Repeated allergen exposure induces histaminergic dysregulation and depression-like behaviors in a non-anaphylactic food allergy mouse model

Danielle L Germundson-Hermanson[1] and Kumi Nagamoto-Combs[2]
Department of Pathology and Biomedical Sciences, University of North Dakota SMHS

UND School of Medicine & Health Sciences
University of North Dakota

Introduction

- Food allergy is often comorbid with neuropsychiatric disorders such as anxiety[1,2], depression[1,2], OCD[3], ADD/ADHD[4], and autism[5].
  - The mechanism of how food allergy may impact brain function and behavior in certain allergic individuals has yet to be elucidated.
- Mast cells (MCs) are not only the effector cell of the hypersensitivity response but are also important in maintaining brain homeostasis[6].
  - MCs have been implicated in neuropsychiatric and neurodegenerative disorders such as multiple sclerosis[7,8].
- We have previously found in a mouse model of cow's milk allergy (CMA) that intracranial MC numbers[9] and histamine 3 receptor (H3R)[10] increased after acute allergic challenge in association with neuroinflammation and behavioral changes.
- **We hypothesized that upon exposure to an allergen, MC-derived histamine would cause dysregulation of the brain's histaminergic system, increasing neuroinflammation and altering behavior.**
- To test our hypothesis, we preformed two studies using our mouse model of non-anaphylactic CMA to 1) observe changes in behavior and brain pathophysiology and 2) determine the role of excess histamine in our mouse model in the development of aberrant behaviors.

Methods

Weeks 1-5: Sensitization

- Sensitized male C57BL/6J mice to a cow's milk whey allergen, β-lactoglobulin (BLG).
- The BLG group was given a weekly oral gavage of 10 µg of cholera toxin and 1 mg BLG for 5 weeks.
- The sham group was given only the cholera toxin for 5 weeks.

Weeks 6-7: Exposure

- Sham and BLG mice were placed on a 0.3% whey-containing diet.
- Allowed to free-feed for 2 weeks and no severe allergy symptoms developed.
- During Week 7, mice underwent behavior testing and were then euthanized.

Results

Study 1: Increased Intracranial MCs and Histamine

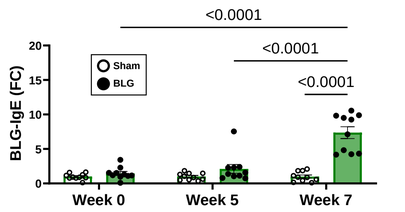

Fig 1. BLG-specific IgE significantly increased after repeated allergen exposure in the absence of allergy symptoms. Plasma samples were collected before, during, and after the sensitization and allergen exposure period to validate their hypersensitivity status by BLG-specific IgE ELISA from which fold change (FC) relative to sham was calculated. No overt allergic symptoms were observed at any time during the 7 weeks. (2-Way ANOVA; Mean ± SEM).

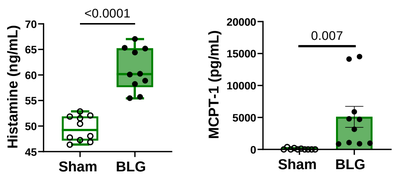

Fig 2. Serum histamine and mast cell protease-1 (MCPT-1) levels were elevated after allergen exposure in BLG-sensitized mice. Histamine levels and MCPT-1 were measured from terminal serum by ELISA as indicators of mucosal MC activation. (Student's t-test; Mean ± SEM).

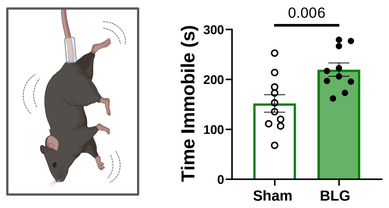

Fig 3. BLG-sensitized mice showed increased depression-like behavior after repeated allergen exposure. Mice were subjected to the tail suspension test for 6 minutes in Week 7, one day before sacrifice, as depicted in the left figure above. BLG-sensitized mice showed significantly increased time immobile compared to their sham counterparts, indicating the development of depression-like behavior (Student's t-test; Mean ± SEM).

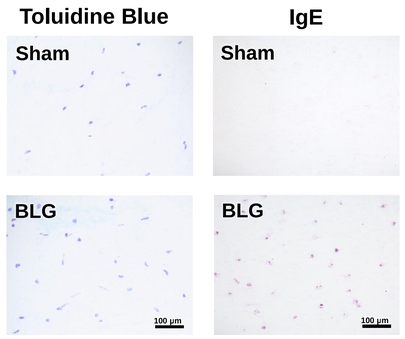

Fig 4. IgE-primed intracranial MCs were observed in the meninges of BLG-sensitized mice. Toluidine blue stained MCs were observed throughout the dura mater of the meninges in sham and BLG-sensitized mice (20x objective). However, only BLG-sensitized mice showed IgE-immunoreactive cells in their meninges (20x objective).

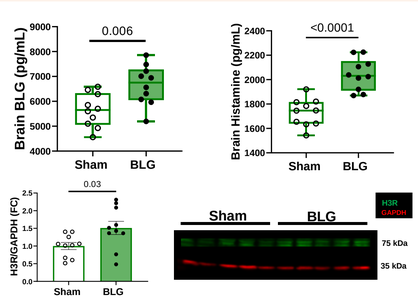

Fig 5. The levels of BLG, histamine, and H3R were elevated in the brains of sensitized mice after repeated allergen exposure. BLG and histamine levels were measured by ELISA from extracted brain lysates. Frontal cortex H3R expression was determined by near-infrared western blotting and normalized to GAPDH reference protein from which fold change (FC) was calculated. (Student's t-test; Mean ± SEM).

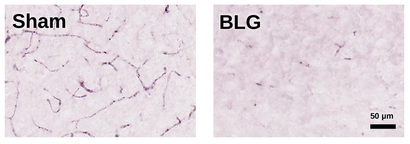

Fig 6. Blood-brain barrier permeability increased in BLG-sensitized mice with repeated allergen exposure. In sham mice, IgG immunoreactivity was found within the blood vessels (40x objective). In contrast, IgG extravasation was observed, indicating an impaired blood-brain barrier.

Study 2: H3R Antagonist Treatment

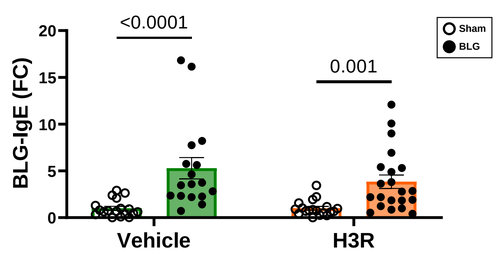

Fig 7. The H3R antagonist did not alter serum levels of BLG-specific IgE levels in BLG-sensitized mice. Terminal plasma was collected from mice at the end of Week 7 confirmed that daily treatment with H3R antagonist thioperamide during the allergen exposure period did not affect the development of hypersensitivity of BLG-sensitized mice (2-Way ANOVA; Mean ± SEM).

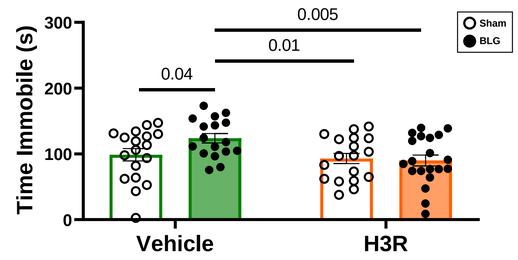

Fig 8. H3R antagonist treatment prevented the development of depression-like behavior in BLG mice. The tail suspension test was performed on vehicle and H3R antagonist-treated mice during Week 7 to determine if depression-like behavior manifested after consuming the allergen containing diet while receiving the H3R antagonist (2-Way ANOVA; Mean ± SEM).

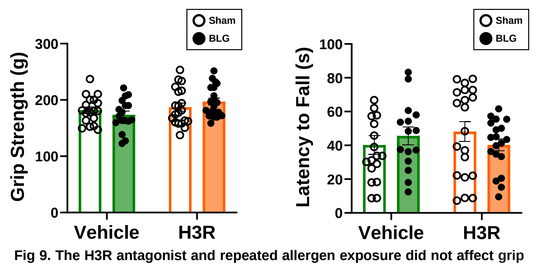

Fig 9. The H3R antagonist and repeated allergen exposure did not affect grip strength or motor coordination. During Week 7, mice were subjected to the grip strength test and rotor-rod test which measure muscle strength and motor coordination, respectively. The left bar graph indicates the average grip strength of all four limbs for each mouse, while the right bar graph shows the average latency to fall from the rotating bar during the rotor-rod test. (2-Way ANOVA; Mean ± SEM).

Discussion

- Our repeated exposure CMA mouse model may better simulate "asymptomatically sensitized" individuals who can consume offending foods without life-threatening reactions[11].
  - This allergic population is more at risk for chronic allergen exposure and inflammation.
- Intracranial MCs were primed with IgE in the dura mater of sensitized mice, and BLG was elevated in their brains after the repeated allergen exposure.
  - MCs are well known to be the first responders in both the hypersensitivity response and brain injuries[6-8]. Thus, activation of intracranial MCs through the allergen/IgE/FcεRI mechanism may be a mediator of the neuroinflammatory and behavioral changes we observed as well as the source of increased brain histamine.
- H3R antagonist treatment of BLG-sensitized mice after allergic sensitization prevented the development of depression-like behavior compared to BLG mice that were given the vehicle during Week 6 and 7.
  - Our results in Study 2 strongly suggests that histamine dysregulation is involved in the food allergy-triggered behavioral changes.

Conclusions

Our findings support the notion that repeated allergen consumption by asymptomatically sensitized individuals can result in the intracranial MC activation, causing histaminergic dysregulation that negatively affects behavior.

Thus, therapeutics targeting MCs and histamine or limiting allergen consumption may be a strategy to reduce the risk of neuroinflammatory or neuropsychiatric conditions in susceptible individuals.

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