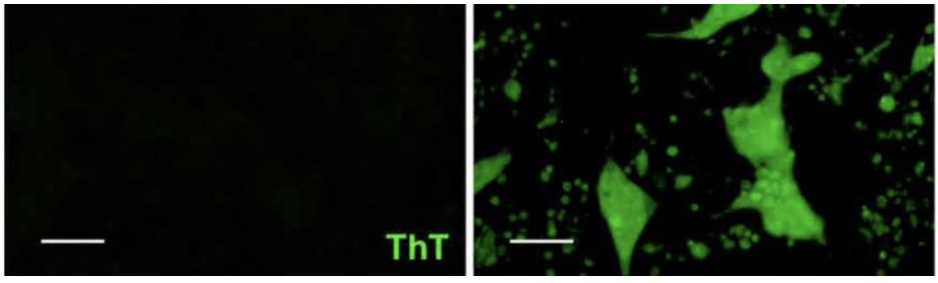

Figure 2. Presence of microglia increases Aβ deposition after HSV-1 infection.

We then dissected the relationship between microglia and Aβ by staining Aβ with ThT and microglia with TMEM119 antibodies. The merged signals suggested that microglia were phagocytosis Aβ in the MC-HBOs (Figure 3). This finding reveals the microglial function of the phagocytic removal of amyloid deposits.

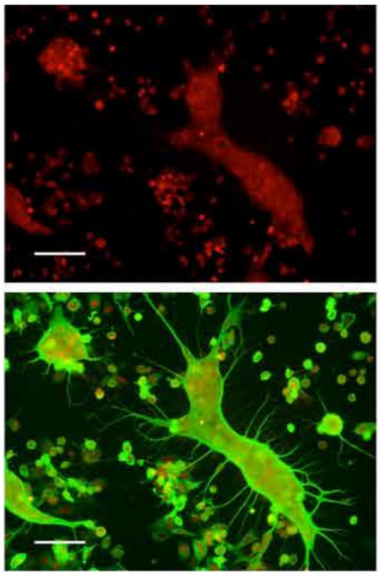

Figure 3. microglia engulf Aβ in the HBO

To evaluate the formation of NFTs, we used an anti-phospho-tau antibody since it is a marker of pathologic tau protein[5]. The microglia infiltration in the MC-HBOs facilitated the rise in NFT formation in the HSV-1 infected group as compared to the control group (Figure 4).This indicates that microglia play a role in tau pathology in this model of Alzheimer's disease.

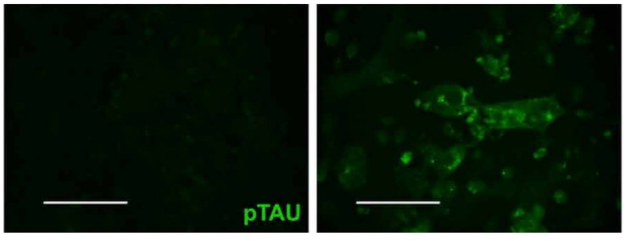

Figure 4. Presence of microglia enhances NFTs generation after HSV-1 infection.

To confirm the changes in the size of MC-HBOs and control HBOs, we performed immunofluorescence staining of neuronal proteins TUJ and MAP2. This implies that there is evidence of neural loss in the presence of microglia after HSV-1 infection (Figure 5). The smaller size and the lower neuron density in the MC-HBOs may indicate that microglia are involved in neurodegeneration in this model.

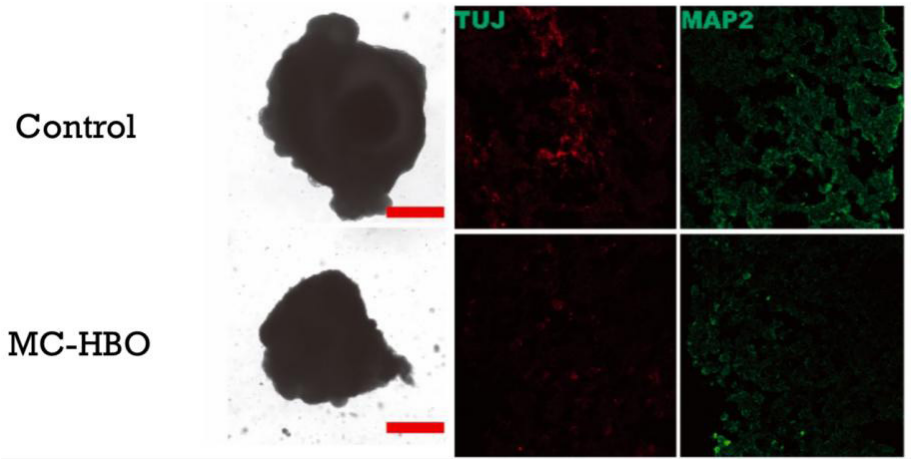

Figure 5. MC-HBO exhibits a more obvious neural loss.

To further investigate the involvement of microglia in gliosis and neuroinflammation we used immuno-fluorescence to stain the tissue for the astrocytic marker GFAP and PCR to assay cytokine levels. These outcomes suggested that the presence of microglia has caused the elevation in reactive gliosis levels and the levels of pro-inflammatory cytokines such as TNF-α and IL-6 which are higher than those of anti-inflammatory cytokines including IL-10 and IL-4 as presented in Figure 6 and 7. This implies that microglia contribute to an inflammatory milieu in the MC-HBOs after an HSV-1 infection.

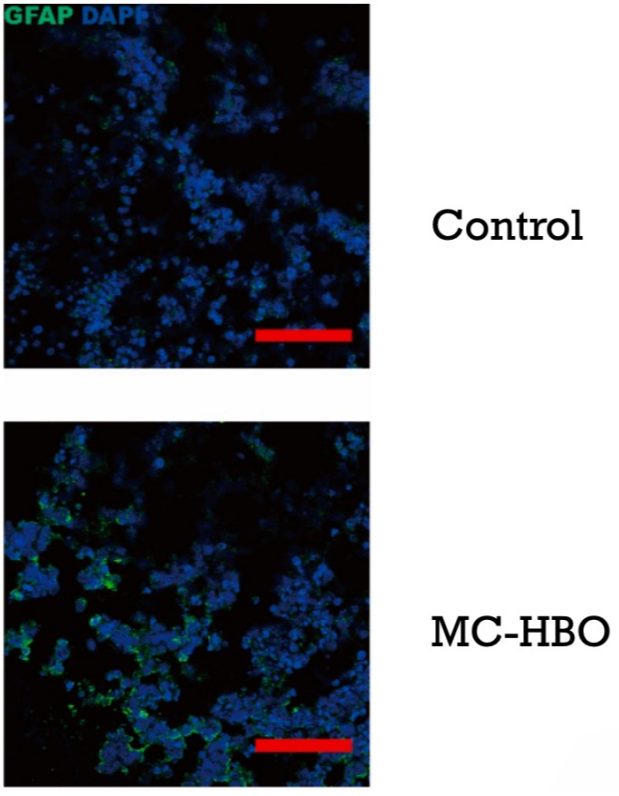

Figure 6. Presence of microgliaresults in increased gliosis.

To evaluate the possibility of using cytokines as therapeutic targets we designed a shRNA sequence targeting IL-6 and delivered it into microglial cells via HSV-1. We confirmed IL-6 deletion by Western blot analysis. The silencing of IL-6 expression resulted in the decrease of Aβ deposition in the HSV-1 infected MC-HBOs, which suggested that this strategy could be applied to Alzheimer's disease for the prevention of amyloid formation.

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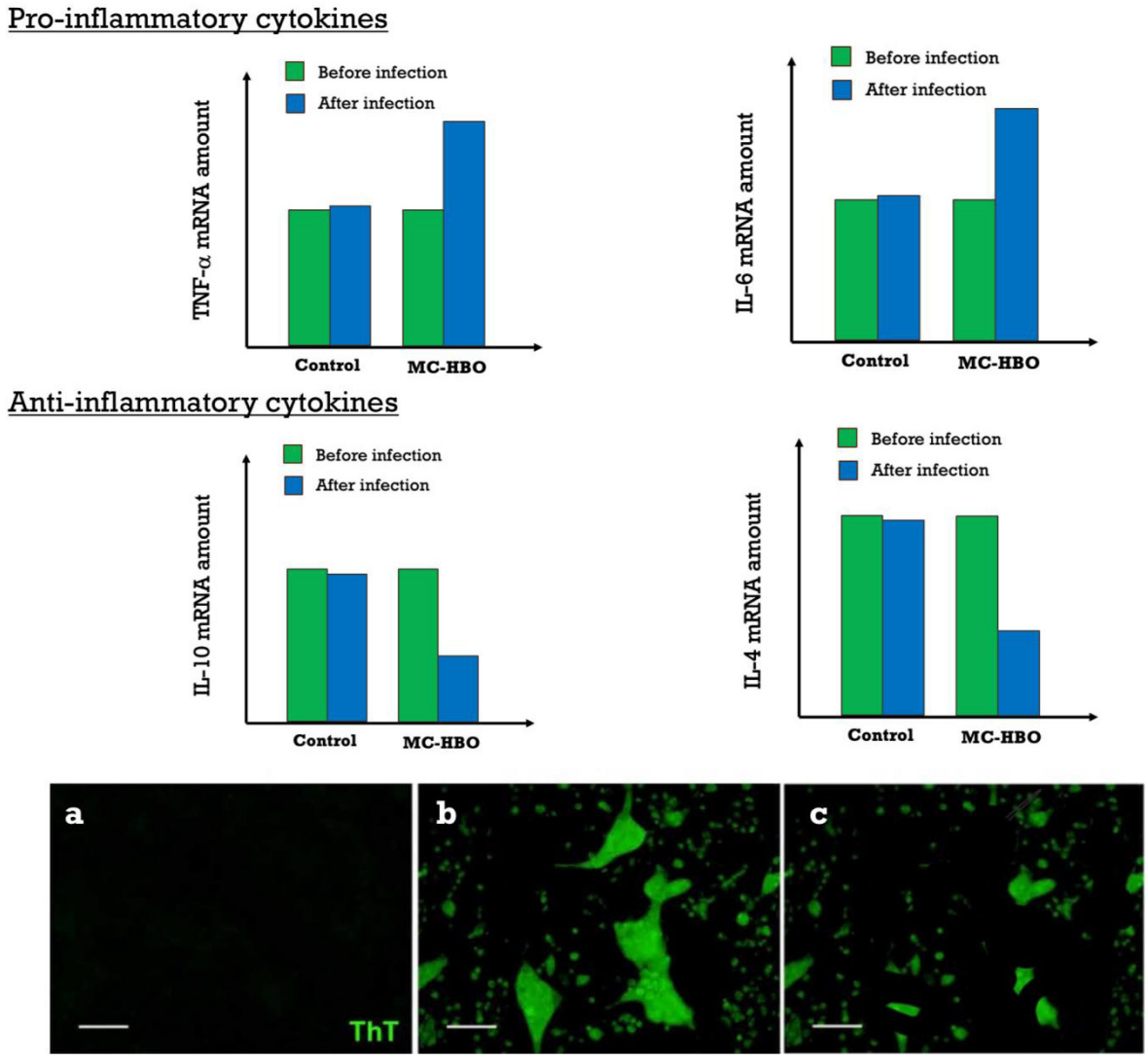

Figure 7. Predicted result of Aβ distribution after cytokines inhibition. Aßdistribution (a) in HSV-1 infected HBO; (b) in HSV-1 infected MC-HBO; (c) in HSV-1 vector infected MC-HBO, with IL-6 knockout

This structure will allow the direct connection of each figure with the corresponding method or result, without confusing the reader of the paper.

4. Discussion

Our study employed human brain organoids containing microglia (MC-HBOs) to show how HSV-1 could contribute to AD. The outcomes of the experiment revealed microglial contribution to the formation of AD characteristics including Aβ plaques, NFTs, neuronal loss, and neuroinflammation.

First, we observed that microglia boosted Aβ burden in the brain organoids following HSV-1 infection[4]. This implies that the microglia may be assisting the virus to cause the brain to be vulnerable to AD. A microglial phagocytic activity where Aβ is engulfed confirms that microglia is not only present but also actively participating in Aβ deposition. This is vital as it raises a possibility that the microglia might be worsening the situation through their efforts of removing the Aβ and in the process enhancing its accumulation. The presence of microglia also correlates with the increase in NFTs in AD thus implying their role in the pathogenesis of AD. Another major feature of AD is the presence of NFTs, which is caused by hyperphosphorylation of tau protein. It also appears that microglia may be involved in this process, perhaps by recognizing viral infection and then allowing for the development of tau pathology. Based on our findings of high neural loss in MC-HBOs as compared to control HBOs, it can be inferred that microglia could be detrimental to AD. The decrease in neuron size and the number of neurons per

unit area may mean that microglia are harming the brain, even as they try to protect it and lead to neuron death. Gliosis and neuroinflammation were more enhanced in the microglial activation as evidenced by the upregulation of GFAP and pro-inflammatory cytokines such as TNF-α and IL-6[5]. This inflammatory response is a two-sided sword; although it is a part of the body's defense mechanism, chronic inflammation exacerbates neuronal damage and aggravates AD development. We also investigated the possibility of blocking cytokines such as IL-6 in the hope of decreasing AD pathology. The inhibition of IL-6 expression in microglia resulted in a decrease in Aβ deposition indicating that targeting the immune system could be an approach to treatment. This is encouraging given the fact that it opens up a possible avenue to halt or reverse the advance of AD by controlling certain parts of the immune system.

In essence, this work demonstrates that microglia could be detrimental to the development of AD particularly in the context of HSV-1 infection. Microglia are not only bystanders in the development of pathological hallmarks of AD but are also involved in the process. These observations suggest that understanding these interactions to a greater extent will enable the creation of potential therapeutic strategies that could neutralize the deleterious aspects of microglia while sustaining the beneficial ones. Further investigations should be directed towards examining other cytokines and the pathways that are related to microglial activation and their effects on AD, thus giving a broader insight into this deadly disease.

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