

## Inflammation-Induced Antibodies in a Murine Model of Alzheimer's Disease

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Alzheimer's disease (AD) is a form of dementia characterized by the presence of damaged proteins (Aβ) in the brain. A week of inflammation stimulates production of this protein in mice. However, a two-week recovery period, followed by a second week of inflammation, results in no additional Aβ. The initial inflammation seems to provide protection from a second exposure. The present study investigated antibodies produced after the second course of inflammation in mice that have human AD. Our data suggests that inflammation exacerbates the antibody response in AD mice, and that these antibodies may specifically target A $\beta$ .

### Introduction

- Alzheimer's disease (AD) is a neurodegenerative disorder that affects nearly 5.3 million Americans, and there is currently no cure (1).
- AD is characterized by A $\beta$  plaques and neurofibrillary tangles in the brain (2).
- Immunohistochemistry of the brains of mice immunized with anti-Aβ antibody reveals increased IgG binding to  $A\beta$  plaques and decreased plaque burden (3,4)
- Our lab has shown that 7 consecutive days of LPS injections result in an increase in hippocampal expression of A $\beta$  along with deficits in learning and memory (5).
- We have also found that 7 days of LPS injections followed by a 2 week break and a second 7-day course of LPS injections does not cause additional A $\beta$ production (6).
- Our hypothesis is that this protective effect seen after repeated LPS exposures is due to increased IgG production.
- Previous studies have demonstrated increased IgG binding to Aβ plaques in the brains of AD patients, which helps to alleviate the plaque burden (7).
- We expected the increased amount of plasma IgG to cross the blood brain barrier and aggregate around  $A\beta$  plaques.

### Methods



- Alzheimer's transgenic (5xFAD) mice were administered LPS or saline per group assignment according the injection schedule above
- 24 hours after the last injection, hippocampal tissue and peripheral blood were collected, and brain tissue was collected for immunohistochemistry
- Hippocampal IgG and plasma IgG and IgM levels were quantifies by ELISA
- Brain sections were immunostained for Aβ and IgG
- IgG and Aβ co-localization was quantified via confocal microscopy. Briefly, mean fluorescent intensity of IgG per plaque was measured, and a general linear model was used to analyze IgG intensity with 6E10 as a covariate

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# Results В 6E10 D 6E10 Figure 1. IgG co-localization around amyloid beta plaques in 5xFAD mouse Tissue hippocampus. Amyloid beta plaque is shown in green in panel A (6E10), and panel B shows the same plaque area with IgG stain in red. Panel C shows both images overlaid, with panel D demonstrating the outlining of the plaque area on confocal software, excluding IgG that is not directly on the plaque 140-



Figure 2. Factorial plot of mean IgG intensity. Figure shows mean intensity for each treatment group, and the central dashed line represents the grand mean. Using a general linear model, mean intensity of IgG was determined for each group, using 6E10 as a covariate. With IgG as the response variable, a significant effect was observed for both 6E10 and treatment groups (p<0.001 and p=0.036, respectively). Treatment group and 6E10 together account for 81.07% of the observed variation in IgG. The control group here indicates an untreated 5xFAD+ group which received no injections.

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#### Conclusion

We hypothesized that administration of LPS would cause an increase in IgG, further leading to an increase in IgG aggregation around Aβ plaques in the hippocampus. Our hypothesis was partially supported by our results. • Increased IgG co-localization in 5xFAD+ hippocampus correlates with 2 bouts of LPS • 2 bouts of LPS did not significantly increase plasma or hippocampal antibodies

Though ELISA data did not reveal differences between treatment groups in hippocampal IgG, confocal microscopy suggested an increase in Aβ-specific IgG after two bouts of LPS, demonstrated by the increased presence of IgG around amyloid plaques.

#### **Future Directions**

Confocal microscopy data showed significantly more IgG co-localization with A<sub>β</sub> plaques in the LPS/LPS treatment group compared to untreated controls, suggesting elevated production of Aβ-specific IgG. Future studies will confirm this antibody specificity with ELISA. Furthermore, the effect of this increased antibody presence in the brain needs to be investigated, specifically determining if IgG is helping to reduce the size or quantity of amyloid plaques in the 5xFAD mice. These results may implicate a role of the adaptive immune response in targeting A $\beta$  plaques in our 5xFAD model of Alzheimer's disease.

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