Decreasing SIL1-R2 concentration could enhance the expression of transcription factors on the gene level

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ABSTRACT

By the time you opened this document, an individual somewhere in the world has developed Alzheimer's disease. With over 6 million cases in the United States alone and a global total exceeding 50 million, Alzheimer's contributes to around one-third of senior fatalities. Horrifyingly, according to the United Nations, the number of patients doubles every two decades if a breakthrough is not discovered. Therefore, it is imperative to the survival of the human species to address and attempt to solve this issue before it can escalate any further. In this work, I have designed a few experiments that could be monumental to developing future Alzheimer's vaccines and medicine. The primary focus of the trials above is to explore the possible role of the IL-1 signaling protein in the Bace-1- 1 deletion process to the potential prevention of DAM-2 formation, which, in turn, could worsen Alzheimer's symptoms. The overall prediction can be summed up in the following: *The activation of the IL-1 signaling pathway will enhance the activity of TF and lead to the up-regulation of TF gene expression.* If my assumption is proven correct, it could pave the way for future Alzheimer's medication to include drugs that moderate IL-1 signaling, which could save countless amount of lives.

Keywords: Alzheimer's Disease, Phagocytic cells, Transcription Factor. Dam-1, Interleukin-1 Inhibitor

1. BACKGROUND INFORMATION

Before mentioning anything else, it is imperative to first show the basis behind the formation of my questions, experiments, and expected results. My approach rested upon the results of three previous research articles, from which I used five tested data points that served as the bedrock for designing my experimental framework.

First, I referred to the article "BACE- 1 Inhibition Facilitates the Transition from Homeostatic Microglia to Dam- 1" by Neeraj Singh. It was primarily used to establish the base relationship of different stages of microglia in the brain and their effects on Alzheimer's disease. I was able to establish three key facts. The first was derived from the following passage, "BACE-1 deficiency elevated levels of transcription factors …, which transition from more homeostatic to highly phagocytic DAM- 1" [1]. This piece explained that when BACE- 1 was removed or inhibited in microglia, transcription factor levels were recorded to rise, and ultimately, the microglia transitioned from a more passive "homeostatic" phase to a more phagocytic "DAM- 1" phase. The next fact was gleaned from the following piece, "Stage 1 DAM represents a more functional, phagocytic subtype of immune cell, while stage 2 DAM may be dysfunctional and contribute to AD pathology" [1]. This piece explained that the previously mentioned DAM- 1 phase of microglia demonstrates heightened functionality as an immune cell, while DAM-2 could potentially

contribute to worsening Alzheimer's symptoms. Finally, I used the following sentences to form my last key point, "Induced expression of this unique set of TFs correlated with the transition from homeostatic microglia to DAM- 1… significantly reduced the expression of DAM-2 marker genes, indicating a reduction of this dysfunctional subtype associated with reduced amyloid plaque deposition" [1]. This information explains how the increased expression of a set of transcription factors helped in two ways: aiding the microglial transition from homeostatic to DAM- 1 and preventing the transition of DAM- 1 to DAM-2. After assessing "BACE- 1 Inhibition Facilitates the Transition from Homeostatic Microglia to Dam- 1", I designed the following table:

Moving on, I reviewed a paper titled "IL- 1 Receptor 2 (IL- 1R2) and Its Role in Immune Regulation" by Vanessa A. Peters, Jennifer J. Joesting, and Gregory G. Freund. This paper served as a foundation for understanding IL receptors, and it was used as a basis to determine if they have any connection with the sequence from Bace- 1 deletion to the suppressed transition of DAM- 1 microglia to DAM-2. The overarching message I needed from this paper can be summarized using two quotes: "IL- 1R1 and IL- 1R2 are competitors of each other, but R1 is the default receptor that binds to IL- 1 as it's much stronger than R2" [2]. This first piece explains the two receptors for the IL-1 protein and labels IL-1R1 as the primary one. "When R2 exists in a more soluble form, sIL- 1R2, it becomes a stronger competitor. In this situation, most IL- 1 proteins

bind to sIL- 1R2." [2]. Now, I add another variable. If the R2 receptor is in a soluble state, it has a higher priority for binding than the IL-1R1 receptor. Finally, I looked at the document "Regulated Intramembrane Proteolysis of the Interleukin- 1 Receptor II by α-, β-, and γ-Secretase." by Peer-Hendrik Kuhn. In this paper, I was able to find a proven relationship between Bace - 1 and IL1-R2. "the β-secretase BACE- 1… increased IL- 1R2 secretion resulting in C-terminal fragments nearly identical to the ones generated by the α-secretase-like cleavage" [3]. It simply proves that increased Bace- 1 would result in higher IL- 1R2 secretion and vice versa. Considering all this information, I developed a full chart (*figure 1*).
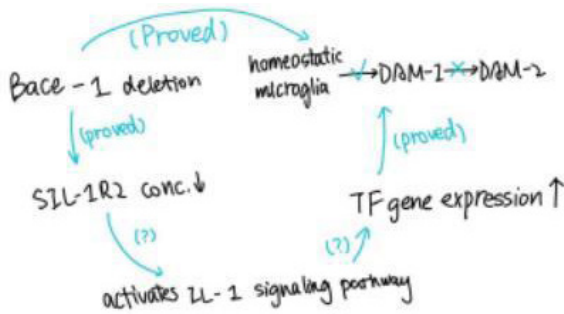

Figure 1. The systems and the assumed systems

With most parts being proven using the sources, I theorized that it could be possible that the way Bace- 1 affects microglia could be through the IL- 1 signaling pathway, as I found no other source denoting how it affected the homeostatic and DAM- 1 stage. Therefore, I developed my hypothesis: As sIL- 1R2 concentration decreases, activity in the IL- 1 signaling pathway could increase, enhancing the expression of transcription factors on the gene level.

2. PROPOSED EXPERIMENTS

Now that I have a solid background, I designed the experiments that should thoroughly test the hypothesis. They are listed in the following chart (*figure 2).*
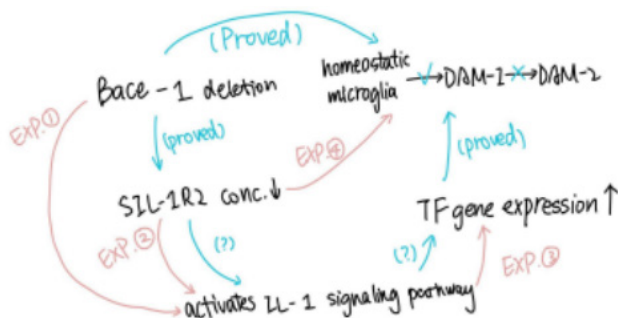

Figure 2. The experiments we will run to prove the assumed system

Experiment 1 should prove that the absence of Bace- 1 affects IL- 1 concentration levels. For this, I designed one in vitro and one in vivo. For the in vitro test, there were three dishes of microglia: 1 with Bace- 1 inhibition, one with Bace- 1 overexpressed, and a control. Inhibition can be done using TAM+ or RNA interference, and the CRISPR method can achieve overexpression via Transfection/Transformation. To test in vivo, three 5xFAD mice, one Bace- 1 inhibited with TAM+ treatment, one control, and one with excess Bace- 1 expression through CRISPR-9 gene editing. Once done, you can use an IL-1 ELISA kit on samples from mice to test the concentration of IL-1 levels in these 3 cases during different times in their lives (i.e., 3 months and 6 months).

Experiment 2 should affirm that as sIL- 1R2 concentration increases, the IL- 1 signaling pathway is silenced through an in vitro and in vivo test. For the vitro test, I can use siRNA to manipulate gene expression to create 3 instances. First, microglia with a low amount of sIL- 1R2, one dish as a control, and microglia with a high amount of sIL- 1R2. Measuring IL- 1 Receptor Associated Kinases (p-IRAK- 1 and p-IRAK-2) using protein extraction and western blotting should be a direct indicator of the activation level of the IL- 1 pathway. For the vivo test, I have mirrored models but with mice, 1 with low IL- 1R2, control, and 1 with high IL- 1R2. This can once again be accomplished with siRNA or induced protein expression. Some testing methods include taking samples from killed mice in different parts of their life cycle and using an ELISA kit on isolated RNA. It can also be done with its bioluminescence.

Experiment 3 will complete the cycle from Bace- 1 deletion to an increased transcription factor (TF) expression. More specifically, it is used to determine if the IL- 1 signaling pathway activation enhances it. In my vitro test, I will again have 3 samples, one with a silenced signal pathway. This can be done in many ways, from overexpression of IL- 1R1, to a high concentration of Bace- 1 if experiment 1 yields that result. Then I have my control microglia sample, and finally, one with an activation of the signaling protein, using IL- 1R1 or Bace- 1 inhibition. TF gene expression results can be measured with Gene-edited fluorescent labeling of TF RNA sequencing. In the vivo version, the mouse types should match the experiment 2, but with IL- 1R1 instead of sIL- 1R2. I can find TF expression levels here by killing the specimen and utilizing RNA sequencing or Chromatin Immunoprecipitation (CHiP).

Now that I have the cycle wrapped up, confirming that IL- 1 R2 inhibition facilitates the transition from homeostatic microglia to DAM- 1 is important. In other words, it proves the cycle follows my predicted path and

not another branch or a different pathway altogether. In this only vivo experiment, I start with 2 5xFAD mice and treat one with targeted deletion of IL- 1R2. After a few months, I tested the state of the microglia, which consists of homeostatic, transition, DAM- 1, and DAM-2.

3. EXPECTED RESULTS

In addition to creating theoretical experiments, an expected result for each has been hypothesized. The purpose and expected results are in the table below *(Table 1)*.

Table 1. Experiment and results table

Experiment	Purpose	Expected Result
1	Examine the correlation between the sIL- 1R2 concentration level and the activation of the IL-1 signaling pathway.	The high concentration of sIL- 1R2 should silence the IL- 1 pathway
2	Explore the impact of BACE-1 expression on the levels of sIL-2 concentration and the activation of the IL-1 signaling pathway.	The overexpression of BACE- 1 should lead to an increase of sIL- 1 R2 concentration and silence the IL- 1 signaling pathways
3	Examine whether the activation of the IL-1 signaling pathway leads to an increase in the activity of transcription factors (TFs).	The activation of the IL- 1 signaling pathway should increase TFs expression
4	Investigate whether the inhibition of IL- 1 R2 promotes the shift from homeostatic microglia to DAM- 1 (disease-associated microglia type 1).	Targeted deletion of IL- 1R2 gene in Microglia should increase the number of DAM- 1 stages and decrease DAM-2s.

4. CONCLUSION

Suppose the experiments and results go as planned. In that case, it will pave the way for drugs and medications that are about to modify IL- 1 signaling pathway rather than Bace-1 inhibition which has been shown to show numerous side effects. The potential that future Alzheimer's medicine has on saving lives is more important than ever, with the number of patients growing every day. It is also important to conduct clinical trials to assess the effectiveness of anti-sIL- 1R2 drugs or sIL- 1R2 inhibitors in treating Alzheimer's disease. Additionally, it also analyzes their potential side effects. Even if the experiment designs may have been vague, not including a specific number of specimens or the specific editing of proteins or pathways, the overarching goal is to contribute to the progress of understanding and addressing Alzheimer's disease in hopes of preventing future loss of human life.

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