The Relation between Gingipain, TREM-2 and the Condition of Alzheimer's Disease

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Abstract: P. gingivalis is a kind of bacterial that causes Periodontitis, and will secrete gingipain, which can help the P. gingivalis to colonize inside the host. It is proposed that the gingipain that is secreted by P. gingivalis will activate TREM-1, which induces the inflammation inside the brain. The inflammation inside the brain will cause the neurodegeneration and cognitive decline, which in a way worthen the AD. Thus, it can be concluded that TREM-1 and gingipain have influences on AD. Beyond this, we found that TREM-2 shares homology with TREM-1, and some studies shows that TREM-2 can help to against inflammatory. In this paper, we came up with a hypothesis that the activation of TREM-2 reduces the level of inflammation and protects neurons from degeneration, mainwhile, P. gingivalis colonizes in the brain and exacerbates features of AD via its gingipains.

1 INTRODUCTION

P. gingivalis is what cause the periodontitis and secrete gingipain. For gingipain, it is essential for P. gingivalis survival and pathogenicity, and plays critical roles in host colonization, inactivation of host defenses, nutrient acquisition and tissue destruction. TREM-1's full name is *The Triggering Receptor Expressed on Myeloid cells 1*. It is a surface receptor on immune cells that amplifies inflammatory processes, which means that once it is being activated, it increases the level of inflammation. This process can be activated by bacterial infection, and studies have shown that it is regulated by gingipain of

P. Gingivalis, inducing chronic inflammation in the brain, leading to neurodegeneration and cognitive decline (Haditsch, Ursula, et al. 2020). TREM-2's full name is Triggering Receptor Expressed on Myeloid cells 2, it shares homology with TREM-1, encoding a receptor expressed on immune cells such as macrophageas and microglia (Dominy, Stephen, et al. 2019). In contrast, studies have shown that TREM-2 defends the liver against hepatocellular carcinoma (HCC), which is a chronic liver injury involving inflammatory and hepatic regenerative processes (Watts, Amber, et al. 2008). Once activated by its ligand, TREM-2 reduces the level of inflammation in the liver, and one of the ligands of TREM-2 is A-beta protein (Esparza-Baquer, Labiano, et al. 2021).
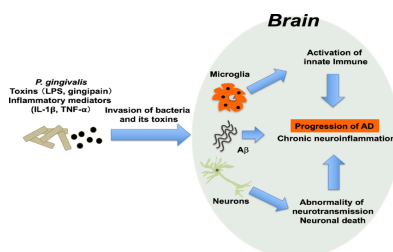

Figure 1: (Matsushita, Kenji & Yamada-Furukawa, Masae & Kurosawa, Mie & Shikama, Yosuke. (2020). Periodontal Disease and Periodontal Disease-Related Bacteria Involved in the Pathogenesis of Alzheimer's Disease. Journal of Inflammation Research. Volume 13. 275-283. 10.2147/JIR. S255309.) It shows the process of how P. gingivalis cause the influences inside the brain.

2 EXPERIMENTS

To prove our hypothesis, we designed experiments follows, proven the function of gingipain, Co-Immunoprecipitation, Gene knockout and Behavior testing. By all these experiments, we are going to prove that the TREM-2 can have a positive result in AD, and it is gingipain that induces the AD (Singhrao, Sim, and Ingar Olsen. 2019). In the first experiment which is the proven of the function of gingipain, we will use the following materials: Wild-type gingivalis which normally exist in the nature, gingipain knock-out mutant K1A which is deficient in Lys- gingipain and E8 strains which is deficient in both Arg-gingipain A and Arg-gingipain B (Bostanci, et al. 2016). We will first cultivate, collect, wash, and suspend the bacterial to get enough copies for the experiment, then, we will control environment for the mouse and develop periodontitis inside mouse. After five weeks, we will collect the brains of the mouse to measure the data. We will measure the concentrations of bacterial endotoxin in the brains and the concentrations of A-beta protein (40 and 42) in the brains so that we can find out whether the gingipain works as expected (Ishida et al. 2017). In the second experiment of Co-Immunoprecipitation, we are going to determine the interactions between Aβ and TREM2, the materials that will be use are the fresh medium containing conditioning molecules and microglia. First step of this experiment is the preparation of cell lysates, we will cultivate bacterial inside of a fresh medium, collect, wash, and suspend the bacterial. After that, we will do the immunoprecipitation, in this step, we will centrifuge the supernatant, and aspirate the residue for use (Maheshwari, Eslick 2015). Then, we will do the western blot. In this step, we will run gel to get data to prove that TREM-2 can be bound to Aβ as it will show a similar result as Aβ is immunoprecipitated with Aβ antibodies (Liu, Yu. 2019). In the third experiment for gene knockout, we are going to prove the function of TREM-1 and TREM-2. In this experiment, we will use gingipain and mouse. The first step is to set two LoxP sites so that it can be clear which part of the gene is going to be knockout (Singhrao, Sim, et al. 2015). After that, we are going to knockout the TREM-1 gene and the TREM-2 gene dividing by group 1 to 4 which with one only knockout TREM-1, one only knockout TREM-2, one knockout both and one does not knockout any gene. Then, we will induce gingipain into the brains, and measure the production of pro-inflammatory cytokines by ELISA. By this experiment and the research done by other scientists, we can conclude that TREM-1 will increase the level of inflammation while TREM-2 will decrease the level of inflammation (Ishida et al. 2017).
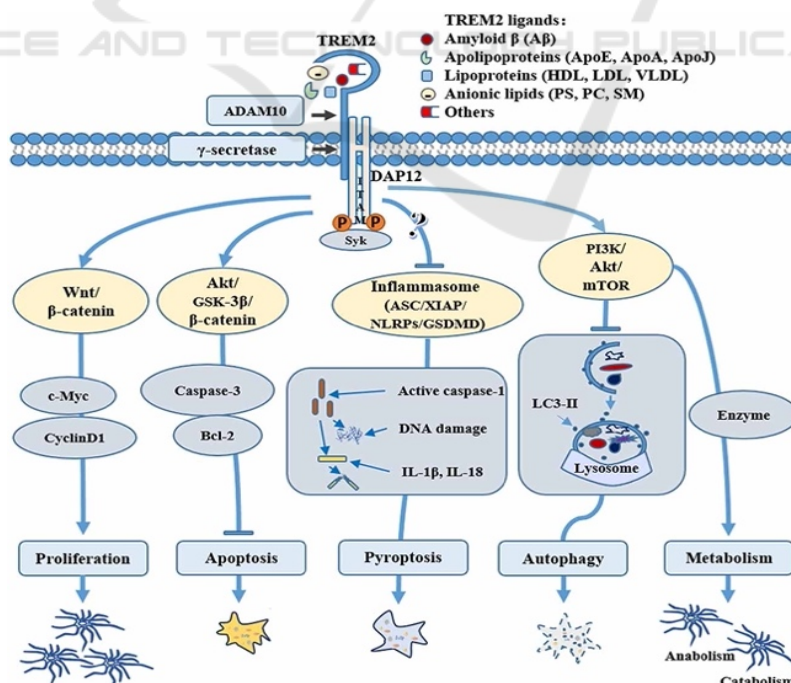

Figure 2: (from TREM2 in Alzheimer's Disease: Microglial Survival and Energy Metabolism) shows the process of TREM-2 and what it caused.

3 BEHAVIOR TESTING

After all these experiments are done, we need to know whether the TREM-1 and TREM-2 have influence on AD, so we designed a behavior testing. We will first divide mouse into 4 groups with group A for the TREM-1 knock-out, group B for TREM-2 knock-out, group C for both knock-out, group D for the one does not do anything. Then, we design three testing to get the result. In the first experiment, we will design a maze with several turns that a normal mouse can walk out. Put each group into the maze individually to measure the time they need for the first time to find way out. Do the same thing for 3 times and measure the time each group need to get out. Compare the change in the need of time between different groups to conclude the result. In the second experiment, we put each group into a box with road A and B that lead to food, if the mice pass through road A, they will receive an electron shock of 5V. Make the mouse empty stomach for 5 hours and put each group into the box so that they will go for the food. Do the same thing for 10 times and measure the time they go for road A and B. In the third experiment, a training for all the mouse to open the box to find food will be done before do the surgery. After the surgery, put two sealed boxes in two directions with two different odor that is not related with mouse in any areas, one of the boxes contains food. Put each group in that area to find food for 10 times. Measure the time it takes for mouse to know the box with which odor contains food. The results of these experiments are that ss the experiments above, we can assume that TREM-1 will exacerbate inflammation during acute inflammation, while TREM-2 will prevent chronic inflammation. According to Naoyuki Ishida, the inflammation is correlated with AD that when inflammation exacerbate, it will exacerbate the symptoms of Alzheimer's Disease (Ishida et al. 2017). So that we can expect that the knock-out of TREM-1 will decrease the symptoms of AD, the knock-out of TREM-2 will exacerbate the symptoms of AD, while knock-out of both TREM-1 and TREM-2 will cause the deterioration of AD afterward. As the assumption we make, we can know that group A will present the state that AD is weakened. In group B, the present of AD will be exacerbated. In group C, the present of AD will be exacerbated. In group D, it is same as group C. For the behavior testing, if our hypothesis is correct, the result will be that in experiment 1, group A will show an obvious decrease in the time it takes to pass the maze as the time of trying increases. Group B will need almost the same time during each time of trying. Group C shows a similar result as group B. Group D shows a constant decrease in time as the time it passes the maze increases. In experiment 2, as the time of the experiment increases, group A shows an obvious decrease in the chance it goes for road A. Group B shows a chance of about 50% that it will go for road A. Group C shows a similar result as group B. Group C shows a decrease in the chance it goes for road A as the time of experiment increases. In experiment 3, group A will show a decrease in the time it needed to find food as the time of experiment increases and can be concluded that group A can link a kind of odor to food. Group B shows a similar time each time it needed to find food as the time of experiment increases so that it can be concluded that group B cannot link a kind of odor with food. Group C shows a similar result as group B. Group D shows a decrease in time as more experiment is done and can be concluded that group D can link a kind of odor to food. If the hypothesis is wrong, then the result will be that in all the experiments, the behavior of all groups of mice are similar and the improvement in time is almost neglectable.
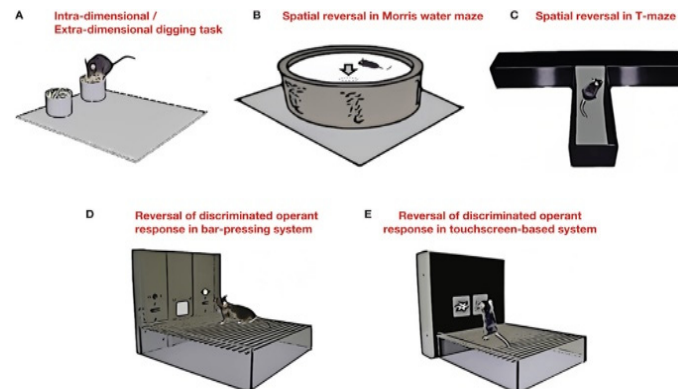

Figure 3: (from Predictably irrational: assaying cognitive inflexibility in mouse models of schizophrenia) It shows some types of behavior testing, the A and C are used in this experiment.

4 CONCLUSIONS

In this paper, it talks about the influence TREM-2 might have on Alzheimer's Disease. After these experiments, the hypothesis of TREM-2 and gingipain can be proved. Though this article and the hypothesis might be wrong, but TREM-2 and gingipain have influences on AD by many factors in different ways. As the Alzheimer's disease is a very serious disease that influence people in so many ways, it is possible to say that the relation between TREM-2 and Alzheimer's disease is crucial to the further research and the development of the treatment in Alzheimer's disease. This article is meant to show a new way of seeing what influences Alzheimer's Disease and point out a factor that have not been tested on. In the future, we will focus on the research of what is the causation of Alzheimer's Disease, what are the factors that make it worse, and how the symptoms Alzheimer's Disease can be decreased.

ACKNOWLEDGEMENTS

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