Up-regulation of the IL-1 signaling pathway enhances gene expression of transcription factors (Jun, Junb, Jund, Fos, and Fosb)

Jiale Mao

Abstract:
This paper focuses on the experiment method to find out the relationship between the IL-1 signaling pathway and the transcription factors; we use 5xFAD mice with targeted deletion of Bace-1 in microglia, We conjecture that the up-regulation of the IL-1 signaling pathway enhances gene expression of transcription factors (Jun, Junb, Jund, Fos, and Fosb), which probably increases the transition microglial signature. The specifically targeted inhibition of BACE-1 in microglia will likely offer a superior therapeutic alternative for improving AD outcomes, circumventing the safety concerns associated with neuronal deletion or global inhibition of BACE-1.

Keywords: Alzheimer’s disease; IL-1 signaling pathway; microglia; IL-1 R2; expression of Bace-1

1. INTRODUCTION
It has long been established that β-amyloid(Aβ) is produced through sequential cleavage of amyloid precursor protein (APP) by β-secretase and γ-secretase, then forms plaques. These Aβ plaques are known to cause synaptic dysfunction and cognitive impairment in Alzheimer’s disease patients. (1) BACE-1 (β-site amyloid precursor protein cleaving enzyme-1) is the major β-secretase generating Aβ peptides. (2) Studies have shown that BACE-1 knockout in Alzheimer’s 5xFAD mice models reduces the amount of Aβ peptides in their brain, which improves synaptic function. (3) However, BACE-1 inhibitor for AD patients did not show cognitive benefits but a rapid loss of hippocampal volume and synaptic dysfunction. (4)

Therefore, we propose that the BACE-1 or BACE-1 gene may have other functions in microglia besides cleaving APP in neurons. Targeted deletion of Bace-1 in mice increases the transition from homeostatic microglia to more functional, phagocytic stage 1 disease-associated microglia (DAM-1). Also, it causes up-regulation of transcription factors such as Jun, Junb, Jund, Fos, and Fosb in the transition signature. It also results in improved amyloid clearance and improved cognitive performance for mice. (5,8) BACE-1 could also cleave the type 2 Interleukin-1(IL-1) receptor (IL-1R2) to form soluble form (sIL-1R2), this sIL-1R2 can bind to pro-IL-1, preventing caspase-1 from enzymatically converting pro-IL-1 to mature IL-1. (6,7)

These findings prompt us to investigate how the expression of Bace-1 impacts microglial transitions. This may provide future directions for future research on Alzheimer’s drugs. We offer some potential strategies to address this query. We believe that the most likely outcome is an increase in the expression of Transcription factors (TFs) caused by the inhibition of the IL-1 signaling pathway by sIL-1 R2, which results in an up-regulation of the DAM-1 signature gene in microglia.

2. METHOD
In our investigation, we will use normal healthy wild-type mice as the control group, 5xFAD mice, 5xFAD mice with targeted deletion of Bace-1 in microglia, and microglia cell cultures. To eliminate Bace-1 in 5xFAD mouse microglia, we could cross Bace-1 flox mice (Bace-1fl/fl) with mice genetically modified to express Cre recombinase under the control of Cx3Cr1 promoter. (8) Western blot will be used to examine phosphorylated IL-1 receptor-associated kinases (IRAK) downstream of the IL-1 signaling pathway, the phosphorylation of this kinase is positively related to the IL-1 signaling pathway.

Enzyme-linked immunosorbent assay (ELISA) will test the sIL-1R2 concentration.

RT-qPCR will be used to measure the mRNA fold-change of transcription factors (Jun, Junb, Jund, Fos and Fosb), it represents the activity of TFs. The scRNA-seq technique will be used to examine the expression of specific genes in different stages of microglia, aiming to identify the stage with a larger amount of microglia.

3. EXPERIMENT 1
The purpose of Experiment 1 is to ascertain whether an increase in sIL-1 R2 concentration is associated with a greater activation of the IL-1 signaling pathway. In our in vitro experiment, we utilized 15 microglial
cell cultures, with each group of 5 cultures treated with varying concentrations of sIL-1R2. siRNA was employed to delete IL-1R2, creating a milieu with reduced sIL-1R2 concentration, while five untreated cultures were used as control groups. Therefore, we employ a western blot to compare the IL-1 signaling in microglia under varying concentrations of sIL-1R2.

For our in vivo experiment, 15 wild-type mice will be utilized: 5 mice with targeted deletion of IL-1R2 in microglia and five additional mice injected with sIL-1R2 will serve as the experimental group. The remaining five mice without treatment will act as the control group. Following a designated period, the mice will be sacrificed for subsequent western blot.

According to our expectation, an elevation in sIL-1R2 concentration was correlated with heightened activation of the IL-1 signaling pathway.

4. EXPERIMENT 2
To validate the upregulation of BACE-1 and its consequential elevation in sIL-1R2 levels while concurrently attenuating or suppressing IL-1 signaling cascade, we fed wild-type mice with targeted deletion of Bace-1 in microglia, wild-type mice with over-expressing Bace-1, and normal healthy mice then use ELISA kit to measure the sIL-12 concentration and western blot examine the IL-1 signaling pathway activity. Subsequently, the sIL-12 concentration was quantified using an ELISA kit. In contrast, the activity of the IL-1 signaling pathway was assessed through western blot analysis after euthanizing the mice at a specific time.

The mice lacking Bace-1 in microglia are expected to demonstrate a decreased concentration of sIL-1R2 and an enhanced activity within the IL-1 signaling pathway.

5. EXPERIMENT 3
In order to determine whether activation of the IL-1 signaling pathway enhances the activity of TFs (Jun, Junb, Jund, Fos, and Fosb), in our in vitro experiment, 15 microglia cell cultures are separated into three groups, Group A) decrease IL-1R1 expression by using CRISPR-Cas9 Group B) control group Group C) increase IL-1 R1 expression by using CRISPR-Cas9. Then, RT-qPCR was used to verify the expression of TFs.

For the in vivo experiment, we will allocate 15 mice into three groups based on varying levels of IL-1R1 expression in microglia using CRISPR-Cas9 technology and subsequently euthanize them immediately to conduct RT-qPCR analysis.

In our expectation, the outcome depicted in Figure 1 demonstrates the expression of TFs (Jun, Junb, Jund, Fos, and Fosb). A significant difference among the three groups indicates a positive correlation between the IL-1 signaling pathway and TF expression.

6. EXPERIMENT 4
We use scRNAsq to examine the signature gene in microglia within homeostatic, DAM-1, DAM-2, and transition states.

According to our hypothesis, targeted knockout of the IL-1R2 gene fragment in microglia decreases the secret of sIL-1R2, and the IL-1 signaling pathway is highly activated, thus enhancing TFs activity, which facilitates the expression of the DAM-1 signature gene. Therefore, the result in Fig.2, comparing the signatures between 5xFAD targeted knockout IL-1R2 in microglia and 5xFAD without knockout, we observed a significant reduction in DAM-2 expression and a notable increase in DAM-1 levels.
If the outcome of experiment 3 deviates from our initial expectations, it suggests a lack of correlation between the IL-1 signaling pathway and TF expression. Perhaps the C-terminal fragment (CTF) produced by cleavage of IL-1R2 inside the cell shields some signal transduction and enhances TF activity. The cleavage of IL-1R2 may possibly generate an intracellular C-terminal fragment (CTF) that may shield certain signal transduction pathways and enhance the activity of transcription factors (TFs). Deletion of Bace-1 leads to reduced cleavage of IL-1R2, resulting in decreased CTF levels in microglia cells. Transcription factor activity is enhanced without CTF shielding, leading to increased DAM-1 production.

If BACE-1 also cleaves IL-1R1, the upregulation of BACE-1 will decrease IL-1R1 expression, thereby inhibiting the IL-1 signaling pathway.

If the observed outcomes are consistent with our expectations, the implication of this finding is still yet to be determined through further experiments on mouse and clinical trials. Future medicine research could prioritize targeting BACE-1 or IL-1R2 in microglia or IL-1 signaling. Future research could focus on investigating the intricate mechanism underlying the impact of TF genes on microglia in relation to DAM-1, with the ultimate goal of developing potential pharmacological interventions for modulating the levels of DAM-1 or DAM-2.

**REFERENCES**


