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The Role of M1 and M2 in Modulating Ab Accumulation and Function

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Abstract. Alzheimer's disease is a neurodegenerative disorder characterized by progressive cognitive decline, memory loss, and the formation of amyloid-beta plaques and tau tangles in the brain. This chronic condition has no known cure, and its progression varies greatly between individuals, eventually leading to severe cognitive impairment, loss of independence, and death. Microglia, the primary immune cells in the central nervous system that serve as the brain's first line of defense, are central to Alzheimer's disease pathology. Microglia play an important role in the response to injury and infection, as well as the regulation of amyloid-beta levels. However, when amyloid-beta accumulates, it activates an inflammatory response via microglia. While this response is initially protective, it may become chronic, contributing to the neuroinflammation that worsens Alzheimer's disease pathology. In this study, I wanted to isolate and investigate the specific effects of M1 and M2 macrophages in the brains of genetically modified mice. Using CRISPR technology, we developed mouse models with selective expression of M1 macrophages without M2, M2 macrophages without M1, and models with both macrophage types. Western blot analysis quantified the levels of amyloid-beta in these mice, revealing how microglial activity influences Alzheimer's disease progression. Our findings show that M1 macrophages primarily regulate amyloid-beta through inflammatory processes, which may increase amyloid-beta production, whereas M2 macrophages are essential for amyloid-beta clearance. These findings emphasize the importance of balancing M1 and M2 macrophage activity when treating Alzheimer's disease.

Keywords: Alzheimer's disease, amyloid-beta plaques, microglia, neuroinflammation, M1 macrophages, M2 macrophages.

1. Introduction

Alzheimer's disease is a neurodegenerative disorder marked by cognitive decline, memory loss, and other neuropathological symptoms. It is the leading cause of dementia among older adults. Alzheimer's disease is a chronic condition with no known cure, and the progression varies greatly between individuals. It eventually causes severe cognitive decline, loss of independence, and, ultimately, death. Ongoing research focuses on better understanding disease mechanisms, early detection methods, and developing new treatments to slow or reverse its progression. A type of cell serves as the primary form of active immune defense in the central nervous system. Amyloid plaques and tau tangles are abnormal protein deposits that accumulate in the brain of Alzheimer's patients, causing neuronal damage and cognitive decline. Microglia are involved in the removal of toxic proteins and debris. The absence of microglia has been shown to result in diverse pathologies and early lethality in Alzheimer's disease

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models, highlighting the critical role these cells play in disease progression that the interaction between amyloid-beta and microglia is crucial, as noted by Selkoe and Hardy (2016), who highlight how microglial activation can influence the progression of Alzheimer's disease, potentially leading to either protective or harmful effects depending on their activation state [1,2]. They are probably related to neuroinflammation, which neuroinflammation is a shared characteristic of neurodegenerative diseases, including Alzheimer's and Parkinson's disease, underscoring the importance of understanding inflammatory mechanisms in both conditions[3]. There are two hypotheses about how MG intensifies AD: Firstly, people don't have enough MG because of unavoidable reasons such as inheritance. Secondly, activated microglia release pro-inflammatory cytokines in response to toxic proteins and debris (e.g., amyloid-beta plaques). Gamma frequency entrainment significantly attenuates amyloid load in the brain, suggesting that neural activity modulation can influence amyloid-beta clearance through microglial activation, and the intricate relationship between beta-amyloid peptides and microglial function underscores the necessity of investigating how these immune cells contribute to Aβ clearance in Alzheimer's disease [4,5]. While this response is initially aimed at clearing toxic substances, chronic activation can lead to neuroinflammation. When Aβ accumulates in the brain, it can trigger an inflammatory response from microglia and astrocytes, the primary immune cells in the brain. When Aβ plaques are present, immune cells activate and release inflammatory molecules like cytokines and chemokines, leading to neuronal damage and progression of the disease. However, far fewer studies have looked into the possible role of microglia in one of the most common AD co-pathologies, cerebral amyloid angiopathy.

In addition, I found that there is a different effect MG caused in two papers.

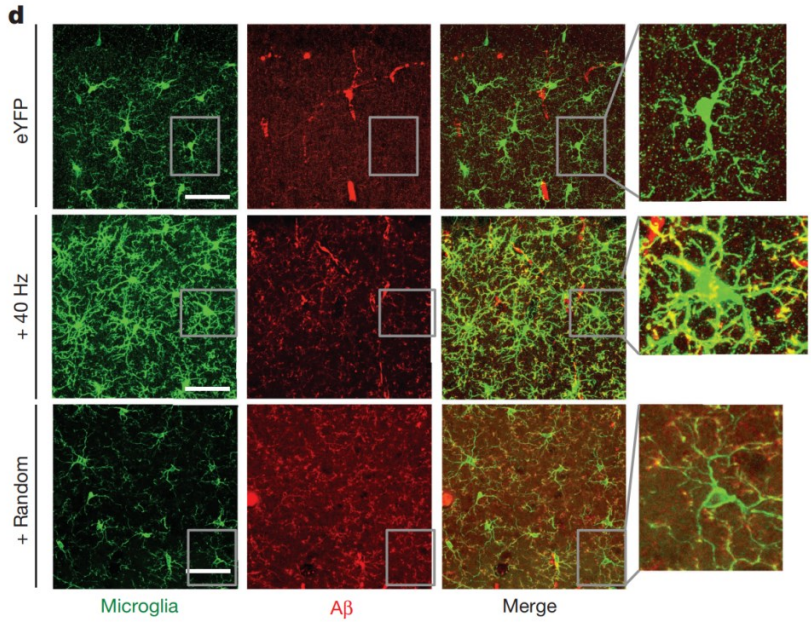

Figure 1. from Gamma frequency entrainment attenuates amyloid load and modifies microglia[4].

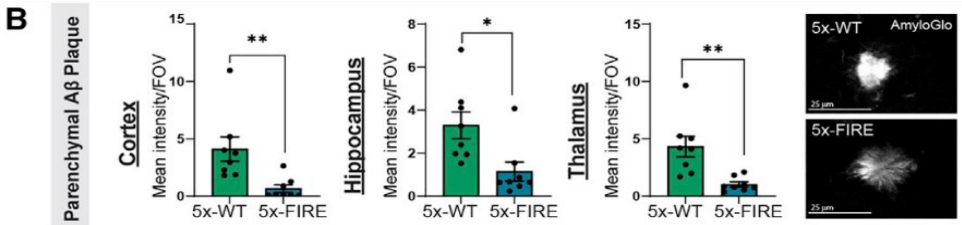

Figure 2. from Absence of microglia promotes diverse pathologies and early lethality in Alzheimer's disease mice[3].

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In Figure 1, the number of MG decreases while the number of Ab increases. In Figure 2, the number of MG decreases while the number of Ab decreases. But in common, these two groups of researchers both didn't clarify the reason. This led me to think of the possibility that M1 and M2, which are two kinds of MG, maybe the main cause of these results. In the present study, I investigated the role M1 and M2 played in causing different effects on the clearance of Ab. And the results show that their effects may be opposite. Specifically, we can propose that M2 will improve amyloid-beta clearance. The balance between M1 and M2 microglia may offer therapeutic avenues for neurodegenerative diseases, indicating that interventions aimed at enhancing M2 activity could improve amyloid-beta clearance in Alzheimer's disease[6].

Secondly, certain substances affect the number of M1 and M2 in the brain. For example, iNOS is involved in the production of nitric oxide (NO), which is linked to neuroinflammation and demyelination. The absence of iNOS in knockout mice reduced neuroinflammation and improved motor function compared to control groups, highlighting the enzyme's role in promoting inflammation in the brain [7]. In addition, the IL-4 receptor plays a crucial role in regulating immune responses and is particularly important for activating the M2 microglial phenotype, which facilitates the clearance of amyloid-beta and mitigates neuroinflammation in Alzheimer's disease[8]. Also, TREM2 plays a crucial role in modulating the balance between M1 and M2 microglia, suggesting that differential activation of microglia through TREM2 signaling could contribute to the varying effects on amyloid-beta clearance seen in Alzheimer's disease[9].

Finally, precise protein detection methods are essential in investigating the role of M1 and M2 microglia in amyloid-beta clearance. Techniques that allow for Western blotting of microscopic areas in histological sections provide a robust approach to analyzing protein distribution and expression in specific brain regions affected by Alzheimer's disease[2]. Also, the CRISPR-Cas9 technology for genome editing is potentially able to create precise knockin mouse models that can be utilized to study complex diseases, including Alzheimer's disease[10,11].

2. Methods

This study aimed to investigate the role of M1 and M2 played to cause different effects to the clearance of Ab using a mouse model. The experimental design included genetically modified mice, specifically engineered to have M1, M2, TREM2, and CSF1R knockout phenotypes. Various parameters were measured to assess the effects of microglia.

iNOS (inducible nitric oxide synthase) knockout mice, Interleukin-4 receptor (IL-4R) knockout mice, CSF1R knockout mice, TREM2 knockout mice, and wild-type mice with no gene defect.

Each of the mice were housed in a pathogen-free environment with a 12-hour light/dark cycle and access to food and water at all times. The animals were acclimated for one week prior to the beginning of the experiments. Mice were 8-10 weeks old and weighed between 20-25 grams. A total of 50 mice were divided into five groups:

Group 1: iNOS Knockout Mice genetically modified to lack inducible Nitric Oxide Synthase (iNOS).

Group 2: Interleukin-4 Receptor Knockout Mice lacking the Interleukin-4 receptor (IL-4R), important in anti-inflammatory signalling.

Group 3: CSF1R Knockout Mice with Colony Stimulating Factor 1 Receptor (CSF1R) knockout, critical for microglia survival.

Group 4: TREM2 Knockout Mice lacking Triggering Receptor Expressed on Myeloid cells 2 (TREM2), which affects microglia activation.

Group 5: Wild-Type Control Unmodified mice serving as the baseline control.

Groups 1-4 and the wild-type control group were genetically engineered to overexpress human Amyloid Precursor Protein (APP) with the Swedish mutation (APPswe), which leads to the development of Aβ plaques. These groups were observed to examine the effects of the respective gene knockouts on Aβ accumulation. After four weeks of treatment, the mice were anesthetized and transcardially perfused with ice-cold phosphate-buffered saline (PBS) to extract blood. The brains were quickly dissected, with the hippocampus and cortex isolated for protein extraction. Each mouse's hippocampus and cortex were

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homogenized in ice-cold RIPA buffer that contained protease and phosphatase inhibitors. The homogenates were centrifuged, and the supernatant was used for protein analysis. Protein samples (30 μg) were loaded onto SDS-polyacrylamide gels, electrophoresed, and then transferred to PVDF membranes. Membranes were incubated with primary antibodies for Aβ, MG markers (such as Iba1), and β-actin (loading control). After incubation with HRP-conjugated secondary antibodies, signals were detected using an enhanced chemiluminescence (ECL) substrate. Aβ and MG marker bands were quantified using ImageJ software, normalized to β-actin, and compared across five groups. The statistical analysis was carried out using GraphPad Prism 9.0 software. The data were presented as mean ± SEM. One-way ANOVA followed by Tukey's post-hoc test was used to compare protein levels among the five groups. A p-value of <0.05 was considered statistically significant. A potential limitation of this study is the use of a single time point for analysis, which may not capture dynamic changes in Aβ accumulation or MG activity over time. The knockout models may also exhibit compensatory mechanisms that could influence the results.[12-14]

3. Results

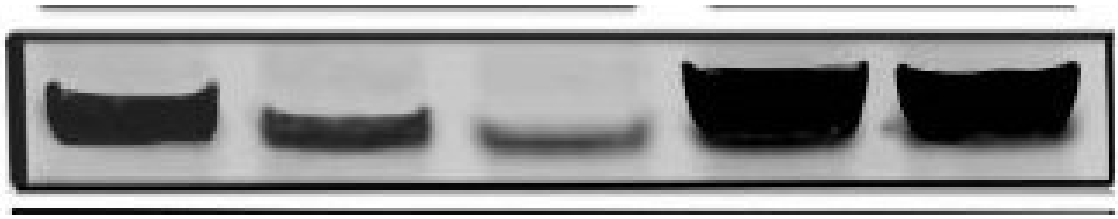

Figure 3. Possible results for MG concentration in different experiment groups by western blotting (From left to right are: Group 1, Group 2, Group 5, Group 3, and Group 4)

For Group 1 (iNOS knockout), the reduction in Aβ levels compared to Group 3 (CSF1R knockout) but higher levels than in Groups 2 (IL-4 receptor knockout) and 5 (wild-type control) suggests that the absence of inducible nitric oxide synthase (iNOS) limits M1 macrophage activity, thereby reducing neuroinflammation and subsequently Aβ production. However, the incomplete elimination of Aβ indicates that M1 macrophages still play a role in sustaining some degree of inflammation that influences Aβ levels. Group 2, which exhibited higher Aβ levels than the wild-type control but lower levels than the other knockout groups, further supports the hypothesis that M2 macrophages are crucial for Aβ clearance. The knockout of the IL-4 receptor, vital for M2 activation, likely impairs the ability of these cells to clear Aβ effectively, even though the reduced pro-inflammatory response from M1 macrophages may prevent excessive Aβ production. Interestingly, when only M1 macrophages were present, Aβ levels were lower than in the complete absence of microglia, yet these levels remained significantly higher compared to conditions where M2 macrophages were active. This finding suggests that M1 macrophages, while capable of managing other immune threats, lack robust mechanisms for Aβ clearance. Their production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β creates an environment conducive to increased Aβ production by neurons, thus exacerbating AD pathology. Conversely, M2 macrophages demonstrated superior capabilities in Aβ clearance, as evidenced by higher Aβ levels in conditions lacking M2 macrophages. M2 macrophages produce anti-inflammatory cytokines like IL-10, which not only suppress inflammation but also promote tissue repair and facilitate the degradation and removal of Aβ plaques through phagocytosis. The presence of M2 macrophages appears to counteract the pro-inflammatory effects of M1 macrophages, suggesting a synergistic relationship where M2 macrophages mitigate the negative impact of M1 macrophage activity on Aβ accumulation. These observations lead to the conclusion that M1 and M2 macrophages jointly regulate Aβ metabolism. M1 macrophages primarily influence Aβ production through their regulation of inflammatory processes, while M2 macrophages play a more direct role in Aβ clearance. The interplay between these macrophage phenotypes is crucial for maintaining Aβ homeostasis, and any imbalance—whether through a reduction in M1 macrophages, leading to unchecked Aβ production, or a decrease in

M2 macrophages, leading to impaired Aβ clearance—can significantly contribute to Alzheimer's disease progression.

4. Conclusion

In conclusion, while the traditional M1/M2 paradigm may not fully capture the complexity of macrophage and microglial functions in Alzheimer's disease, their plasticity opens up new avenues for therapeutic investigation. Furthermore, advances in early detection via biomarkers, as emphasized by Hansson [15], could be critical in designing timely interventions to modulate microglial activation and reduce amyloid-beta accumulation. These findings highlight the potential for more targeted and effective approaches to Alzheimer's disease pathology.

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