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Continued food-allergen consumption exacerbates beta-amyloid accumulation in allergen-sensitized *App*$^{NL-G-F}$ mice

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Abstract

Alzheimer's disease (AD) is the most common neurodegenerative disease, with β-amyloid (Aβ) plaque deposition being one of the hallmark pathologies. However, the etiology of AD remains elusive. While chronic inflammation from recurrent infections or injury seems to contribute to AD development, it is unclear whether atopic diseases, such as allergies, are associated with AD. We previously reported that continuous consumption of a whey protein (WP)-containing diet led to lasting neuroinflammation in C57BL/6J mice that were sensitized but tolerant to a bovine milk allergen, β-lactoglobulin (BLG; Bos d 5). Thus, we hypothesized that the persisting neuroinflammation due to repeated allergen consumption would exacerbate AD pathology over time in genetically predisposed, allergen-tolerant individuals. To address this hypothesis, we sensitized 1-month-old male and female transgenic *App*$^{NL-G-F}$ knock-in mice to BLG and subsequently fed them either a whey-free control or a WP diet for 6 months. Despite their lack of overt allergic reactions, BLG-sensitized mice showed elevated plasma levels of BLG-specific IgE and IgG1. Leukocyte infiltration was observed in the hippocampus of sensitized mice, and WP-fed sham mice to a lesser extent. In contrast, mast cell accumulation was apparent in the dura of sensitized mice regardless of the diet. Importantly, Aβ plaque load and Aβ peptide levels were greater in the hippocampus of BLG-sensitized mice on the WP diet compared to the respective sham group. These results indicated that continuous allergen consumption exacerbated AD-like pathology in BLG-sensitized *App*$^{NL-G-F}$ mice, suggesting that chronic food allergen exposure may promote the progression of AD in susceptible individuals.

Introduction

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease and a leading cause of death among the elderly. AD pathology is characterized by the presence of β-amyloid (Aβ) plaques and neurofibrillary tangles, although the etiology of AD remains elusive.

While chronic inflammation from recurrent infections or immune dysfunction is associated with AD [1], whether inflammation from allergic diseases contributes to AD pathology has not been examined.

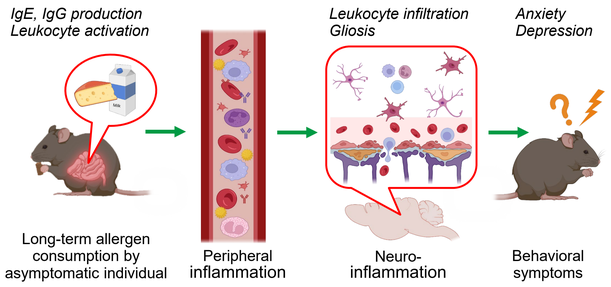

Fig 1. Previous findings on wild-type mice

Hypothesis

We hypothesized that uncontrolled exposure to an offending food allergen would maintain low-grade inflammation and exacerbate or accelerate AD pathology development over time in genetically susceptible individuals.

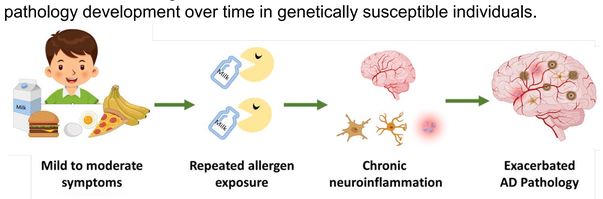

Fig 2. Working hypothesis

Methods

Sensitization of mice: One-month-old male and female *App*$^{NL-G-F}$ mice were given a bicarbonate buffered vehicle with 10 μg cholera toxin without or with 1 mg β-lactoglobulin (BLG; Bos d 5) weekly for 5 weeks. To maintain their sensitization status, mice received their respective sensitization regimen every other week until the end of the experiment. All procedures were approved by UND IACUC.

Chronic allergen exposure: Sham or BLG-sensitized mice were subsequently fed a whey-free control (CTL) or a 0.3% whey-protein (WP) diet for 24 weeks (Fig 3).

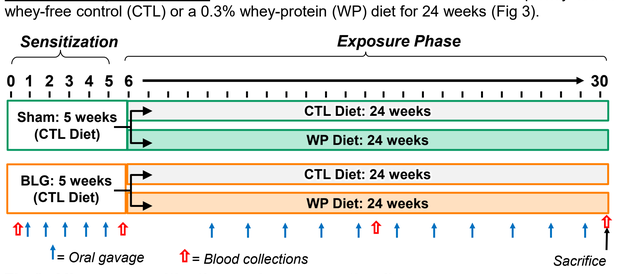

Fig 3. Allergen sensitization and exposure timeline

BLG-specific immunoglobulin ELISA. Plasma samples collected at each timepoint were diluted 1:40 before determining BLG-specific IgE and IgG1 levels by ELISA [2].

Acidic toluidine blue staining: Dura mater was peeled from each skull cap, mounted on glass slides, and stained for mast cells with 0.1% toluidine blue (pH 2.8) for 1 h at RT.

Aβ peptide ELISA: Aβ peptide levels in the hippocampal lysates were quantified using Human Amyloid β40 and β42 brain ELISA kits (MilliporeSigma).

Immunohistochemistry: Paraformaldehyde-fixed brains were cryosectioned at 40 μm and stained with antibodies against CD45 (1:500, eBioscience) [3] and Aβ (1:500, Cell Signaling Technology) at 4°C overnight. Immunoreactivity was visualized with Vector VIP as the chromogen with the Vector Elite ABC kit (Vector Laboratories).

Western Blotting: Western blotting was performed using PSD95 antibody (Cell Signaling Technology) at 1:1,000 dilution [2].

Results

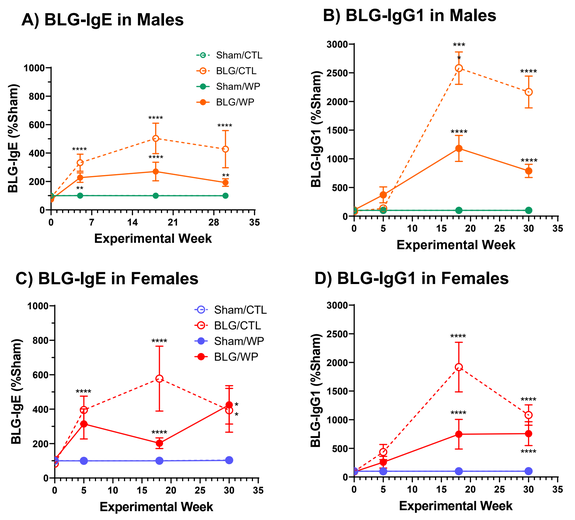

Fig 4. Continued WP consumption lowered BLG-specific IgE and IgG1. The plasma levels of BLG-specific IgE (A, C) and IgG1 (B, D) were quantified by ELISA to determine the sensitization status of male (A, B) and female (C, D) mice at different time points. Mice that were placed on the WP diet showed declines in BLG-specific antibody levels compared to mice that stayed on the whey-free CTL diet. Mean ± SEM, multiple Mann–Whitney's *U*-test at each time point; $^{****}p < 0.00001$ $^{**}p < 0.001$, $^{*}p < 0.05$. n=14-18.

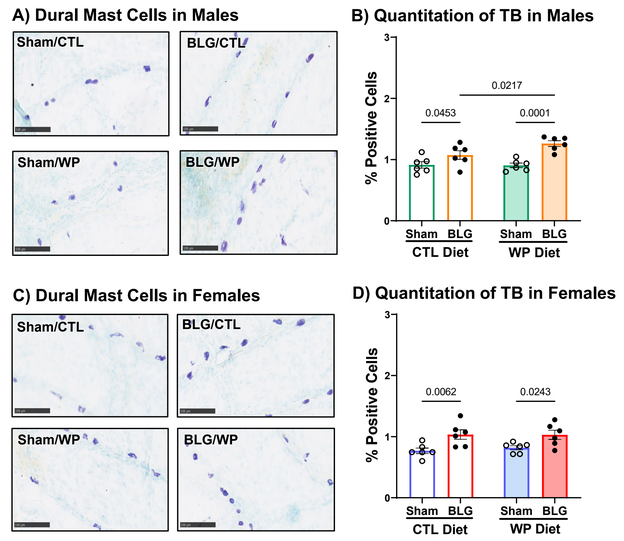

Fig 5. BLG sensitization increased mast cell numbers in the dura. Mast cells identified with acidic toluidine blue staining were present in the dura of sham and BLG-sensitized mice. However, greater numbers of mast cells were found in the latter male (A, B) and female (C, D) groups. 40x objective; scale bar = 100 μm. Quantitative analysis confirmed the qualitative observations (B, D); Mean ± SEM, n=6, two-way ANOVA.

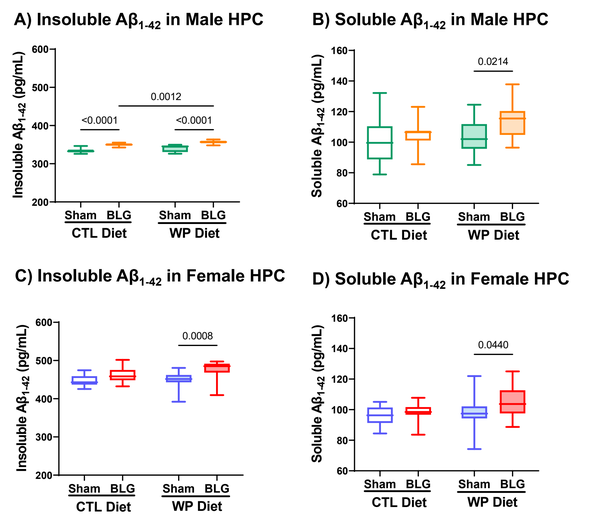

Fig 6. Continuous WP consumption increased hippocampal Aβ$_{1-42}$ levels in BLG-sensitized male and female mice. Insoluble and soluble Aβ$_{1-42}$ peptides in hippocampal tissue lysates were quantified by ELISA in males (A, B) and females (C, D). Aβ$_{1-42}$ levels in both lysate fractions were significantly elevated in all BLG-sensitized groups with the WP diet. Mean ± SEM, n=14-18, two-way ANOVA.

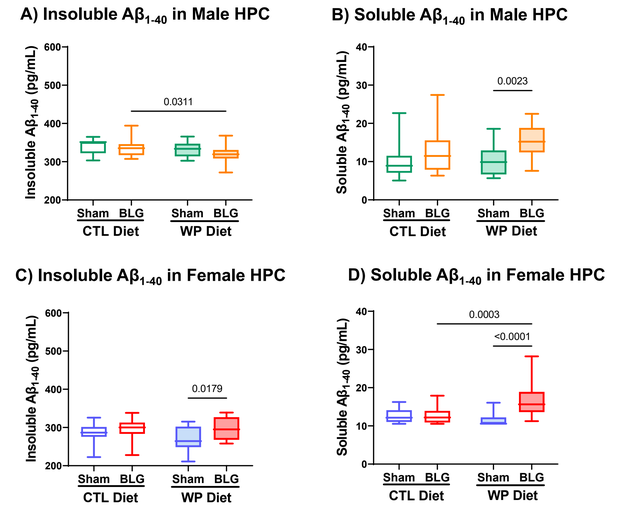

Fig 7. Continuous WP consumption increased hippocampal Aβ$_{1-40}$ levels in BLG-sensitized female mice and soluble Aβ$_{1-40}$ in males. Insoluble and soluble Aβ$_{1-40}$ peptides in hippocampal tissue lysates were quantified by ELISA in males (A, B) and females (C, D). Aβ$_{1-40}$ levels in both lysate fractions were elevated in all BLG-sensitized groups with WP diet, except Aβ$_{1-40}$ in the insoluble fraction in male mice. Mean ± SEM, n=14-18, two-way ANOVA.

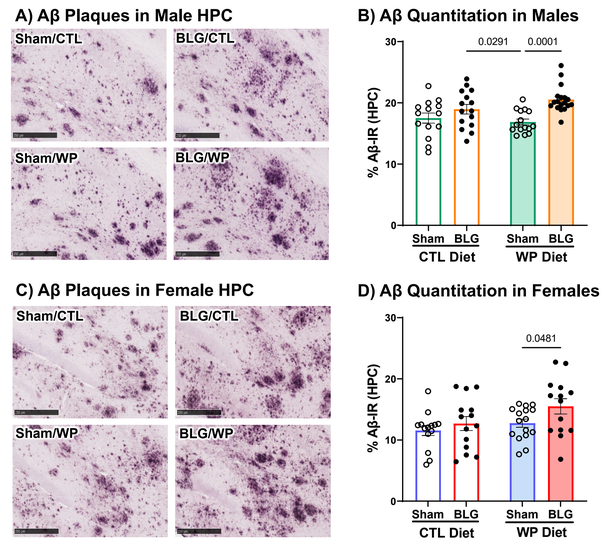

Fig 8. Aβ plaque loads increased in the hippocampus of BLG-sensitized mice with the consumption of the WP diet. While Aβ plaques were present throughout the brains of all *App*$^{NL-G-F}$ mice, the loads were significantly greater in the hippocampus of BLG-sensitized mice with the WP diet for both males (A, B) and females (C, D). Photomicrographs (A, C) were taken with a 40x objective. Scale bar=250 μm. Aβ immunoreactivity (IR) was quantified using QuPath software (B, D). Mean ± SEM, n=14-18, two-way ANOVA.

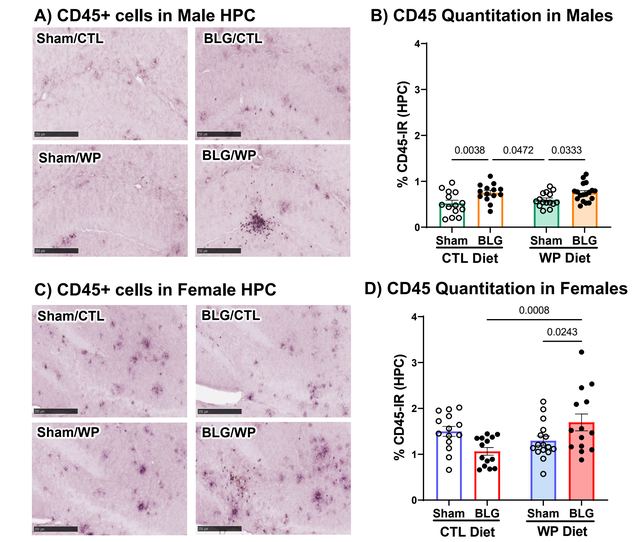

Fig 9. Clusters of CD45-immunoreactive leukocytes were more frequently found in the hippocampus of BLG-sensitized mice. Immunohistochemical staining for CD45 revealed round and ovoid immunoreactive (IR) cells distinct from microglia in the brains of both sham and BLG-sensitized mice. These small CD45-IR cells were more frequently found in BLG-sensitized mice, regardless of their diets, although their presence was more pronounced with the WP diet for both male (A, B) and female (C, D) mice. Scale bar=250 μm. CD45-IR was quantified using Qupath software (C). Mean ± SEM, n=14-18, two-way ANOVA.

Results (cont'd)

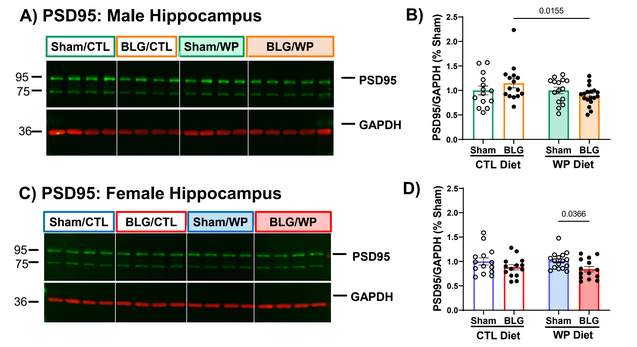

Fig 10. Chronic allergen exposure resulted in the loss of postsynaptic density protein, PSD95. The levels of PSD95 were detected in hippocampal lysates of both male (**A**, **B**) and female (**C**, **D**) *App*$^{NL-G-F}$ mice by western blotting. Representative blots are shown in **A** and **C**. PSD95 immunoreactivity was quantified using the LI-COR Image Studio software (**B**, **D**). PSD95 levels were significantly decreased in the hippocampus of BLG-sensitized mice on the WP diet of both sexes. Mean ± SEM, n=14-18, two-way ANOVA.

Discussion

- Decreased BLG-specific IgE levels in sensitized male mice after the consumption of the WP diet for 30 weeks indicated that chronic exposures to small amounts of the offending allergen led to desensitization in these mice. In contrast, the WP diet regimen did not effectively decrease IgE in females and IgG1 in both sexes.
- Increased numbers of mast cells in the dura mater of BLG-sensitized mice suggest that these cells may play a role in mediating neuroinflammation. Mast cells are the first responders to both allergy and brain injury [4].
- The increased leukocyte presence in the hippocampus of BLG-sensitized mice with the WP diet suggests that brain-infiltrating leukocytes may serve as additional mediators of neuroinflammation.
- Increased levels of Aβ peptides and plaque loads in the hippocampus of BLG-sensitized mice with the WP diet indicated that chronic allergen exposure exacerbated AD pathology development in these mice, despite the lack of physical allergic reactions and decreased IgE.
- The loss of postsynaptic protein, PSD95, was significant in the hippocampus of BLG-sensitized female mice on the WP diet. A decreasing trend in PSD95 was also observed in male mice with the WP diet. These results suggested that chronic allergen consumption might lead to synaptic losses in sensitized individuals.

Conclusions

These results demonstrated that prolonged allergen consumption exacerbated some aspects of AD pathology in the hippocampus of BLG-sensitized *App*$^{NL-G-F}$ mice, supporting our hypothesis. Meningeal and parenchymal mast cells and other CD45-IR leukocytes may maintain neuroinflammation during allergen exposure, serving as peripheral-to-central mediators. Phenotyping of these cells and cytokine profiling will clarify the nature of the immune responses to the allergen and elucidate the mechanism of AD pathology exacerbation.

References

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[2] Germundson D.L. *et al. (2022) Cells*, 11:738.
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Acknowledgments

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