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Histamine H3 receptor antagonism mitigates food-hypersensitivity-associated depressive behavior and neuropathology in a mouse model of cow's milk allergy

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Introduction

Background
Histamine is best known for causing allergy symptoms, but it also regulates blood-brain barrier permeability[1] and oligodendrocyte differentiation[2]. Thus, brain histamine levels are tightly controlled.

Previous Findings
We demonstrated that histamine and histamine H3 receptor (H3R) levels were elevated in a mouse model of cow's milk allergy (CMA) in association with intracranial mast cell activation, depression-like behaviors, and cortical demyelination[3,4]

Gaps in Knowledge
In humans, food allergies are often associated with neuropsychiatric disorders[5-7], but the involvement of allergy-induced histamine in triggering behavioral changes is unclear.

Hypothesis
Repeated allergen consumption can lead to central histaminergic dysfunction through H3R, ultimately resulting in cortical demyelination and aberrant behaviors.

Study Objectives
We aimed to elucidate if antagonizing H3R could improve the behavioral and neuropathological outcomes in our mouse model after induction of CMA with repeated dietary allergen exposure.

Methods

Animals and Treatments
Four-week-old male C57BL6/J mice (n=17-20) were purchased from Jackson Laboratories and underwent sham or CMA sensitization once a week for 5 weeks as shown in **Fig 1**. Both sham and CMA-sensitized treatment groups were then placed on a diet containing 0.3% whey proteins for two weeks with 30 mg/kg of the H3R antagonist thioperamide (thio) or saline (vehicle) 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 IACUC.

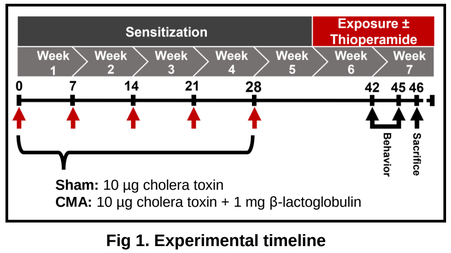

Fig 1. Experimental timeline

Enzyme-Linked Immunosorbent Assays (ELISAs)
The allergen-specific IgE ELISA was performed as described previously[8] with 5 µL of plasma. The competitive histamine ELISA was carried out according to the manufacturer's instructions using 10 µL of plasma.

Immunofluorescence Staining
Whole mount dura underwent antigen retrieval in a tris EDTA buffer (pH 9) with 0.2% tween-20 overnight at 37 °C. Dura and 40-µm brain sections were blocked in PBS containing 0.5% BSA, 0.5% NGS, and 0.1% Triton-X 100 and incubated in a primary antibody against IgE (1:200) and CD117 (1:200) or neurofilament heavy chain (1:3000) at 4 °C overnight, respectively. Antigens were visualized using fluorescently conjugated secondary antibodies and/or dyed with FluoroMyelin™ Red. All tissues were coverslipped with mounting media containing DAPI counterstain.

Statistical Analysis
Results were compared using GraphPad Prism v9.5.1 software. Statistical tests used are indicated in figure legends. A p <0.05 was considered significant.

Results

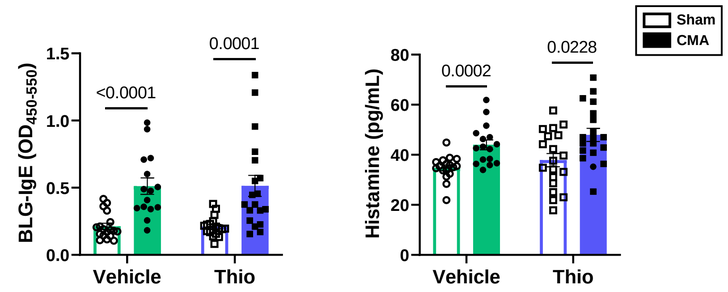

Fig 2. Thioperamide did not affect the sensitization-induced allergen-specific IgE or histamine levels of CMA mice. Terminal plasma collected at the end of Week 7 confirmed that daily treatment with thioperamide did not significantly influence the hypersensitivity to the whey-containing diet (Multiple Mann-Whitney tests and 2-way ANOVA; Mean ± SEM; Outliers were removed by the ROUT method (Q=1%) from the histamine ELISA).

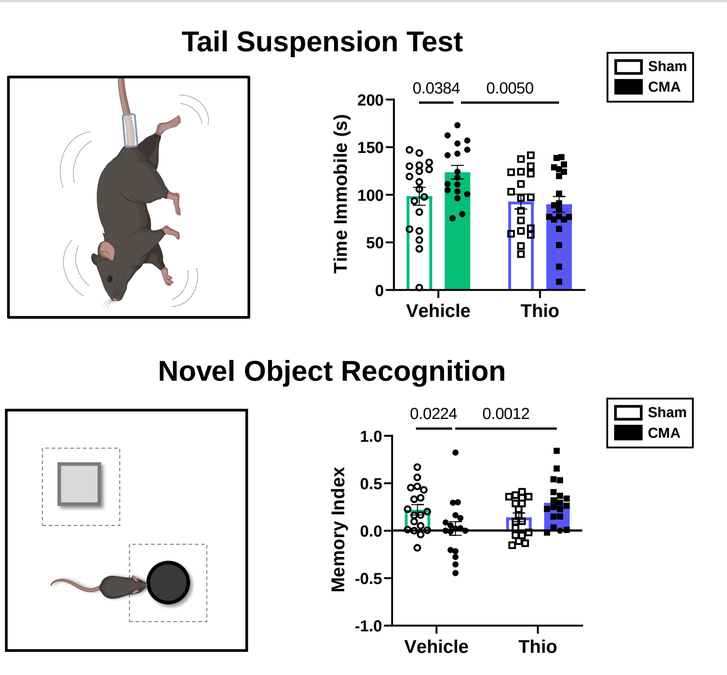

Fig 3. Thioperamide treatment prevented depression-like behavior and memory impairment in CMA mice. During Week 7 mice were subjected to the tail suspension test and novel object recognition to measure their depression-like behavior and recognition memory, respectively. Vehicle-treated CMA mice had significantly increased immobile time and a decreased memory index compared to sham mice. Thioperamide-treated mice had no significant behavioral or memory changes when tested. (Multiple Mann-Whitney tests and 2-way ANOVA; Mean ± SEM; Outliers were removed by the ROUT method (Q=1%) from behavioral testing).

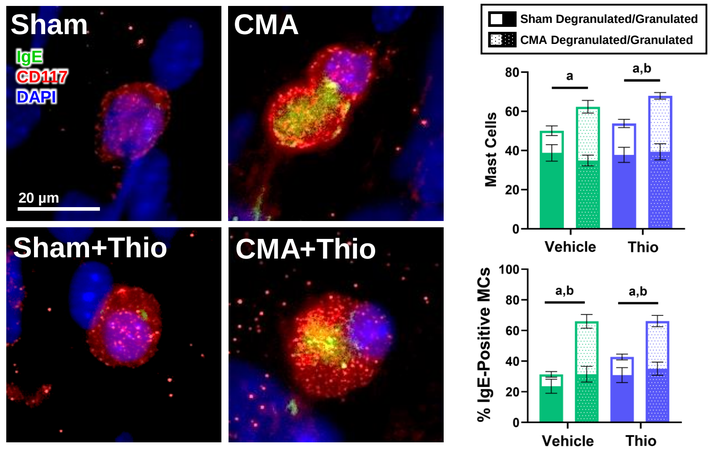

Fig 4. Thioperamide did not change IgE-sensitization or activation of intracranial mast cells. CD117-immunopositive mast cells were abundant throughout the dura of all groups. However, when the number of mast cells per 20 high-powered fields were counted, there was an increase in the number of degranulated (open bars) but not granulated (closed bars) in CMA mice compared to sham mice. Furthermore, in CMA mice, most degranulated mast cells were IgE-immunopositive. Photomicrographs were taken using a 100X objective. (**a** indicates significance of the total mast cells between groups and **b** indicates significance of only degranulated mast cells between groups; Multiple Mann-Whitney tests and 2-way ANOVA; Mean ± SEM).

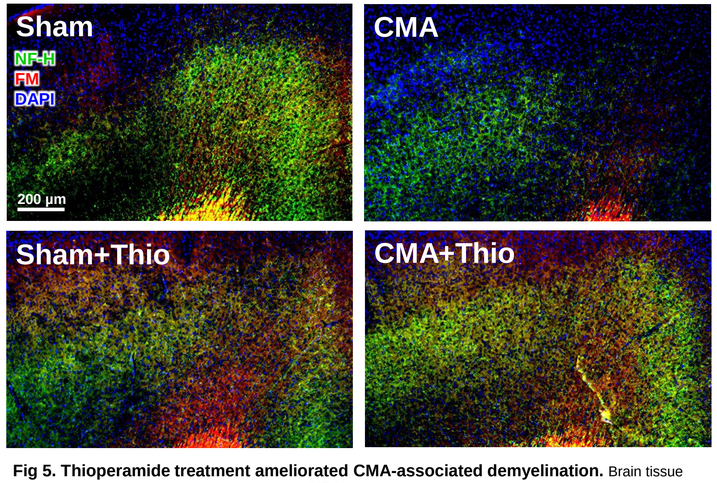

Fig 5. Thioperamide treatment ameliorated CMA-associated demyelination. Brain tissue was immunostained with neurofilament-heavy (NF-H) for neuronal cell bodies and axons, dyed with FluoroMyelin™ (FM) for the myelin sheath. Cortical demyelination was apparent after repeated allergen exposure in saline-treated 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.

Discussion

- Thioperamide effectively prevented allergy-associated depression-like behavior and cognitive impairment without altering the hypersensitivity status of the CMA mice or intracranial mast cell activation, signifying the involvement of H3R in food allergy-triggered neuropsychiatric disorders.
- Thioperamide treatment also appeared to prevent changes in food allergy-associated cortical demyelination.
- We have previously observed cortical demyelination after CMA sensitization[3]. The loss of myelin seemed to have also affected the axonal structure suggested by the decreased NF-H immunoreactivity in the cortex of vehicle-treated CMA mice, although further confirmation is needed.
- Histamine signaling through H3R has been demonstrated to decrease oligodendrocyte differentiation and myelination[2]. Additional investigation is needed to confirm the role of altered oligodendrocyte functions in the progression of cortical demyelination in our mouse model.

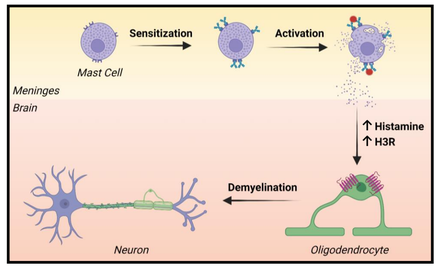

Fig 6. Working hypothesis

Conclusion

Our findings strongly suggest that that excessive signaling through H3R is involved in the development of food allergy-associated neuropsychiatric disorders and neuropathology.

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

References

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