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Cortical demyelination and depression-like behavior are associated with histaminergic dysregulation in a mouse model of peripheral inflammation

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NO – PHOTO / RECORD

INTRODUCTION

- Demyelinating diseases of the central nervous system are increasing in prevalence world-wide[1,2] and manifest as motor, behavioral, and/or cognitive defects[3]. The etiology and pathophysiology of demyelinating diseases remain unclear.
- We have previously observed cortical demyelination in our mouse model of non-anaphylactic cow's milk allergy[4]. The demyelination was associated with depression-like behaviors and region-specific increases in brain histamine and H3 receptor (H3R) levels[4,5].
  - ❖ The brain's central histaminergic system is tightly controlled and regulates many behaviors. Additionally, signaling through H3R plays a crucial role in oligodendrocyte differentiation, and thus, demyelination and remyelination[6].
- **We hypothesized that excess histamine produced during the hypersensitivity response would influence behavior through dysregulation of the central histaminergic system, resulting in neuroinflammation and demyelination.**
- To test our hypothesis, we treated our food allergy mouse model with thioperamide, an H3R antagonist, and examined whether blocking histaminergic signaling would ameliorate the aberrant behaviors and demyelination.

METHODS

Animals and Treatments

Four-week-old male C57BL6/J mice (n= 18-21) were purchased from Jackson Laboratories. Sham mice received an intragastric gavage of a bicarbonate buffer (pH 9.0) vehicle containing 10 µg cholera toxin (List Biologicals). Sensitized mice received an oral gavage of the vehicle with 1 mg of a bovine whey allergen, β-lactoglobulin (BLG; Millipore-Sigma). Both sham and BLG-sensitized treatment groups were then placed on a diet containing 0.3% whey proteins (Envigo) for two weeks with 30 mg/kg thioperamide (Millipore-Sigma) or saline given daily by oral gavage. During Week 7, mice were subjected to a series of behavioral tests and then euthanized via CO_2 asphyxiation. All animal use was approved by UND Institutional Animal Care and Use Committee.

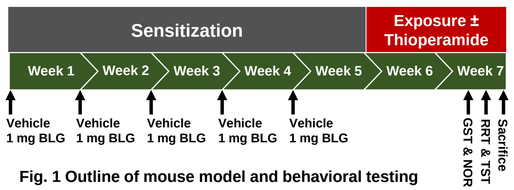

Fig. 1 Outline of mouse model and behavioral testing

Behavioral Testing

Grip Strength Test (GST): Mice were lowered onto a metal grate attached to a force gauge (San Diego Instruments) and allowed to grasp the bars with all four limbs and were then gently pulled backwards by their tail. The force applied before mice lost their grip was recorded as grip strength. The average value from 3 trials was used for the final analysis.

Novel Object Recognition (NOR): Mice were placed in an open apparatus with two identical objects for 10 min. After 1 hr, mice were returned to the apparatus with one familiar and one novel object. Their interactions with the objects were recorded with AnyMaze software.

Tail Suspension Test (TST): Mice were suspended by their tail in a tail suspension apparatus (Bioseb). Their attempts to escape from the upside-down position were video-recorded for 6 min, and their time immobile was tallied by a blinded observer as an indicator of depression-like behavior.

Rotor-Rod Test (RRT): Mice were placed on a rod rotating at 4 rpm (San Diego Instruments). After acclimating for 10 sec, the rod accelerated from 4 rpm to 40 rpm until the mouse fell off the rod. The time mice stayed on the rod was recorded as latency to fall, and the average values from 3 trials were used for the final analysis.

Enzyme Linked Immunosorbent Assays (ELISAs)

The allergen-specific IgE ELISA with 5 µL of plasma was performed as described previously[7]. The competitive histamine ELISA was carried out according to the manufacturer's instructions (Abcam) with 10 µL of plasma.

Immunofluorescent Staining

Formalin-fixed left-brain hemispheres were embedded in gelatin and sectioned at 40 µm as previously described[8]. Tissues were blocked in PBS containing 0.5% BSA, 0.5% NGS, and 0.1% Triton-X 100 and incubated in a primary antibody against neurofilament heavy chain (1:3000, Novus Biologicals) at 4 °C overnight. The antigen was visualized with a goat anti-rabbit antibody conjugated with alexa-488 and dyed with FluoroMyelin™ Red (Invitrogen). Slides were coverslipped with DAPI-containing mounting medium (Vector Laboratories).

Statistical Analysis

Results were compared using GraphPad Prism v9 software. P values <0.05 were considered significant.

RESULTS

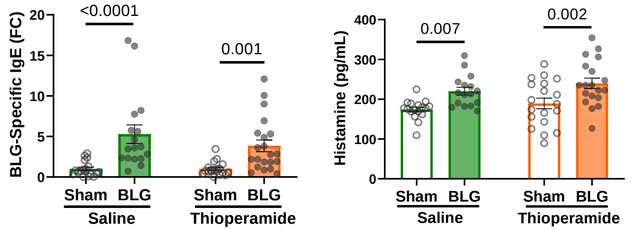

Fig 2. Thioperamide did not affect the sensitization-induced BLG-specific IgE or histamine levels in the sera of sensitized mice. Terminal plasma collected at the end of Week 7 confirmed that daily treatment with thioperamide did not influence the immune response to the whey-containing diet (two-way ANOVA; mean ± SEM; Four outliers were removed by the ROUT method (Q=1%) from the histamine ELISA.

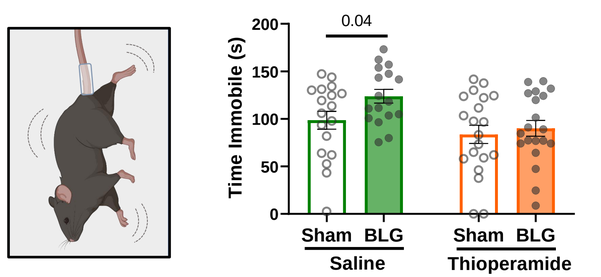

Fig 3. Thioperamide prevented the development of depression-like behavior in sensitized mice. The TST (left) showed that saline-treated BLG mice exhibited significantly increased immobile time compared to their sham counterparts. No difference in immobility was observed due to sensitization when mice were treated with thioperamide (two-way ANOVA; mean ± SEM).

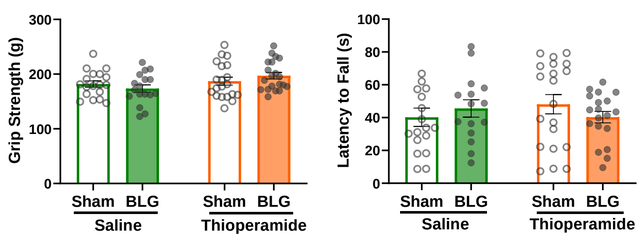

Fig 4. Repeated allergen exposure did not affect grip strength or motor coordination. GST (left) and RRT (right) showed that there were no significant changes in motor function between sham and BLG-sensitized mice. Similarly, the thioperamide treatment did not influence the performance of mice in either test (two-way ANOVA; mean ± SEM).

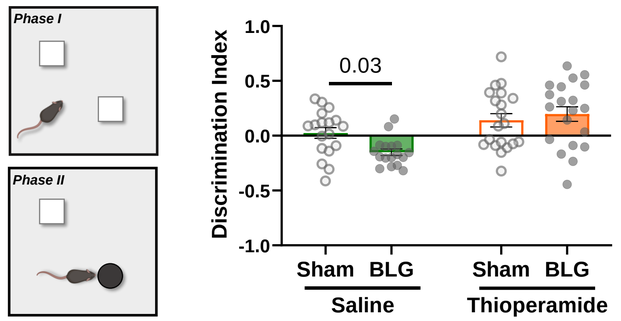

Fig 5. Recognition memory was restored in BLG-sensitized mice after treatment with thioperamide. Mice subjected to the NOR (left) revealed that sensitization significantly decreased the discrimination index between the familiar and novel object. However, thioperamide administration increased the interaction of BLG-sensitized mice with the novel object in phase II of the testing (two-way ANOVA; mean ± SEM).

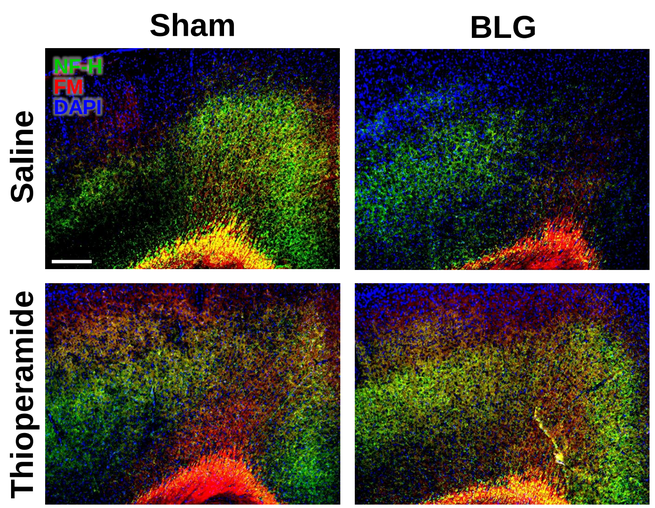

Fig 6. Thioperamide treatment ameliorated sensitization-associated demyelination in the brain. Brain tissue was immunostained with neurofilament-heavy (NF-H) for neuronal cell bodies and axons, dyed with FluoroMyelin™ (FM) for the myelin sheath, and nuclear counterstained with DAPI. Cortical demyelination was apparent after repeated allergen exposure in saline-treated BLG mice. Diminished NF-H immunofluorescence was also observed in the cortex of this group. BLG-sensitized mice given thioperamide did not have a notable decrease in myelin or NF-H staining. Photomicrographs were taken from the anterior cingulate and primary motor cortex using a 10X objective (scale bar=200 µm).

DISCUSSION

- Saline-treated BLG-sensitized mice have significantly increased depression-like behavior and impaired recognition memory associated with cortical demyelination.
  - ❖ It is unclear why mice did not exhibit motor impairment when demyelination and axonal loss were apparent in the motor cortex. A similar observation with widespread cortical demyelination and loss of neurofilament without the development of motor dysfunction has also been demonstrated in a rat model of multiple sclerosis[9].
- We have previously observed cortical demyelination after BLG sensitization[4]. The results of our current study indicated that the loss of myelin was also accompanied by axonal loss.
  - ❖ The degeneration of axons is the major determinant of irreversible neurologic injury in demyelinating diseases[1,2]. It is possible that BLG mice could still display aberrant behaviors after the discontinuation of allergen exposure.
- Thioperamide prevented allergy-associated depression-like behavior and cognitive impairment without altering the sensitization status of the BLG mice. Furthermore, thioperamide mitigated cortical demyelination and axonal loss.
  - ❖ Histamine signaling through H3R has been demonstrated to inversely correlate with oligodendrocyte differentiation[6]. Further investigation is needed to confirm the role of altered oligodendrocyte functions in the progression of cortical demyelination in our mouse model.

CONCLUSIONS

Our findings suggest that immune responses to an allergen still elicit peripheral inflammation in asymptomatic individuals and result in central histaminergic dysregulation and associated demyelination and behavioral abnormality.

Therapeutics targeting histamine production or signaling may be a strategy to reduce the risk of demyelinating and neurodegenerative disorders in susceptible individuals.

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