Proceedings of the 2nd International Conference on Modern Medicine and Global Health
DOI: 10.54254/2753-8818/45/20240582

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

Xiang Li

Department of Integrative Biology and Physiology, University of California, Los Angeles, Los Angeles, 90024, United States

lxlxlx0730@g.ucla.edu

Abstract. Preventing he accumulation of Beta-amyloid (Aβ) plaques has been explored as main treatment of Alzheimer's disease. Microglial deletion of the gene encoding β-site APP cleaving enzyme (BACE-1), an important enzyme facilitating the formation of Aβ, leads to the transition from homeostatic microglia to stage 1 disease-associated microglia (DAM-1) while preventing DAM-1 from further converting into stage 2 disease-associated microglia (DAM-2). Therefore, investigating the pathway of this process can be insight of targeted drug development. In the review part of this paper, the Interleukin-1 (IL-1) pathway is discussed. Based on previous research, a proposal hypothesizing that the activation of the IL-1 signaling pathway enhances the activity of TF and leads to the up-regulation of TF gene expression is designed. The goal is to prove that the up-regulation of IL-1 Signaling pathway can enhance the gene expression of Transcription factors including Jun, Junb, Jund, and Fos, therefore stabilizing the microglia at the stage of DAM-1. Based on the expected experimental results of this study, targeted drugs such as Soluble Type II receptor of Interleukin-1 (sIL-1R2) inhibitors can be developed for AD treatment.

Keywords: Alzheimer's disease, Beta-amyloid, BACE-1, Microglia, Interleukin-1 signaling pathway, sIL1-R2, TF gene expression

1. Background & Introduction

Preventing the accumulation of β-amyloid (Aβ) plaques has emerged as a primary target in the quest to mitigate Alzheimer's disease (AD). A pivotal player in this process is β-site APP cleaving enzyme (BACE-1), a key enzyme facilitating the formation of Aβ. Research has revealed that microglial deletion of the BACE-1 gene leads to a distinctive transformation: the transition from homeostatic microglia to the stage 1 disease-associated microglia (DAM-1) while concurrently preventing DAM-1 from further progressing into stage 2 disease-associated microglia (DAM-2). This intriguing dynamic presents a promising avenue for targeted drug development in the treatment of AD [1-3].

In the review section of this paper, we delve into the Interleukin-1 (IL-1) pathway, a critical element in the context of AD. Drawing upon previous research, we propose a hypothesis positing that the activation of the IL-1 signaling pathway amplifies the activity of transcription factors (TF), ultimately resulting in the up-regulation of TF gene expression. We seek to demonstrate that this up-regulation of the IL-1 signaling pathway can enhance the gene expression of key transcription factors, including Jun, Junb, Jund, and Fos, thereby stabilizing microglia at the DAM-1 stage [4][5].

DOI: 10.54254/2753-8818/45/20240582

The primary goal of our study is to elucidate the complex interplay between BACE-1, microglia, and the IL-1 signaling pathway in the context of AD. By doing so, we aim to provide a scientific foundation for the development of targeted drugs, such as inhibitors of Soluble Type II receptor of Interleukin-1 (sIL-1R2), as potential treatments for Alzheimer's disease [6]. This research opens up new possibilities for therapeutic strategies and contributes to the ongoing efforts to combat this challenging and prevalent neurodegenerative disorder.

2. Literature review

2.1. Alzheimer's Disease (AD)

Alzheimer's disease is one of the most frequently occurring diseases in the worldwide aged population and leads to dementia. The two most typical causes of Alzheimer's disease are the deposition of β-amyloid plaque in the brain and the formation of neurofibrillary tangles of hyperphosphorylated tau [1].

β-amyloid plaques, referred to as Aβ in the rest of this paper, are unusual plaques in the brain and are resulted from the oligomerization, or accumulation, of β-amyloid with the help of APP (amyloid precursor protein) [7]. Therefore, the prevention of Aβ formation was explored as the main logical approach for AD treatment.

2.2. BACE-1

BACE-1 (β-site APP cleaving enzyme) facilitates the formation of Aβ plaques by acting as β-secretase and cutting APP to make it functionalized. In previous research, it has been proved that the BACE-1-targeted inhibition in microglia, a specific type of glial cell in the brain, by using TAM (tamoxifen) to delete the Bace-1 gene, enhances the clearance of Aβ and cognitive performance [2].

Normally, most microglia exist in the form of homeostatic ones. However, in certain situations, the homeostatic microglia would develop into disease-associated microglia at different stages, DAM-1 and DAM-2, where DAM-1 is more functional and phagocytic, and DAM-2 may be dysfunctional and contribute to AD pathology. It was proved that the inhibition of BACE-1 facilitates the transition of homeostatic microglia to DAM-1 [3]. In addition, the expression of the sets of transcription factors, including Jun, Junb, Jund, and Fos, can promote the transition from homeostatic microglia to DAM-1, while significantly reducing the expression of DAM-2.

2.3. IL-1 Receptor 2

Interleukin-1 (IL-1) is a kind of cytokine that is overexpressed in Alzheimer's brain and directly relates to the Aβ formation [5]. It has two types of receptors on the membrane of microglia, IL-1R1 and IL-1R2. As shown in Figure 1 below, IL-1R1 and IL-1R2 are competitors of each other. According to a previous study, when IL-1 protein exists, it would preferentially bind with R1 as IL-1R1 is a much stronger competitor than R2 [5]. After the binding of IL-1 and IL-1R1, the IL-1 signaling would be activated, and the following cascade will be generated.

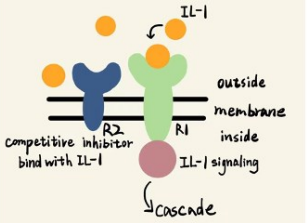


Figure 1. IL-1R1 and IL-1R2

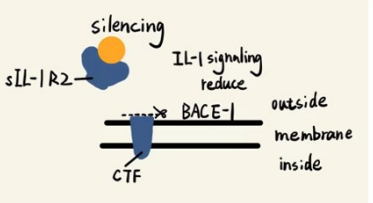


Figure 2. sIL-1R2 Formation&Function

However, as shown in **Figure 2**, according to the same group of researchers, when BACE-1 present, the IL-1R2 can be turned into a soluble form, sIL-1R2 [5]. BACE-1 cleaves the R2 and separates it into two parts, sIL-1R2 and CTF (C-terminal Fragment). The processed R2, in the form of sIL-1R2, is a stronger competitor than R1. Therefore, when IL-1 presents, it would preferably bind to sIL-1R2 instead

DOI: 10.54254/2753-8818/45/20240582

of IL-1R1. In this way, when BACE-1 presents and cuts R2 into sIL-1R2, the signaling pathway induced by IL-1R1 will not happen, therefore reducing the IL-1 signaling cascade.

2.4. Hypothesis

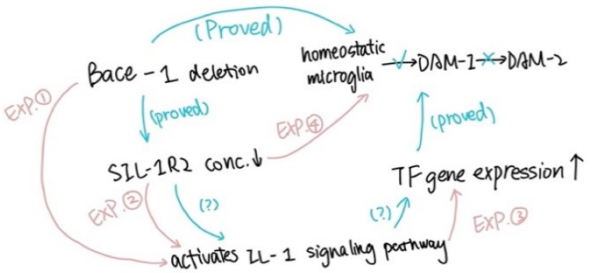


Figure 3. Experiment logic chain

According to previous background information, BACE-1 has the function of preventing the transition from homeostatic microglia to DAM-1 and promoting the transition from DAM-1 to DAM-2, and the function of cleaving IL-1R2 to form sIL-1R2. Therefore, as shown in **Figure 3**, the deletion of the Bace-1 gene, in other words, the inhibition of the production of the BACE-1 enzyme, can promote the transition from homeostatic microglia to DAM-1 while preventing DAM-1 from turning into DAM-2, and reduce the SIL-1R2 concentration. It is still not clear if there is a direct causal relationship between the reduction in the concentration of sIL-1R2 and the transition of microglia forms.

Therefore, based on this problem, the hypothesis of our experiment is: The activation of the IL-1 signaling pathway enhances the activity of TF and leads to the up-regulation of TF gene expression.

2.4.1. Significance

Our experiment provides future directions for developing medicine for Alzheimer's disease. For example, in the future, Anti-sIL-1R2 drugs or sIL-1R2 inhibitors can be designed as microglia-targeted medicine to treat Alzheimer's disease patients.

3. Methodology

In our experiment, the 5xFAD mice model is used. It is a typical type of trans-genetic mice used to study Alzheimer's disease. According to **Figure 3**, in total, four experiments were designed to prove our hypothesis:

3.1. Experiment 1: The effect of the absence of Bace-1 on the IL-1 concentration

In **Experiment 1**, the relationship between Bace-1 and IL-1 concentration will be determined.

For the in vitro experiment, we divide the cells into three groups: Group O, Group P (control), and Group Q. For Group O and Group Q, we use the siRNA technique in microglia to control the concentration of sIL-1R2 in microglia. In Group O (+siRNA), there is a low concentration of sIL-1R2. In Group P (-siRNA), the concentration of sIL-1R2 stays at a normal level. In Group Q (+sIL-1R2), the sIL-1R2 solution is directly added to the experimental dish, meaning that there is a high concentration of sIL-1R2. Since IRAKs (IL-1 receptor-associated kinases) are downstream of the IL-1 signaling pathway [6], we test its concentration by using the Western blot, noted as p-IRAK-1 and p-IRAK-2. Thus, the status of the IL-1 signaling pathway can be determined.

For the in vivo experiment, three groups of mice are used: Group O (wild-type mice with microglial IL1-R2 gene deleted), Group P (normal wild-type mice), and Group Q (wild-type mice with direct IL1-R2 injection or viral infection to raise the level of microglial sIL-1R2). After the experimental mice are killed, Western blot, testing p-IRAK-1 and p-IRAK-2, will be used, similar to the in vitro experiment.

DOI: 10.54254/2753-8818/45/20240582

3.2. Experiment 2: The increase in the sIL-1R2 concentration affects the IL-1 signaling pathway

In **Experiment 2**, whether the increase in the concentration of sIL-1R2 silences the IL-1 signaling pathway will be verified. Experiment 2 involves both in vitro and in vivo experiments.

For the in vitro experiment, there are three groups of microglia: Group E (Bace-1 inhibited), Group F (Bace-1 normally expressed), and Group G (Bace-1 excessively expressed). Group E is where microglial Bace-1 is inhibited, thus leading to BACE-1 deficiency in the microglia. Group F is set as the control group, where the Bace-1 gene is intact with the absence of the BACE-1 inhibitor. Group G has the Bace-1 gene over-expressed, leading to a high concentration of BACE-1 in the microglia. ELISA kit will be used to determine the concentration of sIL-1R2. In addition, the Western blot will be used to test the p-IRAK-1 and p-IRAK-2 to determine the status of the IL-1 signaling pathway.

For the in vivo experiment, three groups of mice will be used: Group E (wild-type mice with microglial Bace-1 gene deleted), Group F (wild-type mice with Bace-1 normally expressed), and Group G (wild-type mice with Bace-1 over-expressed by using CRISPR-Cas9). The sIL-1R2 concentration can then be determined by using Western blot to test the presence of CTF after mice are killed. Similar to the in vitro experiment, the status of the IL-1 signaling pathway will be determined by also using the Western blot, testing p-IRAK-1 and p-IRAK-2.

3.3. Experiment 3: The effect of activation of IL-1 signaling pathway on the activity of TF (transcription factors), including Jun, Junb, Jund, and Fos

Experiment 3 determines whether the activation of the IL-1 signaling pathway promotes the activity of the transcription factors including Jun, Junb, Jund, and Fos.

For the in vitro experiment, microglia are divided into three groups: Group A (IL-1 signaling pathway silenced), Group B (control, IL-1 signaling pathway at normal state), and Group C (IL-1 signaling pathway activated). For Group A, the IL-1 signaling pathway is silenced by decreasing IL-1R1 expression using CRISPR-Cas9. For Group C, the IL-1 signaling pathway is activated by increasing the IL-1R1 signaling pathway, also using CRISPR-Cas9. By using RNA sequencing after the mice are killed, the activity of transcription factors (TF, i.e., Jun, Junb, Jund, and Fos) can be determined.

For the in vivo experiment, we still use the wild-type mice and divide them into three groups. For Group A, the wild-type mice have their microglial IL-1R1 deleted by using microglia specific promotor, with a CRISPR-Cas9 KO DNA. Therefore, their IL-1 signaling pathway are silenced. For Group B, the wild-type mice have a normal state of the IL-1 signaling pathway. For Group C, by using CRISPR-Cas9, the wild-type mice have microglia-targeted deletion of the Bace-1 gene. After the mice are killed, by using RNA sequencing, the activity of TF can be determined.

3.4. Experiment 4: The effect of IL-1R2 inhibition on the transition from homeostatic microglia to DAM-1

According to our hypothesis, the targeted knockout of the IL-1R2 gene in microglia can enhance the activation of the IL-1 signaling pathway as the strong competitor of IL-1R1, sIL-1R2 cannot be formed. Therefore, the TF activity can be enhanced, facilitating the transition from homeostatic microglia to DAM-1 while preventing the transition from functional DAM-1 to dysfunctional Dam-2.

Therefore, in **Experiment 4**, we designed and bred two types of wild-type mice and 5xFAD mice with targeted knockout of the microglial IL-1R2 gen. Since Aβ plaque accumulation in 5xFAD mice begins at around 2 months of age, mice with targeted deletion of IL-1R2 in microglia were treated with either TAM (tamoxifen) for 5 days beginning at the age of 3 months old. Therefore, we can analyze scRNA-seq results of CD11b+ immune cells purified from 4-month-old mice with targeted deletion of IL-1R1 in microglia, with and without TAM treatment.

4. Result and interpretation

Based on the hypothesis and background introduction, these are our expected experimental results:

DOI: 10.54254/2753-8818/45/20240582

4.1. Experiment 1

For Experiment 1, as shown in **Figure 4**, similar results are expected for both in vitro and in vivo experiments. As sIL-1R2 is a stronger competitor than IL-1R1, the presence of sIL-1R2 results in more IL-1 binding to sIL-1R2 instead of binding to IL-1R1 and activating the iL-1 pathway. Thus, the increase of sIL-1R2 concentration correlates with lower activation of the IL-1 signaling pathway.

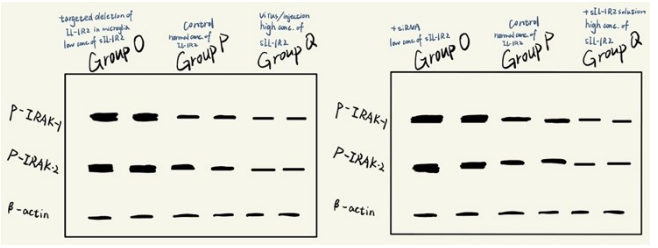
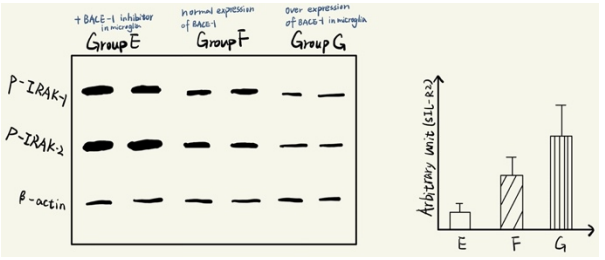


Figure 4. Experiment 1 Western blots (Left: in vitro; Right: in vivo)

4.2. Experiment 2

For Experiment 2, as shown in **Figure 5**, for the in vitro experiment, there is a lower level of p-IRAK-1 and p-IRAK-2 in group G. And according to **Figure 6**, for the in vitro experiment using ELISA to determine the level of sIL-1R2, there's an overall increasing trend from Group E to Group F to Group G, as the level of Bace-1 gene expressed increases. This indicates that the IL-1 signaling pathway is silenced when Bace1- is over-expressed.



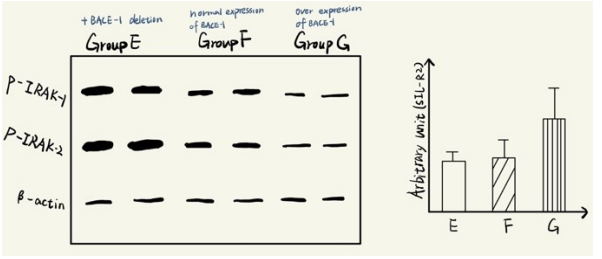


Figure 5 (left). Experiment 2 in vitro results (Left: Western blot; Right: ELISA)
Figure 6 (right). Experiment 2 in vivo results (Left: Western blot; Right: ELISA)

Thus, the over-expression of BACE-1 leads to increased sIL-1R2 secretion and completely or largely silenced IL-1 signaling pathway.

4.3. Experiment 3

For **Experiment 3**, as shown in **Figure 7**, the RNA sequencing results indicate that the activation of the IL-1 signaling pathway enhances the activity of the TFs (Transcription factors including Jun, Junb, Jund, and Fos).

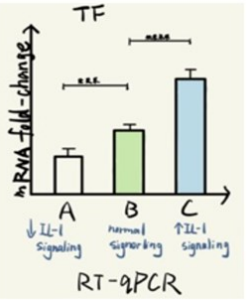


Figure 7. Experiment 3 Results

DOI: 10.54254/2753-8818/45/20240582

4.4. Experiment 4

For **Experiment 4**, as shown in **Figure 8**, there is a larger proportion of DAM-1 in the 5xFAD mice with microglial targeted deletion of IL-1R2 compared to normal 5xFAD mice.

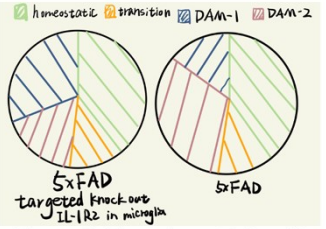


Figure 8. Experiment 4 Result

5. Conclusion & Future Directions

5.1. Conclusion

Experiment 1 proved that elevated sIL-1R2 concentration silences the IL-1 signaling pathway. Experiment 2 proved that the over-expression, or high expression, of BACE-1 increases the sIL-1R2 concentration. Experiment 3 proved that the activation of IL-1 signaling pathway leads to the up-regulation of TF gene expression. Experiment 4 proved that the IL-1R2 inhibition facilitates the transition from homeostatic microglia to DAM-1.

In conclusion, these experiments proved that the activation of the IL-1 signaling pathway enhances the activity of TF and leads to the up-regulation of TF gene expression, which corresponds with the hypothesis stated previously.

As these are expected results, there is also the possibility that the actual results do not follow our hypothesis, or even contradict our expectations. Therefore, two conjectures are formed to explain different results: If the results of **Experiments 1 and 2** are consistent with the hypothesis while **Experiment 3** does not, the first conjecture is hypothesized: **BACE-1 may also cut the IL-1 type I receptor**. Through high expression of BACE-1, the number of IL-1 type I receptors is significantly reduced, thus closing the IL-1 signaling pathway. If the actual results of **Experiment 1 and 3** are consistent with the hypothesis, but no direct relationship between the up-regulation of the IL-1 signaling pathway and TF activity is observed in **Experiment 2**, it is the second conjecture is hypothesized: The state of the IL-1 signaling pathway does not relate with the activity of TF. CTFs (C-terminal fragments), formed by the cleavage of IL-1R2 by BACE-1, are able to shield some signal transduction and inhibit TF activity to some extent.

5.2. Limitations and Future Directions

The main limitations of the experiment are the sample size and the mice model used. The number of mice used in the experiment is not defined yet in our experiment. However, the statistical power and reliability of the results would be influenced if the actual experiment do not have enough samples. At the same time, as AD is a complex and multifactorial disease that specifically owns by humans, the 5xFAD mouse model, as an animal subject, may not fully explain all aspects of human AD pathology. In addition, the ethical issue needs to be considered as animals are involved in the experiment.

In the future, based on the experimental results of this study, targeted drugs such as Anti-sIL-1R2 drugs and sIL-1R2 inhibitors can be developed for AD treatment. Then, clinical trials need to be designed in the future to evaluate the efficacy and possible side effects of Anti-sIL-1R2 drugs/ sIL-1R2 inhibitors. Furthermore, since the mechanism of the effect of the TF gene on microglia transitions (from homeostatic ones to DAM-1, and then to DAM-2) is still unclear, future experiments can also be designed and conducted to determine this detailed process.

DOI: 10.54254/2753-8818/45/20240582

References

[1] Weller, Jason, and Andrew Budson. "Current understanding of Alzheimer's disease diagnosis and treatment." *F1000Research* vol. 7 F1000 Faculty Rev-1161. 31 Jul. 2018, doi:10.12688/f1000research.14506.1

[2] Singh, Neeraj et al. "Targeted BACE-1 inhibition in microglia enhances amyloid clearance and improved cognitive performance." Science advances vol. 8,29 (2022): eabo3610. doi:10.1126/sciadv.abo3610

[3] Singh, Neeraj et al. "BACE-1 inhibition facilitates the transition from homeostatic microglia to DAM-1." Science advances vol. 8,24 (2022): eabo1286. doi:10.1126/sciadv.abo1286

[4] Mrak, R E, and W S Griffin. "Interleukin-1, neuroinflammation, and Alzheimer's disease." Neurobiology of aging vol. 22,6 (2001): 903-8. doi:10.1016/s0197-4580(01)00287-1

[5] Peters, Vanessa A et al. "IL-1 receptor 2 (IL-1R2) and its role in immune regulation." Brain, behavior, and immunity vol. 32 (2013): 1-8. doi:10.1016/j.bbi.2012.11.006

[6] Jain, Ajay et al. "IL-1 Receptor-Associated Kinase Signaling and Its Role in Inflammation, Cancer Progression, and Therapy Resistance." Frontiers in immunology vol. 5 553. 17 Nov. 2014, doi:10.3389/fimmu.2014.00553

[7] Gouras, Gunnar K et al. "β-Amyloid peptides and amyloid plaques in Alzheimer's disease." Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics vol. 12,1 (2015): 3-11. doi:10.1007/s13311-014-0313-y